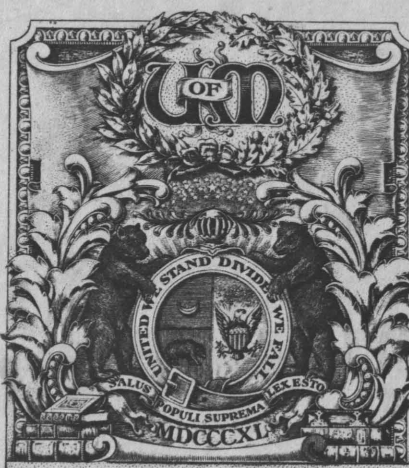
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THE INFLUENCE OF VARIOUS FACTORS, PRINCIPALLY TRANSPIRATION, UPON INFECTION OF WHEAT AND BARLEY PLANTS BY THE POWDERY MILDEW

by

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Erysiphe Graminis is the powdery mildew which grows upon the Gramineae (Grass family). Something like fifty-five species of the family are affected by this fungus. Among these species are the cereals wheat, barley , oats, and rye, so naturally this mildew has attracted much attention.

Many interesting facts have been brought to light concerning the power this mildew posesses to infect the different host plants.

When the conidia of Erysiphe graminis are placed upon the same kind of a plant as the original host , they will readily germinate and in three or four days , if conditions are favorable, produce mycelium and conidia. Mildew from a wheat plant will produce conidia in about four days after it has been placed on a plant of the same variety. Conidia are produced in great abundance. They appear on the blades of the host plants as a white powder. The slightest breeze or jar will send a cloud of them through the air. A supply of the mildew can be kept at hand in the green house by growing its respective host and placing it near the plants already infected. Young seedlings planted every two weeks will replace the older ones as they die. It might be said here that unless inoculation is very heavy the mildew harms the host tissue very little. But usually after about two weeks the mildew has spread over the surface of most of the leaves to such an extent that their growth is retarded. The percentage of germination of the mildew spores is between seventy-five and ninety-five.

This, and the fact that numerous spores are produced, is responsible for the rapid spread of the mildew under favorable conditions. The following table will show the percent of germination of barley mildew conidia.

The spores were germinated in water for six hours. They were then examined under the microscope. The number of spores, germinated and ungerminated, which could be seen in the field with out moving the slide was counted. Few enough conidia were placed on the slide so they could be readily counted. Twenty-five observations were made. These data are the sum of all the observations made.

No. of Obs.	No. of Ger. Spores.	No. of UnGer. Spores.	% of Germination
25	259	66	80.0-%

As a result of this high percent of spore germination, one blade of wheat will, when inoculated, produce hundreds of conidia and these spores, if they lodge on a wheat plant of the same variety, in turn will soon be producing hundreds more.

However in spite of the readiness with which the spores from wheat will infect plants of that variety they will not grow upon any other genus of the grass family, and indeed their growth is limited to certain species and varieties of the genus Triticum. This peculiar fact, when it became known, attracted the attention of Botanists, some of whom have been trying to find out to what extent the mildew is limited in its power of infection. Marchal (3,4,5,) , Salmon (10,11,12) and Reed (6,7,8) have obtained valuable results along this line. Their experiments have been to ascertain to what extent the the grass mildew is specialized as to the hosts it infects, and how many forms there are as a result of this specialization. The results of these men's work all show that the mildew from a certain host plant is limited as to the number of different species it can grow upon. The spores of barley mildew, for instance , will not grow when sown upon wheat , rye, oats, or in fact any ather genus than Hordeum. The mildew seems to have become specialized with respect to that particular host. The same is true of the forms found on wheat , rye, and oats; all are restricted to their own particular genus or species as a host. Morphologically, no difference can be found between the mildew on wheat and that on barley. The spores are the same size and shape ; they germinate in the same manner; in fact , with regard to their structure they are identical. The only distinction that can be made between these mildews is in their power of infection. Wheat mildew has no power to grow on barley , neither can barley mildew infest wheat plants. The same is true for oats and rye. These facts seem to point to the existance of certain biological forms which are distinct on each of these four cereals. The fact that the mildew growing on one of these cereals will not pass over on to a species of any of the others shows evidently that the mildew has become specialized in these cases to its own particular host and that each form of the mildew has become specially adapted to to its host. Reed(7) found in experiments on the immunity of certain species to Erysiphe graminis that often the mildew was limited to one genus or one species of that genus. He found, for instance , that rye mildew infected only the genus Secale and even certain species of this genus were immune. Some species of Hordeum (barley) were immune to barley mildew. In the case of Triticum (wheat) Reed(8) found that three varities of the species T. dicoccum were immune to the wheat mildew , i.e. ,Common emmer, Russian emmer and Spring emmer. Also four varities of the species T. vulgare (10) showed almost no infection , namely; caesium Alef.,ferrugineum Alef., pyrothrix Alef. and alborubrum Kcke.

