A TOMATO CANKER.

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(With 5 Text-figures and Plate XXVI.)

Introduction.

A disease which may be described as a canker of tomatoes was first noticed in fruit on the Pretoria market during the summer of 1914. It is most prevalent from January to March, and during that period each year (1914–20) a large percentage of the fruit offered for sale was disfigured. Affected fruit rapidly becomes attacked by soft rot organisms which enter the fruit through cracks caused by the scab lesions and destroy it within a few days.

The incidence of the disease has a direct relation to the rainfall. Tomatoes which ripen in the early summer are seldom attacked, being grown under irrigation, and the disease is seldom observed up to the end of November. From November to January the temperature is high, and there is usually a considerable amount of rain; moist, humid conditions are favourable to the development of the disease. The fruit can only become seriously scabbed when it is attacked by the organism in the early stages of its development; this would account for the non-appearance of the disease before December or January. A few lesions are to be found on fruits as late as June, but the disease can be regarded as serious only during the summer months.

The occurrence of canker in fruit on the Pretoria market was suggestive of the fact that the disease occurred in market gardens in the district; several tomato growers were visited and the surmise found to be correct. Canker is a very common trouble in market and private gardens in the Pretoria district; it is reported to occur also in the Rustenburg district, but I have seen no specimens from there, and beyond this nothing is known of the distribution of the disease. It is not regarded as being of a very serious nature, but a large percentage of the fruit is disfigured, and much of it decays if it is kept for a few

days after ripening. The summer of 1919–20, when most of the observations were made, was exceptionally dry: another season with heavier or more continuous rainfall the losses would probably be far more serious, and might ruin the whole crop.

The "canker" lesions differ from those caused by other tomato diseases attributed to bacteria. The wilt disease caused by *Bacterium solanacearum* Erw. Sm.(3) is a distinctly vascular trouble and causes a characteristic wilting of the plants. *Aplanobacter michiganense* is also found in the vessels(2). "Streak," described by Paine and Bewley as caused by *Bacillus Lathyri*, is characterised as the name suggests "by the formation of dark brown or black sunken patches on the stem," varying from small spots to long furrows or blazes(1)." On the fruit it forms light or dark brown sunken patches with round or irregular outline.

The effects of the canker organism on leaf, stem and fruit are widely different from any of these as will be evident from the detailed descriptions to be given later.

Symptoms of Disease.

On the leaves the first indication of infection is the appearance of numerous dark green, semi-translucent, water-soaked points on the under surface. In cases of artificial infection in autumn weather this occurred seven to eight days after inoculation, under summer conditions the progress of the disease may be more rapid. The spots increase in size and become round or irregular and about 2 mm. in diameter; they are slightly sunken and are often present in such numbers in the neighbourhood of the lateral veins and leaf margins, that they coalesce, and produce irregular, discoloured streaks. The colour soon changes from dark green to deep quaker drab[1] (Ridgway 51) or vinaceous slate (50). The discolouration penetrates to the upper surface, and the spots eventually consist of a smoke grey centre, which is then membranous and semi-translucent surrounded by a deep brownish drab margin.

Where the spots are numerous the intervening leaf tissue becomes dry, brown and brittle, the original lesions being still plainly visible in the dead areas. In this way the affected portions of the leaf, especially the edges and the tips, become dead and dry and break away, giving the leaves a very ragged appearance, and many of the smaller leaflets are

[1] The numbers quoted after name of colours refer to plates in Ridgway's *Colour Standards and Nomenclature.*

altogether destroyed. Affected leaves show a tendency to curl inwards, and are more or less twisted and distorted.

Spots on calyces, pedicels and young parts of the stem are similar in character to the leaf spots. On the calyx they may be numerous, but very minute and scattered, or less numerous and up to 2–3 mm. diameter, forming elongated streaks up to 5 or 6 mm. long.

Cankers are produced on older parts of the stem, especially where the tissues have been somewhat injured by friction or otherwise. At first there appear irregular, dark green water-soaked areas, which later become corky-looking, slightly raised, roughened and with numerous small longitudinal cracks. They are nearest tawny olive in colour. The surface has the appearance of having become blistered or raised by abnormal tissue development underneath, with subsequent cracking of the blistered areas. Cankers of irregular form and 1–2 cm. in diameter are not uncommon. The discoloration does not penetrate into the wood; it is apparently confined to the cortex and is quite superficial.

In the field, infected fruits are usually found immediately below diseased leaves and are doubtless infected during rainy weather by raindrops which fall on infected leaves and subsequently drip on to the young fruit. The majority of the fruit spots are at the stalk end, but they are also found scattered over the sides and, less frequently, on the blossom end.

A very minute green or brownish blister is the first indication of infection: this blister may remain minute, about 1 mm. diameter, or may increase in size up to about 3 mm. and become considerably raised above the normal fruit tissue. Occasionally, presumably when weather conditions are unfavourable, infection does not proceed further, and when the fruit is ripe these minute blister-like spots have almost the appearance of fly-specks (Plate XXVI, fig. 2).

In the large majority of cases the point of infection becomes surrounded by a dark green, water-soaked area which spreads considerably and then begins to discolour from the centre. The centre becomes deep slaty brown, merging into wood brown at the edges; a water-soaked margin about 1 mm. wide is still apparent whilst the organism is active. Finally the epidermis ruptures in the centre, showing whitish brown over the discoloured tissues like the broken edges of a blister. The spots are hard and scabby in texture and usually slightly convex, although in mature fruit they may lie in slight depressions owing to arrested growth at the point of infection (Plate XXVI, fig. 1).

Single scabs are usually not more than 5 mm. diameter, but they

are often so numerous and close together that they coalesce, forming large, scabby areas several centimetres in extent. As the fruit ripens the tissues round the infected areas remains green, forming a green rim round the scabs which is conspicuous on the red fruit. The rifts in the epidermis become extended in cases of severe infection and whitish brown cracks are formed, many of them over 1 cm. in length and extending into unaffected tissue. These open the way for putrefactive organisms and the fruit usually rots within a few days after ripening. Thus the disease not only disfigures the fruit and reduces its market value, but seriously affects its keeping qualities.

