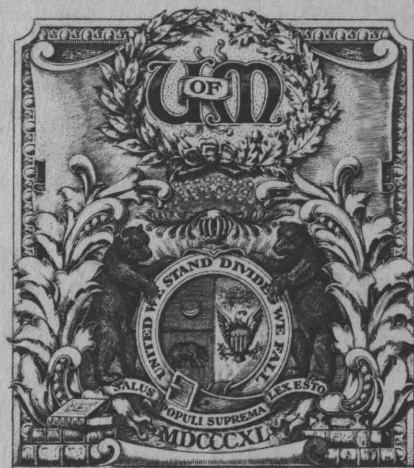
UNITED WE STAND DIVIDED WE FALL
SALUS POPULI SUPREMA LEX ESTO

INFLUENCE OF SOME EXTERNAL CONDITIONS UPON INFECTION OF HOSTS WITH POWDERY MILDEWS

by

Earle Long Overholser, B. S. A.

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INFLUENCE OF SOME EXTERNAL CONDITIONS UPON INFECTION OF HOSTS WITH POWDERY MILDEW.

It has long been observed that one condition or another of the environment is favorable to the perpetuation and spread of fungous diseases. For example, it is very easy to recognize the close relation which exists between the humidity of the atmosphere and certain fungous diseases. Indeed, the relation of the host and the parasite or the dependency, directly or indirectly upon external conditions, of the one to resist the attacks of the parasite, and of the other to invade the hosts tissue, and to firmly establish itself, offers a field of valuable research.

The effects of conditions of growth upon the susceptibility to fungous diseases have been reviewed in two papers by Duggar (5) and Reed (29). The brown rot of stone fruits, the black rot of grapes, the late blight of the potato, are examples of diseases which are very closely associated with abundant precipitation of a humid atmosphere. The powdery mildew of strawberries is favored by sudden alternations of temperature, and it is believed that sudden cooling of roses in greenhouses is an important factor in the development of

the rose mildew.

The club root of cabbage and related plants is influenced by soil conditions. It has been found that liming the soil is very effective in controlling the disease. The disease has always proved more severe in acid soils and losses from club root may be prevented by making the soil alkaline by the use of sufficient quantities of lime.

There are fungous diseases such as the canker-producing fungi and those which cause serious injury to forest and shade trees which, under ordinary circumstances, seem to be wholly dependent for penetration into the host upon wounds.

Many of our most important plant diseases are due to fungi which are endowed with the ability to enter relatively vigorous growing organs of the host plant. The downy mildews, peach leaf curl, rusts, smuts, and the powdery mildews are good illustrations of such. These fungi for the most part do not kill the tissues which they invade, or at least do not kill for a considerable period of time. They establish a relation with the living cells such that when the tissues which they invade are killed the fungus must cease its growth very promptly.

It is with the Erysiphaceae, parasitic fungi which are to be placed in this last group, that experiments have been conducted to determine the effect of some external conditions upon the host's susceptibility.

DeBary (3) in 1887 wrote: ".... facts .... would lead us to expect that there must be differences in the aggressive behaviour of a parasite to the different varieties and individuals of a host; or, to express the matter in the converse way, in the predisposition of the individuals for the attacks of the parasite......The physiological reason for these predispositions cannot in most cases be exactly stated, but it may be said in general terms to lie in the material composition of the host, and therefore to be indirectly dependent on the nature of its food."

The belief has existed that fungous diseases of any host plant are in some way dependent upon a lack of vigor in the plant. The evidence at hand, however, seems to indicate that in the case of strictly parasitic fungi it is very doubtful if vigor alone is sufficient to prevent diseases. It is the belief of a number of men (1), (6), (28), that resistance to strictly parasitic fungi seems to be more commonly a character; a character like earliness, wholly independent of vigor, and any condition of the environment may operate to assist the fungus or operate to make the host more susceptible.

The difference between the direct and indirect influence of environment upon the fungus should be clearly distinguished. The direct effect of a dry atmosphere may mean that the fungous spores cannot germinate. The direct effect of too high a temperature would perhaps be in drying out the

germ-tube after germination had taken place, or in otherwise destroying the fungus so no infection could take place. The host plants would thus escape the disease because unfavorable external conditions had killed the parasite.

In addition to the direct effect of environmental factors upon parasitic invasion, such as sufficient moisture for the germination of the fungous spores, a suitable temperature for growth, etc., the attacks of the parasite may be hindered or aided indirectly, due to the effects of the external conditions on the host.

Smith (36) concluded that there was both a direct and an indirect relation of water to the spread of asparagus rust, Puccinia asparagi. The indirect relation is the effect of moisture acting on the parasite through its effect upon the host and is limited to the soil water. With plenty of water, the host develops vigorously and is able to resist invasions by the rust. Conversely, the vitality of asparagus plants may be much reduced in dry seasons and on the drier soils. As a consequence, the rust is able to invade the host more successfully. There is a direct relation, however, in the fact that dew is necessary for spore germination and subsequent infection by the parasite.

It is this indirect effect which offers a particularly interesting field for investigation.

Reed (28) suggests that it must be remembered that

the immunity which plants enjoy against a given fungus is relative, not absolute, and that plants resistant to a particular fungus in one region may be quite susceptible to the same fungus if transplanted to another locality. He (25) has become convinced that various factors, as water supply, nutrition, relative abundance of spores, etc., play an important part in the susceptibility or immunity of a particular plant to a fungus parasite.

Many students have brought to light the existence of closely related plants which differ markedly in the extent of their susceptibility or immunity to fungous parasites. For example, the emmers have proved to be quite resistant to both the powdery mildew and rust of wheat, while most of the common wheats are susceptible. The development of disease resistant varieties offers one of the most hopeful methods of combatting some destructive diseases. Reed (27) and Orton (23) have reviewed the most important results obtained.

It has often been suggested that immunity is correlated with definite anatomical characters of the host. Immunity is independent, however, of any discernible morphological character, and it has been found practicable to breed varieties morphologically similar to one another, but immune or susceptible to the attacks of certain parasitic fungi. Ward (40) working with the Bromes and their brown rust which attakks them has found that resistance to infection of the 'immune' or 'partially

immune' species and varieties is not to be referred to observable anatomical or structural peculiarities, but to internal, i.e. intra-protoplasmic, properties beyond the reach of the microscope, and similar in their nature to those which bring about the essential differences between species and varieties themselves.
Ward (42) suggests, that infection and resistance to infection, depend on the powder of the fungus-protoplasm to overcome the resistance of the cells of the host by means of enzymes or toxins; and reciprocally, on that of the protoplasm of the cells of the host to form anti-bodies which destroy such enzymes or toxins, or to excrete chemotropic substances which repel or attract the fungus-protoplasm.

Ward (42) seems to have become convinced that chemotropism is an important factor in determining the relative immunity or susceptibility of plants, thereby agreeing with Massee.

Massee (19) believes that the entrance of the germ-tubes of a parasitic fungus into the tissues of a living healthy plant, depends on the presence of some substances in the cells of the host, which is attractive to the fungus. In other words, infection is due to positive chemotropism.

According to Massee a saprophytic fungus can be gradually adapted to become an active parasite on a given host-plant by means of introducing a substance positively chemotropic to the fungus into the tissues of the host. By similar means a

parasitic fungus can be induced to infect a new host.

Biologic Forms: Reed (26, 28) has very completely summarized the work that has been done on the physiological specialization of the Erysiphaceae. Neger is given the credit of being the first to determine that the ordinary morphological species of mildews may consist of a number of physiologically specialized forms which are limited in their occurrence to a single host plant or to a group of closely related host plants. Neger's (21) first experiments were made during the fall of 1901. His general conclusion was that in the mildews he studied, specialization has proceeded to such an extent that conidia from one species of host will not infect a species of any other genus. In some cases the specialization has proceeded still further so that conidia from one species of host is incapable of infecting another species of the same genus.

Marchal (18) during the spring of 1902, attempted the infection of several grasses with the conidia of Erysiphe graminis. He found that conidia from wheat would not infect rye, oats, and barley; conidia from rye would not infect wheat, oats, and barley; conidia from oats would not infect wheat, rye, and barley; and conidia from barley would not infect the other cereals.

Salmon (31) found that, contrary to Neger's (21) belief, the ascospores corresponded in their infecting powers to the conidia from the same host. Furthermore, they proved unable to attack any host that the conidia failed to infect.

Reed (26) assumed in 1905 that the results of the investigations at that time were sufficient to prove that there are physiological differences between the mildews of one morphological species by which it is split up into a number of forms, each limited to one or few host plants.

Biologic specialization, similar to that found in the mildews has been reported by various investigators as occurring in other groups of parasitic fungi. In fact this phenomenon was first observed in the rusts. Eriksson (8) in 1894 showed that although the morphology of Puccinia graminis Pers., on its different cereal hosts varied but slightly, there was a distinct specialization in parasitism. He therefore divided this morphological species of rust into subdivisions which he termed "formae speciales." These are quite analogous to the "biologic forms" of the Erysiphaceae. A large number of more recent investigators have greatly extended our knowledge along this line.

In most cases investigated, the biologic specialization of the parasite is quite sharply limited to specific hosts. A few workers, however, have claimed to find what they have designated as "bridging species." Ward (42), working with species of Bromus, found that Puccinia dispersa growing upon host A would not infect host C, but would infect host B. After it had grown upon host B, however, the spores could then infect host C. Ward called this a case of "bridging species," and believed it to be the clue to an explanation of a phenomenon which must be assumed

to occur in nature, whatever hypothesis is accepted regarding the origin and significance of adaptive parasitism, viz., the passage of the fungus from the species of one circle of alliance to those of another in spite of the fact that it is usually closely adapted to species of one section of the genus only. Ward believes that the host reacts upon and affects the physiological powers of the fungus and that in some cases very slight morphological changes follow. "Bridging" hosts would be the means of an ever-widening cycle of adaptation.

Reed (30) writes in 1913 that thus far one or more species of five genera, Uncinula, Erysiphe, Microsphaera, Phyllactinia, and Sphaerotheca, have been tested for host specialization, no results as yet having been recorded for Podosphaera. Most of the data obtained, however, are very incomplete except possibly in the case of three species of Erysiphe, namely E. Polygoni, E. cichoracearum and E. graminis, and one species of Sphaerotheca, Sph. humuli.

The morphological species, Erysiphe graminis, occurs on approximately sixty different grasses. So far, however, the work indicates that in practically every case the biologic forms are restricted to the species of a single genus. Reed (30) in some unpublished data has been able to transfer the wheat mildew to different species of Aegilops. He tested several species and nearly all of them proved highly susceptible. Some systematists regard Aegilops as a distinct genus, and if this is true here

would be a case of one biological form of grass mildew occurring on the species of two different genera. A similar relation is found in the case of the oat mildew. Reed has been able to confirm Marchal's statement that the conidia from oats are able to infect seedlings of _Arrhenatherum elatius_, although the percentage of infection was not very high.

Not all species of a particular genus, however, may be susceptible to the mildew. For example, the mildew on barley will infect the common barley and also _Hordeum decipiens_, _H. hexastichon_, _H. intermedium_, _H. bulbosum_, _H. distichum_, _H. maritinum_ and _H. zeocriton_, but will not pass over onto _H. jubatum_, _H. murinum_, _H. secalinum_ nor _H. sylvaticum_. Young plants of _Hordeum nodosum_ are easily infected with the barley mildew but older plants are immune. A similar case has been found by Salmon (33) among the brome grasses. Salmon, as a result of his work upon the mildews of the brome grasses, believes that there are four or perhaps five biologic forms within this one genus alone.

The wheat mildew, while able to pass over onto one or more varieties of every species of Triticum tested, is not capable of infecting all of the varieties of wheats. Reed in a recent paper (25) has published the results of tests with seventy-eight varieties distributed among nine different species of this genus. Of these seventy-eight varieties four proved to be immune, two belonging to _T. dicoccum_ and two to _T. vulgare_.

In a few other cases the percentage of infection was rather low but in a great majority of cases the percentage of infection approached one hundred.

Well developed biologic forms have been found on other grasses. The mildew on species of Agropyron are confined to the hosts of this genus. Similarly the orchard grass mildew is confined to Dactylis glomerata and the blue grass mildew to species of Poa.

Salmon (34) in 1905 published some of his results on the behavior of "biologic forms" under certain methods of culture. He found that the restriction in power of infection characteristic of"biologic forms" is broken down if the vitality of the leaf on which the conidia are sown is interfered with in certain ways.

The first method of culture adopted was the following: the leaf to be inoculated was cut off from the plant, and a minute piece of the leaf tissue was cut out with a razor. In this operation the epidermal cells on one surface, and all or most of the mesophyll tissue were removed at the cut place, but the epidermal cells on the other surface (opposite the cut) were left uninjured. By this means a transparent window-like spot 1-2 millimeters across, was made in the leaf. The conidia were sown on the cuticular surface of the uninjured epidermal cells over the wound, and the leaf placed, with the cut surface downwards, on damp blotting paper at the bottom of a closed Petri

dish. As the epidermal cells on which the conidia were sown were rendered transparent by the removal of the underlying tissue, the development of the fungus could be followed in detail under the microscope.

Using this mode of culture, over fifty successful experiments were made in which the conidia of certain "biologic forms" were sown on "cut" leaves of species which are normally immune to their attacks. If the conidia of Erysiphe graminis occurring on wheat are sown on uninjured leaves of wheat and barley, the result will be the infection of wheat but never of barley. But if the conidia of this "biologic form" on wheat are taken and sown on barley leaves which have been "cut" as described above, full infection results, and conidiophores with ripe conidia are produced in 6-8 days.

Injuring the leaf by touching it with the red-hot point of a knife gave the same result. Conidia of "biologic forms" which are unable to infect normal uninjured leaves of certain host species proved able to do so when sown at these injured places.

Stakman (38), however, has not been able to confirm these results for the rusts. He has found that leaf injury apparently had no effect in making an immune plant slightly more susceptible to the rust.

Salmon (35) further found that an exposure of a host to ether vapor for 1½ to 2 minutes rendered it susceptible. Ex-

posure to chloroform vapor for 10 seconds also induced some degree of susceptibility. Immersion in a mixture of alcohol and water, and also exposure to vapor of alcohol, rendered leaves in many cases remarkably susceptible. McGill (20), however, found that when leaves of wheat and barley, still attached to the plants, were soaked in a ten per cent alcoholic solution for two hours they did not become infected when cross inoculated.

Adaptation of Biologic Forms to New Hosts.

Magnus (1894) (16) was one of the first to suggest that a particular biologic form might, by constant association with one host, change its physiological capabilities to such an extent as to make a new race out of it. This view was also expressed by Dietel (1899) (14) who gave it as his opinion that a given rust formerly attacked a number of plants, but by long association with one form became narrowed to this form more closely, possibly retaining also a somewhat weakened capability of attacking other forms. These authors distinguish between adaptative races (Gewohnheitsrassen) and true biologic forms, the tendency being, under favorable conditions, for the former to develop into the latter. Eriksson (8) (1902) also expressed this view in a somewhat modified form. Ward (1902-1) showed that adaptation of *Puccinia dispersa* occurred. Klebahm (1904) (15) has cited numerous experiments to show that this may be the case. Miss Gibson (12) (1904) grew a number of successive generations of rust on resistant host varieties, but, in that time, found

little adaptive tendency. Massee (19) (1904) explained the resistance and susceptibility of various plants to parasitic fungi on the basis of the presence of absence of chemtotactic substances in the host. He contended that saprophytes can be educated to become parasites. Stakman believed this would apply in a large measure to biologic forms. Salmon (1905) grew Erysiphe graminis from wheat on Hordeum sylvaticum for five generations and found no diminution in the power to infect the original host. Freeman and Johnson (1911) (10) concluded that "the host plants exercise a strong influence, not only on the physiological and biological relationships, but in some cases even on the morphology of the parasite."

