V. *Cultural Experiments with "Biologic Forms" of the* Erysiphaceæ.

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1. *Introductory.*

During the past two years the existence of "biologic forms" in the *Erysiphaceæ* has been definitely proved. We know now that there exist in this group of parasitic fungi races of individuals which are morphologically identical, but which differ physiologically—or biologically—in possessing distinctive and sharply defined powers of infection. Thus we find a fungus A growing on host-plant *a*, and a fungus B growing on host-plant *b*. Now the fungus A belongs to the same species as fungus B, and is identical in every morphological character, yet the fungus A cannot infect the host-plant *b*, nor can the fungus B infect the host-plant *a*. The two fungi A and B thus prove to be "biologic forms" of the same morphological species.

This specialization of parasitism was first observed, (1) (2) (5), as regards the *Erysiphaceæ*, in the conidial (*Oidium*) stage of these fungi. At the beginning of the present year I published a paper "On the Infection-powers of the Ascospores in the *Erysiphaceæ*" (4), in which I gave the results of experiments which proved that the ascospores of "biologic forms" are similarly specialized in their powers of infection. Soon afterwards Professor E. Marchal published a summary of experiments in which the same results were obtained (3).

Since, then, restriction in power of infection to one species of host-plant, or to a group of closely allied species, has been proved to exist in these fungi in the sexual ascigerous stage as well as in the asexual conidial stage, we must admit the claim of these "biologic forms" to be considered as distinct entities.

2. *The Behaviour of "Biologic Forms" under Certain Methods of Culture.*

In the course of experiments carried on during the past summer, I have found that *the restriction in power 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.*

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 millims. 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. The following case may be given to illustrate the point. If the conidia of *Erysiphe Graminis* occurring on wheat are sown on uninjured leaves of wheat and of barley, the result will be the infection of wheat but never of barley. That is to say, the form of *E. Graminis* on wheat is a "biologic form" confined under usual conditions to a few species of the genus *Triticum*. But if now we take the conidia of this "biologic form" on wheat and sow them on barley leaves *which have been "cut" as described above*, full infection results, and conidiophores with ripe conidia are produced in 6–8 days.

Other experiments described below seem to prove, further, that plants which are immune in nature can be artificially rendered susceptible. For instance, the two species of wheat, *Triticum monococcum* and *T. dicoccum* have been stated to be immune against mildew, both in the field and in laboratory infection-experiments (2). If, however, leaves of these two species are "cut" as described above, and inoculated with the conidia of the "biologic form" of *E. Graminis* on wheat, or even of a species of barley (*Hordeum sylvaticum*), infection readily occurs, and ripe conidiophores are produced in 4–6 days.

A summary of the experiments with "cut" leaves is given below (p. 114). The experiments described in this summary give conclusive evidence that under certain cultural conditions the distinctive characteristics of "biologic forms" break down, or in other words, that the range of infection of "biologic forms" becomes increased when the vitality of the leaf on which the conidia are sown is affected by injury.

It may be noted here that the conidia of the fungus produced on the "cut" leaf possess full germinating powers. For instance, conidia of the first generation produced on "cut" leaves of *Hordeum sylvaticum* by inoculation with conidia occurring on *H. vulgare*† readily infected "cut" leaves of *H. sylvaticum*; the conidia

* "Cut" leaves = leaves cut as described above. The word "cut" is used to designate the whole area from which mesophyll has been removed.

† All attempts to infect uninjured leaves of *H. sylvaticum* with conidia from *H. vulgare* failed.

thus obtained again readily infected "cut" leaves of *H. sylvaticum*, and so on to the third generation. Again, the conidia of the first generation produced on "cut" leaves of *H. sylvaticum* by inoculation with conidia occurring on *Triticum vulgare* infected, in eleven cases out of twenty-two, "cut" leaves of *T. vulgare*.

In all the experiments hitherto mentioned, leaves removed from the plant, and injured by "cuts," were used, and the question naturally presented itself: Was the susceptibility shown under the above conditions caused directly by the injury due to the "cut," or was it, in part at any rate, caused by the cutting off of the leaf from the plant?