Since it appeared that the mildew on wheat , oats , rye, and barley had become specialized into biological forms the question then came up: could one"biologic form" be made to grow upon the host of another? Evidently there must be some factor entering in to prevent cross inoculation which is difficult to remove. Salmon (13) tried to obtaim infection from cross inoculation , by several methods. He tried wounding the host tissue in a variety of ways. Anesthetics, as ether and chloroform vapors , were used. Heat was applied to the host plant, and alcoholic solutions were used in which to soak the plant leaves. After an application of one of these methods he cross inoculated the leaves. Some results were obtained but none very conclusive, and as yet securing cross infection by bringing external conditions to bear upon the host has not been worked out to any extent.

The problem which is to be discussed in this paper is: The effect various conditions have on the growth of the mildew on wheat and barley plants.

Most of the data presented here are on the effect of transpiration of the host plants as an influencing factor. Other experiments from which data were obtained were (1) the Cross inoculation of wheat and barley with the mildew, (2) the inoculation of the Common emmer with wheat mildew and (3) the effect of alcohol on such inoculations. Although the real problem is the conditions which affect the growth of the mildew on its own host, still the results of these studies throw (indirectly) a light on this problem.

THE EXTENT TO WHICH INFECTION OF THE MILDEW WILL TAKE PLACE ,(I) ON WHEAT AND BARLEY PLANTS WHEN CROSS INOCULATED (2) ON COMMON EMMER WHEN INOCULATED WITH WHEAT MILDEW,(3) ON THESE PLANTS WHEN TREATED WITH SOLUTIONS OF ALCOHOL.

Wheat plants when inoculated with mildew from barley will not produce mildew on their leaves, but when inoculation is heavy little light patches can be observed on the surface of the leaves where the tissue seems to have been injured. These same injured spots are noticed when barley plants are inoculated with wheat mildew and also when the Common emmer , a variety of spring wheat, is inoculated with the wheat mildew.

The object of this experiment was to ascertain to what extent the mildew spores grow on the strange hosts. Evidently some growth takes place since the mildew produces those light patches on the leaves , although no mycelium or conidia were ever observed.

For this work wheat , barley, and Common emmer seedlings were grown in flower pots in the green house. When they were about ten centimeters high the wheat and barley seedlings were cross inoculated with mildew,wheat mildew being placed on the barley seedlings and barley mildew on the wheat. The Common emmer plants were inoculated with wheat mildew. The pots were then placed under bell jars. At the same time controls of wheat and barley were inoculated with their respective mildews.

After forty-eight hours a leaf was taken from each plant and the mildew examined under the microscope.

The epidermis of the leaf was carefully removed at the place where inoculation had been made. This epidermis tissue was then treated with a solution of acetic acid (50 per cent) and alcohol(50 per cent) in order to remove all chlorophyll if any of the mesophyll tissue was present. This solution was allowed to act about ten minutes, then it was washed off with water. Iodine was added next, which stained the mildew spores a light brown but did not affect the host tissue. When the iodine had acted for about five minutes the tissue was mounted in water and examined . This method of preparing the slides was simple and ,at the same time , effective for it brought out the mildew plainly so it could be easily distinguished from the host tissue.

On examination of the leaf tissue forty - eight hours after inoculation, the spores on all the leaves had germinated at about the same rate regardless of the host on which they were placed, with the possible exception of some of the Common emmer leaves , where the spores had clung to the hairs which cover the surface of the leaf, and no germination had taken place. But where the spores had come in contact with the leaf surface they germinated almost as well as those on the wheat leaves. The question might be raised here as to what importance these hairs on the surface of the leaf have in preventing the growth of the mildew. Several times it was observed that the spores clung to them , and when this was the case the spores of the mildew did not germinate. It would seem that possibly the pubescence of the Common emmer may be , in a way, responsible for its immunity. But it cannot be said that the hairy condition of the leaf is the only factor which prevents infection , for in many cases the spores which were placed on the leaf came in contact with the epiderm al cells and germinated in the characteristic manner. Although pubescence may prevent germination of the spores by keeping them away from the surface of the leaf , it does not seem probable that they prevent infection to any great extent.

On the third day after inoculation, other leaves were removed from the plants and examined. Here marked differences could be seen in the way the mildew had grown. Where the wheat mildew was growing on wheat plants the germinated spores had sent out germ-tubes penetrating the host tissue. Haustoria could be seen within the cells , some in a divided condition. They developed from the appressoria which arose from the germ-tubes outside the cell walls. Mycelium and conidia could be seen on the surface of the epidermis . The spores of barley mildew growing on barley leaves grew practically in the same manner.

The result of cross inoculation was quite different. The per cent of germination was almost as high as when each form of mildew was placed on its own host. In some cases the germ-tube had penetrated the host tissue and appressoria were formed against the cell wall. But the haustoria failed to develope from these , and no mycelium or conidia were produced. The spores of wheat mildew on Common emmer leaves germinated quite well when they were on the surface of the leaf.
No germination took place , however, when the spores clung to hairs covering the surface of the leaf , as frequently happened.

