A BACTERIAL BLIGHT OF PEAR BLOSSOMS OCCURRING IN SOUTH AFRICA.

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(With 7 Text-figures.)

During the seasons 1914—15 it was observed by fruit growers in the Stellenbosch district that a large percentage of the pear blossoms blackened and then died, and that in some varieties only a very small number of mature fruits were produced. The blackening was at first attributed to *Fusicladium*, but winter spraying seemed to have no effect on the prevalence of the trouble and specimens were therefore sent to this Laboratory for examination. The discoloured tissues were found to be swarming with innumerable bacteria; from these a pure culture was readily obtained of an organism which caused blackening in pear blossoms, artificially inoculated within a few days. The organism was re-isolated, and studied with a view to comparing it with the two organisms which are known to cause blight in fruit blossoms and which will be referred to in detail presently; one of these is the well known "Fire Blight" organism, *Bacillus amylovorus*, which occurs commonly in America, and the other a *Bacterium* recently described by Barker and Grove, as causing a blight of fruit blossoms in England. The South African organism proved to be distinct from either of these.

It is interesting to note that during some experiments carried out at the Elsenberg Agricultural College, in an orchard which has since been found to be heavily infected with the blossom blight, it was found that if the flowers were covered with paper bags before they opened almost 100 % set, as many as 32 to one truss in some cases; whereas when the flowers were not covered a large percentage fell. This was attributed to the fact that the blossoms were sheltered by the bags from high winds and sudden changes of temperature, but it seems more probable that it was due to the flowers being protected from the

visits of bees and other insects which have been shown both in this case and in the case of other diseases of a similar nature to be the principal carriers of infection.

Comparative Schedule.

	Bacillus amylovorus	Barker and Grove's Organism	*Bacterium nectarophilum*
Dimensions	·9—3 μ × ·7—1 μ	2—4 μ × ·5—·8 μ	·5—3 μ × ·45—·7 μ
Flagella	Several peritrichous	Polar 3—6	Polar 1—5
Capsule	None	None	Always present
Optimum Temp.	25—30° C.	15—18° C.	25—30° C.
T. D. P.	43·7° C.	—	40° C.
Pigment	None	Some fluorescence in old cultures	Fluorescent
Agar colonies	White, circular, elevated, wet, shining, margins irregular	Whitish, circular, smooth margins	Spreading, irregular margins
Nutrient Broth	Clouding not heavy, pellicle and rim slight, moderate amount of grey deposit	Well clouded, appreciable deposit and slight rim and pellicle	Very turbid, deposit heavy, rim and pellicle present
Gelatine Stab	Slow crateriform liquefaction	Liquefaction rapid, first crateriform then stratiform	No liquefaction
Milk	Coagulated in 3—4 days, later digested to pasty condition	Slowly peptonised	Slowly peptonised
Vegetable Cylinders (Potato, Carrot, Turnip, Beet)	Good growth on all, best on beet, weakest on turnip, liquid heavily clouded	Fair on potato, feeble on carrot, none on turnip	Fair growth on all but heavy on none
Uschinsky's solution	Growth copious, not viscid	No growth	Heavy, viscid growth
Indol	Considerable quantity produced	Reaction only obtained after warming	None produced
Diastatic activity	Strong	Feeble	Feeble
Group numbers	221,232201	221,3332123	222,2332123

During September, 1916, I was able to visit the pear-growing district, and to study the disease in the orchard; it was too early for the late-flowering varieties, some of which are the most susceptible, but considerable infection was found in the Keiffers and other varieties which were then in flower. Information as to the susceptibility of the different varieties was also gleaned from various growers, all of whom mentioned the same varieties as the most liable to the disease.

Pear trees flowering in the Wellington District and in the Pretoria District show no signs of the blackening: a trouble of a somewhat similar nature has been reported from Potchefstroom, but it has not

yet been ascertained whether this is identical with the blight at the Cape or not. With the exception of a case of infection reported from Wynberg, therefore, the disease is only known to occur up to the present in the Stellenbosch District and at Elsenberg. It is particularly interesting to find that there are three organisms showing a parasitism, in many respects similar, in different parts of the world; it is therefore my intention to describe the South African disease, and to compare it with the blossom blight occurring in England and with that caused by the fire blight bacillus in America.

Through the courtesy of Professor Barker and Mr Grove who supplied me with a culture of their organism I have been able to make a detailed comparison of the South African and English bacteria. My knowledge of the American blight is less complete, being gathered from the literature to which I have access, and which is by no means exhaustive; sufficient information has been gathered by this means, however, to establish the main points of difference between *Bacillus amylovorus* and the other organisms causing blossom blight in pears.

I. Fire Blight.

Bacillus amylovorus (Burr) de Toni.

The information summarised in the succeeding paragraphs has been derived from the publications listed in the "Literature cited" (numbers 4—11).

"Fire blight" was one of the first bacterial diseases of plants to be recognised as such, and consequently has been studied in considerable detail. It is very widely distributed in the United States and in Canada, and has recently been recorded(8) from several places in Italy. It causes very serious losses, amounting in California in the last fifteen years to one-third of all the full-grown orchards, and to a money loss estimated at $10,000,000 for the five years preceding the efforts for its restriction begun in 1905 by the United States Department of Agriculture(8).

In 1912 a variety of pear in Switzerland was severely injured by a bacterial invasion(7). It is thought that the disease may be similar to the pear blight in America caused by *Bacillus amylovorus*, but complete identification of the organism has not yet been found possible.

Although most common and most disastrous on the pear (*Pyrus communis*) *Bacillus amylovorus* is also found as a parasite on the apple (*Pyrus malus*), Quince (*Cydonia vulgaris*), a number of species of *Prunus*

including the plum and apricot, and on numerous plants indigenous to America. Of the varieties of Pear which was attacked, the Bartlett is said to be very susceptible; other varieties are bracketed with the Bartlett as susceptible, but there seems to be considerable divergence of opinion on this point. The Keiffer, Duchess and Winter Nelis, and the oriental group in general are more resistant.

Symptoms.