Two series of experiments were carried out with the object of answering this question,

In the first, leaves of barley attached to plants were "cut" and inoculated with conidia from wheat. In one experiment a leaf of a plant of barley, 7 days old. was "cut" in two places (one each side of the midrib) and over one "cut" conidia from *Triticum vulgare* were sown, the other "cut" serving as a control. This "cut" leaf, still attached to the plant, growing in a pot, was then inserted through a hole into the bottom of a closed Petri dish and kept pressed down on wet blotting-paper. On the fifth day mycelial hyphæ and large lobed haustoria were evident at the place of inoculation over the "cut." By the tenth day, five conidiophores with chains of ripe conidia, and numerous younger conidiophores, had been produced; while the control "cut" showed no trace of any fungus. The leaf was now beginning to turn yellow towards the apex. On the seventeenth day the leaf was nearly dead throughout its length; three conidiophores were still visible at the inoculated spot, while the control "cut" was still free. In another experiment two "cuts" were made on one barley leaf attached to a plant in a pot, and conidia from *Triticum vulgare* were sown over both "cuts," the other plants in the pot serving as controls. The "cut" leaf was inserted into a closed Petri dish in the same manner as before. On the fourth day mycelial hyphæ had developed over both "cuts"; on the tenth day three conidiophores were formed; on the sixteenth ten conidiophores were visible over one "cut," and six over the other; the leaf-tissue surrounding the "cuts" was now turning yellow. By the twelfth day the leaf in its upper two-thirds was nearly colourless and almost dead, a few conidiophores were still visible; no signs of any fungus appeared elsewhere on the "cut" leaf, nor on the control leaves in the pot. In a third experiment a "cut" was made on a growing barley leaf attached to a young plant in a pot, and a drop of water placed on the "cut." On the dry epidermis over the "cut" conidia of the "biologic form" on wheat were sown. Conidia of the same "biologic form" were sown also on the epidermis of another uninjured leaf of the same plant. The pot was then placed under a bell-jar. On the sixth day the "cut" leaf, which still retained the drop of water hanging in the concavity of the "cut," was infected virulently with vigorous mycelial hyphæ and numerous large lobed haustoria. On the ninth day the "cut" leaf bore a few conidiophores. No infection resulted on the uninjured leaf.

In the second set of experiments leaves of barley and wheat were removed from growing plants, and some of each species placed, uninjured, in Petri dishes under the same conditions as in previous experiments. Conidia of the "biologic forms" on wheat and barley were sown at marked places on these leaves. No infection resulted when the conidia from wheat were sown on barley, nor in the reverse experiment, while infection never failed to occur on the wheat and barley leaves when conidia of the respective "biologic form" were sown.

It seems clear, therefore, that the effect caused by the "cut" on the vitality of the leaf is the factor which brings about increased susceptibility of the leaf to the attacks of the fungus.

It still remained to be seen whether the conidia produced on a "cut" leaf by inoculation with conidia of a "biologic form" which is unable to infect uninjured leaves of that species, possessed the power of infecting uninjured leaves of the plant in question; or whether the restricted infection-powers characteristic of the "biologic form" are retained, under these conditions, in the conidia of the first generation. The following experiment was made to determine this point. Conidia obtained on "cut" leaves of *Triticum vulgare* by inoculation with conidia of the *Oidium* occurring on *Hordeum sylvaticum* were sown on the uninjured damp upper surface of two leaves of *Triticum vulgare* attached to growing plants in a pot. On one leaf fairly numerous conidia were deposited; on the other only a very few. The inoculated plants were kept continuously covered over with a bell-jar for 6 days. The two leaves were then examined, and it was found that the leaf which had been sown with numerous conidia now bore at the place of inoculation a well-grown patch of mycelium, with a group of densely clustered conidiophores—about fifty in number—with chains of ripe conidia. The control leaves (six) were all free. By the ninth day a large and very vigorous *Oidium*-patch, powdery with masses of ripe conidia, was produced on this inoculated leaf. No infection resulted on the other inoculated leaf.

Further, conidia obtained on "cut" leaves of *Hordeum sylvaticum* by inoculation with conidia of the "biologic form" on *H. vulgare* were sown on the under surface, between inked lines, of two leaves of *H. sylvaticum* removed from the growing plant, but otherwise uninjured. These two inoculated leaves were placed by the side of two control leaves on damp blotting-paper in a Petri dish. On the ninth day the two inoculated leaves were covered continuously over the space between the inked lines (= 0·5 centim. wide) with vigorous mycelium bearing densely clustered conidiophores and powdery masses of ripe conidia. The two control leaves remained free.

A variation was now made in the method of culture. In three experiments conidia of *E. Graminis* were sown on leaves which had been "cut" as described above, but the conidia were now sown on the internal tissues of the leaf exposed by the cut.

In the first experiment, thirteen "cut" leaves of barley were inoculated with

conidia of the "biologic form" on barley. In some cases the leaves were "cut," and the conidia were at once sown on the cut surface, which was moist with sap exuding from the cut cells; in others, the cut surface was dried by exposure to air in a Petri dish for 3 hours. By the fifth day all thirteen leaves were virulently infected, the cut surface being covered by interwoven hyphæ bearing numerous little groups of conidiophores.

