A BACTERIAL DISEASE OF SWEDES.

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INTRODUCTION.

IN the early part of April, 1910, specimens of swedes were sent from the neighbourhood of Taunton to the Botanical Department at the University of Bristol for examination.

Some of the specimens sent were attacked by *Plasmodiophora brassicae*, the cause of the common finger and toe disease, and showed the appearance characteristic of this attack, but others, while often in an advanced stage of decay, showed no indication of the presence of this disease and the roots retained their normal shape.

In the latter case the first indication of attack was described as being the appearance of a small crack on the side of the root, almost as if made by a hoe; this crack gradually widened, the interior being filled with slimy rotting tissue; the continued progress of the disease in many cases caused the death of the plant.

In the previous autumn the field had been infested with surface caterpillars, and eelworm had lately been noticed in the swedes, while some showed traces of attack by wireworms. The prevalence of finger and toe in the same field seemed to point to an absence of lime in the soil.

The disease appeared chiefly in one large field in which two varieties of swede had been sown: the soil is a loam, in most parts well drained, dry and shallow, but with local poorly drained damp areas. Both varieties were affected by the disease and apparently to the same extent, and the disease was spread evenly over both dry and damp areas of the field.

The tissue exposed in the cavities in the roots, the formation of which has been described, was discoloured and very soft, having the

appearance of a light-brown slime. On microscopical examination the slime was found to consist of numerous motile bacteria and protozoa. Sections of the diseased root were cut by hand and some material was fixed and embedded in wax for the microtome.

The hand sections showed the gradual decay of the cells and disintegration of the cell wall; some sections were obtained where the bacteria were swarming in the intercellular spaces and in a cavity in the cell wall formed by the decay of the middle lamella. This gave very much the appearance of the disease described by Potter and identified by him as *Pseudomonas destructans*. The action on the cell wall and the motility of the bacteria, facts upon which he lays stress, were both very marked.

To prove that the destruction of the tissue was due to bacterial action and not to animal attack alone, some of the slime from an infected root was added to pieces of fresh swede and turnip, kept moist in Petri dishes. In the course of a few days the infected pieces of root developed a decaying area of slimy tissue which showed the presence of abundant bacteria. Attempts were then made to isolate the organisms, and with a view to doing this, a medium, consisting of turnip extract and gelatin was prepared as follows. One large turnip was pared with a knife, cut in pieces, and put through a mincing machine, the juice was collected and the minced turnip pressed in a screw press. The extract thus obtained was made up to 500 c.c. with distilled water, and allowed to stand in the incubator at 25° C. for some hours, it was then filtered. Half was neutralized with sodium carbonate, the other half was left naturally acid, each quantity was then made up to 10% gelatin, and poured off into tubes, which were sterilized in the usual way. A second turnip was steamed till soft, then cut into blocks, which were placed in tubes and sterilized at the same time as the gelatin medium. The following method was then used for the isolation of the organisms:

A tube of naturally acid medium, previously melted, was inoculated with a loopful of liquid slime taken from a diseased swede. After shaking well this tube was used to pour a series of eight plates in the usual manner. After a few days the colonies developed, the first three plates very soon liquefied as the number of colonies was very great. On the last four plates the colonies were sufficiently separated to enable isolations to be made.

Four colonies were isolated from this series of plates. Three of these colonies produced liquefaction of the gelatin, and on examination

were found to consist of motile bacteria. Each of these colonies showed a characteristic colour, and was distinguished by a separate reference letter—*B(a)*, *B(b)*, *B(c)*.

B(a) produced a magenta secretion which coloured the medium on which it was cultivated. The gelatin became bright magenta and formed a brilliantly coloured liquid after nine days growth. On potato blocks the colour was most brilliant, and on turnip blocks the secretion coloured the whole block and stained the cotton wool plug at the bottom of the tube.

B(b) formed a whitish colony and grew rapidly on gelatin streaks, on neutral tubes liquefaction took place in 4—5 days, but in naturally acid tubes liquefaction was not produced till after 7 or 8 days at room temperature. It grew well saprophytically on sterilized turnip blocks.

B(c) produced liquefaction in gelatin streaks but more slowly than the other forms, the colonies had a yellow colour.

The fourth organism, *C*, appeared as a dense white colony growing on the surface of the gelatin, and producing no liquefaction; it was found to be a yeast, small, spherical, and with a single highly refringent granule.

From a second series of plates two more organisms were obtained and isolated as *A* and *D*. *A* formed an opalescent semi-transparent colony, growing well on gelatin streaks and producing liquefaction only after some time. On the sterilized turnip blocks it formed a slimy film, which on examination was found to consist of non-motile bacilli, possibly a zoogloea condition with a gelatinous matrix.

The last organism, *D*, formed a salmon pink colony and grew well on gelatin streaks and on turnip blocks, forming a smooth surface of salmon pink colour. On microscopic examination it proved to be a yeast with somewhat elongated cells.