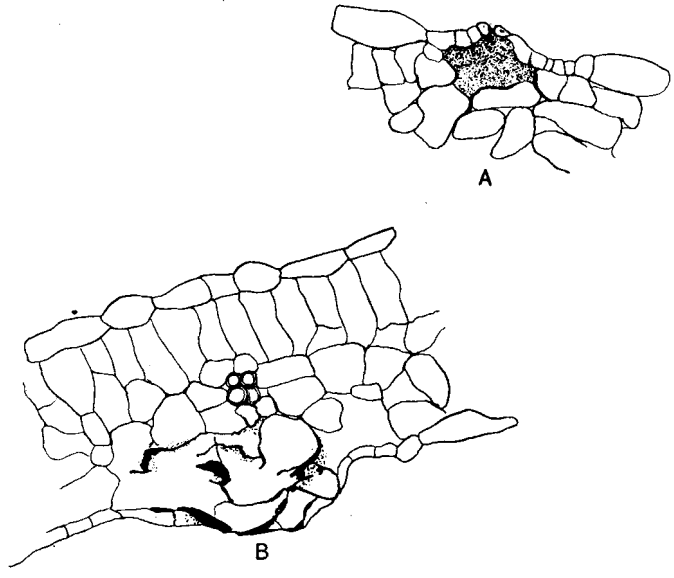

Fig. 1. Early stages in leaf infection.

Morbid Anatomy.

In inoculation experiments tomato leaves and fruit readily became infected without wounding, so that in all probability infection usually takes place through the stomata. This was confirmed by an examination of a large number of sections of spotted leaves: in the earliest stages of infection the bacteria are to be found in the sub-stomatal cavity (Fig. 1 A), and later make their way to the adjoining intercellular spaces. The middle lamella of the cells becomes much swollen, and stains an intense red in sections treated with carbol fuchsin and light green, the cells in the surrounding healthy tissue staining green (Fig. 1 B). As described by

Paine in connection with the "streak disease(1)," the cells become torn asunder by shrinkage of dead cells and by the tension set up by growth of the surrounding healthy tissue and eventually large cavities are produced. There are not many living bacteria to be found in the disorganised tissues, but at the edges of such lesions, where the organism

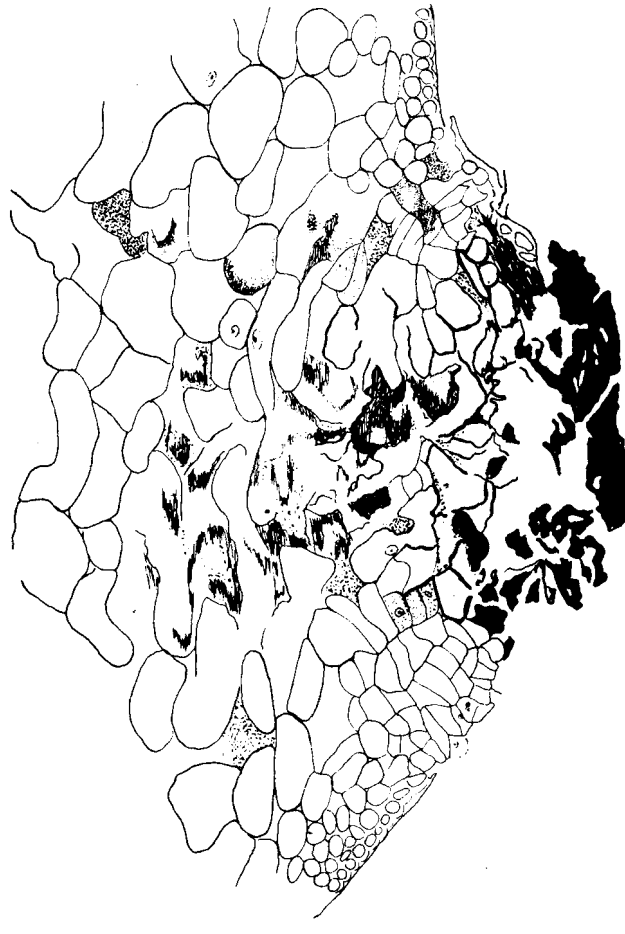

Fig. 2. Section through small lesion on fruit.

is still active, large pockets of bacteria may be found. In the leaf the organism penetrates through the thickness of the leaf, entirely disorganising the tissue, but it does not travel far in a lateral direction. Stem lesions are very similar, but the organism has not been found to penetrate beyond the cortex.

In the fruit a similar disorganisation of cells takes place, but the hypodermal cells at the edge of the injured tissue begin to divide actively (Fig. 2) and sometimes form a complete layer of new cells under the disorganised tissue (Fig. 3); this accounts for the convex contour of the canker. Sorauer (5) has illustrated a similar condition as a result of hail injury; it is probable therefore that the multiplication of cells is not due directly to the action of the organism but to the presence of dead tissue in the rapidly growing fruit.

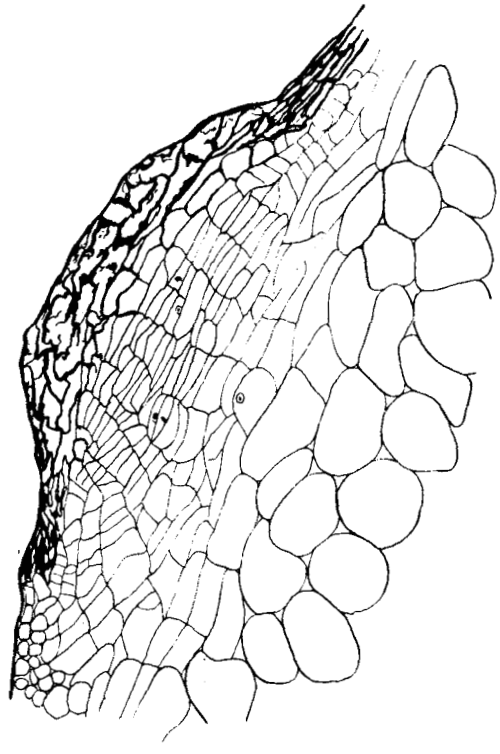

Fig. 3. Section through lesion on fruit, showing complete layer of actively dividing cells.

The nuclei in the affected cells become much hypertrophied, becoming five or six times the size of nuclei in healthy cells. The nucleolus becomes very large and vacuolate, and the nucleolar membrane disappears. There are frequently two nuclei in a cell or two nucleoli in one nucleus, the latter frequently irregular or lobed, being similar in shape to nuclei dividing amitotically as found by Smith (4) in crown gall lesions; but there was no clear evidence that the nuclei were dividing in this way.

