R E P O R T

of

COMMITTEE ON THESIS

THE undersigned, acting as a committee of the Graduate School. have read the accompanying thesis submitted by Mr. Frank J. Piemeisel for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

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Life History and Parasitism of Ustilago zeae (Beckm.) Unger.

A thesis submitted to the Faculty of the Graduate School of the University of Minnesota by Frank J. Piemeisel in partial fulfillment of the requirements for the degree of Master of Science.

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Life History and Parasitism of Ustilago zeae (Beckm.) Unger.

Table of contents.

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Table of contents. (cont'd)

SUMMARY

M615-S-70

Life History and Parasitism of Ustilago zeae (Beckm.) Unger

INTRODUCTION

HISTORICAL

The smut of corn has been known for about two hundred years. The earliest mention of it is to be found in a treatise on the functions of leaves written by the French botanist Bonnet[3] in 1754. Aymen[2] in 1760 described it briefly and again more fully in 1763. Tillet,[24] 1760-1761, was the first to conduct experiments with corn smut, in which he concluded that the black powder found in the smut balls does not cause the disease and that it is not contagious. This opinion was generally held until DeCandolle's[7] work appeared in 1832, inaugurating a period of study of the smut which revealed its true fungus character and its parasitic nature. The most important works were those of Meyen[17] (1838), Leveille[15] (1839), Tulasne Brothers[25] (1847), DeBary[6] (1853), Kühn[14] (1858 and 1874), Fischer von Waldheim[26] (1869) and Brefeld[4] (1883).

Brefeld's[4b] further researches finally gave us the complete life history of the fungus and the course and propagation of the disease. The works of Hitchcock and Norton,[12b] of Clinton,[5] of Arthur[1] and of Stakman[22] in this country have given us added details concerning the fungus.

NOMENCLATURE

The fungus has been placed at various times in the following genera: Lycoperdon, Uredo, Caeoma, Erysibe and Ustilago. It is now known as Ustilago zeae (Beckm.) Unger. A complete synonymy has been given by Stakman[22] in his study of spore germinations of cereal smuts.

THE LIFE HISTORY OF THE FUNGUS

The fungus, as is well known, attacks the corn plant upon which it produces the characteristic boils or smut balls which are the spore beds of the fungus. These boils may appear upon any part of the plant, but are usually largest and of course most destructive when they appear upon the ears of the plant.

In the spring the spores, produced during the previous growing season, germinate upon the ground, in

refuse material, or in manure piles. Upon germination these spores send out a delicate tube or thread, the promycelium, from which are produced small, spindle-shaped, secondary spores, the sporidia. These sporidiamay then be carried to the host plant, where, under favorable conditions, they may germinate by sending out infection threads which penetrate into the tissues of the host and there give rise to the mycelium of the smut. About 10 to 14 days after infection the boils appear in which are found the mature spores of the fungus. These spores may remain in a resting condition until the following spring, or they may germinate and infect the surrounding corn plants. They may germinate on the ground or on the host plant itself. In either case it is probable that sporidia are first produced and that these subsequently infect any young growing part of the plant. When much food material is available, the sporidia may propagate in yeast-like manner, producing an immense number of secondary sporidia and thus increasing the chances for infection many times. As the medium approaches exhaustion the sporidia produce the infection threads. It is thus ap-

parent that a single spore under favorable conditions can indirectly cause hundreds of infections.

PREVIOUS INVESTIGATIONS

Following the first period (1754-1832) of study of corn smut in which little actual scientific work was done and in which the smut was regarded as a symptom of a disease of the plant, as a disease itself, as a product of the plant or as a sting from an insect, came the more exact researches upon which rests our present knowledge of the fungus.

Meyen,[17] 1838 and 1841, was the first to describe the spore forming threads and the formation of the spores from them. This was further investigated by DeBary[6] 1853, Fischer von Waldheim[26] 1869, and Wolff[28] 1874, so that the facts are now well established.

The microscopical characters of the mycelium have been studied by Meyen,[17a] DeBary[6] and Von Waldheim,[26] while its growth and development within the host tissues has been especially well worked out by DeBary and Von Waldheim. Knowles[13] (1889) described the histology of the abnormalities produced in the host plant.

Kühn[14b] was the first to study carefully and delineate the germination of the spores of Ustilago zeae. Further observations were made by Von Waldheim,[26] Brefeld,[4a] Norton[19] and Stakman,[22] The details of spore germination are now well worked out. However, not much attention has been paid to the ecological relations of spores with the exception of the work of Stewart[23] who made a brief study of the thermal relations.

A study of spores naturally includes also a study of the sporidia. Very little definite information on this phase of the problem is available. The general characteristics have been well described but, except the results of a few experiments by Brefeld,[4a] nothing is known in regard to their vitality, resistance to dessication, or germination under unfavorable conditions. Brefeld[4a] states that they are short lived, perishing after five weeks when dry and remaining viable but four weeks when kept in a liquid culture.

The method of infection was first studied by Kühn[14c] who found the corn smut entering at the root node and the lowest stem node. He did not, however, consider

this to be the only place where infection can take place, but seems to have considered it the most likely place, a conclusion which was probably influenced by his own researches and those of others upon some of the other smuts of cereals. Brefeld[4b] demonstrated that infection in the field can take place in any young growing part of the plant. These results were confirmed by the experiments of Hitchcock[12b] and Norton at Kansas, and by Clinton[5] at Illinois. The exact method of entrance of the germ tube is in doubt except for Kuhn's[14c] statement that it pierces the epidermis at the root node. Whether the method of entrance is the same in other parts of the plant, or whether the entrance is through stomata, is still unknown.

Despite the extensive investigations the control of corn smut is still a difficult problem. The obscure points in the life history and course of development of the fungus must be brought to light before any remedy, if such there be, can be found. The present investigation was undertaken for the purpose of determining the relation of these previously unknown facts to the

epidemiology and control of the disease.

EXPERIMENTAL

STATEMENT OF THE PROBLEM

There are four general phases to the problem:

First: The ecological factors influencing spore germination. Second: The ecological factors affecting the growth and vitality of the sporidia. Third: The study of the phenomena of infection. Fourth: Control measures.

The spore germination studies were made with the object of determining the effect of silo fermentation processes upon the vitality of smut spores. A study was also made of the thermal and chemical relations of the spores as an indication of their fate in the silo.