Stakman attempted to test this out. The object was to determine the change, if any, in the physiology of the rust as evidenced by changes in spore dimensions. For this purpose Puccinia graminis tritici was grown on Minnesota No. 163, a susceptible wheat, and on einkorn 2433. The einkorn, in the first few trials, was apparently one of the most resistant of the wheat stem rust. The rust was transferred to einkorn and grown on this host through successive generations for 19 months, transfers being made, on an average, once every three weeks.

As a result of his work Stakman concluded that by confining Puccinia graminis tritici to einkorn for successive generations throughout a year or more the rust adapts itself somewhat to its new host and loses, at least to a slight degree,

its power to infect the original host. It must be noted, however, that this new character is not so firmly fixed that it cannot be overbalanced by environmental factors. The experimental production of new forms is apparently possible, but a long period of time is required.

Stakman found that the change in the fungus manifests itself not merely in the parasitic tendency toward the host but in the morphology as well. Wheat and einkorn were inoculated with spores from the same plant. The uredospores after growing for a year on wheat averaged 37.85x22.76 μ while those grown on einkorn for a year measured 33.58x21.79 μ . When einkorn was inoculated with aecidiospores of Puccinia graminis tritici, the resulting uredospores were more nearly identical with the wheat-rust spores in length. The width, however, remained practically the same. The average size of these spores was 35.92x21.69 .

The External Conditions Varied.

The external conditions, which were varied to learn the effect which changed environment might have in promoting or inhibiting the attack of the Erysiphaceae upon the hosts, were as follows: (1) humidity and soil moisture; (2) light, acidity and alkalinity of the soil; (3) excess of certain salts in the soil, and mineral starvation.

In all the experiments conducted the same varieties of plants were used throughout, namely:

Wheat, Treadwell.

Barley, Oderbrucker.

Sunflower, Large Russian.

Cocklebur, *Xanthium speciosum*.

Cucumber, White Spine Extra Early.

Oats, Wide Awake.

The Effect of Light and Darkness on the Susceptibility of Wheat and Barley to Their Powdery Mildews.

In order to determine the effect of darkness in delaying the infection and inhibiting the rate of development of the powdery mildews upon wheat and barley, the following experiments were carried out a number of times:

Twelve five inch pots of wheat and the same number of barley were planted, and six pots of each were placed in the dark-box during germination, while the remaining pots of each were left in the light of the greenhouse and allowed to grow.

After five or six days the wheat and barley seedlings grown in the light averaged in height, 5-8 cm., while those grown in the dark averaged 10-14 cm. in height. The seedlings were thinned until each pot contained about twelve seedlings. The pots of plants were then heavily inoculated with their respective mildew.

All of the inoculated pots of seedlings except two each of barley and wheat were placed in the dark-box. Each of these sets of two contained one pot of plants grown in the dark up to the time of inoculation, and one pot which had been in the light. These two sets were at this time placed under bell-jars in the light.

At intervals of twenty-four hours two pots each of inoculated barley and wheat seedlings were taken out of the dark-

box and placed under bell jars in the light of the greenhouse. In each pair, one of the cultures taken out had been grown in the dark up to the time of inoculation, and one culture had been grown in the light for a similar period.

The effect of darkness in delaying the evidence of infection is shown in the following tables, which are summaries of the results of a number of experiments:

TABLE NO. I

Conidia from Barley on Barley.

Plants in light or darkness previous to inoculation	Date of inoculation 1913	No. of days in darkness after inoculation	Date when first infection is visible 1913	No. of days infection was delayed
Darkness	Nov. 14	none	Nov. 17	
Light	Nov. 14	none	Nov. 17	
Light	Nov. 14	1	Nov. 17	
Light	Nov. 14	2	Nov. 17	
Light	Nov. 14	3	Nov. 17	
Darkness	Nov. 14	1	Nov. 18	1
Light	Nov. 14	4	Nov. 18	1
Light	Nov. 14	5	Nov. 19	2
Darkness	Nov. 14	2	Nov. 20	3
Darkness	Nov. 14	3	Nov. 20	3

The average height at the time of inoculation of the plants grown in the light was about 9.7 cm., while the plants which had been kept in the dark averaged about 14.5 cm. in height. The seedlings were seven days old.

TABLE NO. II

Conidia from Barley on Barley.

Plants in light or darkness previous to inoculation	Date of inoculation 1914	No. of days in darkness after inoculation	Date when first infection is visible 1914	No. of days infection was delayed
Light	Feb. 18	none	Feb. 22	
Darkness	Feb. 18	none	Feb. 22	
Light	Feb. 18	1	Feb. 22	
Darkness	Feb. 18	1	Feb. 22	
Light	Feb. 18	2	Feb. 23	1
Darkness	Feb. 18	2	Feb. 23	1
Light	Feb. 18	3	Feb. 23	1
Darkness	Feb. 18	3	Feb. 24	2
Darkness	Feb. 18	4	Feb. 25	3
Light	Feb. 18	4	Feb. 26	4
Light	Feb. 18	5	Feb. 26	4
Darkness	Feb. 18	5	Feb. 26	4

Average height, at time of inoculation, of plants grown in light, 5 cm.; grown in dark, 12 cm. Age of plants seven days.

TABLE NO. III

Conidia from Wheat on Wheat

Plants in light or darkness previous to inoculation	Date of inoculation 1913	No. of days in darkness after inoculation	Date when first infection is visible 1913	No. of days infection was delayed
Light	Oct. 10	none	Oct. 14	
Darkness	Oct. 10	none	Oct. 14	
Light	Oct. 10	1	Oct. 14	
Darkness	Oct. 10	1	Oct. 14	
Light	Oct. 10	2	Oct. 14	
Darkness	Oct. 10	2	Oct. 15	1
Light	Oct. 10	3	Oct. 15	1
Darkness	Oct. 10	3	Oct. 16	2
Light	Oct. 10	4	Oct. 16	2
Darkness	Oct. 10	4	Oct. 17	3
Light	Oct. 10	5	Oct. 17	3
Darkness	Oct. 10	5	Oct. 17	3

The average height of the plants grown in the light was about 2 cm., at the time of inoculation; the height of the plants grown in the dark averaged about 3.7 cm. Age of plants, four days.

TABLE NO. IV

Conidia from Wheat on Wheat.

Plants in light or darkness previous to inoculation	Date of inoculation 1913	No. of days in darkness after inoculation	Date when first infection is visible 1913	No. of days infection was delayed
Darkness	Nov. 14	none	Nov. 17	
Light	Nov. 14	none	Nov. 17	
Darkness	Nov. 14	1	Nov. 17	
Light	Nov. 14	1	Nov. 17	
Light	Nov. 14	2	Nov. 17	
Light	Nov. 14	3	Nov. 17	
Darkness	Nov. 14	2	Nov. 18	1
Light	Nov. 14	4	Nov. 18	1
Light	Nov. 14	5	Nov. 19	2
Darkness	Nov. 14	3	Nov. 20	3
Darkness	Nov. 14	4	Nov. 20	3
Darkness	Nov. 14	5	Nov. 20	3

Average height, at time of inoculation, of plants grown in light, 6.5 cm.; the plants in the dark average 12.7 cm. in height. Age of plants, seven days.

TABLE NO. V.

Conidia from Wheat on Wheat.

No. of culture	Plants in light or darkness previous to inoculation	Date of inoculation 1914	No. of days in darkness after inoculation	Date when first infection is visible 1914	No. of days infection was delayed
L0	Light	Feb. 18	none	Feb. 22	
D0	Darkness	Feb. 18	none	Feb. 22	
L1	Light	Feb. 18	1	Feb. 22	
D1	Darkness	Feb. 18	1	Feb. 22	
L2	Light	Feb. 18	2	Feb. 22	
L3	Light	Feb. 18	3	Feb. 22	
D2	Darkness	Feb. 18	2	Feb. 23	1
L4	Light	Feb. 18	4	Feb. 23	1
D3	Darkness	Feb. 18	3	Feb. 24	2
L4	Darkness	Feb. 18	4	Feb. 24	2
D5	Darkness	Feb. 18	5	Feb. 25	3
L5	Light	Feb. 18	5	Feb. 25	3

The average height of plants, at the time of inoculation, was 12 cm.; the plants grown in the dark averaged 18 cm. in height. The plants were nine days old.

The preceding tables do not show completely the extent to which infection was delayed. They show when infection was first apparent or when infection was first noted, with any degree of certainty. The fungus may have made nearly twice as much progress, as far as mycelia development was concerned, when compared to another, although infection was first observed in both cases simultaneously.

Even when infection had been apparent on all the plants for five or six days, the cultures which had been among the last to be removed from the dark-box, were relatively not so heavily infected, and the infection was not so markedly advanced as on the plants which had been in the dark only two or three days. Evidently the absence of light has had some inhibiting effect upon the infecting power of the mildew, or upon the susceptibility of the host plant.

In order to illustrate this difference the details of the observations of one experiment are given as an example.

Observations on Wheat Cultures, the Results of Which are Tabulated in Table No. V.

In the following observations on the wheat cultures of one experiment, showing the effect of darkness upon the susceptibility of wheat to the powdery mildews, the date of the observation is given as the heading. L indicates that the plants have been grown in the light up to the time of inoculation; D means that the plants have been grown in the dark up

to the time of removal from the dark-box. Following the letter is a number which corresponds to the number in the table, and also shows the number of days the culture had been in the dark-box after inoculation. The other conditions under which the experiment was carried out can be learned from Table No. V and the general description given of all the experiments.

<u>Date of Observations Feb. 22, 1914</u>.

L0, light colored areas showing commencement of infection.

D0, The infection here, it was judged, had progressed to about one-half the degree that it had in L0.

L1, shows infection about like L0.

D1, The infection is not quite so far advanced as D0.

L2, shows good evidences of infection.

D2, shows no definite evidence of infection. A few light colored areas are visible. Leaves somewhat etiolated.

L3. Infection slightly evident but the development was delayed when compared with L2. The leaves are somewhat etiolated.

D3. No infection is apparent. The leaves are very much etiolated.

L4 when removed from the dark-box might possibly have shown commencement of scattered infection, by light colored areas on the leaves.

D4 shows no infection. The leaves are very much etiolated.

Observations Feb. 23, 1914.

The infection on L0 and L1 seems to be just about the same, but the infection on L0 is perhaps a little further advanced.

The infection on D0 and D1 is approximately the same, but the infection in either case is not quite so far advanced as the infection on L0 and L1.

L2. Good infection, about like L0 and L1.

D2. Slight infection apparent.

D3 and D4. No infection apparent. The leaves are etiolated.

L3 shows infection, but the infection is not so far advanced as L2.

L4. Very slight infection apparent.

L5. When these plants were removed from the dark-box a few light areas were visible on the leaf as if the commencement of infection had taken place.

D5. No infection apparent.

Observations Feb. 24, 1914.

L0 and D0; L1 and D1. There is apparently no difference between these two cultures, in the degree of infection. Conidia have been produced by the mildew growing upon

both sets.

D2 shows infection. The leaves are still somewhat etiolated.

L2 shows splendid infection.

D3 shows commencement of slight infection, but it has not advanced so far as on D2. The leaves are somewhat etiolated.

L3. Infection is apparent, but is not so far advanced as D2.

D4. Very slight infection is seemingly apparent, but it is not so far advanced as D3. The plants are all etiolated.

L4. Very slight infection. Plants are somewhat etiolated. A microscopic examination showed scattered groups of rather abundant growths of mycelium, with haustoria established in the cells but no conidia were found. Structures which seemed to be conidiophores were pushing out but no conidia had been abstricted.

Observations Feb. 25, 1914.

L4. Infection.

D4. Infection.

L5. No infection apparent.

D5. No infection apparent.

Observations Feb. 26, 1914

L5. Infection.

D5. Infection.

A final experiment was conducted with wheat mildew upon wheat to learn if infection would take place in the dark. The cultures used were nine days old. The plants were removed every forty-eight hours instead of every twenty-four hours. The following table summarizes the results of this experiment.

TABLE NO. VI

Conidia from Wheat upon Wheat

Plants in light or darkness previous to inoculation	Date of inoculation 1914	No. of days in darkness after inoculation	Date when first infection is visible 1914	No. of days infection was delayed
Light	March 27	none	April 1	
Darkness	March 27	none	April 1	
Light	March 27	2	April 1	
Darkness	March 27	2	April 2	1
Light	March 27	4	April 4	3
Light	March 27	6	April 4	3
Darkness	March 27	4	April 5	4
Darkness	March 27	6	April 5	4
Light	March 27	8	April 6	5
Darkness	March 27	8	April 6	5
Light	March 27	10	April 6	5
Darkness	March 27	10	April 6	5

All of the plants which were removed from the dark-box after ten days were infected when taken out. The following notes were taken upon these cultures. The date of observation is given as the heading; L signifies that the plants had been grown in the light; and D in the dark. The number following these letters signifies the number of days the plants had been kept in the dark-box after inoculation.

L10 and D10 both showed infection when taken from the dark-box. D10 showed a little more marked infection than L10. The infected areas of L10 are green, showing the presence of chlorophyll, while the non-infected parts of the leaves are very much etiolated.

D12 when removed from the dark-box showed good infection with abundant production of conidia. The leaves were very much etiolated.

L12 showed slight infection and production of conidia when removed from the dark-box. The infected areas tend to remain green in color, although the other parts of the leaves are much etiolated.

To Determine the Effect of Darkness Upon the Susceptibility of the Sunflower to *Erysiphe cichoracearum*.

In order to determine the effect of darkness in delaying development of the powdery mildew upon sunflowers two experiments were conducted in much the same manner as described for wheat and barley.

An equal number of pots of plants were grown in the dark and in the light. The plants of both sets were then heavily inoculated by scraping off some of the conidia from an infected sunflower with a knife and applying them to the cotyledons.

After inoculation one pot of seedlings from the set grown in the light and one pot from those which had been in the dark were immediately placed in the light under bell-jars. The remaining pots of sunflowers were placed in the dark-box.

Thereafter, in the first experiment one pot of seedlings from each set was removed from the dark-box and placed in the light at intervals of twenty-four hours. The following table summarizes the results of this experiment:

TABLE NO. VII

Plants in light or darkness previous to inoculation	Date of inoculation 1914	No. of days in darkness after inoculation	Date when first infection is visible 1914	No. of days infection was delayed
Light	Jan. 12	none	Jan. 22	none
Darkness	Jan. 12	none	Jan. 22	none
Light	Jan. 12	2	Jan. 22	none
Darkness	Jan. 12	2	Jan. 22	none
Light	Jan. 12	3	Jan. 22	none
Darkness	Jan. 12	3	Jan. 22	none
Light	Jan. 12	4	Jan. 22	none
Darkness	Jan. 12	4	Jan. 22	none
Light	Jan. 12	5	Jan. 22	none
Darkness	Jan. 12	5	Jan. 22	none
Darkness	Jan. 12	6	Jan. 22	none
Darkness	Jan. 12	7	Jan. 22	

The plants were ten days old. Those which had been grown in the light averaged 8 cm. in height, while those grown for a similar period of time in the dark averaged 9.2 cm. in height.

The second experiment with sunflowers was conducted very much like the first except that one pot from each set was removed from the dark-box every three days instead of every twenty-four hours. The plants in the dark box had only the cotyledons with the first pair of leaves scarcely starting to develop. The plants in the light had the first pair of leaves developed. The cotyledons were inoculated with the mildew by means of a scalpel.

TABLE NO.VIII

No. of culture	Plants in light or darkness previous to inoculation	Date of inoculation 1914	No.of days in darkness after inoculation	Date when first infection is visible 1914	No. of days infection was delayed
L1	Light	March 30	none	April 4	
D1	Darkness	March 30	none	April 4	
L2	Light	March 30	3	April 4	
D2	Darkness	March 30	3	April 4	
D3	Darkness	March 30	6	April 6	2
L3	Light	March 30	6	April 7	3
D4	Darkness	March 30	10	April 9	

The sunflower seedlings were eleven days old. The plants grown in darkness averaged 17 cm. in height. The height

of the plants grown in the light was about 8 cm.

D1 and D2 both showed a more pronounced infection than L2.

D3 showed infection one day before L3. The infection upon L3 was very slight.

