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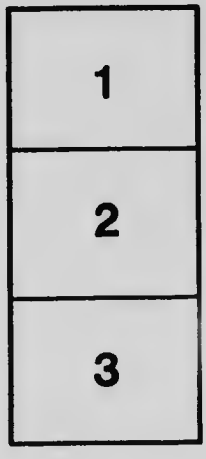


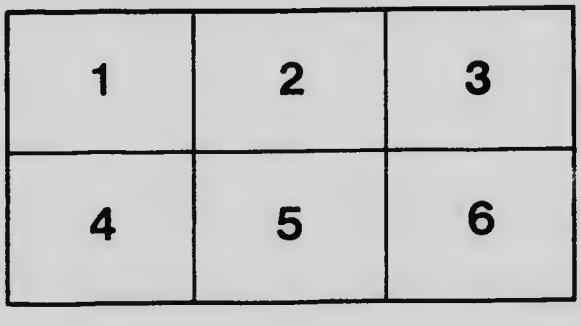

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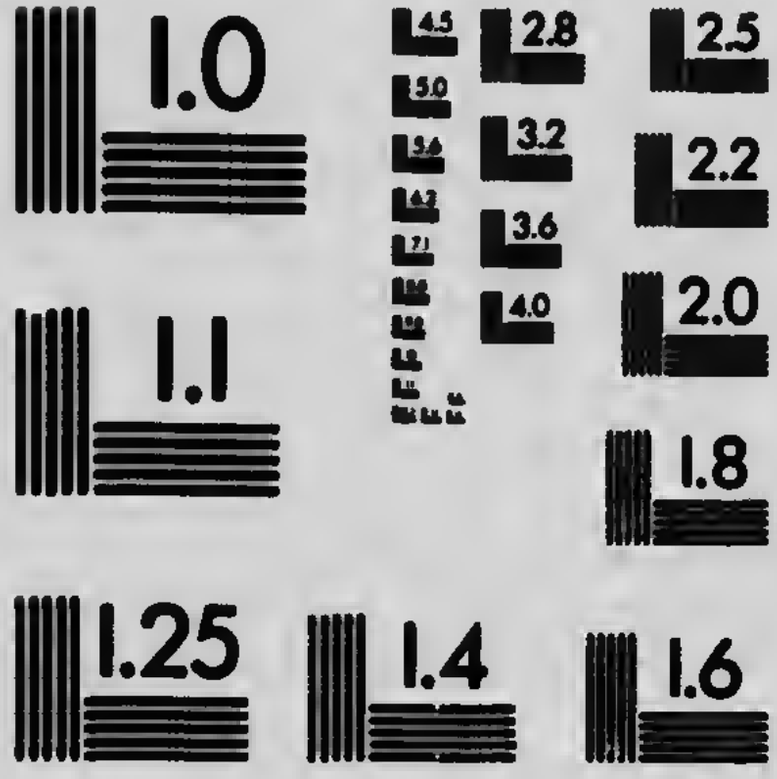


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Ontario Agricultural College and Experimental Farm

# A Bacterial Disease of Cauliflower (Brassica oleracea) and Allied Plants.

By F. C. HARRISON, Professor of Bacteriology.

In the summer of 1901, a market gardener, in the vicinity of Guelph, who made a specialty of growing cauliflowers, complained of a disease which was affecting his plants. Shortly afterwards the cauliflowers in the garden department of the College were also found to be infected, while further investigation in the neighborhood showed that a disease, or rot, of cauliflowers, cabbages and white turnips was quite general and had done considerable damage to these crops.

In the case of the market gardener referred to, more than half of his plants were affected, while in the College garden, about 5 per cent. of the plants were diseased.

Some 40 varieties of white turnips were tested on the trial grounds at Guelph, and most of them were more or less affected with the rot, the percentage of decayed roots varying with the variety, in some cases reaching as high as 64 per cent. The few farmers in the Province, who experimented with the varieties of white turnips that were sent out from this Experiment Station, reported a considerable amount of soft rot.

Later in the same summer I visited a number of farms in the vicinity of Woodstock, and found a varying percentage of white turnips rotting in the fields, although the Swede turnips were not affected, and from conversation with a number of farmers who visited us during the past season, I also gathered that wherever white turnips were grown there was considerable rot during the season of 1901.