Experiments were made to show the effect of dessication and temperature on the viability of the sporidia and to show their reactions to media.

Artificial inoculations were made in the field and in the greenhouse to determine the optimum conditions for infection and the method of entrance of the fungus into the host plant. Careful observations were

made to determine whether the smut infection is systemic or local.

Such remedial measures as suggested themselves from results secured in the above investigations were tried. Attention was especially centered on the fate of corn smut spores when put into the silo, with a view to determining the possibility of the use of the silo as a means of at least partial control of the disease.

FACTORS AFFECTING THE GERMINATION AND VITALITY OF SPORES

The Silo

A study was made of the effect of the fermentation processes that take place in the silo upon the viability of smut spores. Two samples of smut spores were collected and placed in silos.

Sample I. The spores, which had just matured, were collected September 11th, 1914, enclosed in a cheesecloth bag and placed about one-third of the way down in a wooden silo. A sample of the same lot was kept in the laboratory in a paper bag. The spores were taken out of the silo on November 2nd, 1914, after having been there about nine weeks. As the spores were frozen, they were

thawed out gradually under a bell jar in the laboratory. Germination tests were then made with the results shown in the table below.

Table I. The effect of the silo on spore germination.

Test No.	No. cells	Kind of Spores	Medium	Temp.	Percent of Germination
I	3	silo	Ster. dist. H_2O	21°C	0
I	3	check	" " "	"	32
II	4	silo	" " "	"	0
II	3	check	" " "	"	32
III	4	silo	" " "	"	0
III	3	check	" " "	"	12
IV	5	silo	Mod. Cohns Sol.	"	0
IV	5	check	" " "	"	90
V	6	silo	Tap water	"	0
V	5	check	" "	"	12.5

Tests IV and V were not made until four months after the removal of the spores from the silo as it was thought that the rest period or the soaked condition of the spores might have been the cause of their failure to germinate. In appearance the spores were perfectly nor-

mal, no differences being perceptible even under high powers of the microscope. That the spores were capable of germinating was shown in tests made at the time they were collected. A single test of two cells showed an average germination of 68% in sterile distilled water. The check spore tests made at the same time as the silo spore tests showed an average of 35% germination.

Sample II. These spores were collected September 2nd, 1914, and were put into a brick silo on that day. The spores were enclosed in a bag and placed about fifteen feet from the bottom of the silo and about two feet from the side. They were recovered March 4th, 1915, after having been in the silo for over twenty six weeks. The spores when recovered were frozen and a part of the sample was kept frozen by placing the spores in glass containers on the window ledge (cold moist). A part of the sample was thawed out gradually in the laboratory under a bell jar (warm moist) and then a portion of this sample was air dried at room temperature. Tests were made from each lot with the results shown in the table below.

Table II. The effect of the silo on spore germination.

Test No.	No. cells	Lot	Kind of Spores	Medium	Temp.	Percent of Germination
I	8		Silo	Mod. Cohns	21°C	0
I	5		"	Dist. water	"	0
I	3		Check	" "	"	5-10
I	4		"	Mod. Cohns	"	95-100
II	7	cold moist	Silo	" "	"	0
II	5	warm moist	"	" "	"	0
II	7	air dried	"	" "	"	0
II	5		Check	" "	"	95-100
III	5	cold moist	Silo	" "	"	0
III	5	warm moist	"	" "	"	0
III	5	air dried	"	" "	"	0
III	5		Check	" "	"	90-95
IV	5	warm moist	Silo	Tap water	"	0
IV	5		Check	" "	"	15
IV	5		"	ster. dist. H_2O	"	15

In this case, as in that of Sample I, a germination test was made at the time the freshly matured spores

were collected, the test showing 26% germination in water. The tests made with the check spores, as shown in the table above, gave an average of 54% germination. Since the temperature during fermentation of the silage does not rise above 86°F., except in the topmost layers where destructive fermentation occurs, the temperature can hardly have affected the viability of the spores. The gaseous exchange in silage fermentation and the action of the gases on the spores is not known. The determining factors are more probably the chemical changes and chemical substances produced in silage.

Acids

The formation of silage from green corn is invariably accompanied by the development of acid. The production of the acids proceeds very rapidly and usually attains its maximum in two weeks. The total acidity of silage, according to Esten and Mason[9], is about 1.0% to 1.5% The principal acids in order of their importance are lactic, acetic and propionic. Lactic acid makes up more than half of the acids and acetic three fourths of the remainder. Traces of butyric, formic and valerianic acid

have also been found. Butyric acid is found in considerable amount only in spoiled silage. Alcohols are also found in appreciable quantities, ethyl alcohol being most abundant. The exact processes involved in the formation of these substances are not definitely known. Esten and Mason[9] state that the silage fermentation proceeds best at 75°F. to 85°F.

In order to determine whether the chemical substances present in the silo are capable of inhibiting spore germination or possibly of destroying the spores themselves, germination tests were conducted as shown in the following table:

Table III. The effect of acids on spore germination.

Test No.	No. Cells	Medium	Temp.	Germ. %	Remarks
I	3	1% sol. acetic acid	21°C.	0	
I	3	" " lactic "	"	0	
I	3	½% " acetic "	"	5	
I	4	" " lactic "	"	20	
I	1	check: ster. dist. water	"	25.6	

Table III. The effect of acids on spore germination.
(continued)

Test No.	No. Cells	Medium	Temp.	Germ. %	Remarks
II	3	½% acetic acid	21°C.	2	Germination in 1 cell only
II	3	" lactic "	"	0	
II	2 check	ster.dist.water	"	0	
II	3 check	" " "	22-34°	0	
III	3	1% lact.& 1% acetic	21°C.	0	
III	2	½% lactic acid	"	11	Germination in 1 cell only
IV	2 check	ster. dist. water	"	4	
IV	3	½% acetic & ½% lact.	"	0	
IV	3	⅓% " " ⅓% "	"	0	
IV	3	1% " " 1% "	"	.1	
IV	1 check	ster. dist. water	36°C.	1	
V	5	Silage juice (diluted somewhat)	21°C.	10	
V	5 check	ster. dist.water	"	15	
VI	7	silage acid mixt.	"	0	
VI	3	ster. dist. water	"	23	

Table III. The effect of acids on spore germination. (continued)