Although infection most frequently takes place through the flowers, the blossom blight is not by any means the most serious phase of this disease. When the blossoms are attacked the receptacle becomes blackened first, infection taking place through the nectaries, but the infection rapidly spreads into the ovary and the flower stalk and invades the twigs. The blossoms and leaves of affected twigs become discoloured, turning light or dark brown, or sometimes red, and finally shrivel up and die. The spread of infection is frequently so rapid as to result in the complete blackening and death of all branches and spurs upon which flower clusters have been borne. The blight may continue to extend down the branch or twig, the branch being entirely killed as it progresses, and in course of time it may extend into the larger limbs. The bark of infected twigs and branches becomes blistered, and on the blistered areas there is often found a gummy exudate which is crowded with the rods of the causal organism; this exudate attracts the insects which are responsible for the further spread of the disease. Immature fruit is frequently attacked; it becomes light brown and finally black, the flesh soft and pulpy, and the skin somewhat wrinkled. Ripe fruit seldom becomes infected.

The Characters of *Bacillus amylovorus* (Burr) de Toni.

Morphology.

Bacillus amylovorus is a short rod with rounded ends, ·9—1·5μ × ·7—1μ in dimensions, longer (nearly up to 3μ) and slightly narrower in old cultures.

It is motile by means of several (4—8) peritrichous flagella; no capsules or spores have been observed. The rods are usually single or in pairs, but in young cultures short chains made up of 3—4 individuals have been noted.

The organism is Gram-positive.

Cultural Characters.

Nutrient agar colonies are evident on the second and attain a diameter of 2—3 mm. by the fourth or fifth day. They are white and granular, or cloudy with a sharply defined white centre; the margins are entire or slightly wavy. Submerged colonies are opaque, yellowish-white, lenticular.

Nutrient agar stab. Growth takes place along the entire length.

Nutrient agar streak. In 24 hours there is a moderate opalescent growth which spreads slowly. It is finally white, wet-shining, thin along the middle, heavier along the sides, margins wavy, eventually spreading over the surface of the slant. More rapid growth is induced by the addition of the agar of 2 % saccharose, dextrose or maltose or 5 % glycerine. The water of condensation becomes turbid, but the growth is not viscid.

Nutrient gelatine colonies are very slow growing, only appearing after 3—5 days. The surface colonies are round, slightly raised, entire, buried colonies spherical, granular. The medium is liquefied very slowly.

Nutrient gelatine stab. Growth is at first filiform, and is slow and feeble; surface growth spreading with irregular margin; slow crateriform liquefaction takes place, later becoming stratiform.

Nutrient bouillon is clouded after 24 hours and this is accompanied by a slight acidity; after 48 hours there is greater cloudiness with more or less persistent flocculi, the medium becoming alkaline, and in time showing a tendency to clear. In sugar-free bouillon the liquid remains clear for 24 hours except for a slight sediment. It is neutral at first, becoming cloudy and alkaline after some days. The clouding is never very heavy as compared with other organisms.

Milk is coagulated in 3—4 days; coagulation is followed by digestion to a pasty or sub-gelatinous condition, with separation of supernatant whey; this is at first acid, later becoming slightly alkaline. Litmus milk is unchanged.

Blood serum. On this medium the growth is similar to that on nutrient agar; there is no liquefaction.

Potato, carrot, turnip, beet. There is good growth on all these media; it is best on beet, weakest on turnip. In all alike a wet-shining white streak forms along the line of inoculation; the liquid is heavily clouded, white and nearly opaque. The tissues are not softened and there is no odour, gas or pigment.

Cohn's solution. No growth.

Dunham's solution. The organism grows rapidly in this solution, but the clouding is not dense; there is no pellicle or rim and the deposit is slight.

Uschinsky's solution. Growth copious but not viscid.

Biochemical and Physical Relations.

Enzyme production: Amylase. Amylolytic activity is indicated by the fact that the organism liquefies starch jelly.

Gas. No gas is produced in fermentation tubes with glucose, saccharose, lactose, glycerine, maltose or mannite.

Pigment. None, organism is white or greyish white on all media.

Indol. A considerable amount of indol is produced.

Acid and *alkali production.* Ordinary nutrient broth shows a slight decrease of alkalinity, then a return to the original reaction. Broth containing 2 % saccharose or glucose, gradually became acid; lactose broth showed little or no change in two weeks.

Nitrates are not reduced to nitrites.

Colour reduction. Litmus milk and rosolic acid peptone water showed progressive bleaching during the first week, but the colour finally returned.

Toleration of sodium chloride. 3 % did not inhibit growth.

Temperature relations. The optimum temperature is 25—30° C.; there is no growth at 5° C.; growth is very slow at 3° C. Thermal death point (wet) is 43·7° C., 10 minutes exposure.

Desiccation. When organism was dried on cover glasses at about 20° C., 5 days had no effect, 76 days was fatal.

Insolations. 10 minutes exposure of freshly poured plates retarded development; 30 minutes was fatal.

II. BLOSSOM BLIGHT IN ENGLAND CAUSED BY BARKER AND GROVE'S ORGANISM.

This disease is very widespread in England, probably occurring at least throughout the midland and southern counties. The most susceptible varieties are the Beurre d'Amanlis and the Catillac.

The method of infection varies; sometimes the sepals turn grey and blacken, the discoloration finally involving the whole of the calyx and flower stalk, and the flower blackens and shrivels up. It may then fall or it may remain attached to the shoot. The whole truss

of blossom eventually dies and the spur may also die back to its point of attachment to the branch carrying it. In other cases infection takes place through the receptacle, which becomes blackened, the discoloration spreading to the ovary.

The disease is carried from flower to flower by bees. An organism has been isolated and the disease reproduced repeatedly by Barker and Grove in the course of their study of the disease; and I was successful in producing black spots on the receptacle with the culture which they sent to me; these latter developed rather slowly as the room temperature was far above the optimum for the organism.

In a recent report(2) it is stated that an organism has been isolated from diseased gooseberry bushes which is in all probability identical with the organism causing the pear blossom blight.

The Organism.

A parallel series of cultures of this and the South African organism have been carried out; the characters of Barker and Grove's organism are described in some detail in their paper(1) but a few additional points of interest have been observed in making the comparative study, which may be added to their description.

Morphology.