D4 showed infection when removed from the dark box.

Conclusions

The results of these two experiments would lead one to believe that darkness has no inhibiting effect upon the powdery mildew in its power to infect the cotyledons of sunflower seedlings.

The second experiment indicates that growing the sunflowers in the dark and then placing them in the light soon after inoculation, makes conditions more favorable for the successful attack and growth of the mildew upon these host plants.

The Growth of Puccinia graminis Upon Plants Kept in the Dark: Fromme (11) working with the cereal rusts determined the effect of light exclusion on spore germination and rate of development. Four culture pots of the same age - seven days - were inoculated simultaneously. Immediately afterwards one of the four was transferred to a physiological dark room which joined the greenhouse. A continuous circulation of air between the two rooms was maintained by an electric fan. Thus the average degree of humidity of the dark room, which he says was about 80 per cent, did not fall below that of the greenhouse although the range of fluctuation, which was from 60 per cent to 95 per cent, was somewhat greater. The other three cultures were placed in the culture box as controls. The culture was exposed in the dark room for three days and at the end of this time was returned to the culture box. The plants at this time were quite as green and fresh as those of the control cultures and could not be distinguished from them. The incubation period for the three controls was eight days, while that of the culture left three days in the dark room was eleven days. At the time of sporulation some of the leaves of this culture showed signs of yellowing at their tips. No pustules were produced on these discolored areas, but on the normal green parts they were as numerous as on the controls. "The difference of three days in the incubation periods is exactly equal to the period of light exclusion and indicates," writes Fromme, "a complete

arrest of the development of the fungus in the dark room."

Fromme also tested the effect of light exclusion during the latter part of the incubation period. Four cultures were inoculated and placed in the culture box. Four days later one of them was transferred to the dark box, where it was left four days, and then returned to the culture box. No signs of infection were visible on it at this time, while the unripe pustules on the controls were plainly visible. The pustules on the controls ripened on the ninth day, while three additional days, twelve days in all, were necessary for a similar development of the culture that had been in the dark room. By excluding light four days in the latter part of the normal incubation period, the maturation of the rust had been delayed three days. "This shows," writes Fromme, "that even after the fungus has become well established in the host, its development is strongly retarded in complete darkness."

Fromme found that the incubation period is not modified by relative degrees of humidity after the first twenty-four hours. He says the difference in degree of humidity could not have been an important modifying factor in these results.

Fromme raises the question as to how this retardation of the growth of the fungus is brought about. He suggests that it may be the direct effect of total absence of light on the fungus itself. Then again, it is possible that the fungus simply suffers from lack of food, since the host is incapable

of assimilation in the darkness. It seems hardly possible, however, that such a complete inhibition in the growth of the fungus should have resulted in the brief time involved unless it is dependent on the transition products in photosynthesis. This latter possibility is by no means inconceivable and should this explanation prove the correct one it could be made the basis for an explanation of the obligate parasitism of the rusts and their inability to develop on any form of artificial medium.

The Influence of Light and Darkness Upon Growth and Development of Plants: MacDougal (17) (1903) writing about the modes of influence of light upon plants says, "at the present time evidence is at hand to show that certain synthetic effects, such as the union of oxygen with some portions of the protoplasmic substances, may be produced in the organism, by the action of sunlight.......It has also been found that light exerts a direct influence upon the enzymes in protoplasm."

MacDougal says it is entirely probable that the action of light may set up chemical processes in the plant in a manner entirely stimulative, and independent of any communication or transformation of energy. He adds that so far as known facts are concerned, the only method by which light might exert an effect upon growth would be by the decrease of the enzymes participating in the various stages of the process. "The stimulative action of light in chemical processes," writes MacDougal, "is well illustrated in the matter of formation and maintenance

of chlorophyll. Protoplasm is capable of constructing this complex and unstable substance in darkness, and of maintaining it in a fairly normal condition for periods extending over many months. In many species, however, the process of formation is not set up except under the stimulation of light, and the entire spectrum appears to participate in the stimulation. Simultaneously, however, the upper end of the spectrum exerts a disintegrating action, which is probably a direct chemical effect of the same character as that by which enzymes are broken down."

MacDougal believes it is undeniable the etiolation must create most serious disturbances in the nutritive system. The photosynthetic power usually exhibited by chlorophyllaceous organs is wholly lacking, and if the plant is autotropic in its method of subsistence it must prosecute its entire development by the aid of reserve food laid up in its storage tissues. The amount of this material, even in seedlings, is usually far in excess of that needed for the stage of development for which it is provided, but when the plant is forced to depend upon this supply by confinement in the dark room, for the construction of organs and tissues usually supplied from the foliar organs, variations may be expected.

According to MacDougal, the extended endurance of etiolated leaves, and of other organs to confinement in darkness, and the fact that fully etiolated members are capable of taking up normal development when brought into normal illumination lead

to the conclusion that etiolation is not necessarily a pathological condition, a statement corroborated by the condition of the protoplasts of the organs in question.

He further writes, "the unusual ductility of the bodies of plants in darkness is a consequence, or accompaniment of the abnormal forms produced by etiolation, not a cause of them, and the extended existence of shoots in darkness and their subsequent behavior when exposed to illumination is signal proof that a shoot absolutely etiolated is not in a pathological condition in the ordinary acceptance of the term, although when a mature green leafy shoot is confined to darkness the leaves and other chlorophyll-bearing members may become more or less pathological, in a manner which might be expected when any active tissue is forced into prolonged inactivity."

<u>Influence of Etiolation Upon Chemical Composition:</u> With MacDougal (17) as the authority again, the constant respiration carried on by etiolated plants must of course results in the combination of a large amount of plastic material, while on the other hand not so much of the plastic substance is converted into a plastic form in the manufacture of cell-walls in permanent tissue. According to the researches of Palladin etiolated stems contain much less proteinaceous material than the normal. Etiolated leaves of "stemless" plants also were much poorer in proteids than green organs of the same species, while etiolated leaves borne on aerial stems were richer in protein than normal

green leaves. These results were obtained from examinations of etiolated and normal specimens of beans and wheat.

From a table by Karsten which gives the number of parts of the principal constituents of the dried material (at 105° C.) in 100 parts of normal and etiolated plantlets, the proportion of fats is seen to decrease in the leaves during etiolation, to remain stationary in the hypocotyl, to decrease in the stem, and increase in the roots.

The proportion of sugar undergoes a marked decrease during etiolation throughout the entire plant, the greatest percentage remaining in the cotyledons. The amount of starch was greatest in etiolated leaves, normal hypocotyles, etiolated first internodes and normal cotyledons.

MacDougal and André demonstrated that etiolated plants can take up mineral substances from the substratum.

Infection Closely Associated with the Presence of Chlorophyll: Infection seemed to be somewhat closely correlated with the presence of chlorophyll. A résumé of the notes taken during the observations shows that in nearly every case where no infection was apparent, the leaves were etiolated and that soon after chlorophyll had been formed infection would be apparent. Sometimes cultures which had been grown in the light up to the time of inoculation and then placed in the dark-box, when removed from the dark-box at the end of three or four days, would show slight infection at the tips where the blades were still

somewhat green, although no infection would be apparent on the parts of the leaves which had become etiolated.

<u>No Delay in Germination of Conidia in the Dark</u>: To determine if there was any delay in the germination of the conidia in the dark-box, plants were heavily infected and some placed in the dark-box and others left in the light of the greenhouse. At the end of twenty-four hours, leaves were mounted on slides, and stained with an alcoholic solution of iodine for five minutes. By using care to avoid washing the conidia off the leaf, the percentage of germination could be determined microscopically. There was no appreciable difference in the amount of germination on the leaves in the dark and in the light.

<u>Sugar is Present in Leaves of Barley and Wheat Seedlings Which Have Been Grown in Dark Six Days</u>: To determine if infection was due to a lack of food supply, a sugar test was carried out upon the leaves of wheat and barley seedlings grown in the dark-box for six days and upon some grown in the light six days. Fehling's solution was used to make the test, and sugar was found to be present in the leaves of the cultures grown both in the light and in the dark.

Judging by the amount of precipitate present, it would seem that there was a little more sugar present in the leaves of the plants grown in the light than in the plants grown in the dark. The food supply stored in the seed, however, it seems would furnish a supply of food, available in large enough

quantities for some time, for the fungus to obtain its required amount of nourishment, provided it could get its nourishment from food in the form of sugars, and was not dependent on the transition products in photosynthesis as Fromme has suggested.

Summary

When wheat and barley cultures were placed in the dark after inoculation and allowed to remain four or five days, infection was delayed 2 or 3 days in the case of the wheat and 3 or 4 days in the case of barley. Five days was the longest that infection could be delayed upon wheat After this period of time infection took place in the dark.

The cotyledons of sunflower seedlings in the dark show infection as soon after inoculation as seedlings in the light.

The delay of infection upon wheat and barley was, for the most part, a little more marked when the plants had been grown in the dark up to the time of inoculation than when the plants had been grown in the light for a similar length of time.

The power of the mildew to produce infection seemed to be somewhat closely associated with the presence of chlorophyll in the leaves.

The conidia of the mildew from wheat and barley will apparently germinate equally well in the light and in the dark.

Soluble sugars are present in leaves of barley

and wheat seedlings, which have been grown in the dark six days.

Conclusions.

The delay of infection of barley and wheat with their powdery mildews does not seem to be due to the direct effect of the absence of light upon the fungus, but rather to an indirect effect upon the plants.

The inhibiting action of the absence of light upon infection of wheat and barley is not complete, infection will take place in the darkness, after a given period of time although the normal incubation period may be doubled.

Darkness seems to have no inhibiting effect upon the power of Erysiphe cichoracearum to infect the cotyledons of sunflower seedlings.

It does not seem that the lack of available food, in the form of sugars at least, would altogether account for the action of darkness in inhibiting or delaying the infection upon wheat and barley. This does not, however, preclude the possibility that, for its best and perhaps normal development, the fungus may need some of the transition products in photosynthesis.

It is evident that in the absence of light a number of complex physiological disturbances are set up; serious disturbances have been created in the nutritive system, chemical processes are carried on in an abnormal manner, the activity of the enzymes has been influenced, etc.

Furthermore it is quite possible that some of these physiological disturbances have been profound enough to act in a deleterious manner upon the power of the fungus to establish itself and to flourish upon the host plant.

It does seem likely, however, that it is due to a combination of factors rather than to any one specific cause that the incubation period of the mildew upon wheat and barley is lengthened.

There might be some pathological change in the host plant which prevents the normal development of the mildew upon it.

Since, however, the power of the mildew to produce vigorous infection upon wheat and barley seems to be rather closely associated with the presence of light and chlorophyll, it may be possible that the fungus is dependent in some way upon the transition products of photosynthesis, as Fromme (11) has suggested.

To Determine the Effect of Mineral Starvation Upon the Susceptibility of Host Plants to Powdery Mildews.

To demonstrate what effect mineral starvation has upon the susceptibility of the host to the powdery mildew, wheat, barley and sunflower seedlings were grown in nutrient solutions in which the different essential mineral elements were lacking.

The culture solutions used were made as described by Duggar (7).

Chemically pure compounds were used and the water was distilled, once in a copper still and twice with glass vessels.

Stock solutions of each main constituent of Pfeffer's solution were prepared in the proportional quantity of water as follows:

Calcium nitrate $Ca(NO_3)_2$	4 gms. in 1000 cc. redistilled H_2O
Potassium nitrate KNO_3	1 gm. in 1000 cc. redistilled H_2O
Magnesium sulphate $MgSO_4$	1 gm. in 1000 cc. redistilled H_2O
Monobasic potassium phosphate KH_2PO_4	1 gm. in 1000 cc. redistilled H_2O.
Hydrochloric acid HCl	0.5 gm. in 1000 cc. redistilled H_2O
Iron chloride $FeCl_3$	trace

1. A full nutrient solution of proper proportions is obtained by taking equal quantities of the above.
2. Minus nitrogen.

   For the $Ca(NO_3)_2$ substitute $CaCl_2$ in the pro-

portion of 4 grams in 1000 cc. of H_2O and for the KNO_3 substitute KCl in the proportion of 1 gram in 1000 cc. of H_2O.

3. Minus phosphorus.

   For the KH_2PO_4 substitute KCl in the proportion of 1 gram in 1000 cc. of H_2O.

4. Minus potash.

   For the KNO_3 substitute $NaNO_3$ in the proportion of 1 gram in 1000 cc. of the H_2O;for the KH_2PO_4 substitute NaH_2PO_4 in the proportion of 1 gram in 1000 cc. of H_2O.

5. Minus calcium.

   For the $Ca(NO_3)_2$ substitute $NaNO_3$ in the proportion of 4 grams in 1000 cc. of H_2O.

6. Minus magnesium.

   For $MgSO_4$ substitute Na_2SO_4 in the proportion of 1 gram in 1000 cc. H_2O.

7. Minus sulphur.

   For $MgSO_4$ substitute $MgCl_2$ in the proportion of 1 gram in 1000 cc. of H_2O.

8. Minus iron.

   For $FeCl_3$ substitute NaCl adding a trace to 1000 cc of H_2O.

Hoffman's (13) paraffin blocks were used to support the growing seedlings in the culture solutions. Paraffin of a

comparatively high melting point is used. The paraffin is melted and poured out in a shallow pan containing hot water, forming a layer of paraffin above the water of the desired depth, and then allowed to solidify. From the layer thus obtained, blocks of the desired size can be cut with various sized cake cutters or with a sharp thin knife.

The blocks of paraffin are then perforated by means of a cork-borer. Two different diameters are used, a smaller perforation is made through the entire block and then a larger hole is made through the upper portion of the block.

The nutrient solutions were placed in ordinary hydrometer cylinders having a capacity of about 500 cc. These cylinders have an enlargement at the upper portion and this supports the paraffin disk and prevents it from sinking as the plants increase in size and weight.

To exclude light from the root system the cylinders were wrapped with black paper. The black side of the paper being next to the glass and the white side out.

All glassware was thoroughly cleansed with cleaning solution and then carefully rinsed, first with tap water, next with distilled water, and then with re-distilled.

The seeds were germinated as follows: A wire screen of one-fourth inch mesh was coated with paraffin and supported in a vessel of water by means of corks, so it just rested on the surface of the water. The seeds were spread out on this and allowed

to germinate. This enabled them to be kept sufficiently moist, and gave the molds very little trouble.

When the roots had attained a length of 2 to 5 centimeters the seedlings were transferred to the culture solutions.

The cultures were always set up in duplicate and when wheat and barley were grown each cork supported three seedlings. When sunflowers were grown each culture solution contained two seedlings.

Wheat Grown in Re-distilled Water.

Six culture cylinders containing redistilled water were set up. Each culture contained three seedlings.

As checks another set of six containing tap-water were set up. These were allowed to grow in the greenhouse and to become infected with mildew naturally. After two weeks some of the leaves of all the plants were infected with mildew. The plants grew for about five weeks and the mildew continued to infect the new leaves as they were formed.

In this case the plants were grown in a culture solution containing no mineral elements and they exhibited just as abundant infection as the plants grown in tap-water.

At different times two sets of cultures where one element was minus in each culture, were set up and wheat plants were grown in them for over a month. The following tables give the results, which the lack of minerals had upon the wheat plants' susceptibility.

TABLE NO. IX

Elements lacking	-K	-Fe	-N	-P	-Ca	-Mg	-S	Full nutrient
After 21 days nat. infection as follows	good infection	slight infection	none	good infection	infected	slight infection	none	good infection
After 30 days infection (nat.) as follows	infection	infection	none	infection	infection	infection	slight infection	infection
After 40 days infection (nat.) as follows	infection	infection	infection	infection	infection	infection	slight infection	infection

A final experiment was set up to show the effect of mineral starvation upon the susceptibility of wheat plants to the powdery mildew. Twenty liter glass jars, filled with pure white sand, were buried sufficiently deep in soil to exclude the light from the roots. Six wheat seeds were planted in each jar with the different essential mineral elements lackings. A duplicate set of each was arranged.

The following tables summarize the results of this experiment.