## Pathogenesis.

In order to positively demonstrate that the organism isolated from the cauliflower and turnip was the cause of the rotting, the usual requirements were worked out.

1. *Constant association of the Bacillus with the Disease (named Bacillus oleraceae and subsequently described).*

The same bacillus was isolated from diseased cauliflowers from the vicinity of Guelph, and from the garden department of the

College; from diseased white turnips of several varieties taken from the trial grounds of the Experimental Department, and from other parts of the Province, and also from cabbages growing next to the diseased cauliflowers in the garden department

This organism was also found in large numbers on the plate cultures, sometimes in pure culture, at other times in mixed culture, the most common contaminating organism being the *Bacillus fluorescens liquefaciens*. The rot bacillus was so numerous that a loopful of the rotted or pulpy tissue had to be very largely diluted in order to reduce the numbers on the culture plates to about 60-100 colonies per plate. In all these cases, no fungi were present and no mycelium was ever seen.

W. Lochhead, Professor of Biology at the College, who also examined some of the cauliflower material, was also unable to find any mycelium of fungi.

2. *Isolation of the organism and study in pure cultures.*

The isolation of the causal bacillus was quite easy, as it grew well in ordinary 10 per cent. beef peptone gelatine. The bacillus, whether isolated from diseased cauliflower, turnip or cabbage, or from different plants and varieties of the above plants, showed the same characteristics when grown on various media. Comparative studies of the various germs, isolated from different sources, were made, but no essential morphological or cultural differences were noticed. Bouillon,* 10 per cent. gelatine, agar, milk, potato, raw and cooked raw cabbage stems, raw turnip and raw cauliflower were used in the comparative study.

3. *The pure culture of Bacillus oleraceae when introduced into susceptible plants produced the characteristic symptoms of the disease.*

A series of inoculation and cross inoculation experiments were made in order to substantiate the relation of the bacillus to the disease. Thus a series of cauliflower, turnip and cabbage plants were inoculated in the following manner:—

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*These terms, when not otherwise stated, refer to media prepared in accordance with the recommendations of the Laboratory Committee of the American Public Health Association.

Inoculated with a pure culture of the *Bacillus oleraceae* from

| | Cauliflower. | Turnip. | Cabbage. |
|---|---|---|---|
| Cauliflower | x | x | x |
| Turnip | x | x | x |
| Cabbage | x | x | x |

Positive results were obtained in each case, with the characteristic symptoms of the disease, viz., rotting and blackening of the leaves and stem.

These plants were all kept under favorable conditions for the spread of the rot. These conditions are described at length later on in this paper.

4. *The diseased, or rotted, tissues contained the Bacillus oleraceae in huge numbers.*

While their distribution and effect on the tissues was the same as that met with under ordinary field conditions, and re-isolation proved beyond doubt that it was identical with the organism which was inoculated.

5. *The chemical products of the organism also produced the characteristic symptoms of the disease.*

The bacillus was grown on raw turnips and cabbage until all the tissues were completely rotted, and the rotted material was then pressed and the juice extracted and forced through a Chamberland filter. This filtrate, which was found to be sterile, produced softening and rotting when placed on cut surfaces of raw potato, turnip, cauliflower and cabbage. Control cultures of these vegetables, kept under the same conditions as the inoculated slices, remained sterile.

## Pathological Histology.

A microscopical examination of the soft pulp from cauliflowers and turnips showed the presence of enormous numbers of bacteria. No mycelium or fungus spores were present. The bacteria were actively motile. In fresh preparations, free plant cells were visible and many were much disorganized.

A large variety of diseased tissues were fixed in a saturated solution of corrosive sublimate in 94 per cent alcohol, and subsequently imbedded in paraffin. Some 400 sections were cut from various

diseased parts of cauliflower and turnip plants, and were stained by various methods. The most satisfactory results were obtained by staining over night in carbol fuchsin, washing out the surplus stain first with water, and then with 97 per cent. alcohol, clearing in oil of cloves and mounting in Canada balsam. A number of sections were also stained with anilin blue. The latter method gave fair results; but the former method was the more satisfactory.