The organism is a rod 2—4 × ·5—·8μ, the cells are mostly single or in pairs, seldom in long chains. It is highly motile in young cultures by 2—5, lophotrichic flagella (Fig. 1) which are four to five times as

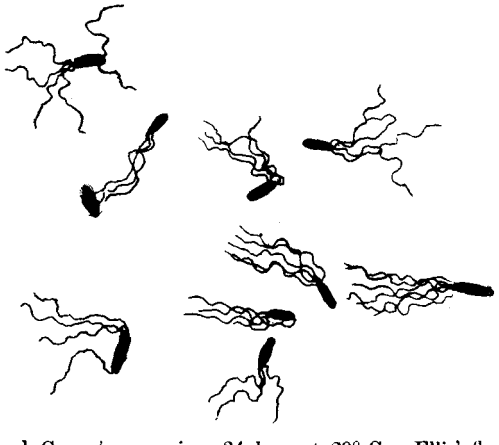

Fig. 1. Barker and Grove's organism 24 hrs. at 20° C. Ellis' flagella stain. Zeiss obj. $\frac{1}{12}$, No. 12 compensating ocular. Drawn with the aid of the camera lucida.

long as the rods. No capsules have been observed. Involution forms are produced in 24 hours in nutrient broth at 30° C. and in old cultures; these take the form of long threads up to 100μ long and irregularly swollen.

It stains well with all the usual stains, especially with Gentian violet and is Gram-positive.

Cultural Characters.

Nutrient agar colonies. At 18°—20° C. the colonies are visible to the naked eye in 48 hours; in three days the surface colonies are ·5 to 3 mm. in diameter, round-irregular, glistening and translucent; in four days they are up to 5 mm. in diameter, of a light coppery tint by transmitted light, creamy white by reflected light. Some of the colonies are inclined to spread and become lobulate, but the majority are more or less circular, with a smooth margin.

The submerged colonies are at first punctiform, afterwards lenticular. There are a few crystals in old cultures.

Nutrient agar streak. Cultures form a flat, whitish glistening growth, spreading out at the base of the slant; very old streaks are slightly fluorescent.

Nutrient agar stab. The best growth is at the top.

Nutrient gelatine colonies. Visible in 48 hours; the submerged colonies are minute white points; those on the surface slightly larger, with an undulate margin around which there is a slight indication of liquefaction. After four days the surface colonies are sunk in small craters of liquefied gelatine, they are moist and glistening semi-transparent, often with a small white nucleus in the centre, surrounded by several concentric rings of whitish, granular matter. Liquefaction of the gelatine is complete in 8—10 days.

Nutrient gelatine stab. In three days at 18—20° C. there is a small crater of liquefaction 4—8 mm. broad and 8—10 mm. deep, and the surface growth has sunk to the bottom of the crater; in seven days the liquefaction involves the whole thickness of the tube and becomes stratiform.

Potato. On this medium there is a raised, creamy-white growth with smooth edges along the needle track.

Turnip. No growth.

Carrot. The growth on carrot is very scanty, thin, and spreading.

Parsnip. A very much raised, shining streak develops, standing

about 2 mm. high, the surface being almost semi-cylindrical; on one cylinder the edges only were raised and the centre depressed.

Beet. On beet the organism grows fairly well, producing a raised, gelatinous-looking growth along the needle track; the growth on the surface of the liquid takes up the colour from the medium to some extent and becomes fairly pink.

Blood serum. The organism does not liquefy blood serum, and the streak is similar to that on nutrient agar, but the growth less copious.

Nutrient bouillon becomes slightly clouded in 24 hours, and in two to three days is fairly heavily clouded. There is a considerable amount of sediment and a very thin pellicle. In very old cultures a slight fluorescence may sometimes be observed.

Milk is not curdled, but is very slowly peptonised. In nine days two-thirds to four-fifths of the liquid is clear; after one month the milk is completely peptonised, the colour is reduced in litmus milk, and the medium is distinctly alkaline to litmus.

Dunham's solution is slightly clouded.

Uschinsky's solution. No growth.

Cohn's solution. No growth.

Nutrient bouillon over chloroform. Growth was unrestrained in the presence of chloroform.

Egg albumen. A medium composed of 1 gram powdered egg albumen and 50 c.c. of ·05 % potassium phosphate was well clouded in five days.

Physical and Biochemical Relations.

Proteolytic activity. The organism is a fairly active proteolytic agent; it slowly peptonises milk and there is a distinct smell of ammonia from liquefied gelatine.

Cultures in nutrient bouillon were tested for ammonia by distillation after five to 10 days at 20° C. In each case 50 c.c. of medium were found to contain approximately ·02 gramme of ammonia nitrogen.

In the egg albumen medium described above there was a positive reaction for peptone after five days. A quantitative test by Sorensen's method showed that 50 c.c. of the medium after five days contained ·0035 nitrogen, in the form of amino acids and ammonia, *i.e.* approximately 2·3 % of the total nitrogen had been broken down. A distillation test for ammonia showed that the whole of this was in the form of ammonia; and cultures tested after 10 days gave an almost identical reading; the amount of ammonia had not increased.

Milk cultures tested in the same way gave a strong reaction for peptone and for tyrosin after 10 days; and there was ·011—·017 grains ammonia nitrogen in 50 c.c. of the medium.

Amylolytic action. Starch is very slowly destroyed; nutrient bouillon containing ·01 gram of soluble starch gave the red-brown reaction of amylodextrin with Lugol's iodine solution after about 10 days, and in three weeks the starch had completely disappeared.

Fermentation reaction. No acid or gas was produced in fermentation tubes containing peptone water, tinted with litmus, and containing 2 % of any one of the following substances:—starch, laevulose, mannite, glycerine, galactose, saccharose, dextrose, lactose.

Indol. No reaction for indol was obtained at room temperature in 10 days old cultures in peptone water and nutrient broth, but after warming a slight, but quite definite coloration appeared after the addition of sulphuric acid and a nitrite.

Nitrates are not reduced to nitrites.

Gas production. It has been mentioned that no gas is produced in fermentation tubes containing sugar solutions. In iron and lead peptone solution and bouillon the precipitate was decidedly blackened, showing that sulphuretted hydrogen had been liberated.

Atmospheric conditions. The organism is a facultative anaerobe; it grows slowly on glucose formate agar in an atmosphere devoid of nitrogen.

Temperature. The optimum temperature lies between 15 and 20° C.; the thermal death point has not yet been determined.