Table No. X

First Set.

Elements lacking	Full nutrient	-Ca	-N	-K	-P	-Mg	-S	-Fe
Infection observed after 17 days growth	Seeds did not germ. new seed planted		Slight infection			Slight infection		Slight infection
Observations after 32 growth		Infection	leaves turning yellow	Infection	leaves turning yellow infection	Infection	Infection	Infection
Observations after 39 days of growth	Infection	Infection	Slight infection	Good infection	Slight infection	Very slight infection	Infection	Infection

Table No. XI

Second Set.

Elements Lacking	Full Nutrient	-Ca	-N	-K	-P	-Mg	-S	-Fe
Infection observed after 17 days growth		Seeds did not germ. new seeds planted		Slight infection	Slight infection	Slight infection	Slight infection	
Observations after 32 days of growth	Infection	Infection	Leaves turning yellow	Infection	Infection	Infection	Infection	Infection
Observations after 39 days growth	Infection	Infection	No infection	Good infection	Slight infection	Very slight infection	Slight infection	infection

The following table shows the effect of mineral starvation upon the susceptibility of the sunflower to its mildew.

Table No. XII

Elements Lacking	Full Nutrient	-Ca	-N	-K	-P	-Mg	-S	-Fe
After 30 days nat. infection as follows	none	Infection	none	none	none	none	Infection	none
After 37 days infection as follows	Slight infection	Infected	plants dead	splendid infection	Infected	Infected	Infected	Infected
Second Experiment.								
After 28 days infection as follows	Infected	Infected	plants dead	none	none	none	Infected	Infected
After 41 days infection as follows	Infected	Infected	plants dead	Infection	Infection	Infection	Infected	Infected

Ward (37) believed if the infective power of the fungus towards different species of host-plant is derived solely from the "nutritive conditions" afforded it by the host-plant it has hitherto been growing upon, two cases are possible - (1) these "nutritive conditions" may be simply the expression of the power of the tissues to yield certain food substances to the parasite in proper proportions and in sufficient quantity, or (2) they may imply some more subtle relations between the mycelium of the fungus and the living contents of the host-cells. For instance, it may not be sufficient that the food substances suitable to the fungus should exist in the cells of the host, but they must be there in a certain superabundance, or presented in a certain manner; or, it may be that the fungus must be vigorous up to a certain standard before it can obtain a hold on such food.

In order to test some of the possibilities referred to Ward planned experiments with Puccinia dispersa on species of Bromus to see whether starving the host-plant of one or other of its necessary food materials would (1) affect its predisposition to infection, or (2) affect the capacity for infection of the fungus grown on the starved plants, or (3) in any other way affect the fungus or its host.

Ward concluded as a result of his experiments that: Lack of minerals in no way secured immunity from infection, though seedlings deficient in phosphorus or in nitrogen tended to show retardation of infection.

Mineral starvation makes itself felt quantitatively in the number of uredospores which can be produced by the fungus in the tissues of the starved leaves.

The pustules yielded spores capable of normal germination, in proportions which showed no relation to the degree or kind of starvation of the seedling which bore them.

The spores, even when reared on starved seedlings, are capable of normal germination and infection when placed on the leaves of other normal seedlings.

Ward found that practically all the infections were successful, showing that not only does mineral starvation not prevent the development of virulent spores on the seedlings so starved if the latter is inoculated with normal spores, but such starvation is also incapable of incapacitating the corresponding seedling for infection by means of spores grown on similarly starved seedlings.

Finally Ward concludes that ( ) the starvation of mineral food substances, although it reduces the size of the host-plant and seriously diminishes the quantity of spores which the mycelium can give rise to on its leaves, does not affect either the virulence of such spores or the predisposition to infection of the leaves of the Brome concerned.

The results of the experiments while not at all conclusive agree closely with those obtained by Ward (37). The tables show that infection was at least delayed on the plants

grown in minus nitrogen,but there was no appreciable differences in the case of the plants grown in minus phosphorus as compared to the other plants. The plants grown in minus nitrogen culture solutions turned yellowish and often died. The general condition of the plants in minus nitrogen was much below par when compared to the plants in any of the other culture solutions.

Stakman (38) working with the rusts upon wheat tried the effect of excluding nitrogen and phosphorus. Sach's modified medium plus one per cent of agar was used and in I no potassium nitrate was added while calcium phosphate was excluded from II. The plants in I were lighter colored from the first than either those in II or the checks. They were inoculated six days after planting. A good vigorous infection resulted, the plants in I being slightly more severely attacked than those in II. The leaves of I began to turn yellow after three weeks, and the rust did not spread farther. The check plants were more severely attacked than those in either I or II. Here again, however, the differences were not very great. There was a slight quantitative difference but qualitatively there was scarcely any difference. This is in accordance with Ward's conclusions reached after his work on mineral starvation.

Stakman found that in general the absence or presence in excessive amounts, of various nutrient substances, such as nitrogen and phosphorus compounds, did not directly affect the immunity or susceptibility of wheats. Conditions favoring a normal devel-

opment of the rust. The action of fertilizers, either natural or artificial, is probably indirect. He believes temperature conditions and relative humidity of the atmosphere are probably more important than soil conditions.

Spinks (37) came to the conclusion that increased immunity does not appear to be due to a lack of food-material available for the fungus in the host, as suggested by M. Ward (41), because he found that plants rendered relatively immune by adding phosphates or potash to their food supply were as healthy and well grown as those receiving no such additions.

Conclusions.

It would seem that quantitative immunity to the mildew is brought about by mineral starvation, indirectly, and only so far as the general health and vigor of the host is influenced. When the plants commence to suffer, as shown by a yellowing of the leaves, etc., the vigor of the mildew is at the same time lessened. Because the host plants suffered most from the lack of nitrogen, the plants grown in solutions from which the element nitrogen was lacking, seemed to exhibit the greatest degree of immunity, but in all cases as long as the plant remained alive, infection took place.

Plants grown in distilled water could become infected with mildew so long as the plants remained alive, and this was true with the plants grown in Pfeffer's nutrient solutions with one essential mineral lacking.

To Determine the Effect of the Addition of Certain Chemical Compounds to the Soil Upon the Hosts Susceptibility to the Powdery Mildew.

Various chemical compounds were added in solution to the soil of the pots in which the different hosts were growing to determine if the hosts' susceptibility to its mildew could in any way be influenced.

In every case control plants watered with tap-water were inoculated at the same time as the treated plants to give a basis of comparison.

The following tables give the results of some of these experiments:

Table No. XIII

Date seeds were planted 1914	Date treatment commenced 1914	Treatment given	Plants treated	No. of pots	Observation Jan. 26 1914	Observation Feb. 4 1914	Observation Feb. 25 1914
Jan. 19	Jan. 21	Dilute KOH	Wheat	6	Infection		
Jan. 14	Jan. 21 plants aver. 2cm in height	tap-water	Sunflowers	3		Infection	
" "	"	5% KNO_3	"	3	Plants wilting	Infection	Dead
" "	"	Solution containing trace of eosin	"	3	dying	dead	
" "	"	tap-water	"	3		Cotyledons show infection	
" "	"	Dilute solution KOH	"	3	Wilting	Cotyledons show infection	Dead
" "	"	5% solution $Ca(NO_3)_2$	"	3	Wilting badly	Infection	Dead
" "	"	5% solution cane sugar	"	3		Infection	

TABLE NO. XIV

Height of seedlings when treated	Date treatment commenced 1914	Treatment given	Plants treated	No. of Pots	Observations Jan. 30,1914	Observations Feb. 25,1914
3.5 cm.	Jan. 22	5% solution KNO_3	Cucumbers	3	Plants drooping	Cucumbers show no
3.5 "	" "	5% solution $Ca(NO_3)_2$	"	3	Plants drooping	relative difference in their susceptibility
3.5 "	" "	tap-water (checks)	"	3	--------	to the mildew as a result
3.5 "	" "	5% solution cane sugar	"	3	--------	of the treatmants
					Feb. 4,1914	
3 cm.	Jan. 26	1% solution of Barium oxide	Barley	3	Infection	Plants show no appreciable immunity
3 cm.	" "	1% solution Manganese sulphate	"	3	Very slight infection	Plants show a good deal of immunity
3 cm.	" "	tap-water (checks)	"	3	Infection	

TABLE NO. XV

Date seeds were planted 1914	Date treatment commenced. 1914	Treatment given seeds	Plants treated	No. of pots	Observations Feb. 25, 1914	Observations Mar. 7, 1914
Jan. 26	Jan. 26	Soaked in a paste of MnO_2 two hours	Barley seeds	10	Slight infection	Good infection
" "	" "	Soaked in dist. H_2O for two hours	"	10	Splendid infection	Good infection
Feb. 25	Feb. 24	Soaked in a paste of MnO_2 24 hrs	"	6	April 4	No appreciable difference between the susceptibility
" "	" "	Soaked 24 hours in dist. H_2O (checks)	"	6	April 4	of the treated and check barley plants

It was believed as a result of the first experiment that the seedlings experienced a slight temporary immunity when the seeds had been soaked for a short time in a paste of Manganese dioxide. The other experiments of a similar nature gave the seedlings which had been treated no appreciable immunity.

The above tables, except the last one, show approximately the result of adding chemicals to the soil after the plants had been allowed to grow for a time and to attain a height of about 3 cm. It was found that plants thus treated could not

adjust themselves to the action of the chemicals as well as when they were treated in a similar manner from the time the seed was planted.

Hence all the above treatments caused the plants to suffer more or less - that is, the chemicals acted in a deleterious manner upon the metabolic processes of the plants.

As has been stated, the object of the above experiments was to determine if the immunity of the host plant could be increased by the addition of certain chemicals.

The tables show that no real appreciable immunity was brought about by any of the treatments except the addition of the 1% manganese sulphate. As has been mentioned if any immunity is brought about by soaking barley seeds in a paste of manganese dioxide, it is only a very temporary immunity while the plants are young. Unless there is a fairly well marked immunity it is extremely hard to say definitely that the immunity has been increased, because of the difficulty of giving a uniform standard dose of conidia to all the plants of both the treated and control sets.

While the barley plants watered with a one per cent solution of manganese sulphate,did not show absolute immunity, they did exhibit a marked immunity to their powdery mildew. The leaves of barley plants, treated in this manner, had small brownish spots on them closely distributed throughout their entire area, as though the tissue had dried. Such leaves were relatively quite free from the mildew while the check plants were heavily

infected.

With the one or two exceptions, so long as the plant remained alive and in a fairly healthy condition, it was susceptible to the attacks of the powdery mildew. As the leaves would turn yellow, dry up, or as the general vitality of the plant would be markedly lowered, the conditions would become less favorable for the growth of the mildew.

In other words, unless as in one or two cases where an age factor entered in[1], the conditions conducive to the health of the plant were at the same time favorable to the infecting power of the parasitic powdery mildew.

In order to further test the influence of manganese sulphate in increasing the immunity of barley to the powdery mildew, further experiments were conducted.

Barley seed was planted in twelve five inch pots containing garden soil. Six pots were watered with a one per cent solution of manganese sulphate and six pots, to serve as controls, were watered with tap-water.

The plants were allowed to grow for ten days when they were heavily and approximately equally inoculated with powdery mildew.

Five days later both sets of plants showed infection but there was a noticeable difference in the amount of infection on the plants watered with a one per cent solution of manganese sulphate and the checks. The latter showed a greater amount of infection than the treated plants.

As the plants grew older and new leaves were formed both sets of plants were heavily inoculated from time to time. The tips of the leaves of the plants watered with manganese sulphate turned yellow and dried. The leaves were also dotted rather thickly with brownish somewhat dried areas. The check plants continued to show splendid infection, but the treated plants showed very little infection, only an infected area now and then on a leaf.

- Four weeks after the plants had been first inoculated, the check plants showed good infection on all of the leaves, while the treated plants showed scarcely any infection, except for a few widely scattered areas on each leaf. The leaves and stems of the treated plants stand erect. The leaves are brownish from the tips back 4 or 5 cm. and the rest of the leaves are dotted with light brownish, dried, areas. They are also slightly narrower than the leaves of check plants.

Watering with a one per cent solution of $MnSO_4$ seems to have a marked influence towards producing immunity.

To test further the effect of Manganese sulphate upon the susceptibility of the host the following experiment was con-

ducted:

Eight pots of barley were planted in five inch pots. Four pots were watered with a two per cent solution of manganese sulphate and four pots, to be used as controls, were watered with tap-water.

Eleven days after the seeds had been planted, the barley plants which had been watered with a two per cent solution of manganese sulphate averaged 15 cm. in height. The leaves were yellowish in color and were thickly dotted with light brown areas. The plants lay out flat in the pots. The plants watered with tap-water were dark green in color and averaged about 17 cm. in height. The plants stood upright in the pot.

At this time two pots of the treated plants and two pots of the check plants were inoculated with barley mildew and placed under bell jars. The rest of the plants were exposed to greenhouse conditions so that natural infection would take place.

Three days after inoculationthe two pots of check plants showed strong evidence of infection. The barley plants watered with a two per cent solution of manganese sulphate showed no commencement of infection.

Five days after inoculation the check barley plants showed splendid infection. The treated plants showed no infection.

Seven days after inoculation the check plants continued to show a good strong infection while the plants watered with a two per cent solution of manganese sulphate showed slight

infection.

As a result of the addition of a two per cent solution of manganese sulphate to the soil the appearance of infection was delayed two days, and the infection was much less vigorous than on the check plants.

The Function of Manganese in Plants: Kelley (14) in his investigation has shown that some plants when grown on mangandferous soils (barley and oats) are stunted in growth and die back from the tips of the leaves, which turn yellow or brown, and sometimes fall off and a general unhealthy appearance results. Microscopic investigations show that in certain instances the protoplasm undergoes changes. Occasionally it draws away from the cell walls and the nuclei become brown. There is a manifest change in the protoplasmic contents of the roots.

The chlorophyll in a number of plants is affected; in pineapples it undergoes decomposition. Simultaneous with the destruction of chlorophyll, starch formation ceases. The activity of the oxidizing enzymes in plants is shown to bear no relation to the destruction of chlorophyll under the influence of excessive manganese. While the oxidases generally contain manganese as a normal constituent, or at least manganese is closely associated with the oxidases, and at the same time the oxygen-carrying power is accelerated by the presence of manganese stalts, Kelly shows there is no correlation between the phenomenon of chlorosis in pineapples and the activity of the oxidizing enzymes. The

decomposition of chlorophyll in this case, therefore, is not due to excessive auto-oxidation. This does not imply, however, that accelerated auto-oxidation in plants is not without effect.

Kelly believes that an important effect of manganese is of an indirect nature, being due to its bringing about a modification in the osmotic absorption of lime and magnesia, and that the toxic effects are chiefly brought about by this modification, rather than as a direct effect of the manganese itself.

The small amounts of manganese in natural soils, therefore, probably perform a two-fold function in plant growth; (I) It acts catalytically, increasing the oxidations in the soil and accelerating the auto-oxidations in plants; and (2) it tends to modify the absorption of lime and magnesia, perhaps by partially replacing calcium from insoluble combinations, but especially, through a direct effect on the osmotic absorption of lime and magnesia, increasing the former and decreasing the latter.

Kelley infers that when plants are grown on manganese soils, the soluble manganese coming in contact with the root hairs is at first absorbed up to a certain point forming combinations with its protoplasms. Once these combinations are established, permeability of the protoplasm becomes altered, whereby the absorption of lime is facilitated, while that of magnesium is hindered. Manganese then, may be looked upon as forming a combination with the protoplasm which materially alters the relative absorption of lime and magnesia.

Further experiments were conducted to determine the effect of the addition of chemicals to the soil, upon the susceptibility of some of the cereals to the powdery mildews. Five inch pots were cleansed, rinsed and filled with fine, pure, white sand. About eight seeds were planted in each pot, and the pots placed in separate clay saucers. The seedlings were watered with distilled water up to the time of the commencement of the treatments. The plants were then watered with the full-nutrient solution used by Detmer and described elsewhere in this paper. To the full nutrient solution was added in each case enough of the specific chemical, with which the plants were treated, to make a one per cent solution of each chemical. The following tables show the relative immunity which seemed to be brought about as a result of the treatments. The number 10 is arbitrarily taken to indicate the most severe infection with mildew and the numeral 1 is used to show very little or no infection. The numbers between 1 and 10 show the relative degree of the severity of infection.

