"On the Parasitism of *Pseudomonas destructans* (Potter)." By M. C. Potter, M.A., F.L.S., Professor of Botany in the University of Durham College of Science, Newcastle-upon-Tyne. Communicated by Sir Michael Foster, K.C.B., Sec. R.S. Received April 7,—Read June 12, 1902.

Since the publication of my paper upon a "Bacterial Disease of the Turnip,"* in which the existence of both a cytase and a toxin secreted by the bacterium was proved, I have pursued my investigations further, studying the action of the cytase and toxin upon the living cells, and have succeeded in tracing the passage of the bacterium into the cells through the cell-wall.

I think it will be sufficient for me to state that the strictest methods of sterilisation have been carefully observed throughout the work, and all sections of turnip were prepared as described in my former paper.

To observe the action of *P. destructans* upon a living cell, a small section of sound turnip was suspended in a hanging drop upon a Stricker's warm-stage, the lower opening of which was closed with a plate of glass cemented to the stage, a little water being introduced immediately before placing the cover-slip in position. The thermometer enabled me to tell the temperature of the preparation (which varied between 15° and 20° C.), and the two tubes leading into the central cavity supplied the requisite amount of air for the growth of the bacterium. Into the hanging drop a small fragment of turnip, inoculated with a pure culture of *P. destructans*, was introduced before the cover-slip was inverted over the Stricker's stage.

The effect of introducing the *Pseudomonas* was most striking and manifested a rapidity of action for which I was hardly prepared. The swelling of the wall could be recognised almost immediately, very soon the position of the middle lamella became visible as a much darker line, and contraction of the protoplasm quickly set in.

To take one particular case, a section was mounted at 10.30 A.M. and a cell selected for observation, which was uninjured by the razor and at the same time near the edge of the section. A wall common to this cell and the adjacent one was measured and found to be 2·5 μ in thickness; at 10.45 the bacteria were hovering round the wall; at 11.0 the wall measured 4·3 μ, and the track of the middle lamella was distinctly defined; at 11.20 the wall measured 6·5 μ; at 11.45 the two parts of the cell commenced to separate, and at 12 o'clock a gap of 2·5 μ separated the two walls.

The first signs of contraction of the protoplasm appeared at 11.15, and by 12 o'clock all the protoplasm had separated from the cell-wall

* 'Roy. Soc. Proc.,' vol. 67.

and formed an irregular bag in the centre of the cell. This contraction was not due to mere plasmolysis, but to the death of the cell under the action of the toxin, as the protoplasm never returned to its original position when placed in water.

Thus, within an hour and a-half of the introduction of the *Pseudomonas*, the cell was dead and its walls well advanced in a process of disintegration. After this the changes were less rapid, and, beyond a slight further separation of the cells, a more watery and rotten appearance of the cell-wall was all that could be observed.

Meanwhile the adjacent cells at the edge of the section were all being attacked in a precisely similar manner; contraction of the protoplasm set in at 11.30, and the decay could be observed gradually spreading inwards.

The original cell was kept under observation for some days and the development watched. The bacteria continued swarming around the cell-walls, and next morning (by which time the cells had been destroyed several layers deep) many bacteria had come to rest in contact with the wall, their long axis being perpendicular to its surface; and one or two had the appearance of being embedded in the wall as if in the act of boring their way through. I next attempted to watch a single individual in the hope of seeing it penetrate the wall; this at first seemed rather a hopeless task, but ultimately I was fortunate in fixing upon an individual which was just coming to rest. This bacterium was then kept continuously in view, and, after assuming a position perpendicular to the wall, it could be distinctly seen slowly forcing its way through until finally it emerged into the cell-cavity. The penetration of the wall was subsequently observed on several occasions, and numerous individuals could be seen in all stages of the process. The time required varied with the thickness of the wall, but on an average occupied about 3 hours. To give a special instance, a particular bacterium which was found to be just entering the wall at 11.30 A.M. emerged at 2.10 P.M. (fig. 1).

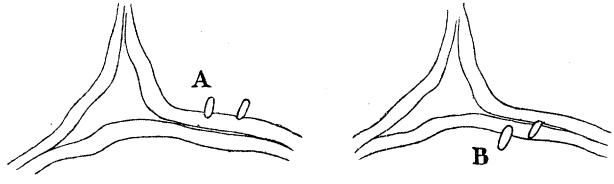

FIG. 1.—*Pseudomonas destructans* passing through a cell-wall. The bacterium was observed to enter the cell-wall at 11.30 A.M. (A) and to emerge into the cell at 2.10 P.M. (B). (Hanging drop; Zeitz obj. 7, oc. 4.)