III. THE SOUTH AFRICAN DISEASE.

It has already been pointed out that so far as is known at present the pear blossom blight only occurs in the Stellenbosch District and at Elsenberg, that is to say in the region where the winter and early spring is the rainy season and in that part of it where pears are extensively grown. Several cases of blackening in blossoms and pearlets grown in other parts of the country have been brought to our notice, but as these occurred in hot dry weather after some six or seven months' drought, it is more probable that the failure of the blossoms was due in these cases to drought than to the bacterial blight, which only spreads rapidly when the atmosphere is moist. Further investigation, however, will be necessary before any definite statement as to the geographical distribution of the disease can be made.

Varieties affected.

Some of the late flowering varieties are the most susceptible. Winter Nelis and Beurre Superfine are very badly affected, also the Kastanje Bergamot; a number of other varieties including the Keiffer, Beurre Diel and Bon Chrétien are also affected but not to the same extent as those above mentioned. The Duchesse d'Angoulême appears to be practically immune, as no sign of the disease could be found on trees standing in an adjacent row to a number of Keiffers which were badly affected.

Symptoms and spread of infection.

So far as can be ascertained up to the present the disease is confined to the flowers, peduncles and very young fruits. No infections have as yet been found on leaves or twigs, and I have failed to produce artificial infections on these parts.

Infection almost invariably takes place through the receptacles; it usually takes place at more than one spot, a number of minute dark spots appear on the receptacle, these rapidly increase in size, becoming black and spreading until the whole receptacle is involved. Less frequently the tissues of the receptacle are very completely invaded before any blackening occurs, the whole assuming a greenish brown, water-soaked appearance and later turning black.

When the receptacle is invaded the infection and blackening frequently spreads to the styles and the ovary, and less frequently to the flower stalk. Infected flowers fall, and in the case of susceptible varieties in such numbers as to seriously affect the crop.

The rapidity with which the disease spreads from flower to flower suggested that the infection is carried through the agency of bees. With the assistance of Mr Neethling, the lecturer in Botany at the Elsenberg Agricultural College, this point was satisfactorily settled. He kindly co-operated with me in this matter by capturing a number of bees which were working in the neighbourhood of the infected trees; some of these were allowed to walk over some nutrient agar and then released; from others the mouth parts were excised, and dropped into tubes of melted agar which were then set on the slant. The tubes thus infected were then posted to me at Pretoria for examination.

Cultures of the causal organism were readily obtained from all five of the tubes containing the bee traces; of the others the organism was found in one into which the head and prothorax of the bee had been

dropped, but not in any of the tubes planted with the proboscis or mandibles alone.

Artificial infections were readily produced with the culture isolated from the bee traces.

It seems possible that ants may also be partly responsible for carrying infections, as quite a number of them were noticed working in the infected flowers at one farm in the Stellenbosch District.

Etiology.

When the organism causing the bacterial blight in pear blossoms was first isolated in October 1915, the blossoming season in Pretoria was almost at an end, but a preliminary infection experiment was carried out with the few flowers which were still to be found on the trees. The blossoms sent from Stellenbosch were shrivelled and quite black, but bacteria were very plentiful in the tissues and no difficulty was experienced in obtaining a pure culture.

The weather was exceedingly hot and dry so that it was useless to attempt any inoculations in the orchard. A number of twigs bearing apple and pear blossoms were therefore carefully cut under water, and conveyed to the laboratory where they were covered over with bell jars; some of these were atomised with a suspension of a culture in sterile distilled water, others kept as controls. The latter remained fresh and showed no signs of drooping or discoloration during the experiment. The inoculated blossoms, however, showed water-soaked spots on the petals, calyx, and peduncle after 24 hours; in 48 hours these had become very numerous and began to turn brown, and in a few days the whole flower had turned black and fallen.

The organism was readily re-isolated and inoculated into some young pears by atomising as before; a few infections were thus obtained in pearlets which had just set, but not on the fruit of the size of a walnut or larger; that is to say, the organism seemed unable to attack the young fruit after it had begun to harden.

After visiting the affected orchards in September, 1916, fresh cultures were obtained from material collected, and a number of inoculations were carried out with the organism thus freshly isolated, the strain isolated the previous year and with Barker and Grove's organism. Inoculations were carried out in one of three ways; the flower was infected by touching the receptacle with a platinum needle, which had been charged with a small quantity of agar culture; a drop of a suspension of an agar culture was placed on the receptacle with a fine pipette;

or the whole inflorescence was atomised with a suspension of an agar culture in distilled water.

When either of the first two methods was employed a number of minute water-soaked spots appeared on the receptacle in 24—48 hours, or, in the case of a heavy infection the whole receptacle became water-soaked in appearance.

The infected areas soon began to turn brown and spread until the whole receptacle was involved. The receptacle was finally quite black, the blackening not infrequently spreading into the styles, ovaries and peduncle; and the slightest movement was sufficient to cause the infected flowers to fall.

When the inflorescence was atomised, infection was equally prompt, discoloured areas appearing on the sepals, petals, ovary and peduncles. In no case have I been able to infect the leaves or fruit spurs, and I have not observed any such infections in the orchards.

All inoculations were carried out in the Laboratory under conditions similar to those employed during the preliminary experiment carried out in 1915; a schedule of the inoculations is appended.

It will be noticed that positive results were obtained in each case when pear and apple blossoms were inoculated, but that attempts to infect cherry, peach and nectarine were all unsuccessful. Up to the present no natural infections on the apple have been found.

In each experiment an equal number of flowering branches were kept as controls; and in no case did the disease appear in these.

No.	Kind of Blossom	Method	Source of Culture	Results
1	Pear	Atomiser	Blossoms from Banhoek, Stellenbosch	Positive
2	Apple	,,	,, ,,	,,
3	Pear (young fruits)	,,	Re-isolated from (1)	,,
4	Pear	Platinum needle	Blossoms from Elsenberg	,,
5	,,	Pipette	,, ,,	,,
6	,,	Atomiser	,, ,,	,,
7	Cherry	Pipette	,, ,,	Negative
8	Pear	,,	As (1) but after 12 months in cultivation	Positive
9	,,	,,	Blossoms from Ida's Valley, Stellenbosch	,,
10	,,	,,	Re-isolated from (4)	,,
11	,,	Atomiser	Same as (4)	,,
12	Peach	Pipette	,, ,,	Negative
13	Nectarine	,,	,, ,,	,,
14	Pear	,,	,, ,,	Positive
15	,,	,,	Bee traces	,,

Pathological Histology.