Tabke No.XVI

Date seeds were plant-ed 1914	Plants	Date treat ment com-menced 1914	Height of plants at com-mencement of treat-ment	Treatment given	Observations 1914	No. of pots
March, 19	Wheat	April, 7	23cm.	1% solu-tion of Lithium Bromide	April, 17 infection ** 2	4
"	"	" "	" "	1% solu-tion of Lead Nitrate	April, 17 infection 7	4
"	"	" "	" "	1% solu-tion of Lithium Carbonate	April, 14 plants dead	4
"	"	" "	" "	Full Nutrient Solution	April, 17 infection 8	4

** The first leaves of the plants watered with lead nitrate are devoid of chlorophyll, except the infected areas, which remain green.

Table No. XVII

Date seeds were planted 1914	Plants	Date treatment commenced 1914	Height of plants at commencement of treatment	Treatment given	Observations April, 20 1914.	No. of pots
March, 20	Oats	April, 7	15cm.	1% solution of $Zn\ So_4$	Infection 5	3
" "	"	" "	" "	1% solution of $Zn\ (No_3)2$	Infection 9	3
" "	"	" "	" "	1% solution of $Fe\ SO_4$	Infection 6	3
" "	"	" "	" "	Full Nutrient Solution	Infection 5	3
" "	"	" "	" "	1% solution of $K\ SO_4$	Infection 3	3

Table No. XVIII

Date of planting seeds and commencement of treatment 1914	Plants	Treatment given	Observations April, 20, 1914	Observations April, 30, 1914	No. of pots
April, 15	Wheat	1% solution of Lithium Bromide	Seedlings not visible above surface of sand	Infection 3	4
April, 15	Wheat	1% solution of Lithium Carbonate	No germination	No germination	4
April, 15	Wheat	1% solution of Lead Nitrate	Seedlings peeping through sand	Infection 8	4
April, 15	Wheat	Full nutrient solution	Seedlings 5cm. in height	Infection 7	4

The plants of the above table were approximately equally inoculated April 24, 1914.

The difficulty in drawing conclusions from the above tables, as has been stated, lies in the fact that it is very hard to give each set of plants, and the plants of each set, a standard uniform, dose of conidia. Furthermore, it is extremely hard to accurately judge the relative severity of the infection upon the different plants of a set. In no case was absolute immunity induced, and with hosts that are already very susceptible, under ordinary conditions, to the attacks of the mildew, one can scarcely say that susceptibility has been induced or increased.

The above tables would indicate that plants watered with zince nitrate and lead nitrate might perhaps be a little more susceptible than normally. It is shown a little more strikingly, perhaps, that potassium sulphate and lithium bromide produce some degree of immunity.

These results agree somewhat closely with those obtained by Spinks ([37]). He found that mineral manures, especially potash salts, decreased the susceptibility of wheat plants to the mildew but could not counteract the effect of large quantities of nitrogeneous manures. He also found that lithium salts were effective in producing immunity, while nitrates of lead and zinc, particularly the latter, rendered plants extremely susceptible. Other salts of lead and zinc had very little effect on the susceptibility of plants.

Stakman ([38]) attempted to determine whether it was possible to confer immunity upon wheat to rust, by means of various

salts. He found that the addition of copper sulphate, copper carbonate, and iron sulphate, to nutrient media in which plants inoculated with rust were grown did not markedly diminish the amount of rust when they were used in such concentrations as to permit of the normal development of the host plants.

Adding $CuSO_4$ to Water Cultures: Salmon (32) by a series of water-cultures which he made, shows conclusively that in the case of cereals copper sulphate supplied to the roots and presumably taken up by root-absorption, is of no effect as a fungicide against the powdery mildew. When the copper sulphate is supplied in a solution of the strength of 1 part of copper sulphate to 13000 parts of water there is a marked deleterious effect on the growth of the plant, causing narrower leaves to be produced, and almost completely checking the growth of the roots. When the strength of the solution used is 1 part of copper sulphate to 2000 parts of water, no root growth at all takes place; the whole plant is dwarfed and only short narrow leaves are produced. Nevertheless the leaves remain susceptible to the mildew.

Duggar (5) discusses, briefly, the possibility of feeding the host plant toxic solutions in order to prevent fungous diseases. He is of the opinion that, in spite of one or two alleged successful experiments, it is not possible at the present time to hold that any value whatever can be expected from this line of work. According to Duggar a great majority of the fungi are far more resistant to toxic or poisonous substances than the

cultivated agricultural plants.

Summary and Conclusions.

Dilute solutions of manganese sulphate when added to the soil brings about a marked immunity of barley plants to the powdery mildew.

Wheat and oat plants when watered with zinc nitrate and lead nitrate seem to be slightly more susceptible to the attacks of mildew than untreated plants.

The addition of potassium sulphate and lithium bromide to the soil produces a more or less marked immunity.

These experiments show that in the case of *Erysiphe graminis*, since it is a very highly specialized, obligate parasite, there is a very intimate relationship between host and parasite, and whatever is conducive to the health of the host is ordinarily conducive to the vigorous development of the parasite also.

The Effect Upon Susceptibility of the Host Plant Due to Wilting Brought about by the Addition of Concentrated Solutions of Potassium Nitrate to the Soil.

An effort was made to keep barley and wheat plants in a wilting condition by adding a ten per cent solution of potassium nitrate to the soil of the pots in which the plants were growing.

In one experiment wheat seedlings twelve days old and averaging about 16 cm. in height were used. Six pots of plants,

as checks, were watered with tap-water and six pots were watered with a ten per cent solution of potassium nitrate. These plants were all inoculated at once, without waiting for the plants to show the effects of the treatment, and placed under bell-jars.

A similar experiment was set up, except that in this case, the plants were not inoculated with the mildew until the effects of the treatment had become evident. The following tables show the results of these experiments:

TABLE NO. XIX

Plants Inoculated Before Effect of Treatment was Shown.

Date seeds were planted 1914	Date treatment commenced 1914	Height of seedlings at commencement of treatment	Treatment given plants	Plants treated	No. of pots	Date of inoculation 1914	Infection 1914
Feb. 27	Mar. 11	16 cm.	Watered with a 10% solution of KNO_3	Wheat	6	Mar. 11	March 16
" "	" "	16 cm.	tap-water	"	6	" "	" "

Four days after inoculation all of the wheat plants watered with tap water and the ten per cent solution of potassium nitrate gave evidences of the commencement of infection. Infection on all of the plants was visible five days after inoculation, where the plants were inoculated before the effects of the treatment was evident.

TABLE NO. XX

Plants Inoculated After the Effect of Treatment was Evident.

Date seeds were planted 1914	Date treat-commence 1914	Height of seedlings at commencement of treatment	Treatment given plants	Plants treated	No. of pots	Date of inoculation 1914	Result
Feb. 27	March 11	16 cm.	tap-water	wheat	6	Mar. 21	Infection Mar. 27
" "	" "	16 cm.	10% sol. $FKNO_3$	"	6	" "	Plants dead Apr. 4

Ten days after the commencement of the experiment the plants were showing the effects of the addition of the ten per cent solution of potassium nitrate by their drooping leaves, which were turning yellowish in color. The treated and the control plants were inoculated with mildew at this time.

Six days after inoculation the control plants showed good infection, but the treated plants showed no infection and the leaves continued to droop, and look yellowish in color.

Two days later the ends of the leaves of the treated plants were yellow. Some of the leaves were completely devoid of chlorophyll for 5 cm. back from the tips. The check plants showed splendid infection with mildew.

Fourteen days after inoculation the treated plants had died with no infection apparent. The control plants continued to show good infection.

The results of a third experiment are summarized in the following table:

TABLE NO. XXI

Date seeds were planted 1914	Date of inoculation & commencement of treatment	Treatment given	Plants treated	No. of pots	Observations Jan. 3, 1914	Observations Feb. 25, 1914
Jan. 24	Jan. 26 plants 3 cm. in height	10% sol. of KNO_3	Barley	6	Leaves are drooping	New leaves show infection; none of leaves kept at wilting point show infection
" "	"	tap-water	"	6	Infection	

Conclusions

When the plants were not inoculated until they had commenced to show the effects of the treatment, infection could be prevented, for a time, by adding such a relatively concentrated solution of potassium nitrate that the leaves wilted and turned yellowish in color. After tap-water had been added and new leaves developed, these new leaves became infected.

To Determine the Effect of Adding Relatively Concentrated Solutions of Salts to the Soil Upon the Host's Susceptibility to the Powdery Mildew.

Experiments were conducted to modify the structure (dwarf) and change the manner of growth of the host-plants by increasing the density of the soil solution in order to determine if such treatment in any appreciable way influenced the host susceptibility to the powdery mildews.

These experiments show the effect of treating the soil with chemicals from the time the seed in planted. In this case the adjustment of the plants to the action of the chemicals is gradual and there is not the more or less violent physiological reaction that is brought about when the chemicals are added to the soil in which plants several centimeters in height are growing.

Pots of barley and wheat were planted in fairly rich soil in six inch pots.

The pots were set in earthen dishes and placed under bell jars in the greenhouse.

Half of each set of the first experiment were watered with a 5% solution of Calcium nitrate and the other half, as controls, were watered with tap-water. The wheat and barley seedlings were allowed to develop until they had attained a height of seven or eight centimeters when they were inoculated with their respective biologic powdery mildews.

The following table gives the results of adding a 5 per cent solution of $Ca(NO_3)_2$ to the soil in which the plants were growing.

TABLE NO. XXII

Plants	Treatment	Average height of seedlings 5 days after planting	Average height of seedlings 8 days after planting	Average height of seedlings 11 days after planting (plants inoculated)	Average height of seedlings at time of appearance of infection 4 days after inoculation	No. of Pots
Barley	5% $Ca(NO_3)_2$	----	1.4 cm.	6.5 cm.	7 cm.	6
Barley	tap-water	1.3 cm	7.8 cm.	14.0 cm.	18.5 cm.	6
Wheat	5% $Ca(NO_3)_2$	----	2.0 cm.	6.0 cm.	8.0 cm.	6
Wheat	tap-water	1.3 cm	8.2 cm.	15.5 cm.	24.5 cm.	6
Wheat	5% $Ca(NO_3)_2$	----	2.1 cm.	6.2 cm.	7.9 cm.	4

The addition of the 5 per cent $Ca(NO_3)_2$ delayed germination of the wheat and barley seeds about two days.

The leaves of the plants watered with 5 per cent $Ca(NO_3)_2$ were a much deeper green color than the control plants, and all the leaves were rolled somewhat. The leaves were slightly more narrow than those of the check plants.

The plants were very much dwarfed by the addition of

the 5 per cent $Ca(NO_3)_2$.

As a result of the treatment some more or less profound physiological disturbance had taken place and with both the wheat and barley the control plants made three times as much growth as the treated plants.

The table shows that infection appeared on both the treated and untreated plants at about the same time, but visible evidence of infection appeared on the leaves of the treated plants a little over twelve hours earlier than on the leaves of the control plants. Also, the treated plants seemed to be much more heavily infected than the control plants, although it was hard to determine absolutely if this was due to increased susceptibility or to the fact that the area of the leaves of the treated plants did not enlarge much after inoculation, while the area of the leaves of the control plants increased to some extent and thus the infection was more scattered and not so dense.

In a similar manner wheat and barley seeds were planted in five inch pots and watered with a 10 per cent solution of $Ca(NO_3)_2$ and an equal number of seeds, to serve as checks, were watered with tap-water.

The following table shows the results of watering the seeds with a 10 per cent solution of $Ca(NO_3)_2$.

TABLE NO. XXIII

Plants	Treatment	Height 4 days after planting	Height 7 days after planting	No. of pots
Wheat	10% solution $Ca(NO_3)_2$	No germination	No germination	4
Wheat	tap-water	8 cm.	17 cm.	4
Barley	10% solution $Ca(NO_3)_2$	No germination	No germination	4
Barley	tap-water	6 cm.	15 cm.	4

When the soil is watered with a ten per cent solution of calcium nitrate wheat and barley seeds will not germinate.

A third experiment was set up where wheat seeds were planted in sand and watered with a ten per cent solution of potassium nitrate.

The following table shows the effect of the addition of a ten per cent solution of potassium nitrate to the soil upon the susceptibility of wheat to its powdery mildew:

TABLE NO. XXIV

Treatment	Height of seedlings at time of inoculation 9 days after planting	Observations 3 days after inoculation	Observations 4 days after inoculation	Observations 5 days after inoculation	Height of plants 16days after planting	No. of pots
10% sol. of KNO_3	12 cm	Plants 13cm in height. Show evidences of infection	Infection with production mycelium.	Infection	14 cm.	6
Tap-water	14 cm	Plants 22 cm in height. No evidences of infection	No infection apparent	Infection	25 cm.	6

The addition of the ten per cent solution of potassium nitrate to the soil checked the growth of the plants considerably, although it did not delay the germination appreciably. The treated plants had a deeper green color and a more narrow leaf than the controls.

Three days after inoculation with the mildew the leaves of the plants watered with a ten per cent solution of potassium nitrate gave evidences of infection as indicated by light colored areas on the leaves. The control plants gave no indication of infection, and these light colored areas were not apparent.

Four days after inoculation the treated wheat seedlings showed unmistakable infection with a rather abundant production of

mycelium. The check plants, watered with tap-water, showed no apparent infection.

Five days after inoculation both the check plants and the treated plants showed infection with mildew. The infection, however, on the check plants was not quite so vigorous as on the plants watered with a 10 per cent solution of potassium nitrate.

Seven days after the plants had been inoculated, the check plants were nearly twice as tall as the treated plants, and, while both sets of plants were infected, as a rule the plants watered with a 10 per cent solution of potassium nitrate showed better infection than the check plants.

The details of a fourth experiment are given, where barley plants were watered with a 10 per cent solution of potassium nitrate. The plants were twelve days old and averaged about 16 cm. in height.

Eight pots were placed under bell-jars, four pots of plants were watered with a 10 per cent solution of potassium nitrate and four pots, as checks, were watered with tap-water. As soon as the plants commenced to show the effects of the treatment they were inoculated with the mildew.

Ten days later the plants watered with the 10 per cent solution of potassium nitrate were showing the effects of the treatment by drooping and turning slightly yellow at the tips. These plants and the controls were inoculated at this time with the powdery mildew.

Seven days after inoculation the treated barley plants were showing good infection. These plants were wilting somewhat and averaged 25 cm. in height. The control barley plants appeared normal, averaged 33 cm. in height and showed no infection.

TABLE NO. XXV

Date seeds were plant-ed 1914	Date treat-ment com-menced 1914	Height of seed-lings at com-mence-ment of treatment	Treatment given plants	Plants treated	No. of pots	Date of inocula-tion 1914	Infection 1914
Feb.27	Mar.11	16cm.	Watered with a 10% solution of KNO_3	Barley	4	Mar.21	Mar.28
" "	" "	16 cm.	tap-water	"	4	" "	no infec-tion

Two days after showing infection the treated plants averaged 26cm. in height and were showing splendid infection. The control plants averaged 34cm. in height, and while they showed no infection, there were light colored areas scattered over the leaves as if the commencement of infection had taken place.

Two days later the treated plants continued to show good infection on the leaves that were green. Some of the leaves were turning yellow, but the infected areas of these leaves were still green. The checks showed no infection.

It was thought that an age factor might have entered in, which prevented infection taking place upon the control plants. And

perhaps watering the plants with a 10 per cent solution of potassium nitrate broke down this limiting factor of age. The control plants, however, were near the west end of the bench in the greenhouse and were in a stronger sunlight, and it was suggested that this might have been the cause of non-infection.