In addition to these organisms, the presence of numerous protozoa was noted in all cases where raw turnip had been inoculated with slime from a diseased turnip: these cannot be obtained in pure culture, but when a streak was made directly from liquid slime the culture invariably showed a mixture of bacteria, yeast and protozoa.

Two forms of protozoa were found, and stained preparations obtained to show the structure. The first form consisted of a rather large colourless body, slightly pointed at the anterior end to which two long equal terminal flagella were attached.

The second form had an elongated colourless body, very contractile,

and apparently only one single flagellum. As nearly all the protozoa are saprophytes, the presence of these forms may be attributed to secondary infection, the decaying cell substance would provide an excellent medium for their development. (These protozoa certainly did not represent the swarmer stage of such a parasitic Myxomycete as *Plasmodiophora*.)

The action of all the organisms isolated was tried on sterilized turnip blocks, in all cases growth occurred, but in no case was any discolouration or sign of decay produced except in the case of *B* (*b*), which after some time produced a decided softening of the tissue, probably due to action on the cell walls. The absence of discolouration may be accounted for by the destruction of all enzymes in the turnip on sterilization, at the same time a watery extract would be formed all over the surface of the block, which would provide a suitable medium for the growth of the organisms as saprophytes over the surface.

More direct evidence as to the parasitic nature of *B* (*b*) was obtained in experiments in which raw turnips were employed. A well-cleaned turnip was cut into thin slices about 1 cm. thick with a knife, which had been cleaned in methylated spirit and then in sterilized water. The pieces of turnip were then quickly transferred to sterilized Petri dishes and moistened with sterilized distilled water; one plate in each set so prepared was kept as a control, and the other plates inoculated with pure cultures of the various organisms. The action of these organisms upon the turnip blocks is given, in tabular form, below:

<i>A</i> after 3 days formed gelatinous mass on surface, tissue remained hard.							
<i>B</i> (<i>a</i>) after 3 days formed coloured mass, tissue remained hard.							
(<i>b</i>)	„	„	„	discoloured patch,	tissue became soft.		
(<i>c</i>)	„	„	„	slight growth,	tissue remained hard.		
<i>C</i> after 3 days formed a slight growth, tissue remained hard.							
<i>D</i>	„	„	„	„	„	„	„

A mixture of all the organisms showed discolouration and softening of the tissues in parts.

The following method of staining was used for comparison of the various organisms as regards microscopical appearance.

A film was spread with a platinum loop and dried on the slide at air temperature; the slide was then covered with weak chrom-acetic acid for 10 minutes, drained, washed in distilled water 3 minutes, drained, then the stain applied to the slide. Aqueous anilin gentian violet was used, and allowed to act for 3 minutes, drained off, washed

in water, drained and dried with filter paper, warmed over a flame, balsam and a clean cover-glass added.

By this method good detail was obtained. The protozoa showed the flagella distinctly and some internal differentiation. The yeast *D* showed granular structure and no vacuoles, while *C* showed a deeply staining granule and a vacuole.

The bacteria *B(a)*, *B(c)*, stained as small simple rods, but *B(b)* showed polar staining.

From the results obtained from inoculation experiments on turnip blocks it would seem that the particular organism causing the disease is that described as *B(b)*, while several facts seem to indicate that the organism isolated as *A* is only a form of *B(b)*, as when *B(b)* is grown on agar streaks, it much resembled the opalescent colonies of the *A* form on gelatin. Also, when the *A* form has been grown on a gelatin streak for some time its appearance is exactly the same as that of *B(b)* when only a few days old. It may be a zoogloea condition of *B(b)*, as cultures of this show this condition in stained preparations. This might also account for the gelatinous slime so often present in inoculated turnip blocks.

A further series of experiments were carried out on seedlings of turnip and swede grown in pots, about five seedlings in each pot. These were inoculated with pure cultures of *B(b)* in the motile form. The cultures were supplied in the form of suspensions in distilled water, to which a quantity of a pure culture had been added by means of a platinum loop. The water tubes were incubated for some hours at 25° C. before using for infection.

Inoculations were made on injured and uninjured seedlings and control sets kept in each case. The injuries were made in three ways: (1) wounding the stem at the ground level with a blunt scalpel, (2) piercing the stem with a needle dipped in the culture liquid, (3) piercing the leaf of the seedling with an infected needle.

The results of these experiments lead to no positive conclusions, although many of the inoculated plants which had been wounded with a blunt scalpel died off, several which had not been inoculated suffered the same fate.

As the seedlings are very delicate and it is impossible to wound each to the same extent this cannot be taken as conclusive.

The reason for the failure of the cultures to obtain sufficient hold on the seedlings is probably that given by Dr E. F. Smith and other workers who assert that only injured roots can be attacked. The

bacteria must then live for some time in the injured tissue as saprophytes, and so attain sufficient strength by rapid growth and increase of numbers to enable them to penetrate the tissue as parasites. In the case of the seedlings the wounded area must necessarily be small, and would probably heal up before the bacteria could obtain sufficient hold.