Infection usually takes place through the nectaries, but the organism sometimes finds its way into the green tissues of the flower and flower stalks through the stomata.

The rods multiply very rapidly in the intercellular spaces, and it is very noticeable that wherever the intercellular spaces are invaded, the contents of the adjacent cells become plasmolysed and stain very deeply with carbol fuchsin. In sections stained with carbol fuchsin and light green these showed up in startling contrast to the normal cells which stained light green and in some of which the nucleus could be plainly seen (Fig. 2).

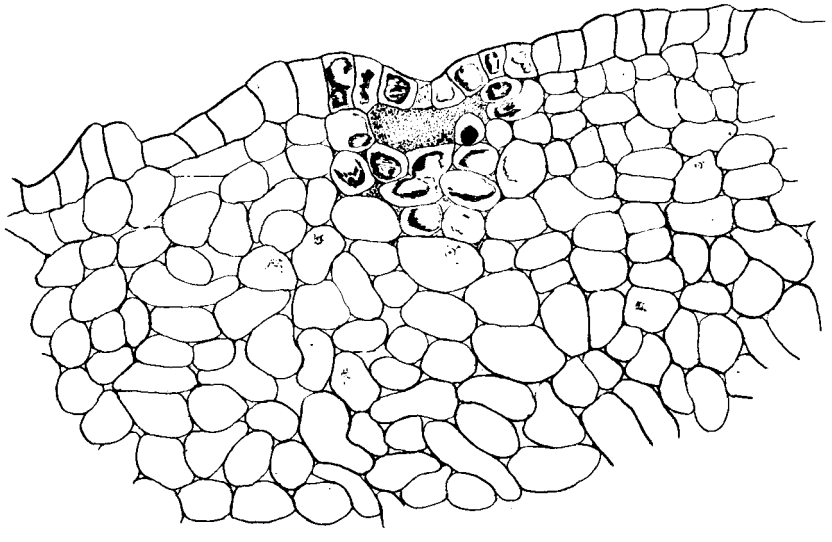

Fig. 2. Section through diseased receptacle, natural infection, drawn with Edinger's projection apparatus. An early stage of infection.

After the cells are plasmolysed and killed they disintegrate very rapidly and collapse, the original cell outline completely disappears and the diseased area consists of a disorganised mass staining intensely with carbol fuchsin.

When the receptacle is infected the flowers soon fall, but in some cases not until the infection has spread into the more deep-seated tissues of the ovary, all of which become blackened and disorganised (Fig. 3).

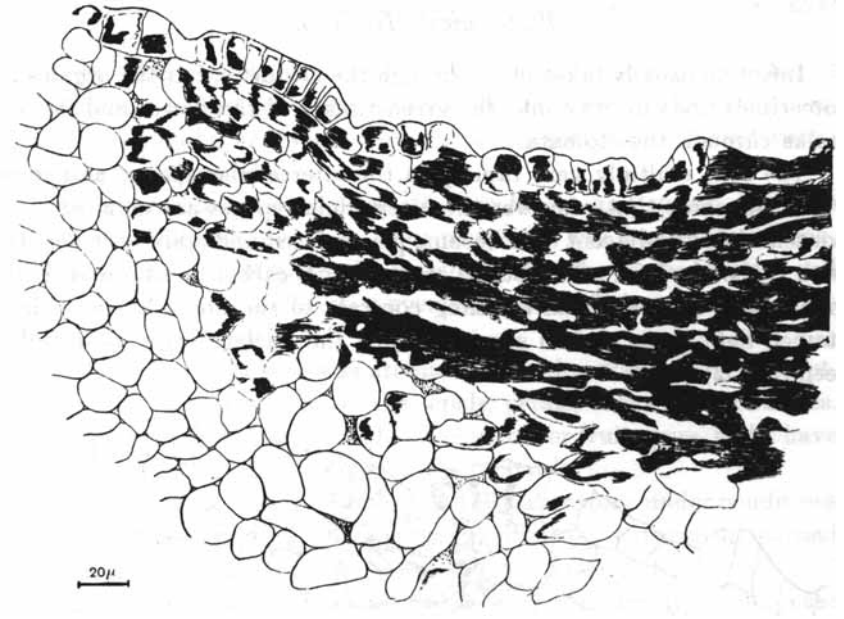

Fig. 3. Section through diseased receptacle, natural infection, drawn with Edinger's projection apparatus, the scale is indicated. A more advanced infection showing the collapsed tissues.

Morphology.

The organism is a short rod of very variable size and shape. A pure culture may be readily obtained from infected tissues, but the milky white masses of bacteria which diffuse out from such tissues consist of rods which exhibit astonishing variations in size and form (Fig. 6). The majority are short thick rods with rounded ends, measuring for the most part ·6—1·5 × ·5—·7 μ, but all the variations found on various culture media may be seen. In addition to these there are undivided rods up to 12 μ long, which are sometimes irregular in shape but which stain intensely with carbol fuchsin; it would seem probable that these are involution forms of some sort, although nothing resembling them has been found in artificial culture media. The same remark applies to fairly numerous rods of various lengths which stain faintly and are only about ·2 μ in diameter. All the rods are distinctly capsuled, and many of them are not stained evenly, showing 1—2 or, in the larger individuals, as many as 5—6 colourless vacuoles.

Young cultures on agar (24 hours at 25° C.) show very similar characters to those described above (Fig. 4) with the exception of the two abnormal forms just mentioned. The size of the rods is very variable, some are almost spherical, the limits of size being ·5—3μ × ·45—·7μ;

Fig. 4.

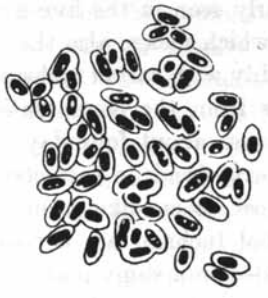

Fig. 5.