In the entirely disorganised cells the nucleus continues to increase in size until the nuclear membrane is ruptured and the chromatin becomes diffused; the nucleolus remains intact for a considerable time, taking a red stain and standing out clearly in the disorganised mass.

ISOLATION OF THE CAUSAL ORGANISM.

The organism was first isolated in January, 1914, from tomatoes purchased on the Pretoria market. On these plates a yellow organism was predominant, but there were also colonies of a spreading, cream-coloured organism. At that time no greenhouse accommodation was available, and attempts to inoculate tomato plants out of doors during a spell of dry weather were unsuccessful; it was quite impossible to keep the atmosphere sufficiently moist. Attempts to inoculate detached fruits in the laboratory also proved a failure.

The disease was noticed on fruit exposed for sale each summer, but there was no further opportunity to investigate the matter until February, 1920. The organism was again plated out from fruit exposed for sale on the Pretoria market; colonies were visible after 48 hours, at 30° C., and in three days assumed their characteristic form and yellow colour. An almost pure culture was obtained direct from the host. The colonies were very similar in appearance to those of *Bacterium citri*, *B. campestre* and other related organisms; there appears to be a large number of plant parasites belonging to this group.

During March, 1920, the organism was repeatedly isolated from scabby fruit and infected leaves collected in a market garden at Daspoort, near Pretoria. In each case a pure culture of the organism was obtained without difficulty, from both leaf and fruit. It is more easily isolated from the fruit since the surface can be sterilised and the culture made from the tissue underneath.

INOCULATION EXPERIMENTS.

Preliminary Experiments.

A.

28. 1. 20. Two well-grown tomato plants were used bearing green fruit, almost full grown. The fruits were inoculated with a culture isolated from tomato fruits purchased on the Pretoria market. The culture was applied with a camel's hair brush, a few tomatoes on each plant being pricked and the rest uninjured. Inoculated fruit was covered for 24 hours with beakers covered with brown paper and plugged with cotton wool.

12. 2. 20. Dark green, water-soaked areas round needle pricks.

16. 2. 20. Two tomatoes ripened and were removed from plant; tissue round needle prick has remained green with a slight tendency to brown discolouration and are crowded with bacteria.

25. 2. 20. Remaining fruit ripened and picked. All calyces show numerous infections, but fruit infections have not gone beyond the green, water-soaked stage. Stems show extensive cankers where they were slightly injured by friction of beaker placed over fruits after inoculation. Control plants remained sound and showed no sign of infection.

B.

10. 3. 20. Three large tomato plants were used, bearing fruit at various stages of development. Some fruits were left uninjured, others pricked or lightly scratched. The culture was applied to fruits and leafy tips with a camel's hair brush, and the plants atomised with water. The leaves were left uninjured. All inoculated portions were covered for 24 hours with butter paper bags, to prevent excessive drying.

13. 3. 20. Numerous minute light and dark brown specks on younger fruits.

18. 3. 20. Numerous minute, water-soaked spots on leaves, also indications of infection round needle pricks on fruit.

23. 3. 20. Tomatoes which were very young when inoculated and which were not pricked show minute brownish blisters all over the surface. One which had only just set when inoculated had an irregular water-soaked zone round the blisters up to 2 mm. diameter. Leaf infections increasing in size and number and assuming characteristic purplish grey colour. Infected spots also visible on calyces, pedicels and stems.

29. 3. 20. Spots on small tomato mentioned above beginning to discolour: water-soaked zone round blisters on slightly larger fruit and round needle pricks on fruit which was inoculated by puncture when almost full grown.

12. 4. 20. Scabs on all fruits now quite typical; they have discoloured, become brown, epidermis has blistered and cracked in several directions. Badly infected leaflets become entirely yellow and dead.

13. 4. 20. Plants discarded. Control plants showed no sign of infection.

C.

29. 3. 20. Eight young plants about 1 ft. high were used. They were inoculated by atomising with culture isolated from artificially infected plant described in experiment B.

6. 4. 20. Numerous minute, water-soaked areas observed on younger leaves.

13. 4. 20. Leaf spots have assumed typical form and colour. Control plants showed no sign of infection.

D.

10. 5. 20. One tomato plant in bearing was inoculated. Fruits of various sizes and leafy tips were inoculated without wounding, and covered with butter paper bags for 24 hours.

19. 5. 20. Small raised spots visible on fruits, water-soaked spots on leaves. (The majority of the affected fruits and leaves were removed for purpose of studying morbid anatomy.)

Cross Inoculations.

It has been mentioned that the organism belongs to what Smith once termed the "yellow *Pseudomonas* group." Since five other organisms of this group were under observation at the time, and all are very similar in culture, it was thought advisable to carry out a series of cross inoculations. All these organisms infect their hosts by way of stomata or water pores; the inoculations were therefore made without wounding the plants.

A.

24. 3. 20. Six tins (1–6), each containing four young tomato plants, were used. These plants were about 1 ft. high and growing vigorously. One set of four plants (1) was inoculated with a culture of *Bacterium campestre*, one (2) with *B. citri*, and one each with (3) *B. Juglandis*, (4) *B. Phaseoli*, (5) *B. malvacearum*, and (6) the tomato organism.

31. 3. 20. Numerous minute, water-soaked spots on tomato seedlings inoculated with organism (6) from tomato scab. Other plants show no infection.

6. 4. 20. Spots on tomato seedlings in tin (6) have now assumed characteristic form and colour. All plants in tins (1–5) perfectly sound and showing no signs of infection.

13. 4. 20. No further development.

B.

24. 3. 20. Six tins of lemon seedlings were used, each containing six plants 10–15 inches high. One group of six seedlings was inoculated with each of the organisms mentioned in experiment A. The tins were numbered (1–6) as follows: (1) inoculated with *Bacterium campestre*, (2) with *B. citri*, (3) *B. Juglandis*, (4) *B. Phaseoli*, (5) *B. malvacearum*, (6) *Bacterium* causing tomato scab.

30. 3. 20. Incipient cankers noticed on lemon seedlings (2) inoculated with *B. citri*.