Test No.	No. Cells	Medium	Temp.	Germ. %	Remarks
VII	10	silage acid mixt.	21°	0	Acid in the arms
VII	9	water	"	0	" " " "
VII	9	silage acid mixt.	"	0	Water in arms
VII	4	distilled water	"	15-20	Only two cells germinated

From the above results it will be seen that spores do not germinate readily in any of the acid solutions. No germination was obtained in 1% of either acetic or lactic acids, nor in ¼%, ½%, and 1% combinations of the two. The silage acid mixture also inhibited spore germination. Although ½% solution of acetic and lactic, ¼% solution of lactic, and diluted silage juice permitted of spore germinations, the results of these tests are not wholly convincing. Water was used in the arms of the Ward cells and as there was often a breaking up of the original drop and considerable condensation of moisture on the cover slip, there is always the possibility that the germination of the spores can be explained either

through a coalition of the acid drop with the water and the consequent dilution or the total dissolution of the drop and its replacement by water. Test No.VII was carried out with this idea in mind, but no germination occurred in any of the cells except in two of the checks, so that the test can hardly be considered conclusive.

The presence of yeasts, bacteria, or other fungi (especially Oidium forms) in the acid drops might bring about a destruction of the acid and hence make the germination of spores possible in those drops. In general, therefore, it can only be said that the presence of acids inhibits or tends to inhibit the germination of corn smut spores.

Temperature

The experiments dealing with the temperature relations of spores were begun after it was found that spores which had been kept in the silo would not germinate. But, according to Esten and Mason,[9] the temperature in the silo never rises above 86°F., except in the first foot of silage where destructive fermentation takes place, owing to the abundance of oxygen. Heidig[18] reports a maximum temp-

erature of 91°F. A temperature of 86°F. is nearer the optimum than the thermal death point. Stewart[23] found that immersion in water at 52°C. for fifteen minutes and a dry oven heat of 105.5°C. to 106°C. for the same length of time killed the smut spores.

In the experiments made by the writer the dry spores were placed in vials which were then suspended in a dry oven at various temperatures. The following table shows the results:

Table IV. The effect of temperature on spores.

Test No.	Temp. to which exposed	Time exposed	No. Cells	Germination*
I	101°- 103°C.	5 minutes	2	(50% (0%
I	100°- 101°C.	7 "	2	0%
I	102°C.	5 "	2	0%
I	101.5°-102.5°C.	5 "	2	0%
I	check		2	(40% (60%
II	103°-104°C.	5 "	2	0%
II	check		2	(12.9% (38%

*The germination tests were all made in sterile distilled water at 22°C.

The results of the experiments seem to show that most if not all of the spores are killed after an exposure to a temperature of 100°C. or more for five minutes. There was but one exception and that was in one lot of spores thus exposed to a maximum of 103°C. In one of the cells, 50% of the spores germinated. The very erratic behavior of smut spores in water makes it difficult to interpret the results or base any conclusions upon them.

Seasonal Factors

Fresh spores were collected from time to time and observations made on their germination. The first trials were made in the summer of 1913 and they germinated readily in tap water at room temperature. The tests made in the summer of 1914 were more complete, beginning with the very first smut spores produced in the field. The first test of fresh smut spores was made June 24th, the last test on October 10th. In all fifteen distinct tests were made. It was found that fresh spores germinated quite readily in water. The average per cent of germination as found for the whole series was 42.8. In-

dividual cells showed a range of from 0% to 85%. Fresh spores germinated much better than did spores from the same lot in winter.

An attempt was made to find out whether spores would germinate more readily in tap than in distilled water. No marked difference could be found. Two cells of each at 30°C. gave 0% germination. Two cells of each at room temperature (22°C.) gave : tap water 37.5%, sterilized distilled water 30.5%. Likewise, no difference was found in the germination of spores in sterile rain water as compared with sterile distilled water.

Incubating the cells at 24°C. to 28°C. did not seem to influence the amount or rate of spore germination.

In one case a difference in germination occurred in the same lot of spores. The tests were made two days apart, eleven cells being used in the two tests. Fifteen per cent of the fresh spores from a young part of a boil which had just matured germinated. Of the spores from the same boil, but taken from a drier and apparently older portion, an average of 61.8% germinated. The difference here might possibly be explained on the basis

of maturity, the maturer and drier spores germinating better. The above tests at least show the erratic behavior of spores when placed in a water solution. In tests with a modified Cohn's solution in all cases 95% to 100% of the spores germinated.

STUDIES OF THE SPORIDIA

Methods

In the studies of sporidia, pure cultures of the smut were used throughout. The spores were sown in poured plates of beerwort agar. In about two days the spores germinated and the position of the colonies was marked. At the end of the four or five days when the colonies had attained about the size of a pin head they were transferred to beerwort agar in tubes. Beerwort agar was found to be the best solid nutrient medium, although a good growth was also obtained on carrot agar.

Morphology

When the colonies first appear they are round, raised, convex, opaque, slightly shiny to dull, light cream colored. As the colonies grow older the edge becomes somewhat lobed and irregular, and the surface be-

comes convoluted, ridged, or sharply papillate. The color deepens with age, turning to a light lavender shade in old cultures. The texture is first soft and ropy, then assumes a mucilaginous to a rubbery consistency or, when kept quite moist, remains butyrous in nature.

The sporidia which are abjointed from the sides and occasionally from the end of the promycelium make up the colonies as they appear on the agar. These sporidia are of the same nature as those produced beneath the surface in liquid media. The sporidia produced in the air from a liquid culture are small, sharply spindle form, and fairly thick walled. They are produced in long chains. Those produced within the liquid or on solid nutrient media are larger than the air conidia, not as thick walled and are somewhat rounded at the end. They are plumper, contain more oil globules and are not produced in long chains. The walls are apparently somewhat mucilaginous. In continuous culture the sporidia produce long germ tubes, only the ends of which are densely protoplasmic and which, therefore, can become resting segments possessing all the properties of sporidia. The

germ tubes become much entangled and give the culture its rubbery consistency, while the disintegration of the empty portions of the hyphae give it its mucilaginous character. A smear from such a culture dries almost instantly and becomes quite brittle. While sporidia produced in culture may not be exactly like those produced in nature, still they must be very similar to those which we imagine are produced in such great abundance in the manure piles. While morphologically they may, therefore, differ slightly from the sporidia produced in nature, physiologically they are identical.