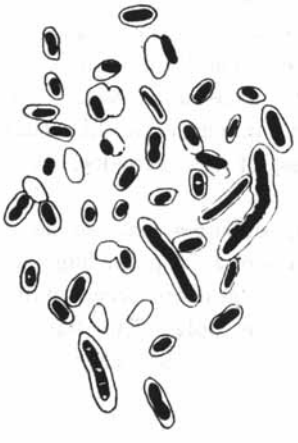

Fig. 6.

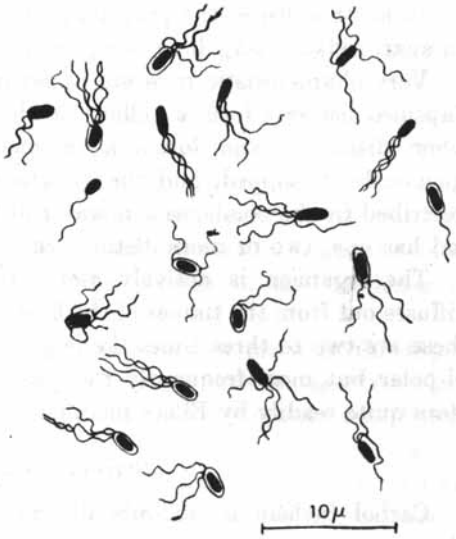

Fig. 7.

Figures 4–7. *Bacterium nectarophilum*; magnification same as Fig. 1. Fig. 4. 24 hrs. at 25° C. on nutrient agar. Fig. 5. Uschinsky's solution, 7 days at 25° C. Fig. 6. Direct from host plant. Fig. 7. 24 hrs. at 25° C., Ellis's flagella stain.

the majority are 1—1·5 × ·6—·65μ. They are usually single or in pairs but chains of 5—10 are fairly frequent, and these are peculiar in that the rods are not as a rule placed uniformly end to end, but are

quite as frequently obliquely, or are attached almost laterally (Fig. 4). One or two vacuoles, the contents of which do not stain with carbol fuchsin, can be seen in many individuals; that these non-staining areas are not due to plasmolysis is proved by the fact that they can be clearly seen in the living condition with the dark ground illumination, by which means also the capsule is plainly visible. The capsule stains readily with carbol fuchsin or gentian violet, and is very obvious around rods from the condensation water in tubes containing nutrient agar cultures about four days old. The growth in the condensation water is very viscid, in consistency almost like egg albumen; short rods and almost coccus-like forms predominate. These stain intensively with carbol fuchsin, are surrounded by a colourless capsule and are embedded in a slimy matrix which stains faintly.

After four weeks at 25° C. on nutrient agar only a small percentage of the rods stain intensely, the staining of the majority being very faint and uneven.

In broth cultures the prevailing forms are slightly longer than those on agar, rods 2—2·5μ long being frequent; the vacuolation is distinct.

Very characteristic rods are found in Uschinsky's solution (Fig. 5). Capsuled bacteria form a pellicle on the surface of the fluid; this sinks when disturbed and forms a sediment. The capsule is very conspicuously developed, and the growth is viscid in character like that described in the condensation water at the base of agar streaks; each rod has one, two or more distinct vacuoles.

The organism is actively motile in young cultures (less so as it diffuses out from the tissues of the host) by means of 1—5 polar flagella; these are two to three times the length of the rod, and are occasionally bi-polar but most frequently they are only at one pole. The flagella stain quite readily by Ellis's modification of Loffler's method (Fig. 7).

Staining reactions.

Carbol fuchsin is undoubtedly the best stain for this organism, although it stains well with all the usual stains. It does not stain by Gram's method, and is not acid fast.

Cultural characters.

Nutrient agar (+15). Colonies are visible after 24 hours at 25° C. as thin milky-white growths 1—3 mm. in diameter and rather irregular in shape; after 48 hours, surface colonies are 1—2 cm. diameter, in thinly sown plates, spreading, sub-circular to irregular in shape, the edges

being auriculate-lacerate; they are coppery by transmitted light, creamy white to light dull, green yellow[1] by reflected light. In crowded plates the colonies are small and somewhat raised; submerged colonies are very small and are lenticular in outline. A greenish fluorescence may always be observed from agar media on which this organism has been grown; frequently this is very marked, and in thickly sown plates can be detected after 24 hours' growth. This characteristic appears to vary with the composition of the medium, and the conditions under which the organism is grown; it is more marked when the organism has been in cultivation for some time than when it is newly isolated, and also appears to be more conspicuous in cultures which have been exposed to the light; slight variations in the composition of the medium are also probably responsible in some degree for variations of this kind. No crystals have been seen in old cultures. Under the microscope the texture of the surface colonies is homogeneous and very finely granular.

Nutrient agar (+ 15). Streak cultures on nutrient agar are wet-shining, yellowish white, flat smooth, undulate at the edges, and inclined to spread over the wetter parts of the medium. No crystals are formed. There is always more or less fluorescence from the agar, but, as observed in connection with the plate cultures, it is variable. Sometimes there is a distinct greenish fluorescence from the agar and the bacterial growth at the end of 24 hours; sometimes it is not marked until the culture is five or six days old; it is more noticeable when the tubes have been exposed to the light. The growth in the condensation water is very viscid.

Nutrient agar. Stab. The best growth takes place at the top.

Dextrose agar. Streaks were also made on nutrient agar to which 2 % dextrose had been added. On this medium the growth was slightly heavier than on ordinary nutrient agar; it was opaque, creamy white, and the medium was rendered opaque, in contrast to ordinary nutrient agar which remains translucent and exhibits a greenish fluorescence.

Hiss glucose medium produced a very similar growth to that on dextrose agar, but it was more noticeably viscid, drawing out into fine threads on the platinum needle.

Nutrient agar + 2 % dextrose and litmus. The streak is similar in character to that described on nutrient agar with dextrose, but striking colour changes may be observed in the medium. On the

[1] Colours are named according to Ridgway's *Colour Standards and Nomenclature* and have been carefully compared with colour charts in that publication.

sixth day the agar, which was a neutral litmus tint when planted with the organism, is Killarney green, when viewed by transmitted light facing the streak; viewed by transmitted light facing the side of the slant the colour is deep red, nearest Nopal red. By reflected light the upper part of the agar is still green, but from the deeper layers there is a distinctly purplish light reflected. There was no further change in colour during the six weeks the tubes were kept under observation.