Completely rotten cauliflower or turnip was difficult—in fact it was almost impossible—to imbed in paraffin, as the whole mass fell to pieces when thrown into alcohol. Portions of petiole, stem, or flower of cauliflower, where the disease was just starting and pieces of tissue in a more advanced stage from which most of the soft parts had been cut away, furnished the best material for study.

Cross sections showed the bacteria in the intercellular spaces, where they increased rapidly and as soon as sufficient enzyme was elaborated, it softened the middle lamella and permitted the bacteria to penetrate between the cells. These enzymes had a marked action on the cell-wall, which gradually swelled up and lost all trace of its striated character. The cell wall at this stage also lost very largely its faculty of taking up the stain, and sections stained with carbol fuchsin showed the enormously thickened cell-wall, faintly stained a pale pink, while adjacent healthy cell-walls were deep red in color and showed very plainly the middle lamella and striations.

The figures 9-10 show the different stages in the destruction of the cells by this bacillus. Fig. 9 shows the bacteria in some numbers in the intercellular spaces, some are just beginning to penetrate along the middle lamella. At this period, the cell-wall is stained deeply. The last stage, just before the absolute collapse of the tissues, may be seen in Fig. 10, in which the lumen of the cells is very small due to the enlarging and softening of the cell-walls which now stain even more faintly than before. The bacteria have also enormously increased.

Sections of pieces of turnips affected with the rot, showed slightly different features; although the action of the bacillus was the same.

Turnips cells have much thinner walls than the cauliflower petiole, or stem; consequently, when attacked with rot they collapse

more rapidly, and the marked swelling which we see in the cauliflower is either absent or less well developed.

Fig. 11 shows the bacteria in the intercellular spaces, the slight swelling of the cell walls and the beginning of the disorganization of the cells.

## Inoculation Experiments.

It was found impossible to perform trustworthy inoculation experiments with plants grown in the field, as in this locality the white turnips and caulitlowers were more or less affected with the rot, hence it was necessary to grow fresh plants, from clean seed, in soil which had never been used for growing these plants. On account of the lateness of the season, the plants were grown under glass and all the following experiments, unless otherwise stated were conducted on pot-grown plants in a greenhouse with an average day temperature of 20-25° C.

### *Series 1.*

Three plants each, of cabbage, caulitlower, rape, white turnip, swede turnip and kale were inoculated with needle punctures through the parenchyma of the leaves. The platinum needle was dipped into a twenty-four hour bouillon culture of the organism, and from three to five punctures were made on one or two leaves of each plant.

Results :—

*Cabbage.* In two days, the inoculated leaves were flaccid and whitish brown in the vicinity of the punctures. This area increased slowly for five days and then dried out.

*Caulitlower.* In two day, there was a flaccid, papery area surrounding the punctures; in five days, all leaves were rotted and had dropped down parallel with the stem of the plant.

There was no subsequent infection of the stems: the leaves gradually dried off at the base of their petioles.

*Rape.* No results followed inoculation.

*White Turnip.* Slight infection was produced around the punctures; but the lack of moisture seemed to hinder further growth.

*Swede Turnip.* No results followed the inoculation.

*Kale.* No results followed the inoculation.

*Control Plants* of each species, pricked with a sterilized needle remained perfectly healthy.

This series, therefore, showed that the inoculation of the germ made with needle pricks in the parenchyma of the leaves, pro luced more or less disease in cauliflower, cabbage and white turnips. The absence of sufficient moisture in the greenhouse however, prevented the disease from becoming thoroughly established.

*Series II.*

This series was a duplicate of Series 1, only an agar culture of the organism was used instead of a bouillon culture.

The results were similar to those in series 1, with the exception of the Swedes, which became slightly infected. In one plant, a whole leaf rotted and fell off the plant; but the petiole subsequently dried off.

*Series III.*

In this series, three plants each of cabbage, cauliflower, rape, white turnip, Swede turnip and kale, were inoculated with needle punctures through the veins of the leaves. The needle was dipped in a 24 hour old bouillon culture, and from 3 to 7 punctures were made on one or two leaves of each plant.

Control plants were punctured in the same manner; but with a sterilized needle.