Hanging drop cultures in Ward's tubes were made with sections of raw turnip and of swede, in water, which were then infected with cultures of *B(b)*, the bacteria were seen actively swarming round the tissue and after some time were found inside the cells, and in the intercellular spaces, although the actual penetration of a wall has not been observed.

Attempts were made to stain the flagella by the Löffler flagella stain, and some by van Ermengen's method. No sign of a single terminal flagellum could be found, but the preparations showed clearly the existence of peritrichous flagella. With the Löffler stain good results were obtained, while the van Ermengen method did not prove satisfactory, our experience being therefore the reverse of that of Potter.

In a recent paper by Johnson a comparative account of the bacteria producing the various forms of rot in turnips and other cruciferous plants is given. They are as follows:

(1) *Brown Rot*. Caused by *Pseudomonas campestris* (Smith). The attack proceeds slowly causing blackening of the tissue, which remains hard and dry. Infection always through a wound or crack, possibly produced through over manuring the crop, the organism follows the course of the vascular system, and is a motile bacillus with a single terminal flagellum; it forms yellowish colonies and liquefies gelatin.

(2) *Soft White Rot*. Caused by *Pseudomonas destructans* (Potter), its attack proceeds more quickly than that of "Brown Rot," causing softening of the tissue which becomes yellowish and strongly smelling. The organism is a motile bacillus with a single terminal flagellum, aerobic, liquefies gelatin (peptonising ferment), softens cell wall (cytase), hydrolyses starch (diastase), gelatin colonies whitish-grey, stab culture produces a funnel-shaped depression filled with liquid gelatin with a cloudy deposit, white glazy growth on agar, parasitic on turnip, potato and carrot, cloudy appearance in turnip broth, produces oxalic acid in turnip juice and in sugar media, residual products acid, gives off CO₂ during fermentation. Stains with anilin dyes, stained by Gram's method shows polar staining.

(3) *Soft Black Rot of Cabbages*. Caused by *Bacillus oleraceae* (Harrison), this organism agrees very closely on all points with *Pseudomonas destructans* (Potter) and only differs in having (1) a white opalescent growth on agar, (2) 7—13 peritrichous flagella instead of a single terminal flagellum, and (3) a slight difference in size, which however seems to be variable.

The organism isolated as *B(b)* has been grown under as many conditions as has been possible in the limited time available for its investigation, and has been found to agree with the two closely allied organisms *Pseudomonas destructans* and *Bacillus oleraceae* on the following points:

Actively motile bacillus, aerobic, liquefies gelatin streaks, forms whitish colonies on plates, produces a funnel-shaped depression in stab cultures with a cloudy appearance in the liquid gelatin, forms a white opalescent growth on agar (and sometimes on gelatin). Grows well on turnip and carrot, and produces softening of tissue showing cytase action on cell wall, produces acid reaction in neutralized media, gives off gas during fermentation, hydrolyses starch, showing diastase action, shows marked polar staining, stains well with anilin dyes, and is stained by Gram's method.

It resembles *Bacillus oleraceae* (Harrison) on two of the points in which this organism is distinct from *Pseudomonas destructans* (Potter), viz.:

- (1) The peritrichous flagella.
- (2) The opalescent colonies on agar (and sometimes on gelatin).

At the same time it hardly seems likely that these distinctions are of permanent value, the size of the organism changes under cultivation, and it seems quite probable that this may be accompanied by changes in the general appearance (opalescence) of the colonies and also, possibly, by changes in the number and attachment of its flagella¹.

The cultures described above show that the organism is capable of existence as a saprophyte, and this means that it may continue to exist in the soil, on rotting tissue, long after the crop itself has been removed. This makes the question of the extermination of the disease a difficult one; one obvious precaution is to lengthen the period as far

¹ Our conclusions seem in agreement with those of Harding and Morse, Bulletin No. 147, Vermont Agricultural Experimental Station, but unfortunately this paper did not reach us until our own work was completed.

as possible between the successive cruciferous crops, when the disease has once appeared. It seems capable of growing both in alkaline and acid media, so that it does not seem clear that any particular dressing applied to the soil, should affect its growth. In this particular case of infection the disease was prevalent at the same time and under the same conditions as finger and toe, conditions which indicate the presence of an insufficient quantity of lime in the soil. The practical question, as to the best treatment of the soil, seems only capable of attack upon a large scale, by actual experiment in the field.

Summary.

A disease of swedes is described and attributed to the action of an organism, probably *Bacillus oleraceae* (Harrison), but closely allied to *Pseudomonas destructans* (Potter). It is suggested after consideration of its appearance on various culture media, that these two organisms may be different growth forms of the same parasitic species.

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