The germinated spores sent out germ-tubes and formed some appressoria , but only two haustoria were ever observed growing into a cell from these appressoria. And these were short and undivided unlike those of healthy mildew. No mycelium or conidia developed.

Apparently , there is something in the strange host tissue which prevents the full develepement of the mildew. From these observations it would seem that the cells of the strange host tissue are able for some reason to resist the mildew and prevent the formation of the haustoria. It seems possible that if some chemical could be used on the plant tissue which would break down this power of resistance which it offers to the mildew, cross inoculations might then be made with success. Salmon (13) found that by injury , action of anesthetics , alcohol, and heat, the leaves of strange host plants could be rendered susceptible to the mildew to a certain extent. The percent of infection he obtained by these methods was low, and since all the leaves he treated were removed from the plants this may have weakened their resistance to the mildew. However , his results showed that possibly these methods of treating the host tissues did affect the growth of the mildew and from them came the suggestion that perhaps by growing the plants in a solution containing a certain per cent of a chemical, alcohol for instance , that cross inoculations could be made successfully. Experiments were carried out in which plants of wheat , barley , and Common emmer were grown in solutions containing different percentages of alcohol.

The experiments were set up in the following manner.

The nutrient solution inwhich the seedlings were grown was a soil extract , made by mixing about four parts of a rich soil with one thousand parts of water. After standing twenty four hours the solution was filtered. This soil solution was clear and the plants placed in it showed very good growth. The different strengths of alcoholic solutions were made by adding the required amount of alcohol to a definate quantity of this soil extract.

These solutions were then placed in wide mouthed bottles of 500 c.c. capacity , which were covered with black paper to prevent the light from injuring the roots. The seedlings were germinated in sand till the plumules were about four centimeters long. These seedlings were then inserted in to the corks of the bottles through holes , usually four plants were placed in one bottle. (There should be left enough space for good ventilation otherwise fungous growths often kill the plants.)

After the plants were arranged in this manner they were inoculated and allowed to grow until the controls showed abundant infection. The first table gives the results obtained from inoculating with wheat mildew , Common emmer which was growing in alcoholic solutions of different strengths, i.e. 1, 2,3,and 5 percents The plants were allowed to grow for two weeks. At the end of that time practically all of them were dead in 5 percent alcoholic solution.

From the ninety-six plants inoculated only one showed infection, more than the characteristic white spots. In this case the leaf which became infected was partially dead . The few conidia that were produced grew on the cell just back of the dead tip. They were not dead but in such an unhealthy condition that they could not withstand the attack of the mildew. The 5 and 3 percent alcoholic solutions retarded the growth of the plants to a great extent, but those in the 2 and 1 percent solutions grew at about the same rate as the controls in the soil extract.

The wheat and barley seedlings were grown in the same alcoholic solutions as the Common emmer. Growth was hindered by the 5 and 3 percent solutions, but not by the 2 and 1. The wheat seedlings were inoculated with barley mildew and barley seedlings with wheat mildew. Controls were grown in soil solution , one part of the plants being inoculated with their own biological form of mildew , the others cross inoculated. After a week all the controls which were inoculated straight had mildew growing abundantly on them, while none of the others had become infected. White spots were observed on the leaves where the spores had been placed but no conidia or mycelium was produced. Evidently , the alcohol when applied to the plant in solution to its roots does not affect the cells of the host tissue in such a way as to produce infection from cross inoculation. Table II. shows the results of the inoculations with the wheat seedlings and table III. gives the results obtained from the barley seedlings which were inoculated.

TABLE I.

COMMON EMMER PLANTS.

Date when Started.	No. of Plants.	Solution Used.	Date Inoculated.	Infection After 2 Wks.
2-3-'12.	8	1% Alcohol	2-3-'12.	None
2-3-'12.	8	2% Alcohol	2-3-'12.	None
2-3-'12.	8	3% Alcohol	2-3-'12.	None
2-3-'12.	8	5% Alcohol	2-3-'12.	None
2-3-'12.	8	Controls in Soil Solution.	2-3-'12.	None
2-10-'12.	16	1% Alcohol	2-10-'12.	None
2-10-'12.	16	2% Alcohol	2-10-'12.	None
2-10-'12.	16	3% Alcohol	2-10-'12.	One Plant Infected.
2-10-'12.	16	5% Alcohol	2-10-'12.	None
2-10-'12.	8	Controls in Soil Solution.	2-10-'12.	None

TABLE II.

WHEAT PLANTS.

Date when Started.	No. of Plants.	Solution Used.	Date Inoculated.	Mildew Used.	Infection After 2 Wks.
1-27-'12.	6	1% Alcohol	2-3-'12.	Barley	None
"	6	2% Alcohol	"	"	None
"	6	3% Alcohol	"	"	None
"	6	5% Alcohol	"	"	None
"	6	Soil Sol.	"	"	None
"	6	Control. "	"	Wheat	Good
2-10-'12.	6	1% Alcohol	2-10-'12.	Barley	None
"	6	2% Alcohol	"	"	None
"	6	3% Alcohol	"	"	None
"	6	5% Alcohol	"	"	None
"	6	Soil Sol.	"	"	None
"	6	Soil Sol.	"	Wheat	Good

TABLE III.