To determine if watering the check plants with a 10 per cent solution of potassium nitrate would induce susceptibility, two pots were treated for a time, and two pots of plants were left as controls.

Both sets of plants showed infection six days after inoculation, but the barley plants which had been watered with the 10 per cent solution of potassium for six days showed much more abundant infection than the control plants. Unfortunately there was no time available to carry out further tests upon this point, but it would seem that the controls were not infected in the first instance because of the direct sunlight upon the mildew rather than any age factor entering in. And the treated plants became infected because they were not exposed to this direct sunlight, rather than because of the indirect effect of the 10 per cent solution of potassium nitrate in breaking down an immunity brought about by age. There appears to be, however, in the case of barley, to some extent at least, an immunity correlated with an age factor.

These experiments indicate that the addition of excessive amounts of potassium nitrate to the soil slightly increases the susceptibility of wheat to its mildew as shown by the shortening of the incubation period and the increased vigor of the infection.

To Determine the Relative Susceptibility of Wheat Plants Grown in Rich Soil and in Poor Sandy Soil.

Wheat plants were grown in rich garden loam, and in sand, to determine if their susceptibility might be influenced by making conditions in this manner favorable or unfavorable for a vigorous growth of the host plants.

Twelve five inch pots were filled with sand, and the same number were filled with rich garden loam. About twelve wheat seeds were planted in each pot containing the rich soil, and three times as many seeds were planted in the pots containing sand. The plants in the rich soil were kept well watered, and frequently a manure solution was poured around the plants. The plants grown in sand were not given much water. The two sets of plants were allowed to grow under these conditions for a little over six weeks.

The crowded, poorly watered, badly nourished plants in the sand did not make as much growth as the less crowded, well-watered, better nourished plants in the rich soil. The plants in the sand were not in as thrifty a condition, of course, being yellowish in color, and in a somewhat wilted condition.

The plants of both sets were approximately equally inoculated with mildew from time to time, by dusting the conidia from infected plants over them. If there was any difference, the poorly nourished plants received the larger doses of conidia at each inoculation.

There was quite a marked difference in the apparent susceptibility of the two sets. The infection was heavier upon the vigorously growing wheat plants than upon the unthrifty plants. Furthermore, the growth of the mildew upon the thrifty plants was much more vigorous than it was upon the yellow, sub-normal, semi-wilting plants.

A similar experiment was conducted where twenty-four pots filled with sand, each containing twelve seeds of wheat were used. One set of twelve pots was kept well watered with a manure solution, and the other set of twelve pots was insufficiently watered with distilled water.

The plants were allowed to grow about five weeks with essentially the same results obtained in the first experiment.

Discussion.

Spinks (37) found that wheat plants which were semi-starved as regards nitrogen exhibited a considerable degree of immunity.

Stakeman (38) grew some wheat plants on ordinary rich loam, others in a rich loam plus fresh barnyard manure, and a third series in rich loam to which barnyard manure and bone meal had been added.

On the wheat plants which were grown on very heavily fertilized soil, Stakman found the infection with rust was clearly more severe. The infected areas were very large as were also the individual pustules. The infection on the check plants re-

mained inferior, although it was very vigorous

Conclusions.

It would seem that well-nourished, vigorously growing, wheat plants are more susceptible to the attacks of the powdery mildew than half-starved, sickly, yellowish, semi-wilted plants. Or in other words, as the host-plants themselves suffer from a combination of outside factors, the fungus is unable to become as successfully established as upon normal well developed hosts.

To Determine the Effect of the Humidity of the Atmosphere Upon the Susceptibility of Wheat and Barley to Their Respective Mildews.

The first experiment was carried out to determine the effect of a comparatively dry atmosphere and a relatively saturated atmosphere upon the infection powers of the barley and wheat mildews upon their respective hosts.

Six pots of barley seedlings averaging 2 cm. in height, were used. The pots were paraffined outside and sealed across the surface by petrolatum to prevent any loss of water except by means of transpiration.

A very satisfactory wax for this purpose, and one used in all the experiments to seal pots of plants, is the one recommended and described by Briggs and Shantz (2). These men found a wax composed of 80 per cent paraffin (melting point 45° C.) and 20 per cent petrolatum to be the most satisfactory for use at ordinary temperatures, exact proportions not being important. This mixture melts at so low a temperature and has such low heat conductivity that it can be poured into a pot around the most delicate seedling without injury. On cooling, this wax adheres well to the glass and to the soil, forming a perfect seal.

Paraffin (45°) when used alone stretches, petrolatum creeps, and beeswax, tallow and the higher paraffins crack. During the winter in the greenhouse the paraffin-petrolatum mixture gave excellent results. During the summer, however, accord-

ing to these men, direct sunlight is likely to melt the wax, and in this way break the seal and cause damage to the plants, due to the creeping over the plant surface. They have used beeswax mixtures which have proven best during warm weather. A mixture of 10-30 per cent beef tallow with beeswax, or of 8-12 per cent of petrolatum with beeswax, has proven an excellent material both in greenhouse and out of door work.

After sealing the pots in the above manner, the plants were placed under large bell glasses.

The air enclosed by the bell jars was kept relatively dry by means of a Petri dish full of calcium chloride and a wide mouthed bottle full of commercial sulphuric acid. The jars were tightly sealed, by means of a heavy vaseline, to the glass plates upon which they rested. Although conditions could not in this case be controlled absolutely, the air surrounding the plants was kept relatively dry.

As a check another set of six pots of barley plants averaging 2 cm. in height was placed under bell-jars which contained no drying agent of any kind. These plants were to be grown in an atmosphere made relatively humid by transpiration.

The plants were allowed to remain under these conditions for eleven days. At the end of this time they were all inoculated with barley mildew. The plants averaged about 17 cm. in height when inoculated.

A similar experiment was carried out exactly as the

one just described excepting that wheat was used instead of barley. The wheat seedlings were eight days old.

The plants were allowed to remain under the same conditions as described for the barley cultures for a period of about 8 days. At the end of this time they were inoculated with wheat mildew. The plants averaged about 20 cm. in height at the time of inoculation.

Along with these experiments was conducted a similar experiment differing in that the plants were changed from a relatively moist atmosphere to a comparatively very dry atmosphere every twenty-four hours.

The wheat and barley plants used were of the same age and practically of the same size as the plants used in the other two experiments.

There were six pots of barley and six pots of wheat Three pots of barley and three pots of wheat were thus changed from humid to dry atmospheres every twenty-four hours for ten days. They were then inoculated and one set of each was placed in a dry atmosphere and one set in a humid atmosphere to remain until infection appeared.

The following table shows the result of this treatment of the plants so far as the development of infection was concerned. The plus sign indicates that infection occurred while the minus sign indicates the absence of infection.

TABLE NO. XXVI

Plants	Age of plants when inoculated	Average height of plants when inoculated	Treatment	Infection	Delay as a result of treatment	No. of plants
Barley	16 days	17 cm	dry atmosphere	+	none	6
Barley	16 "	18 cm	damp "	+	"	6
Wheat	16 "	20 cm	dry "	+	1 day	3
Wheat	16 "	20 cm	" "	+	none	3
Wheat	16 "	20.5 cm	damp "	+	"	6
Barley	16 "	17 cm	dry and damp	+	"	6
Wheat	16 "	20 cm	atmosphere on alternate days	+	"	6

It should be kept in mind that when a dry atmosphere is spoken of in the above experiments it does not mean an atmosphere that is anywhere near absolute dryness, but only relatively dry as compared to the atmosphere outside the bell-jars.

The results of these experiments would indicate that there is an exceedingly wide range in the degree of humidity of the air surrounding the plants inoculated with mildew in which the infection of the host is not to any appreciable extent affected.

A second series of expefiments was carried out to determine if infection would take place when the air surrounding the plants was kept fairly near absolute dryness.

The plants were about the same age and size as the seedlings used in the preceeding experiments. The plants averaged about 2 cm. in height.

In the first set six pots of barley plants were kept in an atmosphere which closely approximated absolute dryness.

The bell-jars under which the pots were placed had openings at the top and in one side near the bottom. Proper connections were fitted up so that air was drawn continuously through each bell-jar by means of a suction-pump.

The air which entered each bell-jar was first allowed to bubble through two bottles of commercial sulphuric acid. In addition a small bottle of commercial sulphuric acid and a small bottle of $CaCl_2$ were placed under each bell-jar. The bell-jars were tightly sealed around the bottom to glass plates by means of wax.

As checks six other pots of barley were put under bell jars and set up in the same manner except that the air in this case was drawn through bottles of water. Two small bottles of water were also placed under each bell jar.

The plants were allowed to remain under these conditions for about seven days, when they were inoculated with barley mildew.

After this experiment had ended another experiment was carried out precisely as this one except that wheat was used instead of barley. These seedlings were inoculated with wheat

mildew after they had been allowed to grow for seven days under conditions similar to those described for the barley plants.

Observations.

The observations taken upon both the experiments with the wheat and barley are combined as follows: The wheat and barley plants in the dry atmosphere would show the effects of the excessive transpiration after three or four days. The tips would lose their chlorophyll and dry up.

For two or three days afterwards the tips of both the barley and wheat plants in the dry atmosphere would continue to lose their chlorophyll and dry up. The plants in the saturated atmosphere were vigorous and looked quite normal.

At the end of seven days the barley plants which had been grown in the dry atmosphere averaged 13.5 cm. in height, while the plants grown in the moist atmosphere averaged 15.5 cm. in height. The leaves of the barley plants in the dry atmosphere were wilted, dried and devoid of chlorophyll for about 1.5 cm. back from the tips.

The wheat plants in the dry atmosphere averaged 11 cm. in height. The buds of the leaves of all the wheat plants in the dry atmosphere were wilted, dried and devoid of chlorophyll back for a distance of about 3 cm. from the tips.

The wheat plants in the moist atmosphere were vigorous and averaged about 11.5 cm. in height.

After inoculation, one of the pots of plants of

both wheat and barley which had been growing in a dry atmosphere, were placed in a moist atmosphere and one of the pots of both wheat and barley plants which had been grown in a moist atmosphere up to the time of inoculation, were placed in a dry atmosphere.

This change was made to determine whether it was the direct action of the relative humidity of the atmosphere or the condition of the plant as an indirect results of the humidity of the atmosphere which in any way might determine the susceptibility of the host to the mildew.

The following table summarizes the results of the several experiments:

TABLE NO. XXVII

Plants	Age of plants when inoculated	Average height of plants when inoculated	Treatment	Infection	Delay as a result of treatment	No. of pots
Barley	12 days	15.5 cm	Saturated atmosphere	+	none	5
Barley	12 "	13.5 cm	Atmosphere absolutely dry	-		5
Barley	12 "	15. cm	Saturated atmosphere up to time of inoc. and dry atmosphere after inoculation	-		1
Barley	12 "	13.5 cm	Dry atmosphere up to time of inoc.and saturated atmosphere after inoc.	+	none	1
Wheat	11 "	11.5 cm	Saturated atmosphere	+	none	5
Wheat	11 "	11 cm	Atmosphere absolutely dry	-		5
Wheat	11 "	11.5 cm	Satur.atmos.up to time of inoc. and dry atmos.after inoc.	-		1
Wheat	11 "	11 cm	Dry atmos.up to time of inoc. and saturated atmosphere after inoculation	+	none	1

The above experiments show that when the plants are grown in an atmosphere closely approximating absolute dryness no infection takes place. It would seem that infection does not take place because of any change brought about in the plants which tend to make them more immune, but because the atmosphere is so dry that the conidia cannot germinate or else the germ tubes dry up and die, so that infection cannot take place.

The wheat and barley plants even when plentifully supplied with moisture in the soil, in the extremely dry atmosphere where transpiration would be excessive, could not apparently take in moisture rapidly enough to replace the loss from the leaves. Hence the leaves wilted and the tips completely dried out.

Thornber (39) writing of the growth corn makes in Arizona, states that even when the plants were irrigated and cultivated twice each week in June the leaves wilted badly, "fired" at the tips, and growth ceased. This condition obtained even when the soil was saturated with water, and only by virtue of the greatly lessened transpiration during the night when the equilibrium was restored, were the plants able to live at all.

It was thought worth while to test out the possibility that the plants which were grown in an atmosphere approaching absolute dryness, had become so weakened that their vitality would be lowered, and thereby the resistance of the host-plant to the attacks of the mildew lessened.

Similar experiments were set up with both wheat and barley, except that they were not inoculated with the biologic forms of mildew ordinarily found growing upon them as parasitic. Wheat was inoculated with barley mildew and barley was inoculated with wheat mildew.

The plants were grown in a dry atmosphere for about eight days, or until the characteristic drying of the tips, followed by the loss of chlorophyll and slight yellowing of the leaves had occurred. Then after inoculation in each case five of the pots which had been growing in the dry atmosphere were placed in a humid atmosphere and five plants which had been growing in a humid atmosphere were placed in a dry atmosphere. One pot that had been in the dry atmosphere was allowed to remain after inoculation in the dry atmosphere, and one pot which had always been in the humid atmosphere was allowed to remain in the humid atmosphere.

There was no infection apparent after twenty days. It would seem, therefore, that growing wheat and barley plants in a very dry atmosphere for a time, and then placing them under conditions favorable to the growth of the fungus in no way influences the host to become susceptible to a mildew of another biologic strain, than the one which normally produces infection.

Another experiment was carried out where wheat and barley plants were grown in an atmosphere free from moisture to determine what influence this external condition would have upon

the susceptibility of wheat to wheat mildew and to barley mildew, and of barley to barley mildew.

This experiment was conducted in a manner quite similar to the other experiments of this nature which have just been described.

The wheat and barley seedlings were about 5 days old. The wheat seedlings averaged 2.5 cm. in height and the barley seedlings 1.7 cm. in height.

The following table gives the results of this experiment:

TABLE NO. XXVIII

Plants	Atmosphere surrounding plants	Mildew used in making inoculations	Infection	No. of pots
Wheat	dry	barley	-	2
"	damp	"	-	2
"	dry	wheat	-	2
"	damp	"	+	2
Barley	dry	barley	-	2
Barley	damp	"		2

In these last two experiments the plants were grown under conditions which very much weakened their vitality as shown by the drying of the tips, loss of chlorophyll, and slight yellowing of the leaves. Then while the plants were in this weakened state, conditions were made very favorable for the growth of the fungus, by placing these weakened plants

in a damp atmosphere, after inoculating them quite heavily, still the mildew could not infect plants other than their normal hosts.

The results obtained are contrary to the idea which sometimes prevails that when a host-plant's vitality is very much weakened it may become susceptible to the attacks of a powdery mildew of a 'biologic form' other than the one which normally grows upon it.

Salmon (34) is of the opinion that the restriction of infection characteristic of "biologic forms" breaks down if the vitality of the leaf on which the conidia are sown is interfered with in certain ways. He (35) assumed that in consequence of the vitality of the leaf-cells being affected by mechanical injury, application of anaesthetics and heat, either the protective enzymes, or similar substances normally present are destroyed or become weakened, or the production of them by the protoplasm is interfered with, and hence the susceptibility of the leaves to foreign 'biologic forms' is increased.

Salmon (36) cites another example in which he believes that _Hordeum secalinum_ when in a normal healthy condition is immune against infection by conidia from _H. vulgare_, but when the health of the plant is gradually impaired by unfavorable conditions such as would be found in a small, hot, badly ventilated greenhouse, it becomes susceptible.

Conclusions

So long as the temperature does not rise too high, infection of barley and wheat plants with their respective mildews, will take place under conditions which allow a wide range of variability in the humidity of the surrounding air. The atmosphere can be comparatively very dry, or very damp and there will be little or no difference in the appearance of infection, as compared to checks placed under what are usually considered optimum conditions for the mildew.

The plants, however, can be grown in an atmosphere which so closely approximates absolute dryness that no infection will take place. And it would seem that in this case no infection results, not because of any changes in the host-plants, as a result of the treatment which tend to make them more immune, but because the atmosphere surrounding the plants is so dry that the conidia cannot germinate, or else the germ tubes dry up and die, and hence infection cannot take place.