7. 4. 20. Cankers on seedlings (2) increasing in size and number. No sign of infection on plants inoculated with other organisms.

15. 4. 20. Numerous typical citrus canker lesions on leaves and stems in tin (2). All other plants have remained sound and show no sign of infection.

C.

24. 3. 20. Six cotton plants 2 ft. to 2 ft. 6 in. high were employed, one plant being inoculated with each of the six organisms mentioned in connection with experiments A and B.

29. 3. 20. Numerous minute, water-soaked spots on young leaves of plant (5) inoculated with *B. malvacearum*.

7. 4. 20. Spots have increased in size and assumed characteristic angular outline.

15. 4. 20. Leaf spots on plant (5) have discoloured, and are now dark brown or black. Cotton plants (1–4) and (6) inoculated with other organisms show no sign of infection.

D.

14. 4. 20. Six young walnut trees planted in separate tins were numbered (1–6) and inoculated with the six organisms tested in experiments A–C.

19. 4. 20. Numerous minute water-soaked spots on young leaves on tree (3) which was inoculated with *B. Juglandis.*

5. 5. 20. Spots on tree (3) have discoloured and assumed typical appearance of walnut blight lesions. All other trees remained sound and showed no sign of infection.

E.

14. 4. 20. Six tins each containing four well-grown cabbage seedlings were used; the tins were numbered (1–6), the four plants in each being inoculated with one of the same six organisms.

24. 4. 20. There is an indication of blackening of veins at edges of some of the leaves in tin (1), plants inoculated with *B. campestre.*

12. 5. 20. Cabbage plants in tin (1) show typical black rot lesions. No sign of infection in any of the other tins (2–6).

F.

14. 4. 20. Four bean seedlings were inoculated with each of the six organisms. Tins again numbered (1–6), four seedlings in each, and inoculated in the same sequence as in experiment A.

19. 4. 20. A few water-soaked areas visible on leaves of plants in tin (4) inoculated with *B. Phaseoli.*

20. 4. 20. Very numerous points of infection now visible on plants in tin (4).

30. 4. 20. Leaves of bean plants in tin (4) now heavily infected and showing numerous typical bean blight lesions. No sign of infection on plants in tins (1–3) and (5–6) inoculated with other organisms.

Inoculation on to other Hosts.

A.

7. 4. 20. 12 tobacco seedlings about six inches high were atomised with a culture of the tomato organism.

13. 4. 20. No sign of infection.

7. 5. 20. Still no sign of infection—plants discarded.

B.

7. 4. 20. Two plants of *Physalis minima* were inoculated by atomising with a suspension of a potato culture.

13. 4. 20. Numerous minute water-soaked spots observed on younger leaves.

17. 4. 20. Spots have increased in size, are somewhat angular, and are becoming brown. Control plants show no sign of infection. Re-isolated organism from infected leaves and found it to be identical with the original.

C.

7. 4. 20. One plant of *Datura stramonium* var. *tatula* was inoculated by atomising with a suspension of a culture of the tomato organism.

13. 4. 20. Numerous minute, water-soaked points on younger leaves: tissues on examination prove to be full of bacteria.

17. 4. 20. On *Datura* plant inoculated, organism has produced very numerous spots about 1 mm. diameter and somewhat angular, these are brown and membranous in centre and have a water-soaked margin: these brown spots show a tendency, especially in the younger leaves, to break away and cause a shot-hole effect. Control plant is healthy and shows a sign of infection; replated organism from infected leaves and found it identical with original.

D.

7. 4. 20. One plant of *Solanum incanum* inoculated without wounding.

17. 4. 20. No signs of infection on this or later date; leaf is protected by thick covering of hairs.

E.

7. 4. 20. One plant of *Solanum nigrum*, inoculated without wounding.

13. 4. 20. Slight indications of infection on younger leaves.

17. 4. 20. Infected spots have developed considerably, but are still small: they are irregular in outline, 1 mm. diameter or up to 2 mm. long, and are light brown and membranous in the centre. Replated organism and found it identical with original. Control plant showed no sign of infection.

F.

24. 7. 20. A number of sweet pea plants were inoculated by needle pricks in the stem. The result was entirely negative.

DESCRIPTION OF THE CAUSAL ORGANISM.

A. *Morphological Characters.*

When taken direct from the tissues of the host the organism is very variable in size and form: the majority are rather stout rods with rounded ends, but some are so short as to resemble coccus forms. They vary from ·6 to 4 μ in length, and from ·5 to ·75 μ in breadth. The majority are 1–1·5 × ·6–·7 μ.

In young agar cultures (24 hours at 30° C.) the organism is a rod with rounded ends, occurring singly or in pairs, or less frequently in short chains of three to seven elements. The rods are for the most part fairly even in size, measuring 1–1·5 × ·6–·7 μ; the extremes observed were ·8–3 × ·6–·7 μ. In old cultures they are less regular, and short forms are numerous.

In young bouillon cultures the rods are similar in form. The organism grows rather reluctantly in neutral bouillon, and in six days old cultures in this medium there are numerous straight or curved rods 4 and 5 μ long and also long undivided threads up to 50 μ long.

On gelatine the average length is slightly greater, being 2–3 μ, and the breadth is ·6 to ·65 μ. Limits observed were 1–4 × ·6–·7 μ. The rods were mostly single but occasionally in pairs or short chains.

In 24 hours old potato cultures the rods are also rather long as compared with those on agar cultures; the majority are 1·5–2·5 × ·6 μ. There are numerous pairs and short chains, and occasionally long threads up to 40 μ long which are only partially or imperfectly segmented. After six days the size and grouping remain the same, with the exception of the long threads which are no longer seen.

Motility. The organism is actively motile in very young cultures. In hanging drop cultures it moves with a forward screwlike action, its progress being frequently interrupted by rotations on its short axis

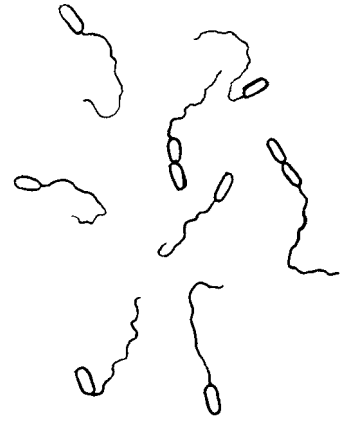

Fig. 4. Rods from streak incubated for 18 hours at 20° C. Stained by Löffler's method.

and other tumbling movements. The rods gradually made their way to the edge of the drop and came to rest. The organism is not motile as it diffuses from the tissues of the host. Rods from a young agar culture examined with the dark ground illumination showed no highly refractive granules, and the rods are apparently motile by means of a polar flagellum on the forward end which whirls like the propellor of an aeroplane.