In the two other experiments conidia of the "biologic form" on wheat were sown on ten "cut" leaves of barley, on the cut surface, and placed by the side of six control leaves of barley, "cut," but not inoculated, on damp blotting-paper in a closed Petri dish. On the ninth day eight of the inoculated leaves were infected at the "cut"; all bore patches of mycelial hyphæ on the surface of the cut cells, and on five leaves the fungus had produced a little group of conidiophores. In some cases the group of conidiophores sprang from the internal surface (exposed by the cut) of the epidermal cells; in other cases from the exposed mesophyll tissue. The controls were everywhere quite free.

At Professor MARSHALL WARD'S suggestion an experiment was now made to see if the same result, *i.e.*, the infection of the host-plant of one "biologic form" by the conidia of another "biologic form," could be obtained by a method which avoided lesion of the leaf. For this purpose eight barley leaves, which had been removed from the plant, were touched on the upper surface for a few seconds with a red-hot knife. These were then sown with the conidia of the "biologic form" of *E. Graminis* on wheat; on three leaves the conidia were placed on the burnt spot on the upper surface of the leaf; on five leaves on the lower surface opposite to the burnt spot. The leaves soon showed the effect of the application of the red-hot knife, a small area of leaf-tissue becoming discoloured, limp, and apparently dead on both surfaces. On the eighth day two of the three leaves bore at the burnt place little flecks of mycelium with a few conidiophores. The flecks of mycelium were at the very edge of the patch of killed cells on what appeared superficially to be uninjured cells. Of the five leaves each one was infected, and bore small flecks of mycelium with a few conidiophores. The mycelium occurred, as a rule, on living tissue at the very edge of the group of dead cells killed by the hot knife, but in one or two cases mycelial hyphæ could be seen on the now dead cells, and extending thence to the surrounding living tissue. No signs of any fungus appeared elsewhere on these seven leaves.

3. *General Considerations.*

One of the first questions that presents itself in connection with the present subject is: Do we meet, *in nature*, with injuries to leaves, such as those caused in the above experiments? A case which has come under my observation leads me to conclude that such injuries do occur, and shows also of what nature the injury may be. This case is as follows. In the course of infection-experiments with the "biologic forms"

of *E. Graminis* on species of *Bromus*, I have found that the "biologic forms" occurring on *B. commutatus* and *B. velutinus* are not able to infect *B. racemosus*. In these experiments, fifty leaves—from fifty different plants—of *B. racemosus* were inoculated, twenty-five with conidia from *B. commutatus*, and twenty-five with conidia from *B. velutinus*; fifty control leaves of *B. commutatus* and *B. velutinus* being inoculated at the same time. In every case, infection resulted on *B. commutatus* and *B. velutinus*, and failed on *B. racemosus*. There is no doubt, therefore, that we have on *B. commutatus* and *B. velutinus* a "biologic form" which is incapable under ordinary conditions of growing on *B. racemosus*. Now, these plants of *B. racemosus* were kept in the laboratory for 6 weeks or so during the experiment, and, at the end of this time, became infested with "green fly" (*Aphis*). These insects, in sucking the juices of the plant, injure the leaf in much the same manner as was done artificially by "cutting" the leaf, that is, by puncturing the leaf they destroy a few of the leaf-cells at little isolated spots. At these injured places on the leaf flecks of mycelium appeared, which eventually produced a few conidiophores. It seems to me that we have here a case of a plant which is immune against a fungus so long as the leaves remain uninjured, but which becomes susceptible when its leaves are injured by an insect.

Hitherto, the result of all experiments with "biologic forms" has been the accumulation of evidence tending to emphasise the fixity and sharply defined nature of "biologic forms." The evidence obtained was compelling us to believe that the numerous "biologic forms," of which we found each morphological species to be composed, were rigidly separated one from the other by immovable limits which restricted each "biologic form" either to a single host-species, or to certain host-species of one genus. At the most, a "biologic form" might, by means of a "bridging species" (see 5, p. 285), pass from the host-species of one section of a genus to those of another. The result of the present experiments, however, shows us some conditions under which "biologic forms" break down—some conditions under which the conidia of one "biologic form" can infect host-plants (of another "biologic form"), which are, as a rule, immune against its attacks. We find that when the vitality of the leaf-cells of a host-species, susceptible, as a rule, to the attacks of only one "biologic form," is affected by an injury—such as a cut or burn—the leaf-cells in the neighbourhood of the injury are rendered susceptible to the attacks of other "biologic forms." It is evident, and we have clear experimental proof of the fact, that such injured leaves will serve as "bridges," enabling a "biologic form," restricted in its parasitism, as a rule, to one genus, to pass over to the host-species of another genus.