Finally the vitality of wheat and barley plants cannot be so weakened by growing them in an extremely dry atmosphere that their powers of resistance to the attacks of the fungus are so lessened that biologic forms, other than the one which normally produces infection, can establish themselves, bring about infection, and produce conidia, even when after cross-inoculation the plants thus weakened are placed under optimum conditions for the growth of the fungus.

To Determine Whether the Lack of Carbon Dioxide Available for Photosynthesis Influences the Susceptibility of the Host-Plant to Its Powdery Mildew.

Experiments were conducted to determine if barley, wheat and sunflower plants, heavily inoculated with mildew, would become infected when placed in an atmosphere free from carbon dioxide.

Control plants were placed under identical conditions except that the air was not deprived of its carbon dioxide.

The plants were placed under bell-jars having openings at the top and in the side near the bottom. By means of rubber stoppers, rubber and glass tubing, air-tight connections were made and the complete set of twelve bell-jars were attached to one suction-pump and the air drawn through them continuously.

The carbon dioxide was removed from the atmosphere enclosed by six of the bell jars as follows: The air was first allowed to bubble successively through two vessels containing concentrated solutions of caustic potash and then, before the air entered the bell-jars, it was finally allowed to pass through a rather weak solution of commercial sulphuric acid, to make sure that any caustic action of the concentrated KOH solution would be neutralized.

As an additional precaution to guard against the presence of any carbon dioxide two glass vessels, each with 100 cc. capacity and containing a concentrated solution of KOH were

placed under the bell-jars enclosing the pots of plants.

The air enclosed by the six remaining bell jars, containing the control plants, was drawn continuously through vessels containing tap-water, before it entered the bell-jars.

After the plants had been inoculated and placed under the bell-jars, the latter were sealed to pieces of plate glass, and all connections were made absolutely air-tight, with a mixture of paraffine, bee's-wax, and vaseline.

The following table gives the results of growing wheat and barley in an atmosphere free from carbon dioxide, so far as the effect upon the parasitic powdery mildew is concerned.

TABLE NO. XXIX

Plants	Height at time of inoculation	Age at time of inoculation	Atmosphere surrounding plants	Observations 4 days after inoculation	Observations 5 days after inoculation	No. of Pots
Barley	4.5 cm.	8 da.	$-CO_2$	----	----	6
Barley	4.5 "	8 "	normal	infection		6
Wheat	5.5 "	8 "	$-CO_2$	----	----	6
Wheat	5.5 "	8 "	normal	infection		6
Wheat	12.9 "	7 "	$-CO_2$	light colored areas on leaves	light colored areas on leaves	6
Wheat	12.9 "	7 "	normal	" "	infection	6
Barley	11.7 "	9 "	$-CO_2$	" "	light colored areas on leaves	6
Barley	11.7 "	9 "	normal	" "	infection	6

The table above shows that four days after inoculation both sets of plants may show what appears to be the commencement of infection, as made apparent by the light colored areas on

the leaves. But a day or two later while the control plants would show infection, the plants in an atmosphere free from CO_2 continued to show only the light colored areas on the leaves.

Eleven days after inoculation, while the control plants showed abundant infection, much mycelia development and production of conidia, the leaves of the plants in an atmosphere minus carbon dioxide showed no infection beyond the appearance of the light colored areas. This would indicate that the commencement of infection had taken place, but that growth and development of the fungus had been arrested soon afterwards.

Furthermore seven days after the plants had been placed in an atmosphere free from carbon dioxide they had commenced to droop, indicating the ill effects of the lack of carbon dioxide.

Four days later the plants looked so unhealthy and drooped so much, apparently as though they had lost their turgidity (there was, however, plenty of moisture available in the pots), that the experiments were brought to a close.

The plants which had been grown in an atmosphere free from carbon dioxide were then placed under bell-jars in the greenhouse, to see if the fungus mycelium would resume its arrested development. No further growth of the fungus took place although the fungus on the control plants continued to produce conidia abundantly.

The wheat and barley plants of both sets were test-

ed to determine if sugar or starch was present in the leaves.

The leaves were cut off close to the surface of the soil and one-half placed in alcohol to extract the chlorophyll so a starch test with a solution of iodine could be made. And one-half of the leaves of each pot were at once crushed in a mortar, boiled in test-tubes, and tested for sugar with Fehling's solution.

TABLE NO. XXX

Plants	Treatment	Test	Result	No. of tests
Barley	$-CO_2$	starch	negative	6
Barley	normal air	starch	negative	6
Barley	$-CO_2$	sugars	slight positive	6
Barley	normal air	sugars	positive	6
Wheat	$-CO_2$	starch	negative	6
Wheat	normal air	starch	negative	6
Wheat	$-CO_2$	cane sugars	slight positive	6
Wheat	normal air	cane sugars	positive	6

While the checks gave tests for sugar which were a little more marked than the test which the plants in an atmosphere free from carbon dioxide gave, the fungus was apparently not suffering from the lack of available sugars obtainable from the treated host plants.

The experiments with the sunflowers were conducted in essentially the same manner as described for the wheat and barley plants. The cotyledons of some of the sunflower seedlings in each pot were removed. One plant in each pot had only one cotyledon removed; three plants had both cotyledons removed; and two plants in each pot had neither cotyledon removed. The cotyledons were removed so the plants thus treated could not draw upon the food stored therein.

The air which was drawn from the bell-jars was tested for the presence of carbon dioxide, every fortyeight hours. The air supposed to be free from carbon dioxide was allowed to bubble through a 5 per cent solution of barium oxide for five minutes, and if no precipitate was formed it was regarded as an indication that no carbon dioxide was present.

In the following experiment the first pair of sunflower leaves were heavily inoculated with sunflower mildew by means of a scalpel just before the pots of plants were placed under the bell-jars.

The tables give the results of growing sunflowers in an atmosphere free from carbon dioxide, so far as the effect

upon the mildew is concerned:

TABLE NO.XXXI

Plants	Height at time of inoculation	Age at time of inoculation	Atmosphere surrounding plants	Observations 4 days after inoculation	Observations 9 days after inoculation	No. of Pots
Sunflower	9.5 cm	19 da.	$-CO_2$	Plants turning yellow	No infection Plants dying	6
Sunflower	9.5 "	19 "	normal	Plants green and healthy	Plants show infection	6

The above table shows that four days after the experiment had been set up the sunflowers in the atmosphere free from carbon dioxide began to show the ill effects of such a treatment by turning yellow in color as compared with the normal green color of the control plants.

Six days after the commencement of the experiment, it was evident that the plants growing in the atmosphere minus carbon dioxide, which had their cotyledons removed were turning very yellow, the leaves were limp, and the plants were making no growth. The plants from which the cotyledons had not been removed were suffering also as compared with the control plants; still they appeared better in this respect than the plants which had their cotyledons removed.

A number of the seedlings growing in the air minus carbon dioxide had had their vitality so lowered that they were rotting just at the surface of the soil. The saprophytic fungi

were able to attack the plant in its weakened condition. Furthermore, the moist atmosphere of the bell-jars made conditions quite favorable for the growth of saprophytic fungi.

There was also a noticeable difference between the control plants which had their cotyledons removed and those plants which could still draw upon the food supply available in the cotyledons. The former were not making as much growth as the plants from which the cotyledons had not been removed.

Nine days after inoculation the first pair of leaves of the control sunflower plants showed infection with the powdery mildew. The plants in an atmosphere free from carbon dioxide showed no infection, and were dying and rotting from the attacks of saprophytic fungi, which were able to attack them successfully in the damp atmosphere, because of the weakened vitality of the plants.

The following table gives briefly the results of a second experiment conducted with sunflower plants:

TABLE NO. XXXII

Plant	Height at time inoculation	Age at time of inoculation	Atmosphere surrounding plants	Observations 6 days after inoculation	Observations 8 days after inoculation	Observations 9 days after inoculation	No. of Pots
Sunflower	16 cm.	32 da	$-CO_2$	Plants are turning yellow and leaves are drooping	No infection; plants turning brown; are being attacked by saprophytic molds	Plants are dead	6
Sunflower	16 cm.	32 da	normal	Plants look healthy	Show infection; seem thrifty	Plants continue to show infection and look healthy	6

The plants used in the above experiment had formed three pairs of leaves beyond the cotyledons. Only the youngest pairs of leaves were inoculated.

The plants grown in an atmosphere free from carbon dioxide showed signs of suffering within six days after the experiment had been started. The plants looked yellowish and the leaves were drooping.

The control plants showed infection eight days after inoculation.

Nine days after inoculation the plants growing in an atmosphere free from carbon dioxide were all dead. They had

turned brown and black in color and were being attacked by saprophytic fungi.

TABLE NO. XXXIII

Plants	Height at time of inoculation	Age at time of inoculation	Atmosphere surrounding plants	Observations 9 days after inoculation	Observations 12 days after inoculation	Observations 14 days after inoculation	No. of Pots
Sunflower	16 cm.	24 da.	$-CO_2$	No infection	No infection Plants look yellowish	No infection Leaves continue to turn yellow	5
Sunflower	16 cm.	24 da.	normal	Slight infection	Good infection:	Splendid infection	5

The table above shows that no infection had appeared on the sunflower seedlings, grown in an atmosphere lacking carbon dioxide, five days after the control plants had shown infection. At this time the treated plants were in a very weakened condition as shown by their yellow drooping leaves.

The following table gives the results of a fourth experiment with sunflowers grown in an atmosphere minus carbon dioxide.

TABLE NO. XXXIV

Plants	Height at time of inoculation	Atmosphere surrounding plants	Observations 7 days after inoculation	Observations 8 days after inoculation	Observations 8 days after inoculation	Observations 11 days after inoculation	No. of Pots
Sunflower	23 cm.	$-CO_2$	not making as much growth as the control plants	Leaves turning yellow & plants in a much weakened condition	Plants have turned very yellow. Four pots of plants dead	Plants dead	6
Sunflower	23 cm.	normal		Plants healthy & leaves infected with mildew	Splendid infection	Splendid infection	6

The above tables show that after the sunflower plants have been growing in an atmosphere free from carbon dioxide, they would commence to suffer, as shown by the yellowing and drooping of the leaves.

Discussion.

Ward (42) working with _Puccinia glumarum_ upon Michigan Bronze, attempted to grow the fungus in leaves from which all atmospheric carbon dioxide had been cut off by potash, but which were normally supplied with water and light.

The plants were inoculated and left for twenty-four hours to initiate infection, then the leaves were cut off close to the base and placed in tower-tubes. Some of the tubes were

linked with potash bulbs, and some of the tower-tubes as controls were not connected with potash bulbs, the air passing over their contained leaves passed through distilled water only.

The histological examination of these infected leaves showed that as soon as the leaf manifests the deprivation of carbon supplies, the fungus-hyphae began to degenerate and starve; and in some cases Ward and Evans (42) found corrosions of the tissues beneath the spores on the epidermis. They also made out numerous interesting details regarding the degeneration of the nuclei, breaking up of the chlorophyll corpuscles, thinning of the cell-walls and so forth.

The results as shown by the above tables agree essentially with those obtained by Ward with the rust.

Both sets of plants gave tests for sugars, so it does not seem likely that such a complete inhibition in the growth of the fungus should have resulted solely from the lack of available food, unless it might perhaps be dependent on the transition products in photosynthesis as Fromme has suggested by way of explaining the delay of infection on plants grown in the dark.

Summary.

Wheat and barley plants grown in an atmosphere free from carbon dioxide would show no infection with their powdery mildews even nine days after the infection was apparent upon the control plants.

When growing in an atmosphere free from carbon dioxide

most of the wheat and barley plants would be dead at the end of fifteen days.

Infection would be apparent upon the control plants at the end of four or five days.

The wheat and barley plants gave tests which indicated the presence of sugars in both the control and treated plants.

While the control sunflower plants show infection eight or nine days after inoculation, the plants grown in an atmosphere free from carbon dioxide would show no infection five days later, which was the longest time the plants could be kept alive.

Conclusions

Wheat and barley plants, seven or eight days old, as a rule, cannot live longer than twelve days in an atmosphere free from carbon dioxide.

Sunflower plants, as a rule, are dead twelve or fourteen days after they are placed in an atmosphere free from carbon dioxide.

During the time the host plants could be kept living infection with the powdery mildew cannot take place upon wheat, barley, and sunflower plants when grown in an atmosphere free from carbon dioxide.

To Determine the Effect of Adding an Extract of Barley Leaves to the Culture Solutions, Upon the Susceptibility of Wheat Seedlings to Barley Mildew.

Into each of six wide mouthed liter bottles, containing 900 cc. of tap-water were placed two wheat seedlings. The seedlings were supported by means of paraffine corks.

To the tap-water of four bottles was added 25 cc. of an extract of barley leaves. The other two bottles were left as controls. These seedlings were grown and protected by bell-jars so there would be no opportunity for chance infections with mildew. From time to time more of the extract of barley leaves was to be added to the tap-water of the four bottles. Later the wheat seedlings in all the bottles were inoculated with barley mildew to determine if the immunity of the wheat to barley mildew could be broken down by the addition of the extract of barley leaves to the culture solution.

The extract of the barley leaves was prepared by crushing 5 grams, green weight, of barley leaves, heavily infected with barley mildew, in a porcelain mortar and then adding 100 cc. of redistilled water. The mixture was filtered and used at once. A new extract was made up each time it was used.

When the plants had been allowed to grow four days after the addition of the barley extract, it was noted that the control plants were making slightly more growth than the seedlings in the culture solutions containing the 25 cc. of barley

extract.

The control plants averaged 12.5 cm. in height; the plants of the treated set averaged about 10 cm. in height.

After the cultures had been growing nineteen days, 25 cc. of barley extract was added to each of the four bottles, and the water lost was restored in all the cultures.

After the cultures had been allowed to grow twenty-five days, 25 cc. more of the barley extract was added and the plants of all the cultures were inoculated. They were then placed under bell-jars again.

Observations were taken for a little over three months and no infection was ever observed.

To Determine if Injury to Leaf-Cells Renders Them Susceptible to the Attacks of Foreign 'Biologic Forms'.

Experiments similar to those described by Salmon (34, 35) were set up. Thin sections of barley leaves were cut out from the epidermis with a sharp razor, the leaf tissue was cut or bruised with a cork borer, and sections were burnt with a hot iron, to determine if such injuries to the leaf-cells of a host species, susceptible as a rule, to the attacks of only one "biologic form", would affect the vitality in such a manner, that the leaf-cells in the neighborhood of the injury would be rendered susceptible to the attacks of other "biologic forms".

Local infections were made, by means of a scapel at the point injured on the side of the leaf, opposite the wound.

Three injuries were made on each piece of a leaf about six or seven centimeters long. Two injured leaves were placed in each large Petrie dish upon moist filter paper, and kept under conditions favorable for infection.

Careful precautions were taken to guard against chance infection with conidia from the same "biologic form" which normally grows upon barley.

The following table summarizes the results of these experiments:-

Table No. XXXV

Cross Inoculation of Injured Barley Leaves.

Date of Inoculation 1914	Treatment given leaves	Inoculation Host	Inoculation Fungus	Observation 1914	Observation 1914	Experiment
Feb., 21	"cut" areas	Barley	Wheat	Feb., 27 conidia germ & production of mycelium	March, 3. no infection	I
Feb., 25	burnt areas	"	"	Feb., 27 spore germ. & production of mycelium	March, 4. no infection	II
March, 4	"cut" areas	"	"	March, 9 spore germ. penet. host tissue, no haust. form	March, 11 no infection	III
March, 4.	burnt areas	"	"	slight germ of conidia	no infection	IV
March, 14.	"cut" areas	"	Barley	March, 18 conidia, germ. slight develop. of mycelium	March, 21. slight infection	V
March, 14.	"cut" areas	"	wheat	conidia have not germinated	no infection	VI
March, 21	uninjured	"	Barley	April, 1 scattered infection & production of conidia		VII
April, 18	bruised areas	"	Wheat	April, 20 spore, germ. & some mycelia development	April, 27 no infection	VIII
April, 21	bruised areas	"	"	April, 23 spore germ. & some mycelia development	May, 1 no infection	IX

Some of the experiments included in the above table are given more in detail below:

Experiment No. I :- Eighteen barley leaves, injured by cutting out a piece of the epidermis, 2-3mm. long on one side, were heavily inoculated, locally by means of a scapel, on the side opposite the cut, with conidia from wheat plants.