This observation was confirmed by the examination of stained preparations. Good preparations were not very easy to obtain as the organism sheds its flagella very readily; fairly good results were obtained with a modification of Löffler's method and by van Ermengen's method. There is a single polar flagellum with a length of about 5–10 μ (Fig. 4).

Spores were not observed.

Capsules. The viscid nature of the growth on agar and other media points to the presence of a capsule. This is most clearly seen in potato cultures. Examined after 24 hours at 30° C. each rod is surrounded by a white halo; when stained with carbol fuchsin the rod stains intensely and the capsule is pale with a feebly stained margin (Fig. 5). As the culture grows older the capsule becomes more evident, and when stained with carbol fuchsin the rod and capsule stained as described above are embedded in a slimy mass which stains pink.

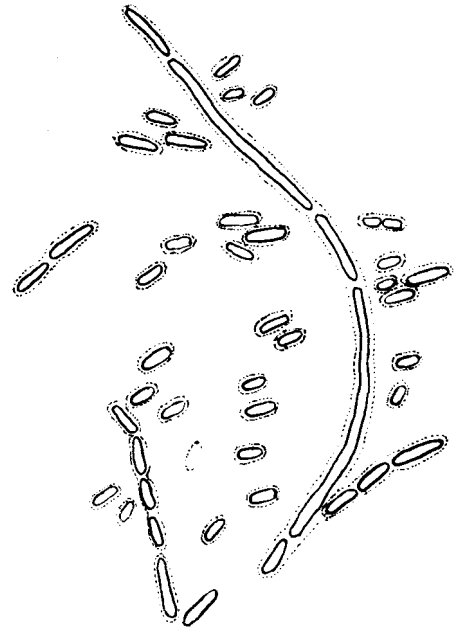

Fig. 5. Rods from potato culture, 24 hours at 30° C. Stained with carbol fuchsin.

Involution forms have been found in old gelatine cultures. They vary much in form; some are stout rods about 1 μ thick in the centre and tapering to pointed ends: others are longer threads up to 35 μ long and varying in thickness, curved and distorted and with or without constrictions at irregular intervals.

Staining reactions. The organism as taken from young cultures stains evenly and intensely with carbol fuchsin and all the ordinary aniline stains. In rods from older cultures all staining is fainter and more irregular. It is not acid fast and stains only faintly with Löffler's blue; it is Gram positive: no granules were observed in rods stained by Neisser's method.

Cultural Characters.

Preliminary cultivation was carried out as suggested by the Society of American Bacteriologists. In all cases, unless otherwise stated, cultures were incubated at 30° C.

Plate cultures on nutrient agar +15. Colonies show as minute, shining points after 48 hours. By the third day they have increased somewhat in size, and are coppery, translucent by transmitted light, and yellowish or somewhat opalescent by reflected light. There is seldom any development after the fourth day; on thinly sown plates fully developed surface colonies are circular or oval, up to 5 mm. diameter, semi-translucent, colour Naples yellow (XVI). The margin is entire and texture homogeneous, finely granular as seen under the low power of the microscope. In some there is a faint indication of concentric zoning, but this is more evident in the bluish white colonies about 2 mm. in diameter, formed between the agar and the bottom of the plate. Long feathery crystals begin to form in the medium after about ten days. Submerged colonies are minute and lenticular, and often form surface colonies by excentric development.

Streak cultures on nutrient agar +15. After 24 hours the streak is smooth, slightly convex, wet-shining, with entire margin, and about 5 mm. broad. The growth increases somewhat up to the third or fourth day, entirely covering the wetter part of the medium at the base of the tube. The colour is Naples yellow, and growth shows irregular, semi-opaque streaks when held up to the light. There is a heavy growth in the condensation water. Crystals begin to form under the streak at the end of the first week.

Stab cultures in nutrient agar +15. Surface growth only; no growth in the depths of the medium.

Shake cultures in nutrient agar +15. Numerous surface colonies develop and become confluent, entirely covering surface. There are some very minute submerged colonies just below the surface but none in the depths of the medium.

Plate cultures on nutrient gelatine +15. All gelatine cultures were incubated at 20–22° C. Colonies were visible on the third day; on the fourth day thickly sown plates were partly liquefied, on more thinly sown plates surface colonies were up to 1 mm. diameter, convex glistening, cream colour, translucent, context homogeneous and finely granular; submerged colonies minute, opaque, lenticular. On the sixth day thickly sown plates were completely liquefied; in more thinly sown plates the

colonies were up to 5 mm. diameter and each sunken in a saucer of liquefaction, the liquefied gelatine was clear and the colonies denser in the centre than at the circumference. On the tenth day the gelatine in the thinly sown plates was completely liquefied, the medium being very slightly clouded. Colonies were still intact but somewhat diffused, up to 15 mm. diameter and often rolled at the edges; they were not tough, but gelatinous in consistency, breaking very readily if an attempt were made to lift them with a needle. Under the low power of the microscope the centre was moruloid to grumose.

Stab cultures in nutrient gelatine +15. After 24 hours there were slight indications of growth at the surface and along the upper part of the stab, and on the third day there was a shallow crater of liquefaction at the surface. On the fourth day liquefaction was crateriform, 7–10 mm. wide and about 5 mm. deep; there was a good surface growth and a slight bacterial deposit on the unliquefied gelatine, but the liquefied part of the medium was colourless. Subsequently liquefaction became stratiform, and when it had proceeded to a depth of about $1\frac{1}{2}$ cm. growth apparently ceased. The gelatine remained clear or very slightly cloudy, and eventually the surface growth sank to the bottom of the liquefied portion.

Streak cultures on nutrient gelatine +15. A whitish streak about 1 mm. wide is visible after 24 hours, which is replaced on the second day by a narrow channel of liquefaction along the needle track; this channel gradually becomes more extensive, the bacterial growth being carried with the liquefied gelatine to the bottom of the tube. This continues until there is 1–1·5 cm. of liquefied gelatine at the bottom of the tube.