Loffler's blood serum is not liquefied, a streak culture on this medium is similar to that on nutrient agar.

Cabbage agar. On this medium the organism forms a ribbon-like streak, honey yellow, 3—4 mm. broad, and with a wrinkled shagreen surface.

Nutrient gelatine (+ 15). Colonies at 20° C. attain a diameter of about 4 mm. in three days; they are thin, spreading, with a slightly irregular margin, in the centre of each colony there is a small, glistening, raised point; the remainder of the growth has a dull, ground glass appearance. Submerged colonies are small, yellowish opaque.

There is no liquefaction, and colonies do not change further, except to increase in size in thinly sown plates. There is no fluorescence in this medium.

Nutrient gelatine stab. There is a growth similar to the gelatine colonies, covering the surface of the medium and a filamentous growth along the upper part of the medium; but no liquefaction during two months and no development in the depths of the medium.

Nutrient gelatine streak is flat, about 4 mm. broad with undulate or crenate margin; here also the growth is dull and thin, and has the appearance of ground glass when held up to the light.

Nutrient bouillon (+ 15) is heavily clouded in 24 hours at 20° C., and afterwards becomes very turbid. There is a tendency to pellicle formation, but when disturbed the pellicle breaks up into flocculi and sinks. A greenish fluorescence may be observed, and is usually very noticeable especially from the surface of the medium. A considerable amount of rather viscid sediment collects at the bottom of the tube or flask.

Nutrient bouillon + 2 % dextrose. The growth in this medium is heavier than in nutrient broth to which no sugar had been added, but the broth does not become fluorescent.

Nitrate bouillon becomes heavily clouded, and there is a thick pellicle, but the fluorescence is not marked.

Dunham's solution is not a very favourable medium and does not

become very densely clouded; a slightly heavier growth was observed in peptone water to which potassium nitrate had been added.

Nutrient bouillon over chloroform; growth was unrestrained in the presence of chloroform; the tubes were clouded in 24 hours, turbid in 48 hours.

Litmus milk is slowly peptonised. After 24 hours at 25° C., the fluid is clear to a depth of about 2 mm. from the surface, the remainder of the milk being unchanged. After 5—6 days the upper third of the medium is clear and translucent, the middle third is partially peptonised, and the remainder still opaque and unchanged; the medium thus exhibits three strata of approximately equal depth. In 8—10 days the whole of the milk has been peptonised, the colour is at first unchanged by reflected light and reddish by transmitted light, but it finally becomes slowly reduced from the top downwards leaving a yellowish translucent fluid. There is a small amount of sediment.

Egg albumen. A medium composed of 1 grm. powdered egg albumen in 50 c.c. of ·05 % potassium phosphate was used to determine the proteolytic activity of the organism. The medium after sterilisation is a colourless liquid in which the insoluble part of the egg albumen has been separated out in white flakes; when planted with the organism the liquid became clouded and then became deep sea foam green in colour. The solid albumen was acted upon and became slimy and soft in consistency.

Uschinsky's solution is clouded in 24 hours at 25° C., after six days it is very turbid, first the upper part of the broth, and gradually the whole of it becoming light, dull, green yellow to clear, dull, green yellow, the colour being much more noticeable by reflected than by transmitted light. There is a fair amount of sediment and the rods are normal and active. There is a ring above the medium, and a pellicle which sinks if the tube is shaken.

At the end of 20 days the pellicle still continues to form, and to sink when disturbed. A very heavy deposit is thus formed which is very viscid and almost like egg albumen in consistency. After some weeks the liquid becomes clear but yellowish; there is some yellowish growth clinging to the sides of the tube and a deposit 1—2 cm. deep in the bottom.

The organism also grows, but less vigorously, in a solution from which the asparagin and ammonium lactate have been omitted and replaced by ammonium sulphate. The organism is therefore able to obtain its nitrogen from a simple salt.

Cohn's solution. No growth.

Potato. A creamy-white, wet-shining streak about 6—8 mm. broad appears along the needle track; the edges are undulate.

Turnip. On turnip there is a thin, wet-looking, whitish growth, almost covering the slant surface.

Beet. On beet the growth is heavier than on turnip and carrot but it is not so spreading; it is yellowish and slightly raised.

Carrot. There is a very thin, whitish, wet-looking growth, in some tubes almost completely covering the slant surface; in others where the cylinder was drier only producing a streak a few millimetres wide along the needle track.

Parsnip. On parsnip there is a good growth along the needle track, 5—10 mm. wide, and slightly raised, shining.

Physical and Biochemical Relations.

Proteolytic activity. It has been pointed out in the section dealing with the cultural characters of the organism that milk is slowly peptonised. If a ten days old culture of the organism is killed by exposing it to a temperature of about 55° C. for half an hour and then 3—5 c.c. of the culture run into each of a number of tubes of sterile litmus milk, it is found that the milk is slowly cleared in precisely the same way as if the organism were growing in the medium.

A series of flask cultivations was carried out with a view to testing for the products of proteolysis. The media used were ordinary nutrient broth, egg albumen (1 gm. in 50 c.c. of ·05 % potassium phosphate) and milk; in each case 50 c.c. of the medium being sterilised in an Erlenmeyer flask of about 150 c.c. capacity. In this way the organism received abundant aeration and growth was fairly rapid.

Cultures in nutrient broth were tested for ammonia by distillation after five days at 25° C. The Nessler test could not be used owing to the presence of an appreciable amount of ammonia in the control flask.

A quantitative test showed that the amount of ammonia in the culture had not increased from the fifth to the tenth day, the difference in each case between the amount of ammonia in the culture flask and the control being ·016 grm. of ammonia nitrogen.

Egg albumen after five days at 25° C. gave a definite reaction for peptone and for tryptophane. The culture was tested by Sorensen's method for amino-acids and ammonia together at the end of the fifth and the tenth day. After five days the result was ·0148 grm. nitrogen

in the form of amino-acids and ammonia: if egg albumen contains ·150 grm. (approximately) total nitrogen, then 2·8 % of the total nitrogen had been broken down; after 10 days the figures were ·007 grm. or approximately 4·6 % of the total nitrogen.