RESULTS:—

*Cabbage.* In two plants there was no apparent change; in the other plant a small cavity 15 m.m. long and 5 m.m. wide had been formed on the mid-rib by the rotting of the cells; but this subsequently dried out and the leaf remained healthy.

*Cauliflower.* No results followed the punctures of the veins and mid-rib.

*Rape.* In ten days, the leaves of all three plants were slightly affected, the vein was split, and a watery exudation was present on the surface. The inoculated leaves began to droop; but the disease progres- no further, and the wound became callused and partly healed.

*White Turnip.* The inoculated leaves behaved in exactly the same manner as the inoculated rape leaves.

*Swede Turnips.* No results followed the punctures of veins and mid-rib.

*Kale.* No results followed the punctures of veins and mid-rib.

This series, as a whole, gave less harmful results than the inoculation of the parenchyma. In cabbage, rape, and white turnip some slight disease symptoms were produced; but there was no general infection of the plant. Lack of moisture seemed again to prevent the rapid development of the disease and perhaps the different composition of the vascular cells hindered the formation of cell-destroying enzymes.

*Series IV.*

In this series, three plants of each of the five species already mentioned were used. A small portion of the epidermis on the upper part of the base of a leaf-stalk was removed and two loopfuls of a bouillon culture were rubbed on the exposed portion.

RESULTS:—

*Cabbage.* The leaf-stalk rotted through in three days and the leaf fell off from its own weight. The rotting did not effect the stem, as the diseased tissue dried out.

*Cauliflower.* There was slight rotting, or softening, in two days, and in five days the leaf rotted off, and the portion next to the stem dried up.

*Rape.* Slight rotting occurred for three days, when the wound dried up.

*White Turnip.* In two days, the softening of the tissues at point of inoculation had extended across the petiole. In five days, the leaf fell off, the rotting extending all through the stalk. The infected base then dried and healed.

*Swede Turnip* Behaved the same way as the white variety.

*Kale.* In three days, the leaves were so much rotted through that its own weight caused it to break off. The wound then dried up.

*Control Plants* with the epidermis removed, but with no inoculation, remained healthy.

In the above account of this series, the results are given for only one plant of each species, the two remaining plants of each lot behaved in a similar manner. These plants showed considerably more disease than those inoculated by vein or parenchyma punctures. This was probably owing to more moisture being present. At the juncture of the stalk with the stem, small drops of water would collect from the leaf surface, thus providing more moisture for the bacteria. As soon

as the leaf had rotted off, or fallen by its own weight, no more water collected in the angle of leaf and stem and the tissues rapidly dried up.

*Series V.*

In this series one plant of each of the species already mentioned, was used.

The lowest leaf of each plant was cut off, about an inch from its juncture with the stem. The cut surface was then rubbed over with a platinum loop, charged from a bouillon culture of the organism. Check plants received the same treatment, without inoculation. The results were very similar to Series IV. and need not be repeated in detail. Rotting usually extended downwards toward the stem for about half an inch, or even as far as the juncture with the stem, and then dried ont. The check plants showed no signs of rotting.

*Series VI.*

Three cauliflower plants were inoculated at the base of the petiole with a bouillon culture by means of two or three needle pricks. In two days, there was rotting, the affected area being 3 x 7 m.m. One of these plants was then placed under a bell-jar and at the end of six days the inceulated petiole and leaf were completely rotted, the leaf fell off in a mushy mass and the rot spread to the stem, infecting the whole plant.

The flower head, which was well developed and quite white, gradually changed to a brown color and then rotted. The plant was practically destroyed 14 days from infection.

The diseased area in the other two plants (kept in the same state except that no bell-jar covered them) gradually dried out, leaving a small hole caused by the rotting of the tissues.

Subsequently, this experiment was repeated several times with the same results, the plant under the bell-jar rotting leaf by leaf, with final rotting of the flower

Fig. 2 shows the beginning of the rot, a leaf (the one inoculated) having rotted through at the base of the petiole and fallen off. The stem of the plant, just below the crown, was darkened, due to the softening and discoloration of the tissues and the lower leaves are beginning to wilt, owing to the cutting off of their supply of nourishment. The leaves of the healthy plant, as shown in Fig. 1, are erect and rigid and comparison with the inoculated plant in Fig. 2 shows

the different position of the infected leaves which gradually declined until they were at a right angle with the stem and finally fell or broke off at the base of the petiole.