Shake cultures in nutrient gelatine +15. Numerous minute surface colonies appeared in the third day, and the gelatine was slowly liquefied from the surface downwards, liquefaction being first noticed on the fourth day. At no time was there any growth in the depth of the medium.

Potato. On steamed potato cylinders a spreading, wet-shining butyrous growth covered the moister parts of the cylinder in 24 to 48 hours; on the sloping surface there was a broad, more or less raised streak surrounded by a narrow white "fermentation zone." The growth was amber yellow, and became somewhat viscid, especially in old cultures; it increased in quantity, covering the whole cylinder unless the medium was dry; the colour deepened somewhat with age and the medium was somewhat greyed.

Carrot. On steamed carrot the organism produces in three days a spreading, wet-shining, cream-coloured growth, practically covering the medium, and a thick shiny growth on the surface of the liquid at the bottom of the tube; the colour deepened after the first few days, becoming straw yellow to amber yellow.

Turnip. On steamed turnip cylinders growth is similar to that on carrot. The cylinder is entirely covered after three days with wet-shining, slimy-looking growth, which may be flat and smooth or somewhat raised, standing out on the surface in large drops. The colour is cream to straw yellow.

Parsnip. On steamed cylinders of parsnip, growth is similar to that on other vegetable cylinders described but rather less copious. The colour is straw yellow to amber yellow.

Beet. A very copious, spreading, wet-shining growth is also obtained on steamed cylinders of beet; the growth is more raised than on the other media and straw yellow in colour. On all steamed vegetable cylinders the amount of growth varies with the amount of moisture present, the best growth being obtained with the maximum amount of moisture.

Steamed rice. The rice grains become thinly covered with a spreading wet-shining growth which is straw yellow on the wetter parts of the medium and becomes light ochraceous buff where there is less moisture.

Sweet potato was a very favourable medium, growth being similar to that on the ordinary potato tubes.

Streak cultures on Loëffler's blood serum. Growth was fairly abundant, in the form of a wet-shining, cream-coloured streak along the needle track; liquefaction was observed on the sixth day and proceeded slowly.

Nutrient bouillon +15. Growth in bouillon varies considerably with the reaction of the medium. In tubes with a reaction of +15 of Fuller's scale the bouillon is faintly clouded after 48 hours; it never becomes turbid. A straw yellow ring 1–2 mm. broad forms above the liquid and there are often minute, whitish flocculent particles suspended in the liquid which eventually sink and form a sediment. In +20 bouillon there is a slight pellicle formed, which sinks when disturbed, and considerable sediment is produced.

Durham's solution becomes lightly clouded. There is no ring, but often a few very minute flocculi in suspension which eventually sink and form a slight sediment.

Uschinsky's solution. There is no growth in this medium; it was kept under observation for 20 days.

Cohn's solution. No growth.

Egg albumen medium containing 1 gm. of powdered egg albumen and 50 c.c. of a ·05 per cent. solution of potassium phosphate became well clouded, but there was no ring or pellicle formation and no discolouration of the medium.

Fermi's solution becomes lightly clouded on the second day. Growth proceeds slowly and after about three weeks there is a good ring above the medium, and considerable sediment at the bottom of the tube. This is Naples yellow in colour and very viscid, rising in a spiral swirl when the tube is rotated.

Cabbage broth clouds fairly heavily: a ring forms above the medium and there is some indication of pellicle formation; there is also a sediment at the bottom of the tube.

Milk shows no change until the third day; separation of the whey takes place slowly in such a way that there is an increasing quantity of clear yellowish whey at the surface, and the lower part of the tube is filled with whitish minute flocculent particles. The clot is not coagulated, and is slowly digested, but had not completely disappeared in cultures which had been under observation for some weeks. The whey is clear and yellowish in colour.

In litmus milk the colour is partially reduced, and the culture becomes slightly more acid than the control. In flasks, where a greater part of the medium is exposed to the air the action of the bacterium is considerably more rapid than in tubes.

Physical and Biochemical Relations.

Proteolytic activity in milk. In describing the cultural characters of the organism, it has been stated that milk is slowly peptonised. A number of flasks each containing 50 c.c. of milk were tested at intervals of five days for peptone, tyrosine and tryptophane. At the end of five and ten days at 30° C. the culture fluid gave positive reactions for each of these compounds. In each case the reaction was stronger on the 15th and 20th day, particularly in the case of tyrosin for which an intense reaction was obtained on the 20th day. There was also a strong reaction for peptone on the 20th day.

A similar set of flasks was tested quantitatively for the production of amino-acids and ammonia. The following figures give the results from one such experiment.

Ammonia by distillation.

	Number of c.c $N/10$ acid neutralised			N as ammonia
	Culture	Control	– Control	approx. figure
5 days	4·27	3·40	·87	·0022 grm.
10 „	14·14	3·07	11·07	·0155 „
15 „	24·90	2·80	22·10	·0309 „
20 „	35·33	4·76	30·57	·0428 „

From these figures it will be seen that the amount of ammonia increases steadily up to the 20th day. Similar results were obtained from other tests carried out on the same lines.

The amount of amino-acids was estimated by the Sorensen method; it increases up to the 15th day and then decreases, the amino-acids being probably broken down to ammonia.

	Amount of $N/10$ NaOH used for final titration				N as
	Culture	Control	– Control	– Ammonia	amino-acids
5 days	9·57	8·33	1·24	·37	·00005 grm.
10 „	34·42	9·61	24·81	13·74	·0192 „
15 „	47·08	4·00	43·08	20·98	·0294 „
20 „	48·42	8·27	40·14	9·58	·0134 „

In *egg albumen* medium (1 gm. egg albumen in 50 c.c. of 0·5 per cent. potassium phosphate) similar results were obtained. The qualitative test showed that less tyrosin was formed than in the milk cultures, but a strong reaction for peptone was obtained all through.

The culture and control were tested for amino-acids and ammonia in the same way as the milk flasks. To an additional flask, zinc sulphate was added to complete saturation, and the total nitrogen in the filtrate determined by the Kjeldahl process; this gave nitrogen as peptone plus amino-acids and ammonia, and by subtraction nitrogen as peptone. It will not be necessary to give detailed figures as in the previous schedules; taking the amount of nitrogen in 1 gm. egg albumen to be ·15 gm. a typical test gave the following figures. In each case the control was tested and figures obtained from control flasks deducted.