Thermal Relations

A complete understanding of the thermal relations of corn smut sporidia would not only be of value in throwing additional light upon the phenomena of infection, but it would also show more clearly the exact methods of propagation of the fungus. An attempt was therefore made to ascertain the minimum and maximum temperatures. In this study as elsewhere pure cultures of the sporidia were used and both dried and actively vegetating sporidia were tested.

The following tests were made to determine the maximum temperature of sporidia in a moist condition:

Test 1 - Sporidia in 2% sugar solution in four cells were exposed to a temperature of 25° to 37°C. They grew, producing more germ tubes than the check at 21°C.

Test 2 - Sporidia in sterile distilled water were exposed to 28.5°C. for one and one half hours. They budded profusely at this temperature. The temperature was then gradually increased to 39°C. and then to 43°C. They grew more slowly as the temperature rose and although greatly vacuolated at 43°C. still they appeared to be alive. Two other cells kept first at 21°C. and then at 39°C. gave similar results.

Test 3 - Sporidia in sterile distilled water in four cells were kept at 28.5° to 31°C. for twenty four hours. They budded abundantly at 28.5°C. for twelve hours. At 31°C. they became somewhat vacuolated and produced slender germ tubes. Then the temperature was raised to 39°C. at which they produced long, thin germ tubes. At 45°C. they apparently were still alive, but contents were greatly vacuolated.

Test 4 - Sporidia in sterile distilled water in six cells exposed to 43°C., the sporidia and germ tubes becoming much vacuolated. At 46°C. some very nearly hyaline and these were probably dead. The contents of the others, while still refractive, were greatly broken up by large vacuoles. At 47°C. they appeared to be all dead. At this stage a 2% sugar solution was added and the cells put out at room temperature. No growth was evident.

Test 5 - Exposed sporidia in drops of water in two cells to 45°C. and one cell to 39°C. for several hours. They subsequently grew weakly at room temperature.

Test 6 - Sporidia in sterile distilled water in cells were exposed to 35°C., then to 43°C., and finally to 46°C. The result was the same as in test 4, i.e. diminished activity accompanied by vacuolation of cells and germ tubes is evident up to 43°C. At about 46°C. the thermal death point is probably reached for most of the sporidia.

Test 7 - Sporidial cultures on beerwort were exposed to 49°C., the one for two days, the other for three days. Transfers made to beerwort showed no growth. The sporidia

placed in water in hanging drops were also dead.

The following tests were made to determine the minimum temperature of sporidia in a moist condition:

Test 1 - Five cells containing sporidia in sterile distilled water were exposed to the cold until the drops were frozen solid. The cells were then kept at 21°C. The sporidia appeared to be unharmed as they grew actively.

Test 2 - Sporidia in suspension in water were kept at 0°F. for four days. The sporidia were then placed in 2% sugar solution and kept at room temperature. A few appeared to be killed and disintegrating. The rest budded, the second generation of sporidia appearing as vigorous as ever. The weaker cells sent out germ tubes.

Test 3 - Sporidia on an agar slant were exposed to 0°F. for three hours, at the end of which time the mass was frozen solid. The sporidia were then placed in sterile distilled water in three cells. They grew.

Test 4 - Two cells with sporidia in 2% sugar solution were made up from a culture on an agar slant that had been exposed to alternate freezing and thawing for

thirty one days. No growth took place.

Transfers from the two tubes were also made to beerwort agar, but no growth took place.

Test 5 - Two culture tubes were put out at a temperature of 30°F. and left at the alternate freezing and thawing temperature for six days. Transfers were then made to beerwort agar, but the sporidia had apparently been killed.

Test 6 - Tubes of sporidia on beerwort agar were packed in snow and placed in a box. The box was then buried in the snow in a shaded place. They were kept in this frozen condition for thirty seven days during which the temperature was never lower than 10°F. At this time the snow had all melted and the agar was just thawing out. The four tubes set out represented the following:

Tube 1 - A smut colony that had been kept in continuous culture for twenty months.

Tube 2 - A smut colony kept in continuous culture nine months.

Tube 3 - A smut colony kept in continuous culture eight months.

Tube 4 - A smut colony kept in continuous culture two months.

Transfers were then made to beerwort and all showed developing colonies, except the transfer from No.1 which showed no growth.

Table V - The maximum and minimum temperatures of dessicated sporidia.

No. Cells	Dessicated	Temp. exposed to	Time	Medium	Results
1	20 days smear	22°-34° F. (alt. freez. & thaw.)	12 days	Ster. dist. H_2O	Grew
1	"	" " "	" "	"	No growth
2	16 days smear	20° F.	12 hrs	"	Grew
1	7 days smear	36°-29° F.	" "	"	"
1	6 days smear	alt. freez. & thaw.	31 days	2% sugar sol.	No growth
1	1 days smear	15°-20° F.	14 "	Ster. dist. H_2O	Grew
3	smear one day	28.5° C.	25 hrs	Ster. dist. H_2O	Grew
3	smear ten hrs	28.5° C.	9 "	"	"
2	smear 20 days	30.5°-35° C.	26 "	"	"

Table V - The maximum and minimum temperatures of dessicated sporidia. (continued)

No. Cells	Dessicated	Temp. exposed to	Time	Medium	Results
1	drop dry: 20 days	28.5°-31°C.	24 hrs	Sterdist. H_2O	a few germ weakly
1	smear 16 days	28.5°-31°C.	24 hrs	Sterdist. H_2O	Grew

The optimum temperature of the sporidia is, therefore, probably 25°C. to 28°C. Maire[16] gives the optimum temperature as 20°C. to 25°C. Sporidial activity in liquid media is greatly decreased by temperatures over 40°C. Increasing vacuolation of the cells results as temperatures go above 35°. The vacuolation in some cases produces a distinct swelling resembling very much a spore forming thread which is enlarging at its end to produce a spore. The thermal death point is probably about 46°C.

Attempts to get the lower limits of the death point of sporidia in liquid media gave negative results as the sporidia withstood the severest cold of the winter. Alternate freezing and thawing, however, kills sporidia in a moist condition. Dessicated sporidia, on the other hand, are not only able to withstand the severest cold,

but in most cases are not severely injured by alternate freezing and thawing. The maximum temperature of dessicated sporidia have not as yet been definitely determined.