We can see, now, more of the factors at work in the evolution of "biologic forms." We find, on the one hand, factors, which we may call "specialising factors," tending to make specialisation of parasitism more and more complete, with the result that there originate from each morphological species a number of "biologic forms," each one of which is incapable of infecting healthy leaves of any but its own host-species

or group of host-species. This breaking-up of a morphological species into "biologic forms" rigidly fixed in the sexual (ascosporic) stage as well as in the asexual (conidial) stage, would, we may believe, lead ultimately to the evolution of new *morphological* varieties or species. I have already (5, p. 264) pointed out that some "biologic forms" show in the conidial stage distinctive characteristics in the colour of the conidia, and, in some cases, a further difference is found in the size of the conidia.

But we find antagonistic factors at work, which we may term "generalising factors." When the vitality of the leaf-cells of a host-plant is affected by injury in certain ways, other "biologic forms" than the one specially adapted to it become capable of infecting it, and, as experiments have shown, conidia produced on these injured places are at once capable of infecting uninjured leaves of the same species. Thus the separate streams of evolving "biologic forms" are liable to flow into each other.

As to the actual manner in which the injury to a leaf causes it to become susceptible to a "biologic form" otherwise unable to infect it, the following explanation may be given tentatively. The phenomena of adaptive parasitism shown by the *Erysiphaceæ* so closely resemble those found in the *Uredineæ* that the hypothesis put forward by MARSHALL WARD (6) (7) as to the factors governing infection and immunity may be advanced for the *Erysiphaceæ* also. This hypothesis assumes the existence of enzymes or toxins, or both, in the cells of the fungus, and of anti-toxins, or similar substances, in the cells of the host-plant.

Now, in connection with the above experiments, we may suppose that the leaf-cells of wheat, barley, &c., contain substances peculiar to each species, which when the cells are vigorous and the leaf is uninjured, are able to prevent the successful attack of any mildew except the one "biologic form" which has become specialised to overcome the resistance. When the vitality of the cells, however, becomes affected by injury, either these substances are destroyed, or become weakened, or the production of them by the protoplasm is interfered with, in the leaf-cells in the neighbourhood of the injury, with the result that the conidia of other "biologic forms" are now able to infect them, since they no longer meet with any special substance able to injure them.

I would point out here that the present experiments furnish conclusive and direct proof that, as regards the *Erysiphaceæ*, the immunity of a certain host-species against the attacks of other "biologic forms" than its own in no way depends on any anatomical or structural peculiarities—such as hairs, ribs, thickness of the cell-wall or cuticle, or the chemical nature of the cell-wall, &c.—since in the experiments where a "cut" leaf became susceptible to a "biologic form" otherwise incapable of infecting it, the conidia were sown on the uninjured epidermis of the leaf.

It seems to me possible also that we may find in the facts shown by the above experiments the explanation of a phenomenon which so frequently confronts plant-

pathologists, viz., the sudden appearance of a parasitic fungus disease on plants which had hitherto proved immune. As the above experiments prove, it needs only an injury to be made to the leaves of certain plants in order to render them susceptible to disease, although otherwise they remain immune; and since, as experiments further proved, when the fungus has gained a footing on the injured parts, it can at once spread to uninjured parts, we can see how plants, the leaves of which have been injured by animals, hail, storms of winds, &c., may become the starting-point of a new disease.

It is worthy of note that the cases in which conidia of one "biologic form" were sown *on the cut surface* of the leaf of a host-species of another "biologic form," and proved under these conditions able to infect a plant otherwise immune to it, present a close and interesting parallel to the biological facts obtaining in the class of parasitic fungi known as "wound parasites"—*Polyporus*, *Botrytis*, *Nectria*, *Cucurbitaria*, *Peziza willkommii*, &c.—which are able to infect their hosts only through a wound.

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SUMMARY OF EXPERIMENTS.

1. *Experiments in which Conidia of one "Biologic Form" of* E. Graminis *were Sown on "Cut" Leaves of a Host-plant of another "Biologic Form."*

In all the following experiments conidia were sown on "cut" leaves belonging to a species whose uninjured leaves had been proved to be immune to these conidia.

Conidia from Triticum vulgare *Sown on "Cut" Leaves of* Hordeum vulgare. (Experiments Nos. *a*, *b*, *c*, *e–j*.)