Milk cultures after ten days tested by the Sorensen method contained ·019—·022 grms. nitrogen in the form of amino-acid and ammonia. When tested by distillation for ammonia alone, practically the same figures were obtained; it was evident therefore that the amino-acids had been reduced to ammonia.

Tested qualitatively the milk culture gave a very decided reaction for peptone and for tyrosin.

The results recorded above are sufficient evidence that the organism is a fairly active proteolytic agent.

Amylolytic action. Potato cylinders on which the organism has been growing for any length of time give the red-brown reaction for amylodextrin when treated with Lugol's iodine solution rather than the deep blue starch reaction.

Tubes containing 10 c.c. nutrient bouillon and ·01 grm. soluble starch were planted with a vigorous culture and incubated at 25° C. It was between two and three weeks before the starch totally disappeared. The action of the organism on starch therefore is comparatively slow.

Invertase and *lactase* are not produced by this organism. This is shown by the fact that although the organism produces acid in solutions containing dextrose and galactose it is unable to do so in those containing lactose and saccharose. Were it capable of reducing these sugars, acid would be formed in solutions containing them.

Fermentation reactions. No gas was produced in fermentation tubes containing peptone water tinted with litmus and 2 % of various carbohydrates. The amount of growth and presence or absence of acid production may be scheduled as follows:

Carbohydrate	Acid production	Nature of growth
Dextrose	Distinctly acid after 3 days	Moderate
Dextrin	None	Very heavy
Galactose	Distinctly acid after 3 days	Moderate
Glycerine	None	Light clouding
Laevulose	,,	,, ,,
Lactose	,,	,, ,,
Maltose	,,	,, ,,
Mannite	,,	,, ,,
Saccharose	,,	,, ,,
Starch	,,	,, ,,

A quantitative test for acid production was carried out with some of the sugars mentioned above. Cultivations were prepared in bulk in flasks containing 50 c.c. nutrient broth and 2 % of the substances to be tested. They were incubated for 10 days at 20° C. and were as follows (expressed in degrees of Fuller's scale).

Sugar	Culture	Control
Dextrose	+35·5	+15·6
Glycerine	+10	+15
Lactose	0	+15
Laevulose	+10	+15
Saccharose	0	+15

It will be observed that except in the case of dextrose the culture was slightly less acid than the control.

No reaction for alcohol or aldehyde was obtained in the distillate from a culture in dextrose bouillon.

Indol. There was no indol in cultures in Dunham's solution or in nutrient bouillon after 10—12 days at 20° C. Tests for phenol were also negative.

Pigment production. It has already been pointed out in the section on the cultural characters of the organism that it produces on certain media a distinct greenish or greenish yellow fluorescence.

Colour destruction. Methylene blue was almost completely reduced in 24 hours; neutral red and rosolic acid were not reduced. Litmus was partially reduced in milk cultures but not in nutrient broth.

Nitrate reduction. Nitrates were not reduced to nitrites during ten days growth in nitrate broth and in nitrate peptone water at 25° C.

Gas production. It has been stated that no gas is produced in fermentation tubes containing various sugar solutions.

Cultivations were prepared in iron and lead peptone solution; the precipitate began to blacken after some days, that in the tubes to which iron tartrate had been added becoming decidedly black, thus showing that some sulphuretted hydrogen had been liberated.

Growth under anaerobic conditions. The organism grows very slowly in glucose formate agar in Bulloch's apparatus from which the oxygen has been absorbed. Control tubes under ordinary atmospheric conditions made a very vigorous growth.

Temperature. The optimum temperature for growth lies between 25 and 30° C. The organism grows much more slowly at 20 than at 25° C. and at 35 than at 30° C.

The thermal death point is 49° C., ten minutes exposure in thin walled test tubes containing 10 c.c. nutrient broth.

Reaction of medium. The bacterium is not specially sensitive to the reaction of the medium in which it is grown. The optimum reaction lies between + 10 and + 20 Fuller, + 15 taken as approximately the optimum for cultural purposes.

The following table will serve to indicate the extreme reactions at which growth will take place and the amount of various substances necessary to inhibit growth.

Acid or alkali	Amount to restrain growth	Amount to inhibit growth
Acetic acid	+20	+25
Citric acid	+45	+50
Hydrochloric acid	+18	+20
Malic acid	+68	+70
Oxalic acid	+35	+40
Tartaric acid	+30	+35
Sodium hydrate	+30	+55

Toleration of sodium chloride. Cultivations were made in nutrient bouillon to which varying amounts of NaCl had been added. Growth was unrestrained in tubes containing up to 4 % NaCl, meagre in those with 5 to 6 % and inhibited in those with 7 %.

Desiccation. The organism is not particularly sensitive to desiccation; cultures are readily obtained from cover slips on which the organism has been dried for six weeks; more prolonged tests have yet to be made.

Insolation. The bacterium is fairly sensitive to the action of direct sunlight. Five minutes exposure is sufficient to destroy a large percentage of the rods and ten minutes to kill them all. The exposures were made on a block of ice, to the mid-day summer sun, the plates being further protected by being covered with glass basins containing about 2 cm. of a 4 % solution of potash alum.

The growth of the organism is not restrained in the diffuse light of the laboratory.

Nomenclature.

The organism appears to be one which has not previously been described. I therefore propose for it the name *Bacterium nectarophilum* n.sp., its chief characters are as follows:

Bacterium nectarophilum n.sp., parasitic in pear blossoms, causing blackening of the receptacle and ovary, and less frequently of the sepals and flower stalks; a short rod ·5—3μ × ·45—·7μ, majority are 1—1·5μ × ·6—·65μ; rods single or in pairs, short chains are fairly frequent,

capsule always present, motile by means of 1—5 polar flagella. Gram-negative.

Forms spreading yellowish-white colonies in nutrient agar, fluorescent; fluorescence absent in media containing dextrose; gelatine is not liquefied; nutrient bouillon heavily clouded; milk slowly peptonised, no change in reaction. Uschinsky's solution clouded, fluorescent, growth viscid; potato growth moderate.

Fairly active proteolytic agent; starch slowly destroyed; acid from dextrose and galactose, no gas or acid from any of the other carbohydrates tested; no indol; nitrates not reduced.

Facultative anaerobe; optimum temperature 25—30° C.; T.D.P. 49° C.

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