Moisture Relations

Methods - In most cases the sporidia were taken direct from the pure cultures on beerwort agar and smeared on sterile cover slips. The slips were then placed in sterile petri dishes and were allowed to dry at room temperature for various lengths of time. In other cases a water solution was first made of the sporidia and drops of this were then transferred to the slips and allowed to dry as above. When the test for viability was made a drop of the desired medium was added and the slips were then mounted on Ward or Van Tieghem cells.

Table VI. - The effect of dessication on the vitality of sporidia.

Test No.	No. Cells	Method	Days dried	Medium	Temp.	Observations.
I	2	Grown in cells in H_2O 2 days slip dried	3	Ster. dist. water	21°C	Grew actively
II	3	Spores germinating in H_2O in cell Slip dried	3	Ster. dist. water	"	Sporidia continued to bud and germinate

Table VI. - The effect of dessication on the vitality of sporidia. (continued)

Test No.	No. Cells	Method	Days dried	Medium	Temp.	Observations.
III	5	In H_2O in cells: Slip dried	1	Ster. dist. water	21°C.	Grew
IV	1	Smears made on slips	12	2% sugar sol.	"	Budded and germinated
IV	1	Smears made On slips	56	Mod. Cohn's	"	Grew
IV	1	Smears made on slips	6 @ 21°C. 31 " alt. freez. & thawing	2% sugar sol.	"	No growth in 7 days
V	1	In water on slips	12	2% sugar sol.	"	Grew
V	2	" "	48	Mod. Cohn's	"	No growth
V	1	" "	20 @ 21°C. 24 hrs. @ 28.5°-31°C.	Ster. dist. water	"	A few germinated weakly
VI	1	Smears on slips	7	2% sugar sol.	"	Grew vigorously
VI	1	Smears on slips	51	Mod. Cohn's	"	Budded & germinated Est.60% dead
VII	1	Smears on slips	16 @ 21°C. 24 hrs. @ 28.5°-31°C.	Ster. dist. water	"	Grew
VII	1	" "	1 da. @ 21°C. 14 " " 15°-20° F.	Ster. dist. water	"	Grew
VIII	3	In water on slips	124	Mod. Cohn's	"	No growth appeared in 8 days

Table VII. - The viability of sporidia dessicated in light and darkness.

No. Cells	Cond. Dried	Method	Days Dried	Medium	Temp.	Observations
2	Light	Smear on coverslip	1	Ster. dist. H_2O	21°C.	Grew
2	Dark	"	1	" " "	"	"
1	L	"	1½	" " "	"	"
1	D	"	1½	" " "	"	"
1	L	"	6	" " "	"	"
1	D	"	6	" " "	"	"
1	L	"	8	" " "	"	"
1	D	"	8	" " "	"	"
1	L	"	11	" " "	"	"
1	D	"	86	" " "	"	"
1	L	"	87	" " "	"	Germinated profusely
1	D	"	149	" " "	"	Grew

From the results shown in the above tables it is apparent that sporidia can withstand long periods of drying without serious injury. Sporidia when direct from pure culture withstood a drying of 149 days at room

temperature. Not all of these sporidia, however, remained alive. Sporidia first placed in water and then dried seemed to be less resistant to dessication. Sporidia thus treated grew after drying for twenty days, but no growth was apparent when dried for forty eight days. The latter result may be somewhat misleading as the number of sporidia in a water drop is much smaller than the number put on by means of a smear direct from the pure culture.

There was no noticeable difference between sporidial smears dried in the dark and in the light. The light, therefore, is probably not a very strong factor in causing the death of sporidia.

The above results are not in accord with those of Brefeld[4a] who found that sporidia were killed when dried five weeks. Nor are they in accord with the statements of Arthur and Stuart[1] who characterize the conidia as "shortlived" and further add "these are borne through the air which must be rather moist or the sporidia will be killed by drying."

Not only were the sporidia alive after twenty-

one weeks as shown above, but when placed in water or a nutrient liquid they germinated very readily and hence in all probability still possessed the power of infection.

Table VIII. - The effects of temperature on sporidia in a moist condition.

Test No.	No. Cells	Temp. dried	Days dried	Temp. to which exposed	Time exposed	Medium	Observations
I	1	21°C.	20	alt. freezing and thawing -5° to 1° C.	18 days	Ster. dist. water	Grew
"	1	"	"	" "	" "	"	No growth
"	2	"	16	-7° C.	12 hrs.	"	Grew
"	1	"	7	3° C.	" "	"	"
"	1	"	5	43° C.	15 min.	"	"
"	1	"	19	40°- 50°C.	16 hrs.	"	A few germ. weakly
"	1	"	12	45° C.	15 min.	"	Grew
"	1	"	18	54°- 55°C.	15 min.	"	No growth
II	1	"	6	alt. freezing and thawing	31 days	2% sugar sol.	"
*II	1	"	20	28.5°- 31°C.	24 hrs.	Ster. dist. water	A few germ. weakly
II	1	"	16	" "	" "	"	Grew
"	1	"	1	-10° to -7°C.	14 days	"	"

*Sporidia in water - all the rest are sporidial smears.

Sporidia dried for some time and then exposed to low temperatures are apparently not injured. When exposed to alternate freezing and thawing, however, there seems to be some injury, as two tests out of three showed no subsequent growth. Smears direct from pure culture were not killed by drying for one day at 21°C. and for fourteen days at a constantly low temperature of 7° to 9° C. But sporidia dried six days and then exposed to alternate freezing and thawing for thirty one days were killed.

Sporidial smears direct on slips from the pure culture were unaffected by drying for sixteen days at 21°C. and then at 28.5° to 31° C. for one day. Sporidia first placed in water, then dried for twenty days, also withstood the same temperature. Sporidial smears were also not killed by 40° to 50° C. for sixteen hours, after drying for nineteen days, but appeared to be killed at 54° to 55° C. for fifteen minutes, after drying for eighteen days. The thermal death point of dried sporidia would, therefore, probably be near 54° to 55° C.

Chemical Relations

Sporidia were placed in various solutions of acetic and lactic acid, in corn silage juice, and in a mixture of various acids in such proportion as to approximate true silage juice. If corn smut spores germinate when placed in a silo, it was thought that by means of tests with various acids commonly produced in silage that the fate of the sporidia might be determined. The sporidia in each case were obtained direct from pure cultures on beerwort agar and were transferred to the solution from which hanging drops were made in either Ward or Van Tieghem cells.