Fig. 3 shows a plant at a later stage. Most of the leaves are affected and the flower has become brown, and a part of it has completely rotted to a pulpy mass.

*Summary.* The experiments made in this series plainly show the relation of a humid atmosphere to the disease. When the air is full of moisture, it affords the best conditions for the rapid growth of the micro-organism on the exterior of the plant and it favors the production of a large amount of cytase-like enzyme which quickly causes the softening and destruction of the tissues.

*Series VII.*

Under field conditions, one frequently noticed that the leaves seemed perfectly healthy; but the flower was affected. This fact seemed to point to the probability that the flower-head might be very susceptible to the disease; or that the organism might be able to penetrate the unbroken epidermis. In order to test these points, three well developed cauliflower plants were infected in the following manner:

No. 1.—Water drops on the leaves were inoculated with a twenty-four hour old bouillon culture.

No. 2.—A small piece of softened tissue, taken from the interior of an affected petiole with a sterilized wire, was laid on the surface of the healthy flower.

No. 3.—A loopful of bacteria taken from the surface of an agar-slope culture 24 hours old, was gently rubbed over a portion of the dry surface of the flower.

Two check plants were well watered with a syringe and kept under the same conditions as the above, viz., in the warm greenhouse, which has a very humid atmosphere, and an average day temperature of 28 degrees to 34 degrees C, and a night temperature some 10 degrees lower.

RESULTS :—

In two days, No. 1 showed slight discoloration of the treated area. In four days, softening commenced; and in 8 days the whole flower was a pulpy mass.

No. 2 behaved in a similar manner, but the discoloration and softening started earlier and the flower was reduced to a pulpy,

blackish mass in 6 days from the time of inoculation. Fig. 4 is a photograph of this plant on the fifth day, the whole flower mass having dropped and turned black.

No. 3 showed no signs of disease even after fourteen days.

N. B.—No water was syringed on the flower of this plant.

The check plants were syringed every day and remained absolutely healthy.

*Summary*: These experiments seem to show that, provided sufficient moisture is present on the interior of the flower of the cauliflower, infection by *Bacillus oleraceae* can and does take place. If small portions of the rotted tissues were placed upon the flower of healthy plants, infection took place, in spite of the mechanical resistance of the cuticle and epidermal cells. Many plants, under field conditions, were found with the flower alone infected.

*Series VIII.*

In this series three healthy white turnips (Greystone variety) were inoculated at the crown with two needle punctures. A check plant was treated in a similar manner, but with a sterilized needle.

Nothing was noticeable for two days; but on the third day, a small drop of water was exuding from each puncture of the inoculated plant and on the fifth day, rotting to a depth of 5 m.m. had taken place. In 14 days, the plants were dead, Fig. 8 is a photo of one of these plants 9 days after inoculation.

The check plant remained perfectly sound and healthy.

Subsequently the experiment was repeated, with the same results. Fig. 6 shows the extent of the rotting process, 6 days after inoculating with one needle puncture while Fig. 7 shows the most complete rotting 10 days after inoculation.

The Greystone turnip in all the inoculations was very susceptible to this disease.

*Series IX.*

Three healthy Swede turnips were inoculated at the crown with two needle punctures. A check plant was treated in a similar manner; but the punctures were made with a sterilized needle.

Two days after inoculation, there was a slight softening of about 2 m.m. in diameter around the puncture. In five days the area was only slightly larger and there was no further increase of the disease; although the plants were kept under observation for three weeks.

The check plants remained sound.

Subsequently this experiment was carried out in the tropical house, in a warmer and moister atmosphere. The results of the inoculation were, however, the same as before, a slight local rotting, followed by a gradual drying up of the infected area.

It seems that although the Swede turnip is not wholly immune; yet it has considerable natural immunity from this disease. This is proved partly from the experimental date above presented, and partly from the fact that we found very little disease among Swede turnips growing in the fields; although on our own grounds, some lots of Swede turnips were growing alongside white turnips which were very badly infected with the disease

*Series X.*

In this series, two white turnip plants and two cauliflower plants were watered with about half a litre of water in which a bouillon culture of the *Bacillus oleraceae* had been poured. This watering was again repeated two days later and all the plants, including two check plants, watered without the addition of culture were kept under observation for about five weeks. No disease developed in any of the plants, which seems to indicate that the *Bacillus oleraceae* does not gain entrance to the plants through the root hairs.