Percentage of egg albumen reduced.

	Total	Ammonia	Amino-acids	Peptone
5 days	44·5	4·4	6·0	36·0
10 „	51·6	8·1	13·2	30·4
15 „	63·9	15·1	2·8	46·0
20 „	82·2	15·0	1·2	66·0

The amount of ammonia and peptone increases gradually; the amino-acid, as in the case of the milk flasks, increases and then decreases, the decrease being correlated with an increase in the ammonia content. The large percentage of albumen reduced shows that the organism is a fairly active proteolytic agent.

A fair amount of ammonia is also produced in media containing peptone, *e.g.* ordinary nutrient bouillon.

Broth cultures after sterilisation cause no peptonisation of milk. If a culture 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 litmus milk, there is no change in the milk during ten days. *Bacterium nectarophilum* gave positive results with this test, the milk being slowly cleared in the same way as when the organism was growing in the medium.

Amylolytic action. The starch of steamed potato cylinders is not destroyed at all rapidly; cylinders on which the organisms had been growing for three weeks still gave a strong blue black reaction with iodine.

Tubes containing 10 c.c. nutrient bouillon and ·01 gm. soluble starch were planted with a vigorous culture and incubated at 30° C. It was only after 14 days that the starch entirely disappeared. When a similar set of tubes was planted with *Bacterium citri* the starch had entirely disappeared at the end of 48 hours; the amylolytic action of the tomato organism is therefore comparatively feeble.

Cultures in nutrient broth which had been incubated for five days at 30° C. were tested for the presence of diastase. A mixture was made of equal quantities of the cultivation and a thin starch paste containing 2 per cent. thymol; this was placed in the incubator at 37° C. for six hours and then tested with Fehling's solution, with negative results. Control cultures of *Bacillus subtilis* tested in the same way gave a good reaction for reducing sugars.

Production of invertase. Nutrient bouillon in which the organism had been growing for five days was tested for the presence of invertase. A mixture was prepared containing equal quantities of the cultivation and a 2 per cent. solution of cane sugar to which 2 per cent. of carbolic acid had been added. After several hours the mixture was tested for reducing sugars with negative results.

When the organism is grown in nutrient broth containing cane sugar, however, a certain amount of reducing sugar is produced. In flasks containing 500 c.c. of nutrient broth with 2 per cent. saccharose, the

organism produced 61 mg. of reducing sugar in three days, and at the end of five days the culture contained 159 mg. of reducing sugar.

Gas formation. No *gas* was produced in fermentation tubes containing nutrient broth and 2 per cent. of following substances: dextrose, laevulose, saccharose, maltose, lactose, mannite, dextrin, galactose, glycerine. In no case was any growth observed in the closed end of the tube. There was a small amount of alcohol in the distillate from cultures in dextrose bouillon. In tube cultivations in iron and lead peptone solution and broth, there was a slight discolouration of the precipitate after three weeks, showing that traces of sulphuretted hydrogen are liberated.

Acid production. The behaviour of the organism in the various sugar broths very closely resembles that of *Bacterium campestre* and other organisms of this group, that is to say, it produces a small amount of acid from certain sugars but the acid production is very rapidly obscured by the formation of alkali, probably in the form of ammonia, which has shown to be produced in appreciable quantities in peptone broth.

In dextrose bouillon the acidity is more marked than in any of the other media, the greatest acidity being observed about the fifth day; the acidity of the medium gradually decreases and after three or four weeks it becomes neutral or slightly alkaline.

The same may be said of cultures in nutrient bouillon with 2 per cent. laevulose, saccharose, lactose, galactose, glycerine, and dextrin, but less acid is produced with any of these substances than with dextrose. No definite acid formation was observed in flasks containing mannite and maltose.

Indol and phenol production. Cultures in nutrient bouillon and Dunham's solution were repeatedly tested for indol on the third, fifth and tenth day, but with negative results. No reaction was obtained with a nitrite and sulphuric acid, nor with the Rosindol test.

Cultures in nutrient bouillon tested on the tenth day also gave a negative reaction for phenol.

Reducing agent formation: colour destruction. A number of tube cultivations were prepared in nutrient bouillon tinted with litmus, rosolic acid, neutral red, methyl orange, and methylene blue: no colour reduction was observed.

Nitrate reduction. A number of tube cultivations in nitrate bouillon were tested after three, five and ten days. Some of these showed a trace of nitrite but in others no reaction was obtained. The controls gave no reaction for nitrite.

Flasks of nitrate bouillon tested after ten days contained slightly more ammonia than similar flasks containing bouillon without potassium nitrate. These tests were repeated several times with no more decided results; it would seem therefore that the organism has a feeble action on nitrates, and that traces of nitrite and ammonia are at times produced in culture solutions containing a nitrate.

Atmosphere. The organism is strictly aerobic and makes no growth in the depth of media nor in the absence of oxygen. No growth took place in Bulloch's apparatus in an atmosphere of hydrogen, nitrogen or carbon dioxide, but except in the case of tubes exposed to CO_2 the organism was not killed and began to grow when removed from the apparatus and placed in the incubator.

Temperature. This bacterium grows through a wide range of temperature, it grows slowly at 5° C. and at 40° C.; the optimum is about 30° C. It is killed by a prolonged exposure to 42° C. The thermal death point (moist, ten minutes' exposure) is 56° C.; in dry tubes the organism did not grow after ten minutes' exposure to a temperature of 64° C.

Reaction of medium. This bacterium does not grow at all in an alkaline bouillon, even if only − 5 of Fuller's scale; it grows very slowly in bouillon neutral to phenol-phthalein. It grows well in bouillon with a natural reaction of + 25, the optimum being about + 20 of Fuller's scale; it grows almost as well at + 15 as at + 20. A number of cultures were also made in broth which had been made neutral and acidified with various acids. It grew well in + 20 (Fuller), malic, lactic, tartaric, hydrochloric and citric acids; in tubes with a reaction of + 25 Fuller, it grew in tubes acidified with malic, lactic and citric acids and not with tartaric or hydrochloric acids; it is much less sensitive to malic and citric acids, growing unrestrainedly in bouillon with a reaction of + 30 Fuller, and clouding bouillon up to + 50 Fuller, malic acid and + 60 citric acid; the inhibition point for these was + 55 and + 65 respectively.