Table IX. - The effect of silage acids on sporidia.

Test No.	No. Cells	Medium	Temp.	Observations
I	2	½% sol. acetic	21°C.	Sporidia apparently larger than in ½% solution growing actively.
"	2	½% sol. acetic	"	Growing
"	2	½% sol. lactic	"	Germinating profusely. Inclined more to germinating than to budding.
"	2	½% sol. lactic	"	" " "
"	1	2% sugar solution	"	Much more abundant growth. Sporidia larger.

Table IX. - The effect of silage acids on sporidia. (continued)

Test No.	No. Cells	Medium	Temp.	Observations
II	3	silage juice	21°C.	Budding and germinating about the same as in the check.
"	1	ster. dist. H_2O	"	" "
III	2	½% acet. ½% lact.	"	Grew actively.
"	1	"	35°C.	Dried up
"	2	1% acet. 1% lact.	21°C.	Grew quite actively.
"	1	" "	35°C.	Dried up
"	2	ster. dist. H_2O	21°C.	Grew actively.
IV	5	silage acid mixt.*	"	No growth apparent. The sporidia after a time appeared starved as compared with those in water or those taken direct from pure culture.

*The silage acid mixture was made up of the following:
295 gm. H_2O (dist.)
2.9 gm. lactic acid
4.1 gm. of 50% acetic acid
Propionic acid-a trace
Butyric acid - " "

Sporidia apparently can grow in acetic and lactic acid solutions of 1% concentration or in a mixture of the two. In the lactic acid the sporidia tend to produce a greater number of germ tubes which probably indicates that the medium is slightly unfavorable for growth.

The sporidia also grow well in expressed silage juice. It seems quite probable, therefore, that if spores do germinate in the silo, the sporidia may continue to live in the silo for some time. Whether long exposure to the action of the acids would be detrimental or not was not determined.

A more concentrated mixture of acids such as was used in the silage acid test proved to be deleterious to the growth of the sporidia. The sporidia appeared starved and became greatly vacuolated, a condition which probably precedes the death of the sporidia. The results here obtained are not in accord with those obtained when silage juice itself was used. The effect of the traces of butyric and propionic acids alone upon the sporidia has not yet been determined.

INOCULATIONS

Spores and sporidia from pure cultures were both used in making all inoculations. Various methods of inoculation were used. Where spores were used they were either dusted directly into the tops of the plants or they were first mixed with moist soil and then applied.

In a few cases the spores were applied in suspension in water. Most of the inoculations were made with sporidia from pure culture. Two general methods were used. In some cases the sporidia were smeared directly from the agar to the part to be inoculated. In the other method, the sporidia were first placed in water and this suspension of sporidia was then applied by means of a dropper or a hypodermic syringe. The hypodermic syringe was used when it was desired to inoculate the very young parts which had not yet been unfolded.

During the summer of 1913 and 1914 a total of 2064 plants were inoculated and 522 plants were used as checks. The highest percentage of infection obtained was 70.8 in the summer of 1913 and 84.2 in the summer of 1914. In both cases suspensions of sporidia in water were injected as near the growing point as possible.

The results of the inoculation showed that infection by artificial means was influenced more by the age of the plants than by the weather conditions. Plants that are healthy and vigorous and have attained a height of about three feet are the most susceptible. It is very

difficult to infect very young or old plants. These results are in accord with those obtained by Brefeld[4b] and by Hitchcock and Norton[12b]. Inoculations were also most successful when made late in the afternoon or evening of humid or cloudy days. This, of course, allowed the sporidia to penetrate into the tissues of the host before they were dried by the heat. Experiments carried on by Clinton[5] at Illinois showed that mutilation of the corn plant, when about ready to tassel, tends to increase their susceptibility. The experiments of the writer likewise showed that injury tends to increase the chances for infection. When young leaves were injured and then inoculated the resulting infection always spreads from the point of injury as a center. It was evident eight to fourteen days after inoculation that infection had resulted. Many infected leaf areas, however, never developed sufficiently to produce mature spores. This was probably due in most part to the rapid maturing of the tissues of the leaf and the consequent inability of the fungus to spread through these older tissues. It was found in all cases that injury is not necessary to the

entrance of the fungus, and that the age and vigor of the plant is by far the most important factor concerned in infection. Arthur and Stuart[1] of Indiana report that early planting, close planting, and moist and rich soil increase the amount of smut. Rainy periods were also closely followed by outbreaks of smut in the corn fields. The observations made by the writer point to the same conclusions.

All the infections produced by artificial means were local in character. Many observations were also made on plants in other fields but no evidence of systemic infection could be found. Many plants that were found smutted when quite young matured healthy ears. If the smut is systemic in nature, it must gain entrance to the plant while the latter is in the seedling stage. The results of the inoculations above cited showed that infection of young corn plants is very difficult. Further inoculations were made in the greenhouse upon germinating seeds. Forty three young plants were dipped into a water suspension of sporidia and then planted in pots. None of the plants were smutted after growing for

a month and a half. Brefeld[4b] and Kühn[14c] both succeeded in inoculating a few seedling plants but in all cases such plants were destroyed by the smut. In order to further ascertain the results of early infection, ten plants in a fodder corn field were selected and marked. All of the plants were about a foot in height and showed signs of smut in varying degrees. Of the ten plants thus marked, eight were killed by the smut in less than a month, and the other two were greatly stunted. These two plants, however, produced healthy ears. The above results seem to indicate rather clearly that the infection is local rather than systemic, and that the corn smut fungus does not exhibit as high a degree of parasitism as do some of the other smut fungi of cereals which are able to live in the growing points without causing injury to the host until the time of seed production.

In the greenhouse and field infection experiments, two other interesting facts were revealed. Successful infection was obtained in the field with spores that were five years old, having been collected in the summer of 1909. There was only two per cent of smut in

the check plot while in the plot inoculated with the spores collected in 1909 there was six per cent of smut. In the greenhouse two plants were inoculated with sporidia that had been kept frozen for a month. The sporidia had been kept in continuous culture for eight months. One, a small plant about two feet high, became smutted in the five leaves inoculated. The other, about five feet high, inoculated in an ear produced there a large mass of smut. These results are significant because they show that sporidia do not lose their infecting power when frozen or when kept in continuous culture for a considerable length of time. Brefeld,[4a] who worked with liquid media, states that the sporidia lose the ability to send out germ tubes and the power of infection when kept in continuous culture for a year. It is not probable that the sporidia behave in the same way when grown upon solid nutrient media for the same length of time.