BARLEY PLANTS.

Date When Started.	No. of Plants.	Solution Used.	Date Inoculated.	Mildew Used.	Infection After 2 Wks.
1-27-'12.	6	1% Alcohol	2-3-'12.	Wheat	None
"	6	2% Alcohol	"	"	None
"	6	3% Alcohol	"	"	None
"	6	5% Alcohol	"	"	None
"	6	Soil Sol.	"	"	None
"	6	Soil Sol.	"	Barley	Good
2-10-'12.	6	1% Alcohol	2-10-'12.	Wheat	None
"	6	2% Alcohol	"	"	None
"	6	3% Alcohol	"	"	None
"	6	5% Alcohol	"	"	None
"	6	Soil Sol.	"	"	None
"	6	Soil Sol.	"	Barley	Good

The data from these three tables shows that alcohol added to the solution in which the plants are growing will not produce infection from cross inoculation even though the percent used is high enough to injure the growth of the plants. If alcohol is able to break down the resisting power which a strange host cell offers to mildew, it is not taken in by the roots in sufficient quantities to produce the result.

Since growing the seedlings in alcoholic solutions was not effective , an other method was tried. Seedlings of wheat , barley , and Common emmer were grown in pots, about twenty-five plants in each pot. When they were about fifteen centimeters high, the leaves were soaked in a solution of alcohol for a time. A 10 percent solution was used and the tops of the plants were soaked for two hours. If they were allowed to remain in the solution longer at the green house temperature the leaves were injured. The plants were not removed from the soil in any of these experiments. To treat the seedlings the alcoholic solution was placed in a jar and the pot with the plants was inverted over it , so that the leaves were entirely submerged. Part of the seedlings were cross inoculated after treatment and the others inoculated with their own respective mildew. Table IV. gives the results of this experiment in tabular form.

TABLE IV.

No. of Pots used.	Plant.	Length Treated	Sol. used to Treat.	Mildew used.	Infection After 10 da.	Condition of Plants.	Age of plants Treated.
1	Wheat	2 hrs.	10% alch.	Wheat	Good	Good	9 da.
1	Barley	"	"	Barley	"	"	"
3	Wheat	"	"	"	None	Tips slightly Yellow.	14 da.
3	Barley	"	"	Wheat	"	Good	"
1	" "	4 hrs.	"	"	Slight growth.	Almost dead	"
1	Common emmer.	2 hrs.	"	"	None	Good	9 da.

These results show that cross inoculation cannot produce infection when the host plant has been soaked in a 10 percent solution of alcohol for two hours. In the case of barley which had been soaked for four hours, the leaves were almost dead and the infection was very slight. Apparently the cells of the leaves infected were so injured that they could not withstand the growth of the fungus. Cross inoculation in the other cases produced nothing more than the characteristic white spots . Straight inoculation produced mildew somewhat different from the normal. The spores were relatively fewer and many had a peculiar black spot on them. The mycelium seemed to be more irregular and the hyphae had a sort of a curled , wrinkled appearance. These spores when sown on their respective hosts produced abundant mildew and when placed on strange hosts caused no infection. However it would seem that these differences in the appearance of the fungus do not affect its ability to infect its own host so no importance was attached to them.

From these experiments it seems that alcohol dees not affect the host tissue in such a way as to allow the mildew from a strange host to infect it or to prevent infection when the mildew is applied to host plants.

The remainder of this paper gives the data and results obtained from experiments on transpiration of the host plants and its effect on infection.

EXPERIMENTS TO SHOW THE EFFECT OF TRANSPIRATION ON THE INFECTION OF WHEAT AND BARLEY PLANTS BY ERYSIPHE GRAMINIS.

It is a well known fact that plants are constantly losing water. The process by which the water is given off the plant is transpiration.

The plant itself is not active in this process but just as a moist piece of blotting paper is unable to prevent the evaporation of the water from it, so the plant is inactive in transpiration.

The conditions which favor transpiration are; 1, an optimum amount of water in the soil in which the plant is growing, 2, a dry, warm atmosphere. On the contrary transpiration is reduced in a plant when the soil is dry and the surrounding atmosphere moist.

The problem to be discussed in the remainder of this paper is the effect that varied rates of transpiration in the host plant has on infection of it by the mildew.

The fact that the spores of the mildew will germinate in water, on a slide in a moist atmosphere, or on the leaves of species of the grass family other than the particular host, was referred to earlier in this paper. The conditions which favor germination are moisture and a cool atmosphere. If these conditions are present the spores will send out germ tubes even if they are not on their particular host plant.

In these experiments the conditions for spore germination were kept favorable , then the effect of different rates of transpiration on infection was studied.

The first series of experiments carried out in this problem consisted in inoculating plants growing where conditions were best for transpiration and also other plants growing where little water was lost. Infection of the plants grown in these two conditions by the mildew was noted.