In these nine experiments fifty-eight leaves of *H. vulgare* were sown with conidia from *T. vulgare*, the leaves being "cut," and the method of culture adopted as described above. Infection resulted on forty-six of the leaves, the conidia producing patches of mycelium with little groups of conidiophores and ripe conidia on thirty-seven leaves, and on the remaining nine leaves patches of mycelium only.* Penetration of haustoria into the epidermal cells took place after 18 hours, and was at once followed by the vigorous growth of the first haustorium (which soon became lobed by the development of finger-like processes), and the production of hyphal branches from the appressorium of the germinating conidium. By about the

* The latter fact I believe to be due to these leaves dying before the fungus had time to produce conidiophores, and not to the occasional inability of the fungus to produce conidia under the conditions of culture used.

fourth day the bulbous bases of the first conidiophores were visible, and by 6–8 days little groups of conidiophores with chains of ripe conidia were formed. These persisted up to the tenth or eleventh day, sometimes becoming covered with little powdery heaps of accumulated ripe conidia. None of the thirty-six control leaves used in these experiments showed any signs ot a fungus, and no fungus appeared on the "cut" leaves except at the place of inoculation. The leaves used in the experiments were taken from young seedling plants, and were usually at once "cut" and inoculated; in a few cases, however, the leaves were kept with their stalks in water 18 hours after being removed from the plant (in the afternoon). The leaves, which had become somewhat flaccid, were then "cut" and inoculated, and nine of fourteen leaves became infected—the fungus on seven of the leaves producing conidiophores. As a rule, the leaf-tissue in the neighbourhood of the "cut" began to change colour in 5–8 days, and the leaves in 11–15 days became yellowish or whitish throughout, and dead, or nearly dead. In some cases several "cuts," or even holes, were made in the neighbourhood of the inoculated place, and leaves so treated were as strongly infected—if not more so—as when only one "cut" was made. In some cases conidia were sown at marked places on uninjured leaves—removed from the plant, but not "cut"—and placed side by side with inoculated "cut" leaves. No infection resulted on the uninjured leaves. An uninjured wheat leaf, however, inoculated and treated in the same way, was virulently infected, and after eight days bore a powdery *Oidium*-patch. In one experiment, six "cut" leaves and six uncut leaves of barley were floated on water in a large Petri dish, and also two uncut wheat leaves. All the leaves were sown with conidia from wheat. On the seventh day five of the "cut" barley leaves showed vigorous patches of mycelium bearing little groups of conidiophores; no signs of infection occurred on the uncut barley leaves, while the wheat leaves were covered continuously at the marked place —1 centim. wide—with powdery *Oidium*-patches. In another experiment six pieces of barley leaves, $\frac{3}{4}$–1 inch long, were "cut" and inoculated. By the sixth day the pieces of leaves had all turned yellow, and were nearly dead; three leaves, however, bore vigorous mycelial patches. On the eighth day one leaf bore a few conidiophores.

Conidia from T. vulgare *Sown on "Cut" Leaves of* Hordeum sylvaticum.
(Experiments Nos. *s*, *z*.)

Nine leaves were sown, and on four full infection took place, resulting in the production, in 5–6 days, of groups of conidiophores, with chains of ripe conidia. The fungus continued to grow vigorously, and gave rise, in 11–14 days, to luxuriant powdery *Oidium*-patches, which persisted for over 3 weeks. In one case, where three leaves were inoculated, a group of conidiophores appeared on one leaf exactly over the middle of the "cut," on the second leaf over the edge of the "cut," and on the third leaf at a spot about 1 millim. from it. (The leaves of this species of grass

possess the power of living for a long time after being removed from the plant. Leaves cut off and placed in a Petri dish on damp blotting-paper remain fresh and green for over 5 weeks.)

Conidia from T. vulgare *Sown on "Cut" Leaves of* Avena sativa.
(Experiment No. *l*1.)

Six leaves were inoculated. On the fourth day all the leaves had turned reddish-green, and were nearly dead. On two leaves lobed haustoria were formed, and small patches of mycelial hyphæ. The six control leaves were all free. No further growth of the fungus was observed, and on the eighth day the leaves were dead.

Conidia from Hordeum vulgare *Sown on "Cut" Leaves of* Triticum vulgare.
(Experiments Nos. *d*, *k*, *l*, *v*1.)

In the first experiment five "cut" leaves and five uninjured leaves of *T. vulgare* and one uninjured leaf of *Hordeum vulgare* were inoculated, and placed side by side in a large Petri dish. By the second day large lobed haustoria were formed on three of the five "cut" wheat leaves. On the seventh day four of the "cut" wheat leaves were infected with vigorous mycelial patches, one of which bore a few conidiophores; the uncut wheat leaves showed no signs of infection; while the uncut barley leaf was covered at the marked place with dense almost continuous *Oidium*-patches. In another case twelve leaves of *T. vulgare* were removed from seedling plants, and placed with the ends in water for 3 days. They were then all "cut," and six of them inoculated and placed side by side in a large Petri dish. On the second day penetration of haustoria had occurred in all the inoculated "cut" leaves. By the fourth day all the six inoculated leaves bore small mycelial patches over the "cuts"; no signs of any fungus were visible elsewhere on these leaves, nor on the six control leaves. By the eighth day all the leaves were nearly dead; mycelial patches were distinct on all the inoculated leaves, but no conidiophores were observed.