CONTROL MEASURES

The losses occasioned by corn smut are not always as evident as those due to other diseases. The loss can be estimated only with difficulty because the smut

attacks all parts of the plant and hence causes a decrease in vigor. Where the smut attacks the plant below the primary ear, the smut hyphae grow at the expense of the ear. It is only when the fungus attacks the ear itself that we can get a definite idea of the loss. Losses of from 0.5% to 25% of the crop have been reported in other states. It is doubtful if the average loss due to smut in any state ever exceeds 5%. Individual fields may, of course, show greater loss, especially the fields of sweet corn and popcorn. Henry[4] of Wisconsin in 1883 reported a loss of from 5% to 25% of the corn crop. Pammel and Stewart[20] of Iowa in 1893 estimated the loss in that state at less than 1%. Taking an average of o.5% as a basis, the loss in money amounted to $500,000. annually. Selby and Hickman[21] of Ohio in 1897 estimated the loss in that state at o.5%, an actual loss of $125,000. Wheeler[27] of Michigan in 1895 also placed the loss of ears at 0.4%. A. S. Hitchcock[12a] of Kansas in 1899 reported that the usual amount of smut present in the fields was 6.2%. From counts that have been made at the Minnesota Experiment Station, the average percentage of ears smutted, for the

three years 1912, 1913 and 1914, was 2.8. The average amount of smut found in the fields ranges from 5% to 10%. Assuming that the actual loss here is 0.5%, the annual monetary loss would be $189,000. The control of corn smut then becomes an important problem.

An experiment was therefore carried out with different fertilizers to determine their effect upon the amount of smut present. A single variety of corn, Minnesota No.13, was used in the test. The corn was planted May 8th, 1914, in five plots of six rows each. The manures and superphosphate were incorporated with the soil. The sodium nitrate was applied soon after the plants appeared above ground. The results were as follows:

Table X. - The effect of fertilizers on the amount of smut present.

Plot	Fertilizer used	No. of plants	No. smutted	% smutted
1	Sodium nitrate: 1# per sq. rd.	198	38	19.1
2	Check plot No fertilizer	195	20	10.2
3	Superphosphate: 3# per sq. rd.	214	36	16.8
4	Old cow-manure:	210	44	20.9
5	Fresh cow-manure:	248	48	19.3

It will be seen that all of the plots show an unusually large amount of smut. This may be due to the earliness of the planting, the effects of the fertilizers, and to the fact that the plots were near a badly smutted field. The results show, however, that fertilizers increase the amount of smut. This is no doubt due to the more succulent growth produced on the richer soils. A healthy, vigorous, succulent plant is much more liable to attack than a plant that is weak and unthrifty, because in the latter the tissues mature rapidly and the chances for infection are consequently diminished. These results also are in accord with those of Arthur and Stuart[1] previously mentioned.

Varietal resistance as a means of controlling the smut was also considered. No definite results however can be obtained from variety tests in a single year. In the test made, ten varieties of field corn and eight varieties of sweet corn were planted on ground that had been fertilized with fairly old cow-manure mixed with smut. There was an average of one hundred plants of each variety. From 2.0% to 8.1% of smut developed on the

field corn varieties. Only one variety remained unsmutted. The amount of smut in the sweet corn varied from 0.9% to 2.0%, only one variety having no smut. These two unsmutted varieties however had had as much smut as any of the others in 1913. Hitchcock and Norton[12b] report little or no varietal resistance. Further investigation is necessary before the final conclusions can be drawn. A seed treatment experiment with formaldehyde gave negative results thus further substantiating the work of numerous other investigators on this point. Crop rotation, the use of old rather than fresh barnyard manures, the ensiling of corn and the destruction of the smut masses themselves are then the chief methods of control.

SUMMARY

1. The fermentative changes in the silo destroy the germinating power of smut spores.

2. Lactic and acetic acids inhibit or tend to inhibit spore germination even when present in lower concentration than in normal silage juice.

3. The temperature of the silo cannot be the factor

which destroys the viability of the spores, since the temperature never rises above 33°C.[18] The thermal death point of corn smut spores in water is 52°C. for fifteen minutes.[23] That of dry spores is 100°C. for five minutes or slightly higher.

4. Fresh spores germinated more readily than older ones.

5. Beerwort agar was found to be the best solid nutrient medium for the artificial propagation of the smut.

6. The optimum temperature of sporidia is 25°C. to 28°C. Temperatures above 35°C. greatly decrease sporidial activity and cause increased vacuolation, a condition which probably precedes death. The thermal death point of moist sporidia is about 46°C., of dessicated sporidia 54°C. to 55°C.

7. Sporidia can withstand temperatures as low as -25°F. without serious injury. Alternate freezing and thawing kills moist sporidia but does not seriously injure dessicated sporidia. Sporidia that had been kept frozen for a month were apparently uninjured and still retained the power to infect.

8. Sporidia dessicated for twenty one weeks at room

temperature were not seriously injured. These results are not in accord with the current opinion that sporidia are sensitive and shortlived nor with the results of Brefeld[4a] who found that sporidia were killed when dried five weeks.

9. Sporidia can apparently grow in one per cent solutions of either acetic or lactic acid, but become starved and vacuolated when placed in a mixture of lactic, acetic, propionic, butyric acids in such concentration as they appear in silage juice.

10. That sporidia can grow in silage juice may be due to the presence of unfermented sugars or to the destruction of the acid by micro-organisms.

11. The best results in artificial inoculations were obtained late in the afternoon or evening of humid or cloudy days. Injury seems to increase the chances for infection. Very young or old plants are difficult to inoculate successfully. The age and vigor of the plant are most important factors concerned in infection.

12. All the evidence at hand indicates that the infection is local rather than systemic.

13. It is much easier to secure infection with sporidia than with spores. Spores five years old and sporidia that had been frozen for a month do not lose their power of infection.

14. Brefeld[4a] reports that sporidia lose the power of producing germ tubes and hence infection, when kept in continuous culture for a year in liquid media. Successful infection was obtained by the writer with sporidia kept in continuous culture for eight months on solid nutrient media.