To obtain maximum transpiration the plants were well watered and placed in a part of the green house where the air was dry and warm. To decrease the amount of water lost, the plants were grown in a rather dry soil and a moist surrounding atmosphere. For this the plants were grown in glass tumblers with just enough moisture for them to develope . When the seedlings appeared through the soil they were not given any more water. When the seedlings were about 10 cm. high the tops of the tumblers were sealed with paraffin so the soil would remain dry. The seedlings were shielded from the hot paraffin by placing pieces of glass tubing over them until it cooled. The tumblers were then placed on glass plates and bell jars inverted over them. Along with the jars containing the plants a tumbler of water was placed . This soon formed an atmosphere saturated with moisture and under these conditions very little transpiration took place. Inoculations were made with the mildew spores on the plants just before they were placed under the bell jars . In all these experiments with transpiration the mildew was placed on its own particular host.

Usually the plants were allowed to grow under these conditions for about two weeks , or till the mildew had become well developed. It might be said here , that if the air ,in which the plants were kept was too warm and dry , the spores of the mildew could not germinate . This occured in one or two instances , and no infection took place. The data given in table V. Will show the results of this experiment.

In some cases the plants under the bell jars became infected with the mildew about one day earlier than those in a dry atmosphere, but this difference seemed to be due to the fact that the air was too dry and warm for the spores to germinate. When the plants were placed where the air was a little cooler and contained a little more moisture , but where transpiration still was rapid , infection took place just as readily as on the seedlings under the bell jars. Practically no difference was found between the time required to produce infection on wheat and barley. Something like two hundred and fifty plants were inoculated and placed under bell jars and about as many kept in a dry atmosphere . Half of these were wheat and half barley, but as infection on both took place at practically the same time they were tabulated together.

In the case of the plants grown in a dry atmosphere , on Nov. fifth the temperature was so high that the spores failed to germinate . But where the conditions were favorable for the growth of the mildew the rate of transpiration seemed to have no effect on infection.

TABLE V.

Date When Inoculated	No. of Plants With reduced Transpiration.	No. of Plants with maximum Transp.	Date of infec. in reduced Trans.	Date of infec. in max. trans.
Nov. 5th	9	15	Nov. 15th	None
Nov. 27th	4	36	Dec. 3rd	Dec. 4th
Dec. 8th	17	33	Dec. 12th	Dec. 15th
Dec. 9th	36	10	Dec. 13th	Dec. 13th
Dec. 11th	44	25	Dec. 15th	Dec. 15th
Dec. 26th	120	75	Dec. 30th	Dec. 30th
Jan. 8th	50	25	Jan. 15th	Jan. 16th

As this means of varying the rate of transpiration of the host plant seemed to be ineffective in producing changes in mildew infection another method was employed. This method was to grow seedlings of wheat and barley in soils and soil solutions containing various amounts of salts and acids.

Livingston 1905 (2) , Harter 1908 (1) and Reed 1910 (9) have published results of experiments in which they found that salts and acids effect the amount of transpiration. As I carried out experiments duplicating in part those of Harter and Reed, it will be well to describe their work in detail.

For the most part , Harter used soluble salts in his experiments , usually in large enough amounts to affect the growth of the plant. A saline soil from the region of Salt Lake City Utah , which contained sodium chloride in most abundance, also some sodium phosphate , sodium bicarbonate and potassium sulphate was mixed with the required amount of garden loam to form the concentrations desired. For his experiments , Harter used soil of three different concentrations,- 2 percent, 1.5 percent and 1 percent soluble salts . By analysis , it was found that these three concentrations would represent respectively, 1.4 , 1.0, and 0.7 percent sodium chloride. These percentages are higher than is ever found in fields where wheat and barley will grow.

Wheat, oats, and barley plants were grown in glass jars containing soil of these three concentrations of salts. Glass pots were used so none of the salts would be taken out of the soil by the water . Controls were grown in garden loam. Harter found that the growth of the plants was retarded by the salts especially when the percentage was as high as 2. In fact growth here was very slow.

The results Harter obtained showed that the structure of the leaves had been modified in that the cuticle had become thicker and a waxy bloom appeared on the surface of the leaf. The thickness of the cuticle increased with the concentration of the soil solution but when there was not enough salts in the soil to retard growth no changes in the plant tissue was observed. It was also found that the plants grown in the saline soil containing 2 percent, 1.5 percent and 1 percent of soluble salts lost less water by transpiration than the controls growing in garden soil. Harter was inclined to think that this retardation in the amount of transpiration was due to the presence of the waxy bloom produced on the surface of the leaf.

Two series of experiments were carried out with wheat in which the percent of salt in the soil was 0.09 and 0.12. It was found that these small amounts of salts stimulated transpiration in stead of retarding it.

From these experiments of Harter's it is obvious that the amount a plant transpires is affected by the presence of salts in the soil. It seemed possible that plants which were grown in saline soils would be so modified as to affect the growth of the mildew on them.