The observation of the movements of the bacteria, though difficult and very trying, was yet considerably furthered by the different re-

frangibility of the cell-wall and the bacteria, this difference enabling the course of the bacteria to be distinctly followed.*

Important evidence that *P. destructans* has the power of perforating the cell-wall was also afforded by the well-known method of paraffin sections. Small sterile pieces of turnip were inoculated with a pure culture of *P. destructans*, and after 12 hours were found to be partially rotten. These were then fixed in Muller's and also in Flemming's fluid, washed, dehydrated, and embedded in paraffin. The sections were then cut, floated in water, and fixed to the slide by means of the white of egg. The paraffin was dissolved in turpentine. The slides were next placed in absolute alcohol, and then in gradually decreasing strengths of alcohol, for the purpose of staining. When fixed in Flemming's solution, it was found that the bacteria did not readily stain, and for this reason Muller's was preferred.

In staining the sections a further problem presented itself, namely, how to differentiate between the cell-wall and the bacterium. After numerous trials it was found effective to employ a weak aqueous solution of ruthenium-red, which was first allowed to act, the cell-walls being stained by this means but not the bacteria: the sections were then washed in water and stained with Ziehl's carbol-fuchsin or other aniline dyes. Subsequently ruthenium-red followed by Löwit's method for staining flagella was found to give the best results.

This method of fixing and double staining distinctly differentiated the cell-wall and bacteria, and showed the latter fixed in the actual process of perforating the wall, and various stages of penetration could be distinguished (fig. 2). These results confirm my observation of the penetration of the wall by *P. destructans*.

An organism which is thus capable of secreting a powerful cytase and toxin, producing such a remarkable effect in destroying living plant cells, and which subsequently has the power of perforating the cell-wall and entering the cell-cavity, must certainly be regarded as producing a true plant disease. Indeed the parasitic action of this bacterium upon living tissues is exactly comparable with that of certain of the parasitic fungi, though differences in detail are naturally presented from the different character of the organisms.

According to my previous observations, an attack of *P. destructans* could always be traced to a wound. I have found that this bacterium has no power to penetrate the cuticle of the mature epidermis. A number of blocks of turnip cut with the usual precautions, and to include a portion of the uninjured epidermis, were inoculated on the internal parenchyma, placed in sterile plugged test-tubes, and incubated at 30° C. for five days. At the conclusion of this period the pieces of

* It might be objected that the cells in the hanging drop were not under normal conditions, and thus their vitality might be impaired, but no such objection can be raised in the case of the cells of the blocks employed for the paraffin sections.

turnip were quite rotten, but the cuticle remained intact, and could be readily separated as a fine membrane. The experiment was also tried upon leaves and young shoots; in this case I employed no sterilising agent, which would have been liable to kill the very young epidermal cells. A small portion of turnip about the size of a pin's head, rotten through the action of *P. destructans*, was placed on the uninjured surface of a number of mature leaves, and also upon very young ones exposed by removing the outer leaves at the growing points, both sets being kept damp at the temperature of 30° C. After 5 days no sign of decay could be detected upon the mature leaves,

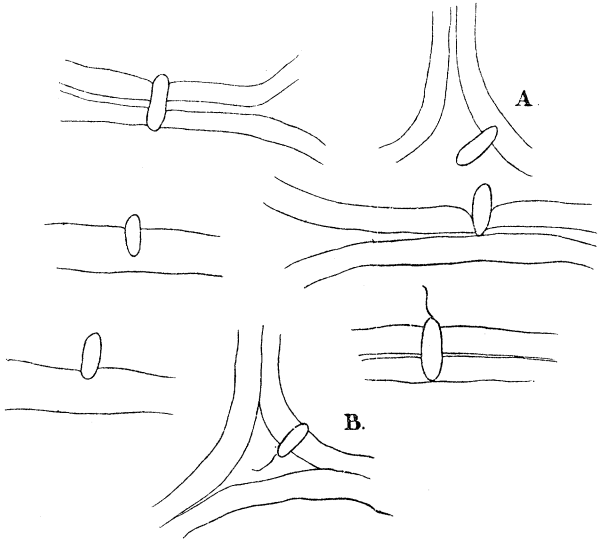

FIG. 2.—Stages in the perforation of the cell-wall by *Pseudomonas destructans*. At A and B the bacterium is seen passing into the cell from an intercellular space. (Paraffin sections; 1/12 oil immersion. Compensating oc. 8.)

but in the young ones the result was very different. These being in a growing condition, and possessing little or no cuticle, offered but a feeble resistance, and after the interval of 5 days had become quite rotten.

The old and fully developed cuticle is apparently proof against the action of the enzymes excreted by *P. destructans*, but this parasite can readily effect an entrance into its host through the undeveloped epidermis of young and tender structures.