15. The average amount of smut in Minnesota is about 5% to 10%. About 2.8% of the ears are affected. Assuming that 0.5% of the ears are destroyed the actual annual loss is $189,000. Fertilizers and close planting increase the amount of smut in the fields. No marked varietal resistance was found. The silo may aid in the control of the fungus. Crop rotation is of the utmost importance.

Literature Cited.

1 Arthur, J.C. and Stuart, Wm.- Corn Smut, Twelfth Rep. Ind. Agric. Exper. Sta. for 1900:84-135.

2[a] Aymen,- Recherches sur le progres et la cause de la nielle, Mem. Acad. Sci. Paris, 3:68-85. 1760.

2[b] Aymen,- Second memoire sur les maladies des bles, Mem. Acad. Sci. Paris, 4:358-398. 1763.

3 Bonnet, Ch.- Recherches sur l'usage des feuilles:327-330. Gettingue. 1754.

4[a] Brefeld, O.- Die Brandpilze, Bot. Unters. Heft. 5:67-75. Leipzig. 1883. Abs. in Oesterr. Bot. Ztschr. for 1883:267; Jour. Royal Micr. Soc. 2:877, 1883; Gard. Chron. for 1889:267-268; Amer. Nat. 30:224-226. 1896.

4[b] Brefeld, O.- Infection mit Maisbrandconidien, Unters. aus. d. Gesamtg. d. Myk. Heft. 11:52-92. Munster, 1895. Abs. in Bot. Centr. 64:273-281. 1895; Hedw. 34:138-140. 1895; Amer. Nat. 30:137-142. 1896.

5 Clinton, G.P.- Smut of Indian Corn and Teosinte, Ill. Agl. Expt. Sta. Bull. 57:321-335. 1900.

6 DeBary, A.- Unters. über die Brandpilze:4-8. 1853.

7 DeCandolle, A.P.- Phys. vegetale 3:1453. 1832.

8[a] Dox, A.W. and Neidig, R.E.- The volatile aliphatic acids of corn silage. Ia. Agl. Coll. Research Bull. 7. 1912.

8[b] Dox, A.W. and Neidig, R.E.- Lactic acid in Corn Silage, Ia. Agl. Coll. Research Bull. 10:1913.

9 Eaton, W.M. and Mason, Christie J.- Silage Fermentation, Storr's Conn. Agl.Expt.Sta.Bull.70.1912.

10 Hart, E.B.and Willaman, J.J.- Volatil Fatty Acids and Alcohols in Corn Silage, Jour.Amer.Chem.Soc. Vol.XXXIV, No.12.Nov.1912.

11 Henry, W.A.- Corn Smut, First Rep.Wis.Exper.Sta.for 1883:25-27. 1884.

12[a] Hitchcock, A.S.- Note on Corn Smut, Bot. Gaz.28:429-430.1899.

12[b] Hitchcock, A.S.and Norton, J.B.S.- Corn Smut, Bull. Kans.Exper.Sta. No.62:162-212. 1896.

13 Knowles, E.L.- Abnormal Structure Induced by Ustilago zeae Mays, Jour. Myc.5:14-18.1889.

14[a] Kühn, J.- Entwickelungsgeschichte des Maisbrandes, Hedw.2:5-7. 1858.

14[b] Kühn, J.- Die Krankheiten der Kultur-gewachse:70-76, 260. Berlin, 1858.

14[c] Kühn, J.- Ueber die Entwickelungsformen des Getreidebrandes und die Art des Eindringens der Keimfaden in die Nahrpflanze, Stzbr.d. Nat. Ges.Halle, 1874: --;Centr.f. Ag. Chem.1874:150-153; Bot.Zeit.1874: 121-124; Just's Bot. Jahresb. for 1874:247-248.1876

15 Leveille, J.H.-Recherches sur le developpement des Uredineas, Ann. Sci. Nat.2nd.ser.11:13-14. 1839.

16 Maire, R.- On the saprophytic development and cytological structure of the yeast like sporidia of Ustilago Maydis, Bull. Soc. Mycol. France,14 (1898), No.4, 161-173,pl.1.

17[a] Meyen, F.J.F.- Ueber die Entwickelung des Getreidebrandes in dem Mays Pflanzen,Wiegm.Arch.3:419-421. 1837.

17[b] Meyen, F.J.F.- Pflanzen Pathologie: 101-104, Berlin, 1841.

18 Neidig,Ray E.- Chemical changes during Silage Fermentation, Ia. Agl.Coll. Research Bull.16:8-10.1914.

19 Norton, J.B.S.- A study of the Kansas Ustilagineae especially with reference to their germination, Trans. Acad. Sci. St.Louis 7:234-235. 1896.

20 Pammel, L.H.and Stewart, F.C.- Prevention of Corn and Oats Smut, Bull. Ia.Exper.Sta.No.20:726-728. 1893.

21 Selby, A.D.and Hickman,J.F.- Corn Smut, Bull.Ohio Exper.Sta. No.78:92-96, 1897.

22 Stakman, E.C.- Spore Germinations of Cereal Smuts, Minn. Agl.Expt.Sta. Tech.Bull.133:34-38. 1915.

23 Stewart, F.C.- Effects of heat upon the germination of corn and smut, Proc. Ia. Acad.Sci.for 1894, 2:74-78.1895.

24 Tillet, M.- Observation sur la maladie du Mais ou ble de Turquie, Mem.Acad.Sci. Paris for 1760:254-261. 1766. Abs. in Hist. Acad. Sci. Paris for 1760:85-89.1766. (The Histoire and Memoires of this year form one volume with a single title page and table of contents, but separately paged. The volume is without number.) German trans. by Beck. Hannov. Mag.6:1329-1340. 1768.

25 Tulasne, L. and C.H.- Ann.Sci.Nat.3rd.ser.7:14-126. 1847.

26 Waldheim,Fischer von -Ustilagineae, Rep. N.Y.State Agl.Soc,1870:280-355. Trans. from German in Pringsheims Jahrbucher fur Wissenschaftliche Botanik, vol.7,pt.1-2, 1869.

27 Wheeler, C.F.- Notes on U.maidis, Mich. Rep.1895:184-185.

28 Wolff, R.- Der Brand des Getriedes, Inaug. Diss:10-11, 18-24. Halle, 1874. Abs. in Just's Bot. Jahresb. for 1873:84-86, 1874.