Six days after inoculation the conidia of the wheat mildew had germinated, and produced some mycelium upon the barley leaves.

Eleven days after inoculation, while the conidia had germinated more or less extensively, it could not be observed that haustoria had been produced, nor that mycelium had become established. Certainly no conidia had been produced.

Experiment No. II:- Areas on barley leaves were burned with a hot iron and inoculated on the opposite side with wheat mildew. Fourteen leaves were treated in this experiment.

Two days after inoculation, spore germination had taken place quite extensively, with the development of mycelium.

Seven days after inoculation no haustoria had been formed, so far as observations couldbe made, and the germination of the conidia had not been so extensive as in experiment No. I with the cut areas. The burnt areas were all a bright green in color, while the rest of the leaf was very much etiolated.

Experiments No. III and IV :- These two experiments were set up at the same time. In experiment No. III eleven leaves were injured by cutting and in experiment No. IV ten leaves were

injured by burning with a hot iron.

Five days after inoculation the conidia upon the "cut" areas had germinated slightly, and apparently the germ-tube in some cases had penetrated the host-tissue, but the finger-like haustoria had not been formed, only a slight enlargement being visible at the end of the hyphae. There was not a very abundant production of mycelium. The conidia upon the burnt areas had not germinated to any extent. Another fungus, a species of Macrosporium, however, was growing very abundantly over some of the burnt areas, and the fruiting bodies of this fungus, under low power of the microscope, might somewhat easily have been confused with the conidiophores and conidia of the powdery mildew.

Experiment No. VII :- Uninjured barley leaves were inoculated with barley mildew by dusting the conidia over them. Ten days after inoculation about 75 per cent of the leaves showed scattered infection with the production of conidia.

Mention has been made of Salmon's (34, 35a) work in which he showed that the range of infection possibility of Erysiphe graminis may be increased under certain cultural conditions. By injuring leaves and subjecting plants to heat and anesthetics he was able to infect normally immune forms. Ray (24a) states that by subjecting maize to ether vapor and then inoculating it with spores of Ustilago zeae, the resulting infection was much more virulent than on plants not so treated. Stakman (38) found that the use of anesthetics had some effect in rendering an immune wheat plant slightly more susceptible to the rust; leaf injury, however, had no effect.

If the different forms of Erysiphe graminis are merely adaptations it might seem possible to change their parasitic tendencies by restricting or chagging their environment. Stakman believes it ought to be possible to break down the biologic forms under conditions abnormal for host or parasite.

Conclusions.

The results of these experiments indicate that the specialization of the mildew has become so sharply restricted that conidia from Treadwell wheat connot produce complete infection in the leaves of Oderbucker barley which have been injured or otherwise had their vitality lowered.

To Determine if the Powdery Mildews of the Cereals Could be Induced to Form Perithecia.

It is sometimes believed that when the host plant is suffering as a result of unfavorable environmental conditions or declining vegetatively from old age, the parasitic powdery mildew is stimulated to produce perithecia. The mildew could thus tide itself over until conditions were favorable again for the growth of its host plant. The mildew upon the cereals rarely forms perithecia and experiments were conducted in which badly infected host plants were subjected to various treatments to ascertain if the mildew could be induced to form perithecia.

Old barley and wheat plants were placed in the ice-box

and chilled from 12 to 48 hours. Plants were allowed to become dry enough to wilt badly for from one to three days. Plants were kept in the dark for from one to five days, and in some cases alternately kept in the dark box and then in the light for different periods of time. Plants were etherized and various combinations of the above treatments were tried without success. No perithecia were observed.

There were barley and oat plants in the greenhouse which had been growing for eight months, and while these plants were infected with mildew, no perithecia had been formed.

To Trace, by Microscopic Examinations, the Process of Infection of Barley with the Powdery Mildew.

Attempts were made to follow the process of infection of barley with the mildew. The plants were heavily inoculated and then placed under bell-jars in the greenhouse. Every twenty-four hours several leaves were removed from the pot of plants and placed in a mixture of equal parts of 95 per cent alcohol and glacial acetic acid. This killed the leaves and extracted the chlorophyll. After remaining for twenty-four hours the leaves were transferred to a 70 per cent solution of alcohol until permanent stained mounts were made.

Until the fungus had become firmly established upon the host great care had to be exercised to avoid washing the mildew from the leaf. In order to prevent this the leaves which had been inoculated for less than three days were killed and decolorized and stained on the slide. The chlorophyll of the leaf was sufficiently extracted

in two hours when the leaf was kept flooded on the slide with the mixture of equal parts of 95 per cent alcohol and acetic acid.

To make the permanent mounts the excess acetic acid was washed away with 95 per cent alcohol and the leaf stained with the Pianeze IIIb stain as follows:

The leaf is placed on the slide so that the infected surface is on the upper side and stained with Pianeze III b stain for 25 or 30 minutes. This stain is made accodring to the following formula:

Malachite green	0.5	gr.
Acid Fuchsin	0.1	"
Martins gelb.	0.01	"
Distilled water	150.	cc
Alcohol 95 per cent	50.	cc

After staining the specimen is decolorized by applying 95 per cent alcohol with a pippette. The time it takes to properly decolorize varies. The specimen should be examined at intervals in order to determine when it has been decolorized to a sufficient extent. By this method the host cells are stained green, and the fungous hyphae pink. All parts are stained green first, but the decolorization with 95 per cent alcohol brings out the pink color in the fungous hyphae.

The leaf, after staining as outlined above, is immersed in absolute alcohol for five minutes; treated with a mixture of equal parts of absolute alcohol and xylol for five minutes; and then immersed in pure xylol for five minutes. The excess xylol is poured off, a drop of balsam added and a cover glass put on.

A careful study of these preparations show that at the end of twenty-four hours in most cases approximately 20 per cent of the conidia have germinated. From some of the germ-tubes haustoria have been developed which have penetrated into the epidermal cells.

Under favorable conditions twenty-four hours after heavy inoculation the conidia of barley sown on barley leaves have germinated, produced some mycelium, sent out one or two haustoria, and thus become established upon the host. The haustoria at this time seem to be produced at the tips of the hyphae. Sometimes there is a longer hyphae from one side and a shorter hyphae growing out in the opposite direction, both with a haustorium developed at the ends.

Often the mycelium follows the slight depression between two cell-walls, until near the end of the mycelium, when it turns and goes towards the center of the cell, or perhaps, crosses one cell to another and there sends in a haustorium.

By the end of 48 hours a larger percentage of the spores have germinated, several germ-tubes have been sent out, two or three haustoria have started to be formed, and the mycelium may have commenced to branch.

At the end of three days the mycelial growth of the barley mildew has increased considerably, and the long finger-like haustoria are to be plainly observed. In some cases no conidiophores are distinctly observable as such. In other cases conidiophores have have been formed but no conidia have been abstricted.

By the end of four days one can observe different stages

in the development of the conidiophores. Some are just pushing out from the mycelium, and have not produced any conidia, other conidiophores have gone far enough in their development to have cut off a few conidia.

By the fifth day there is usually an abundant production of conidia.

To Determine if Infection With "Bunt" in Anyway Influences the Wheat's Susceptibility to Powdery Mildew.

Six pots of wheat seeds which had been heavily inoculated with the spores of bunt were planted. When the seedlings were eleven days old, and averaged about 14 cm. in height they were inoculated with the mildew and placed under bell-jars. Each pot contained about three dozen plants.

As controls, six pots of wheat of the same size and age which had not been inoculated with bunt, were inoculated at the same time with mildew.

Four days after inoculation the wheat plants, the seeds of which had been inoculated with bunt, showed infection with mildew.

Five days after inoculation the controls showed splendid infection with mildew.

A second experiment was conducted just as the first one. There were twelve pots altogether, six as controls. The seedlings were 10 days old and 12 cm. in height.

Five days after inoculation, both sets showed splendid infections with mildew.

Conclusions.

The fact that the mycelium of the bunt was evidently growing in abundance throughout the tissue of the seedlings seemed in no appreciable manner to influence the susceptibility of the wheat to the mildew. It is also a well known fact that

rust and mildew can both grow vigorously upon wheat plants at the same time.

To Determine if the Mildews which Infect the Cocklebur, the Sunflower, and Cucumber are the same Physiologically.

Cocklebur Mildew upon the Sunflower; Experiments were conducted to determine if the mildew which infects the cocklebur can also infect the sunflower. The cotyledons of sunflower seedlings in some cases, and the first pair of leaves, in other cases, were inoculated with mildew from the cocklebur. The sunflower plants averaged about 11 cm. in height, and were heavily inoculated by means of a scalpel and then placed under bell-jars.

In the following tables which show the results of these experiments, the plus sign indicates infection:

TABLE NO.XXXVI

Age of plants when inoculated	Average height of plants when inoculated	Part of plants inoculated	Length of inoculation period	Infection	No. of plants
12 days	7 cm	cotyledons	9 days	+	20
15 "	11.5 "	"	9 "	+	15
19 "	15 "	1st pair of leaves	9 "	+	17
14 "	11 "	1st pair of leaves	9 "	+	19
18 "	15 "	1st pair of leaves	9 "	+	14

In the above experiments about ninty-five per cent of the leaves and cotyledons inoculated showed splendid infection with the mildew nine or ten days after inoculation.

Sunflower Mildew upon the Cucumber: The cotyledons of a number of young cucumber plants were heavily inoculated with sunflower mildew by means of a scalpel and then placed under bell-jars in the greenhouse. The plants averaged 4.5 cm. in height.

The following table shows the results of these experiments:

TABLE NO. XXXVII

Age of plants when inoculated	Aver. height of plants when inoculated	Part of plants inoculated	Length of inoculation period	Infection	No. of plants
7 days	4.5 cm.	cotyledons	7 days	+	7
9 "	4.7 "	"	7 "	+	14

Seven days after inoculation each cotyledon of every cucumber plant showed splendid infection as a result of inoculation with the sunflower mildew.

Cocklebur Mildew upon the Cucumber: To determine if the mildew growing upon the cocklebur can infect the cucumber the cotyledons of a number of cucumber plants were inoculated with cocklebur mildew and placed under bell-jars. The plants averaged 15 cm. in height and had formed one leaf after the cotyledons. A scalpel was used to make the inoculations.

The following table gives the results of these experiments:

TABLE NO. XXXVII

Average height of plants when inoculated	Part of plants inoculated	Length of inoculation period	Infection	No. of plants
15 cm.	cotyledons	8 days	+	7
14 "	"	8 "	+	8

As shown by the table eight days after inoculation with the cocklebur mildew the cucumber seedlings showed infection. Three or four days later the infection would be very marked.

Conclusions.

From the above experiments it would seem that the mildew which grows upon the cucumber, sunflower and cocklebur is the same mildew physiologically, and as yet, upon these plants it has not become sharply restricted to one host by "biologic specialization."

SUMMARY AND GENERAL CONCLUSIONS.

Darkness has no inhibiting effect upon *Erysiphe cichoracearum* in its powder to infect the cotyledons of sunflower seedlings.

The inhibiting action of the absence of light upon infection of wheat and barley with *Erysiphe graminis* is not complete. Infection will take place in the dark, after a given period of time, although the normal incubation period may be doubled.

The delay of infection of barley and wheat with their powdery mildews is not due to the direct effect of the absence of light upon the fungus, but rather to an indirect effect upon the plants. Furthermore, it does not seem likely that the lengthened incubation period is due to any one specific cause, but to a combination of factors.

The power of the mildew to produce infection seems to be somewhat closely associated with the presence of chlorophyll in the leaves.

Partial immunity to the mildew is brought about by mineral starvation indirectly, and only so far as the general health and vigor of the host is influenced. Because the host plants suffered most from the lack of nitrogen, the plants grown in solutions from which the element

nitrogen was lacking seemed to exhibit the greatest degree of immunity, but in all cases, as long as the plant remained alive, infection took place.

Dilute solutions of manganese sulphate when added to the soil bring about a marked immunity of barley plants to the powdery mildew.

Wheat and oat plants, when watered with zinc nitrate and lead nitrate, seem to be slightly more susceptible to the attacks of mildew than untreated plants.

The addition of potassium sulphate and lithium bromide to the soil produces an immunity to some extent.

When wheat and barley plants were not inoculated until they had commenced to show the effects of the treatment, infection could be prevented by the addition of such a relatively concentrated solution of potassium nitrate to the soil, that the leaves wilted and turned yellowish in color. After tap water had been added and new leaves developed, these new leaves became infected.

Well-nourished, vigorously growing wheat plants are more susceptible to the attacks of the powdery mildew than half-starved, sickly, yellowish, semi-wilted plants; or, in other words, as the host plants themselves suffer from a combination of outside factors, the fungus is unable to become as successfully established as upon normal, well-developed hosts.

Contrary to an idea sometimes held, it does not seem possible to so weaken the vitality of the host plant, by growing it under extremely unfavorable conditions, that it will become more susceptible, even when placed, after inoculation, under conditions very favorable to the growth of the mildew. As Stakman states, in regard to *Puccinia graminis*, so too, *Erysiphe graminis* is a very high specialized, obligate parasite, and there is a very intimate relationship between host and parasite. Hence, whatever is conducive to the health of the host is ordinarily conducive to the vigorous development of the parasite also.

There is an exceedingly wide range in the degree of humidity of the air surrounding the plants inoculated with mildew, in which the infection of the host is not to any appreciable extent affected. If the air, however, which surrounds the inoculated plants closely approximates absolute dryness, it has a direct effect upon the fungus in preventing infection. The conidia either cannot germinate, or the germ-tubes dry out, and hence the host is disease escaping.

Wheat and barley plants seven or eight days old, as a rule, cannot live longer than twelve days in an atmosphere free from carbon dioxide. Sunflower plants, similarly treated, died after twelve to fourteen days.

With the control plants infection took place in the case of wheat and barley in four or five days, and in the case of the sunflowers in eight or nine days; but in an atmosphere free from carbon dioxide, during the time the host plants could be kept living, no infection with the powdery mildew took place.

The addition of an extract of barley leaves to culture solutions has no effect upon the susceptibility of wheat seedlings to the barley mildew.

Specialization of the mildew has become so sharply restricted that conidia from Treadwell wheat cannot produce complete infection in the leaves of Oderbucker barley which have been injured or otherwise had their vitality lowered.

Unsuccessful attempts were made to induce the mildews of the cereals to form perithecia by submitting the host plants to unfavorable environments.

The process of infection of barley with the mildew was traced microscopically, as follows:

First day: At the end of twenty-four hours, in most cases approximately twenty per cent of the conidia have germinated. From some of the germ-tubes haustoria have been developed which have penetrated into the epidermal cells.

Second day: By the end of forty-eight hours, a larger percentage of the spores have germinated, several germ-tubes have been sent out, two or three haustoria have started to be

formed, and the mycelium may have commenced to branch.

Third day: At the end of three days, the mycelial growth has increased considerably, and the long, finger-like haustoria are to be plainly observed. In some cases, no conidiophores are distinctly observable as such. In other cases, conidiophores have been formed, but no conidia have been abstricted.

Fourth day: By the end of four days, different stages in the development of the conidiophores can be observed. Some conidiophores have gone far enough in their development to have cut off a few conidia .

Fifth day: By the fifth day there is usually an abundant production of conidia.

Wheat may be infected with perhaps "bunt", rust, and mildew at the same time, and there be no influence exerted over the host's susceptibility to any of these diseases.

Erysiphe cichoracearum DC growing upon the cucumber, sunflower and cocklebur, seems to be the same mildew physiologically, and successful cross-inoculation can be made with the mildew of each of these hosts upon the other two.

The work on this thesis has been done under the direction of Professor G. M. Reed, and I am greatly indebted to him for his kindly criticisms and valuable suggestions.

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