Toleration of sodium chloride. Growth is inhibited by 4 per cent. of sodium chloride; the organism grows freely in nutrient bouillon containing 3 per cent. sodium chloride.

Resistance to fungicides. Tests were made in nutrient bouillon containing various percentages of copper sulphate, phenol, mercuric chloride and formalin. Tubes were planted fairly heavily and after ten minutes a plate was poured from each dilution. In this way the inhibition coefficient and lethal coefficient were determined.

The organism is killed by ten minutes' exposure to copper sulphate

1 : 25; phenol 1 : 500; mercuric chloride 1 : 500 and formalin 1 : 200. It is extraordinarily resistant to copper sulphate, the lethal coefficient for *Bacillus mangiferae* being about 1 : 400. The inhibition coefficient was not determined very exactly; the bacterium grows in a bouillon containing 1 : 1000 copper sulphate, but was inhibited by 1 : 800; the same result was obtained with formalin. The organism grows in bouillon containing phenol 1 : 800 but not in 1 : 600; similarly it grows in mercuric chloride 1 : 3000 but not in 1 : 2500.

Desiccation. A number of sterile cover slips were covered with a film of the organism and placed in sterile ventilated petri-dishes in a desiccator; one of these was removed each day and dropped into a tube of nutrient bouillon. No growth was obtained from cover slips which had been exposed to desiccation for more than ten days.

Nomenclature.

The name *Bacterium vesicatorium* n. sp. is suggested for this organism, which has apparently not previously been described: its chief characters are as follows:

Bacterium vesicatorium n. sp. A parasite of the tomato plant, causing spots on leaves and stems and raised blisters or cankers in the fruit. A motile rod averaging 1–1·5 × ·6–·7 μ, with rounded ends and a single polar flagellum, occurring singly, in pairs, or in short chains. On nutrient agar colonies appear in 48 hours at 30° C. and are finally circular, about 5 mm. diameter, semi-translucent, Naples yellow in colour. On potato produces a copious yellow, spreading growth, butyrous or rather viscid in consistency. Liquefies gelatine and blood serum; no growth in Uschinsky's and Cohn's solutions: grows slowly but well in Fermi's solution. In milk there is a separation of whey, and casein becomes slowly peptonised. Is a fairly active proteolytic agent, destroys starch very slowly. There is no gas formation in sugar bouillon; there is a slight increase in acidity but bouillon ultimately becomes more alkaline. No definite evidence of nitrate reduction was obtained; no indol or phenol is produced in bouillon or Dunham's solution. The organism is strictly aerobic; grows through a wide range of temperature from 5° C. to 40° C.; the optimum temperature for growth is about 30° C.; thermal death point 56° C. Does not grow in alkaline media, but tolerates a fair amount of acid; growth inhibited by 4 per cent. sodium chloride. Group number 211, 2332523.

Two other yellow organisms have been described as causing diseases

of the tomato, *Aplanobacter michiganense* Erw. Sm.(2) and an organism believed to be identical with *Bacillus Lathyri* Manns and Taubenhaus(1).

Aplanobacter michiganense is non-motile, tolerates considerable alkali, growing in − 25 and − 30 peptone bouillon and does not grow in peptone beef bouillon acidified to + 20, + 25, or + 30 with malic or citric acid.

The second organism has four to six peritrichous flagella and grows in Uschinsky's solution: cultures in nitrate broth give a strong reaction for nitrite on the second day.

The three organisms appear to be quite distinct, and *Bacterium vesicatorium* differs considerably from the other two in its effect on the host.

CONTROL OF THE DISEASE.

The organism is not very sensitive to the fungicides usually employed in spraying solutions, and experience has shown that spraying is of little use in combating plant diseases caused by bacteria. This is particularly the case with organisms disseminated chiefly by rain splash and infecting the plant through the stomata.

It appears to be the custom of the market gardeners in the Pretoria district to save seed from their own plants for the following season. This custom is probably in part responsible for carrying over the disease from one season to another. Certain varieties are more susceptible than others, but it has been found practically impossible to discover the name of the varieties usually grown. The following methods are therefore suggested for the control of the disease.

(1) Selection of resistant varieties.

(2) Sterilisation of the seed by means of formalin or mercuric chloride.

(3) A long crop rotation.

(4) Destruction of diseased fruit and of affected plants at the end of the season.

In connection with (3) it will also be of importance that the irrigation furrows shall not be allowed to flow through the old tomato bed to the site selected for tomatoes in the following season.

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EXPLANATION OF PLATE XXVI

Fig. 1. Tomato fruits severely attacked by *Bacterium vesicatorium*.

Fig. 2. A milder form of the canker showing the characteristic blistering in early stages of infection.

Fig. 1.

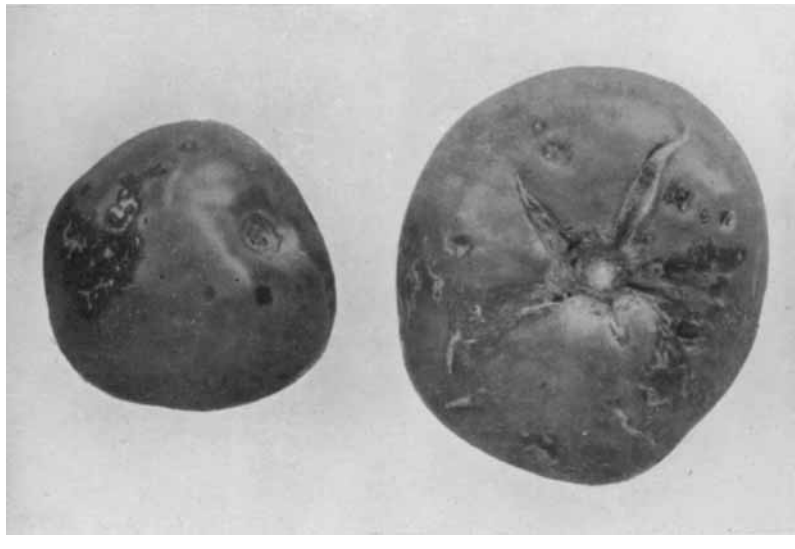

Fig. 2.