In the other experiments five uncut barley leaves and sixteen uncut wheat leaves were inoculated at marked places. By the sixth day all the barley leaves were virulently infected with *Oidium*-patches; no signs of infection appeared on the wheat leaves.

Conidia from H. vulgare *Sown on "Cut" Leaves of* H. murinum.
(Experiment No. *n*.)

Seven "cut" leaves were inoculated. After 18 hours penetration of haustoria had occurred on three leaves. At the end of 7 days all the leaves had turned yellow and were nearly dead; patches of interwoven mycelial hyphæ occurred on four leaves, but no conidiophores were observed.

Conidia from H. vulgare *Sown on "Cut" Leaves of* Avena sativa.
(Experiment No. *k*1.)

Six "cut" leaves were inoculated. By the fourth day all the leaves had turned

reddish-green and were nearly dead; on one leaf a small but vigorous patch of mycelial hyphæ occurred. Leaves dead on the eighth day.

Conidia from H. vulgare *Sown on "Cut" Leaves of* H. sylvaticum.
(Experiments Nos. 67*, 82*.)

In a previous infection-experiment (No. 67*b*) conidia of the "biologic form" on *H. vulgare* were sown on three uninjured leaves of growing plants (in a pot) of *H. sylvaticum*. No infection resulted. Twelve leaves from this pot of *H. sylvaticum* were then removed from the plants, and of these six were "cut" and sown with conidia from *H. vulgare*; the remaining six were not "cut" and were sown at marked places on the lower and upper epidermis with conidia from the same source—one of the originally inoculated leaves of Experiment 67*b* being used in each lot.

The twelve leaves were placed side by side in a large Petri dish. On the third day four of the six "cut" leaves were infected, numerous haustoria and hyphal branches proceeding from the appressorium being present; one of these infected leaves was a leaf previously used in Experiment 67*b*. On the seventh day all the six "cut" leaves showed vigorous mycelial patches. On the seventeenth day four of the "cut" leaves (including the leaf from Experiment 67*b*) showed groups of conidiophores.

No infection took place of the six uninjured cut-off leaves. In the second experiment three "cut" leaves of *H. sylvaticum* were inoculated. After 16 hours several haustoria had penetrated into the epidermal cells. On the fourth day the three leaves showed small patches of mycelium. On the sixth day small groups of conidiophores appeared on two of the leaves; by the eighth day little *Oidium*-patches powdery with conidia were visible over the "cuts" on all three leaves.

Conidia from H. sylvaticum *Sown on "Cut" Leaves of* Triticum vulgare.
(Experiments Nos. *s*1, *x*1.)

Seven leaves were taken direct from young seedling plants of *T. vulgare*, and "cut" and inoculated; three control leaves were placed at their side. On the fourth day virulent infection, as shown by the formation of very numerous large lobed haustoria and hyphal branches had taken place on five of the leaves over the "cuts." On the sixth day the mycelial patches on these five leaves bore little groups of densely clustered conidiophores, and on the tenth day *Oidium*-patches quite powdery with little masses of conidia. (These conidia were sown on an *uninjured* leaf of *T. vulgare*, and caused full infection.) No trace of any fungus appeared elsewhere on the "cut" leaves, or on the control leaves.

Conidia from Bromus commutatus *Sown on "Cut" Leaves of* Hordeum sylvaticum.
(Experiment No. *o*.)

Seven leaves were inoculated. By the fifth day small patches of mycelial hyphæ were formed on two leaves. By the fifteenth day fairly vigorous but barren mycelial patches were visible on two leaves over the "cuts."

Conidia from B. commutatus *Sown on "Cut" Leaves of* B. racemosus.
(Experiment No. *a*1.)

Nine leaves were inoculated. On the seventh day the leaf-tissue was nearly dead in the neighbourhood of the "cut" places; on one leaf a distinct mycelial patch was evident over the "cut." By the tenth day all the leaves were dead.

Conidia from B. commutatus *Sown on "Cut" Leaves of* B. asper.
(Experiment No. *m*1.)

Twelve leaves were inoculated. On the fifth day the beginning of a mycelial patch was visible on one leaf. On the ninth day this leaf showed a vigorous patch of mycelium bearing a few conidiophores. On the eleventh day a few conidiophores were still visible on the one leaf, but all the leaves were nearly dead in the neighbourhood of the "cuts."

Conidia from B. secalinus *Sown on "Cut" Leaves of* Hordeum vulgare.
(Experiment No. *y*.)