Experiments were carried out duplicating , in part , those of Harter , and when the plants were of sufficient size , they were inoculated with the mildew . About two hundred plants of wheat and barley were grown in soils containing different concentrations of sodium chloride . The soil used was a good rich, sandy soil and sodium chloride was added to it in the percentages of 1.4 , 1, and 0.7 . The plants were grown in glass tumblers of about 500 c.c. capacity.

It was found that soil containing 1.4 percent and 1. percent sodium chloride produced small plants, in some cases almost no growth taking place at all in the soil with 1.4 percent sodium chloride. The leaves on these plants were different from the controls. They seemed to be more rigid and thicker, and there was as Harter reported, a bloom on the surface of the leaves.

Along with the plants grown in the soil , others were placed in soil solution containing sodium chloride in the percents as was used in soil. Also controls were grown in the soil solution without sodium chloride. These water cultures were started in the same manner as those in which the alcoholic solutions were used, (referred to earlier in this paper) except in these as little space as possible was left where evaporation could take place. About one hundred and fifty plants were grown in these aqueous solutions but those in the 1.4 percent sodium chloride solution lived only about ten days.
The plants were inoculated with the mildew and as a result, the plants grown in the soil and aqueous solutions became infected

uniformly , except when the plants were almost dead. The average infection for both wheat and barley growing in soil and aqueous solution which contained 1.4 percent sodium chloride was about 50 percent of the total number of plants inoculated, but of the number which were living after inoculation had been made the percent of infection was 80.

The average infection of the seedlings growing in 1 percent sodium chloride solution was 92 percent but several plants which were almost dead did not become infected, no doubt because of their unhealthy condition. The plants growing in the 0.7 percent sodium chloride and the controls showed perfect infection . No difference was observed in the length of time it took the plants to become infected no matter in what solution they were growing . Infection on all the plants showed up well in four days.

One series of these experiments was weighed to determine the amount of water lost by transpiration. The results confirmed Harter's statement that soluble salts , when present in the soil or solution in large enough quantities to produce a waxy bloom , caused a decrease in transpiration. Table VI. will show the data obtained from this experiment. The plants were grown for ten days; until they began to show signs of wilting and those in the 1.4 percent salt solution had begun to die. The initial weighing was carefully done when the seedlings were put in the the solutions, then other weighings were taken at intervals in the ten days. The green weight of the plants was taken immediately after the final weighing. The difference between the initial and final weighing showed the amount of water lost by transpiration during the ten days . In this table the average of eight plants was taken in each case.

TABLE VI.

	Water loss in grams.	Green Wt. in grams.	Water loss per gram of Green Wt.	Percent of Transp. using controls-100.
Wheat				
Controls	38.5	.61	63.0	100.0
1.4% NaCl	12.0	.31	38.0	60.3
1.0% NaCl	12.5	.285	43.0	68.2
0.7% NaCl	11.5	.25	47.0	74.6
Barley				
Controls	45.0	.69	65.0	100.0
1.4% NaCl	10.5	.33	32.0	49.2
1.0% NaCl	25.0	.485	51.0	78.4
0.7% NaCl	23.0	.505	47.0	72.3

The data show that there is a marked decrease in the amount of green weight produced in the solutions containing the NaCl as well as a decrease in transpiration. The mildew grew uniformly on all these plants except some of those in the 1.4 percent salt solution which died before the mildew had time to develope.

It seems from these experiments that the rate of transpiration of wheat or barley does not affect the rate at which the mildew will grow on them. It is quite evident that transpiration is reduced when salts are added in large enough quantities, but the rate of growth of the mildew on plants grown in solutions of sodium chloride of these percents is neither increased or diminished. Only where the plant itself is injured by the salts is the infection inhibited. It appears that the mildew does not grow readily on plants which are unhealthy or almost dead.

The last series of experiments to be described in this paper is that in which Reed's(9) work on transpiration was partially duplicated. In his experiments he used solutions of salts and acids in quantities small enough not to retard growth, and noted the effect on transpiration . Only those which showed a marked increase or decrease of transpiration were repeated and these will be described briefly.

Reed grew wheat in both soil and aqueous solutions. The two experiments of his which I wish to describe are those with salts and acids . Four different salts were used , sodium nitrate , potassium sulphate , mono calcium phosphate and calcium carbonate. The first three were applied to both soil and aqueous solutions in the proportion one hundred parts to a million; the calcium carbonate was added one thousand parts to a million . Where soil was used the plants were grown in paraffin wire pots for three weeks. When the seedlings appeared above the soil the top of the pot was sealed with oiled paper; the seedlings were allowed to grow up through narrow slits made in the paper. This reduced evaporation to a minimum.

After the plants were covered , the initial weighing was made . The total transpiration was determined by adding up the total water loss from the pots during the experiment. Reed obtained the green weight of the plants by cutting them off just above the surface of the soil and then weighing them immediately.

The water cultures were prepared by using culture jars of 250 c.c. capacity and fitting ten seedlings into the corks of these. The soil extract was prepared by stirring one part soil with five parts of distilled water. After standing thirty minutes it was filtered through a Pasteur - Chamberland filter. The required amount of the different salts were added to the right quantity of this extract and the same proportions of the salts were obtained as were used in the soil. The seedlings were grown in these solutions for two weeks and the green weight and loss of water were calculated as in the experiments where soil was used.