This bacterium is incapable of manipulating the hard and tough rind of the sound turnip, but when brought in contact with a wounded surface it at once flourishes as a saprophyte upon the remains of the injured cells; very soon the number of individual bacteria becomes

largely increased, each one contributes its share of toxin and cytase, and in a very short time these products have sufficiently accumulated to kill the first cell. With the death of its protoplasm the cell-contents are liberated, and an additional supply of nutriment is thus provided;* the bacteria continue to multiply, cytase and toxin continue to be set free, and thus each cell succumbs in turn. It is not, however, until the protoplasm has been killed and the cell-wall very much softened that the bacteria have the power of perforating the walls and passing into the cell-cavity. It would hardly be supposed that a single bacterium, through its own excretions, could soften the wall and pierce it at one definite point after the manner of a fungus germ-tube. The extreme minuteness of the bacteria and the rapidity of their multiplication lead them to act, as it were, in concert, and the wall becomes softened by the cumulative action of many bacteria before the penetration of a single individual.

A comparison of the parasitism of *Botrytis cinerea* as demonstrated by the recent investigations of Nordhausen† presents an exact parallel. He has shown that the spore of this fungus excretes a powerful toxin in its initial stages of germination before any trace of the germ-tube can be detected. Its manner of effecting an entrance into a host-plant is first to kill the cell by the emission of the toxin; the germ-tube then penetrates the dead cell and is nourished saprophytically upon it; with the vigour thus gained it destroys the neighbouring cells and passes from one to another without further difficulty. The fungus hypha has the power of perforating the cuticle, but only in young and tender structures; old and hardened membranes could only be entered when the cuticle had been injured, or when it had gained strength by special saprophytic nutrition.

Whether in the case of *P. destructans* the toxin or cytase is the first excretory product I cannot say; the latter produces the first visible effect, and doubtless it prepares the way for a more rapid action of the toxin. But this is immaterial; though differing, as we have seen, in detail, the behaviour of *P. destructans* and *B. cinerea* is the same in principle. The main point is established that this bacterium has the power of destroying the living cells of the turnip, and, subsisting upon their dead contents, continues to work its way through the host, and it thus acts in precisely the same manner as one acknowledged parasitic fungus.

How far this kind of parasitism may be typical of bacterial diseases generally remains to be proved. Another form of *Pseudo-*

* A proof of the escape of the cell-sap was afforded by subjecting cells with a coloured sap to the action of this bacterium, in a hanging drop, when the coloured sap could be detected slowly percolating outwards through the cell-wall.

† M. Nordhausen, "Beiträge zur Biologie parasitärer Pilze," 'Jahrbücher für wissenschaftliche Botanik,' vol. 33, 1899.

monas producing a brown rot (not to be confused with *P. campestris*, E. F. Smith), which I am at present studying, works in a totally different manner; its action is very much slower, and the rapid swelling of the cell-wall, as described above, is not a conspicuous feature.

"The Influence of Varying Amounts of Carbon Dioxide in the Air on the Photosynthetic Process of Leaves and on the Mode of Growth of Plants." By HORACE T. BROWN, LL.D., F.R.S., and F. ESCOMBE, B.Sc., F.L.S. Received April 28,—Read May 29, 1902.

[PLATES 5—10.]

In a paper recently laid before this Society dealing with the physical processes which regulate the entry of atmospheric carbon dioxide into the leaves of plants,* we incidentally described a series of experiments relating to the rate of absorption of dilute gaseous carbon dioxide by surfaces of solutions of caustic alkali, when air containing definite small amounts of this gas is drawn over the liquid. Contrary to what might be expected from the perfect absorbing nature of the solution, and the known laws of gaseous diffusion, the amount of CO_2 absorbed by unit area of the liquid surface in unit time ceases sensibly to increase when a comparatively low velocity of the moving air current has been reached. This, however, only holds good when the proportion of CO_2 in the air stream is maintained quite constant, any slight variation in the amount at once affecting the rate of absorption. On investigation it was found that for dilutions of carbon dioxide lying between 0·6 part and 6 parts per 10,000 of air, the rate of absorption of the carbon dioxide *is strictly proportional to its partial pressure.*†

In determining the rates of gaseous diffusion of atmospheric carbon dioxide through multiperforate diaphragms extended over chambers containing perfect absorbents, the same relations between partial pressure of the gas and its absorption were found to hold good: under these conditions the amount of carbon dioxide passing through the

* 'Phil. Trans.,' B, 1900, vol. 193, p. 278.

† So accurately is this the case that the process, which is described in detail in the above-mentioned communication, may be used for determining the varying amounts of CO_2 in air without the necessity of measuring the volume of air which passes through the apparatus. It is merely necessary to pass the air over the absorbing surface at a sufficient rate to ensure maximum absorption, and to compare this amount of absorption in a given time with that produced from air of a known content of carbon dioxide, a process of standardisation which is done once for all with the apparatus. The ratios of the absorptions give at once the ratios of the partial pressures of the CO_2 in the two cases.