Six leaves were inoculated. On the fourth day a small mycelial patch was visible on one leaf over the "cut." By the sixth day the patch of mycelium had increased, but all the leaves were beginning to die rapidly. On the ninth day all the leaves were dead.

Conidia from B. secalinus *Sown on "Cut" Leaves of* B. asper.
(Experiment No. *i*1.)

Five leaves were inoculated. On the third day one leaf bore a patch of mycelium; on the sixth day four young conidiophores appeared on this leaf, over the "cut"; the leaf-tissue in the neighbourhood of the "cuts" was dead on all other leaves. On the twelfth day the one infected leaf was also dead.

Conidia from B. velutinus *Sown on "Cut" Leaves of* Hordeum vulgare.
(Experiment No. *j*1.)

Twelve "cut" leaves were placed in a large Petri dish, and six inoculated. On the third day three leaves were infected, and showed numerous lobed haustoria and the beginnings of mycelial patches. On the fifth day these three leaves showed vigorous mycelial patches over the "cuts"; no fungus was visible elsewhere on these leaves, nor on the six controls. On the thirteenth day two of the infected leaves bore respectively two and three young conidiophores. No further growth was made by the fungus, in consequence, apparently, of the leaves now rapidly dying. By the eighteenth day all the leaves were dead.

Conidia from Avena strigosa *Sown on "Cut" Leaves of* Hordeum vulgare.
(Experiment No. *u*1.)

Seven leaves were inoculated. On the second day haustoria were visible on three leaves. On the fourth day a vigorous mycelial patch was formed on one leaf. By the sixth day three leaves bore vigorous patches of mycelium; no conidiophores were observed. On the ninth day the mycelial patches were still evident, but all the leaves were nearly dead.

Conidia from A. strigosa *Sown on "Cut" Leaves of* Triticum vulgare.
(Experiment No. *u*2.)

Seven leaves were inoculated. On the second day lobed haustoria formed on three leaves. On the fourth day vigorous mycelial patches formed on two leaves. By the sixth day these patches were overrun by other fungi, stopping further growth of the *Oidium*. On the ninth day the mycelial patches were all killed by the growth of other fungi.

2. *Experiments in which Conidia of* E. Graminis *were Sown on "Cut" Leaves of "Immune" Species of Plants.*

Conidia from Triticum vulgare *Sown on "Cut" Leaves of* T. dicoccum.
(Experiments Nos. *m*, *u*, *w*.)

In the first experiment six of the seven inoculated leaves became fully infected, and from the first the fungus behaved as though sown on its own host-species. On the second day virulent infection was shown on six leaves by the abundant formation of large lobed haustoria and the production of numerous hyphal branches. On the third day the bulbous bases of young conidiophores appeared on the mycelial patches, and on the fourth day groups of densely clustered ripe conidiophores. In the second experiment five of the eighteen inoculated leaves were infected and produced conidiophores. In the third, four of nine leaves were infected. The leaves of *T. dicoccum* die in 11–12 days, usually smelling fœtidly of putrefaction. Frequently the infected spots remained of a vivid green colour when the rest of the leaf had turned pale yellow or brown.

Conidia from T. vulgare *Sown on "Cut" Leaves of* T. monococcum.
(Experiment No. *p*.)

Seven leaves were inoculated. On the second day lobed haustoria were formed on five leaves, and by the sixth day conidiophores, on vigorous mycelial patches, were produced on these leaves. By the fourth day the leaves began to die and turn brown round the "cuts," and by the eleventh all the leaves smelt of putrefaction and were nearly dead.

3. *Experiments in which Conidia produced on "Cut" Leaves were Sown on other "Cut" Leaves.*

Conidia Produced on "Cut" Leaves of Hordeum vulgare *by Inoculation with Conidia from* Triticum vulgare *Sown on "Cut" Leaves of* H. vulgare. (Experiment No. *o*1.)

Six "cut" leaves were placed side by side, and three were inoculated (only a very few conidia were available for this experiment). On the sixth day a vigorous mycelial patch was formed over the "cut" on one leaf; the control leaves were all free. On the tenth day this patch bore two conidiophores with chains of ripe conidia, and several young conidiophores. On the sixteenth day a little group of conidiophores with an almost powdery mass of ripe conidia was visible on the one leaf; all the leaves were now rapidly dying.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum *by Inoculation with Conidia from* Triticum vulgare *Sown on "Cut" Leaves* of T. vulgare. (Experiments Nos. *b*1, *e*1, *g*1, *h*1, *r*, *r*1.)