Reed has given the following figures which show the effect of these four salts on transpiration. They are the averages of plants grown in one hundred and eighty- nine soils from different localities. The untreated pots(controls) gave off in transpiration 103.3 g. of water for each gram of green weight produced. In the pots where the salts were added the amounts of water transpired per gram of green weight were: $NaNO_3$, 93.36 gms. ; K_2SO_4 , 97.95 gms.; $CaH_4(PO_4)_2$, 104.07 gms.; $CaCO_3$, 101.09 gms. If the transpiration of the controls is used as a basis and is represented as 100, these salts show the following percents

$NaNO_3$, 90.4% ; K_2SO_4, 90.8% ; $CaH_4(PO_4)_2$; 100.8%; $CaCO_3$- 97.9%.

The first two salts show an inhibiting effect on transpiration but the other two do not show much difference from the controls.

Turning now to the experiments which Reed carried out with different acids , it is found that they also have an effect on transpiration. The acids he used were oxalic, acetic hydrochloric , nitric and sulphuric. Each was added to soil extract in five different strengths; n/2500 , n/5000 , n/10000, n/ 20000, n/40000. The plants were grown in these solutions for about two weeks. The jars were weighed at intervals and,at the end of the experiment the green weights were taken.

His results show that the inorganic acids retarded transpiration. Organic acids were somewhat variable, but on the whole they showed a slight increase.

Other chemical agents were used by Reed which affected transpiration some , but as these two experiments just described are those shich I repeated ,the others will not be referred to.

In repeating the experiments of adding the four salts to the soil I used instead of paraffin wire pots , glass tumblers which held about 400 gms. of soil. Instead of $Ca\ H_4(PO_4)_2$ which gave the same amount of transpiration as the controls I used $NaHPO_4$. Aside from these differences the experiment was conducted very much as Reed performed it. Each pot contained six plants and sixteen pots of soil containing each salt was set up, eight of wheat and eight of barley. Paraffined paper was used to cover the top of the tumblers to prevent evaporation. The plants were allowed to grow up through holes made in the paper . The initial weighings were made when the paper was put on and the final and green weights were taken when the plants began to wilt.

when the plants were about ten cm. high , they were inoculated with the mildew. In four days after inoculation all the plants shewed perfect infection and no difference in growth of the mildew could be detected.

The results I obtained on the amount of transpiration were quite different from those of Reed. They show that K_2SO_4, $CaCO_3$ and $NaNO_3$ increased the amount of transpiration while Na_2HPO_4 decreased it decidedly , while Reed's figures show a slight decrease in transpiration when K_2SO_4, $CaCO_3$ and $NaNO_3$ were used. The following table (VII) will show the results of thes experiment. The data given here are the averages of eight pots each containing six plants.

This table (see following page) shows that salts (except $NaHPO_4$) when added in small amounts has a stimulating effect on transpiration. But this increase in transpiration does not in any way affect mildew growth on the seedlings. It might be said that all the plants in this experiment grew at practically the same rate ; no difference in their size could be observed.

At the same time this experiment was being worked out , another was started in which plants of wheat and barley were grown in solutions containing two concentrations (n/2500 ,n/5000) of the following acids: acetic , oxalic, hydrochloric, sulphuric

TABLE VII.

Wheat.

Salts added to soil.	Amount of H_2O lost by Trans. in grams.	Green weight of plants in grams.	Water loss per gm. of green Wt.	% of Trans. Controls-100 %.
K_2SO_4	151.5	2.07	71.2	112.2
$Ca\ CO_3$	182.0	2.76	66.0	104.1
$NaNO_3$	167.0	2.50	66.5	105.2
$Na_2H\ PO_4$	175.0	3.08	53.5	90.3
Controls	196.0	3.09	63.4	100.0

Barley.

K_2SO_4	180	2.87	62.6	106.1
$CaCO_3$	168	2.79	60.5	102.5
$NaNO_3$	193	3.27	59.0	100.0
N_2HPO_4	184	3.37	54.5	92.3
Controls	183	3.10	59.4	100.0

and nitric. There were four jars of each solution , two containing four plants each of barley and two with four plants each of wheat. The experiment was run for two weeks. Two days after the seedlings were placed in the solutions they were inoculated with mildew . It might be well to state here that all these inoculations were made straight. In four days good infection was observed on all the plants.

The inorganic acids caused a decrease in transpiration while the organic acids were somewhat variable, usually though with a higher water loss than the inorganic acids and slightly less than the controls. These results are similar to those of Reed. Table VIII. will show the data collected from this experiment.

The last studies , on the effect of transpiration on the growth of mildew , were made with solutions of oxalic and sulphuric acids . (n/500 and n/1000). The plants of wheat and barley were grown in solutions of these acids for ten days. On the fourth day after these plants were inoculated with the mildew almost perfect infection was observed.