In these experiments twenty-two leaves were inoculated, and infection resulted on eleven leaves. In two cases quite powdery *Oidium*-patches appeared in 5–6 days on some of the inoculated leaves, and—all the experiments being safeguarded by controls—it is quite clear that under the conditions created the form of *E. Graminis* on *Hordeum sylvaticum* can fully and virulently infect *Triticum vulgare*. To illustrate the course of growth a summary of one experiment may be given here. Conidia of the fungus growing on "cut" leaves of *H. sylvaticum* (which had been inoculated on August 18 with conidia from *T. vulgare*, and which bore at the date of the present experiment—August 29—small powdery *Oidium*-patches) were sown on four "cut" leaves of *T. vulgare*, and four "cut" control leaves placed by their side. On the third day three of the inoculated leaves showed signs of virulent infection, the conidia having produced numerous lobed haustoria and hyphal branches. In fact, the virulence of infection shown here was as great as any observed in the Petri dish cultures. On the fifth day one leaf bore over the "cut" a large and vigorous *Oidium*-patch, quite powdery with little masses of ripe conidia; the two other leaves, also, bore each a smaller group of ripe conidiophores. All the controls remained perfectly free. On the eighth day two of the three infected leaves bore powdery *Oidium*-patches; the four controls showed no signs of a fungus. On the eleventh day all the leaves were dead.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum *by Inoculation with Conidia from* Triticum vulgare *Sown on "Cut" Leaves of* T. dicoccum. (Experiment No. *n*1.)

Six "cut" leaves were placed side by side, and three inoculated. On the fifth day

two of the inoculated leaves bore over the "cuts" little groups of 3–7 conidiophores with chains of ripe conidia; the third inoculated leaf bore a vigorous patch of mycelial hyphæ. All the controls were perfectly free. On the eighth day all three leaves bore vigorous little patches of *Oidium* almost powdery with massed conidia; controls all free. On the fifteenth day a few conidiophores were still visible, but the leaves were all nearly dead.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum *by Inoculation with Conidia from* Triticum vulgare *Sown on "Cut" Leaves of* Hordeum vulgare. (Experiment No. *t*1.)

Ten leaves were "cut" and five inoculated. On the seventh day small but vigorous patches of mycelium were formed over the "cuts" on two leaves. On the ninth day three of the five inoculated leaves bore over the "cuts" mycelial patches with young conidiophores; the five control leaves all free. On the thirteenth day the three infected leaves bore little groups of conidiophores with chains of ripe conidia. On the fifteenth day the same; but all the leaves were now nearly dead; controls still free.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum *by Inoculation with Conidia from* H. vulgare *Sown on "Cut" Leaves of* Secale cereale. (Experiment No. *p*1.)

Six leaves were inoculated. After 6 days two conidia had produced on one leaf short hyphal branches from the appressorium, after penetration of the haustoria, and penetration of several other haustoria had occurred. (What appeared to be failure of the haustorium to develop in the epidermal cell after penetration was observed in several instances.) After 13 days, one small mycelial patch was visible over the "cut" on the one leaf, no conidiophores were observed; all the leaves were now dead.

Conidia Produced on "Cut" Leaves of Triticum dicoccum *by Inoculation with Conidia from* T. vulgare *Sown on "Cut" Leaves of* T. dicoccum. (Experiment No. *q*.)

Three leaves were inoculated. On the second day vigorous infection was evident on two leaves, large lobed haustoria and vigorous mycelial hyphæ being present. On the fifth day the mycelial patches were still growing vigorously, but all the leaves were turned yellow, and nearly dead.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum *by Inoculation with Conidia from* H. vulgare *Sown on "Cut" Leaves of* Triticum vulgare. (Experiments Nos. *d*1, *f*1.)

Eight leaves were inoculated. On four of these the germinating conidia produced

in 7 days fairly vigorous mycelial patches and numerous large lobed haustoria. No conidiophores were observable on the eleventh day, by which time all the leaves were dead.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum *by Inoculation with Conidia from* H. vulgare *Sown on "Cut" Leaves of* H. sylvaticum. (Experiments Nos. *t*, *v*, *x*.)

Nine leaves were inoculated, and infection resulted on four. Conidiophores were produced in 4–6 days, and after 14 days small powdery *Oidium*-patches were produced, which persisted, keeping powdery with accumulated conidia, for as long as a month from the date of the sowing of the conidia.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum *by Inoculation with Conidia on "Cut" Leaves of* H. sylvaticum *Produced by Inoculation with Conidia from* H. vulgare *Sown on "Cut" Leaves of* H. sylvaticum. (Experiments Nos. *c*1, *q*1.)

Two leaves were inoculated and both became infected. A small but vigorous patch of mycelium, bearing groups of conidiophores, appeared in 10 days, and this became powdery with the massed conidia by the twelfth day. A large continuous powdery *Oidium*-patch, measuring 6 millims. by 3 millims., was produced over the "cut" on one leaf at the end of 4 weeks from the sowing of the conidia.

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