The stronger solutions of acids increased the transpiration somewhat, although in two cases it was decreased. It is possible that this variation was due to some desturbing factor. Table IX. gives the data on this experiment.

The growth of the plants was somewhat retarded in the n/500acid solutions , but in the n/1000 there was very little retardation in the growth.

TABLE VIII.

Wheat Seedlings.

Solution	Transpiration in grams.	Green Wt. in grams.	Trans. per gram of Green Wt.	% of Trans. controls-100.
H_2O	94.0	1.64	57.3	100.0
CH_3COOH,n/2500	95.0	1.75	54.5	95.1
" ,n/5000	95.0	1.71	56.05	97.8
$C_2H_2O_4$, n/2500	38.0	.785	49.25	87.7
" n/5000	94.5	1.63	58.0	101.0
H Cl , n/2500	53.5	.985	53.05	93.5
" , n/5000	114.0	2.03	56.2	98.0
H_2SO_4 ,n/2500	66.5	1.18	56.00	97.7
" , n/5000	34.0	.68	49.0	87.2
HNO_3 , n/2500	75.0	1.32	54.30	95.0
" , n/5000	123.0	2.22	55.7	97.2
Barley Seedlings.				
H_2O	106.0	1.37	56.5	100.0
CH_3COOH,n/2500	97.5	1.79	54.2	96.0
" ,n/5000	115.0	1.96	58.85	104.0
$C_2H_2O_4$, n/2500	62.0	1.12	59.45	105.2
" ,n/5000	104.0	1.81	57.5	101.7
HCl, n/2500	105.0	1.89	55.4	98.0
" , n/5000	114.0	2.11	54.5	96.4
H_2SO_4, n/2500	94.0	1.69	55.0	97.3
" , n/5000	79.5	1.44	55.2	97.7
HNO_3, n/2500	97.5	1.88	52.7	93.2
" , n/5000	120.0	2.21	54.3	96.1

TABLE IX.

Wheat Seedlings.

Solution	Transpiration in grams.	Green Wt. in Grams.	Trans. per gm. of Green Wt.	% of Trans. controls-100.
H_2O	38.5	.61	63.0	100.0
$C_2H_2O_4$, n/500	41.0	.405	89.5	142.0
" , n/1000	36.0	.59	60.5	96.0
H_2SO_4, n/500	42.0	.64	66.0	104.7
" , n/1000	35.5	.59	60.0	95.2

Barley Seedlings.

Solution	Transpiration in grams.	Green Wt. in Grams.	Trans. per gm. of Green Wt.	% of Trans. controls-100.
H_2O	45.0	.69	65.0	100.0
$C_2H_2O_4$, n/500	42.0	.55	76.0	116.8
" , n/1000	31.0	.445	70.5	108.4
H_2SO_4, n/500	66.0	.465	71.0	109.2
" , n/1000	40.5	.57	71.0	109.2

It appears from the data taken from these experiments just described , that soluble salts and acids when added to the nutrient solution of wheat and barley affect the amount of water given off by the plants , but this increase or decrease in transpiration does not affect the ability of the mildew to infect those plants.

Evidently transpiration is not a factor which influences in any way the growth of the mildew.

SUMMARY.

The experiments presented in this paper seem to point to the following conclusions:

1. Wheat and barley plants will not become infected when cross inoculated,- that is when barley plants are inoculated with mildew growing on wheat and visa versa, more than the appearance of small light colored areas on the surface of the leaf, where inoculation was heavy. Also Common emmer is immune to wheat mildew. These statements are confirmations of the results obtained by previous workers along this line.

2. Alcoholic solutions as concentrated as 5 percent when used as nutrient solutions will not bring about the growth of the mildew on wheat and barley plants when they are cross- inoculated; neither do they destroy the immunity which Common emmer has for the mildew. Leaves of wheat and barley still attached to the plants , when soaked in a 10 percent alcoholic solution for two hours will not become infected when cross inoculated.

This is different from the conclusion which Salmon comes to from his experiments.

3. When plants of wheat and barley are grown under conditions where transpiration is greatly reduced and then inoculated with their respective forms of mildew, infection takes place on them as on plants grown where transpiration is very much greater.

4. Plants of wheat and barley grown in soil or aqueous solution containing 0.7%, 1.0%, and 1.4% sodium chloride transpire less than plants grown in media which have no salt, but they will become infected with the mildew just as readily, (except in the case of 1.4% NaCl where the plants are unhealthy or almost dead).

5. The presence of small amounts of the salts K_2SO_4, $NaNO_3$, and $CaCO_3$ in the soil in which wheat and barley plants are growing causes an increase in transpiration. Na_2HPO_4 causes a slight decrease in the amount of water lost; but the mildew infects these plants as readily as controls growing in soil with out these salts.

6. Inorganic acids added to a soil solution in small quantities (n/2500 and n/5000) cause a decrease in transpiration, while organic acids seem to produce variable results. The growth of the mildew on these plants is not affected by the acids.

7. Transpiration is slightly increased in plants that are grown in solution of n/500 and n/1000 oxalic and sulphuric acids but infection by the mildew is not affected.

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