One of the turnip plants of this series was subsequently inoculated at the crown and rotting followed in the course of a few days, thus showing that the turnip plant is susceptible to the disease.

*Series XI.—The Virulence of Old Cultures.*

In order to test the pathogenic power of old culture, both a cauliflower and a white turnip were inoculated with an agar culture of the rot bacillus, 2 1-2 months old, being the seventh transfer after isolation.* The cauliflower was inoculated, by means of needle pricks in the leaf, and kept in warm, moist place. In three days the first signs of rotting were noticed and the disease subsequently ran its usual course, ending in the complete destruction of the plant.

The turnip was inoculated with a puncture at the crown, which gave rise to the rotting and final destruction of the plant.

*Summary*: These experiments prove that the bacillus is able to retain its virulence, for a considerable length of time, in artificial agar cultures.

* I have since tried the virulence of cultures which have been on agar for more than 18 months. The cultures produced the characteristic rot in inoculated plants.

## Inoculation of Freshly Gathered Vegetables.

In all the following experiments, the vegetables were obtained fresh from the garden. These vegetables were thoroughly washed in running tap water and then, by means of sterilized cool knife, slices were cut and placed in Petri dishes. These slices were immediately inoculated by rubbing a platinum loop, (which had been charged with a bouillon culture) over their surface. In all cases, uninoculated slices of the different vegetables were also kept in order to check any contamination from germs growing on the surface, or from those which might develop from insuffiecent care in the preparation of the slices. In no cases did such uninoculated slices decay or rot.

All the cultures were kept at room temperature, which varied from 20-28° C.

*Cauliflower.* The whole plant, after rinsing, was cut down the centre and placed in a large dish, and then inoculated. In two days, the stem and flower had discolored to a dirty brown, and softening extended downwards about 20 m. m. There was a very disagreeable smell. In 7 days, the whole of the plant was completely rotted could be cut down and across with a platinum needle and the dirty brown color was darker. Gas bubbles were present in all the decayed parts,

*Cabbage.* Cabbage plants, treated like the cauliflower, underwent the same change and in 7 days there was a complete softening of the whole plant, with the production of a very bad odour.

*Turnip* (White). In two days a whitish wet growth spread over the surface of the slice. There was a pale brown discoloration of the infected part. The rot extended downwards to a depth of 10 m. m. In 5 days, complete softening had occured. The smell was slightly disagreeable.

*Turnip* (Swede). Decay in the yellow turnip was usually slower than in the white, but depended largely on the amount of moisture present. Where the turnip was very moist, decay advanced rapidly; but on drier surfaces decay was slower, and at times no growth took place.

There was considerable exudation of water on the inoculated part and abundant brown-black growth which softened to a depth of 4 m.m. After two days growth, gas bubbles were present. Frequently, a whitish moist growth was noticed instead of the brownish-black, due either to difference of water content or to difference of

variety. Observations were madeon some thirty turnips of several different varieties.

*Rape.* In two days, there was a water soaked appearance, slight smell and slight softening. After 7 days, the slice, 12 m. m. thick, was completely softened, the odour was bad and on the surface a white, moist growth was present.

*Radish.* In 24 hours, the surface was covered with a copious watery exudate. The affected area was darkened and softened to a depth of 6 to 7 m. m. In two days, the radish had completely softened, was blackish in appeance with a thin, dirty white skin on the surface. The pigment of the skin was dissolved and colored the condensation water. In 6 days, the radish was completely dissolved, forming a thick, dark, liquid.

*Parsnip.* In two days, softening to a depth of 10 m. m had occurred. There was considerable water lying in the form of drops on the inoculated portion. The growth on the surface was moist and yellowish white in color and around the growth the parsnip was brownish black. In 7 days, the slice, 12 m. m. thick, was completely softened. There was no smell.

*Carrot.* In two days, there was abundant growth (both on the red and yellow portions of the carrot) which was transparent and very wet, and the carrot had softened to a depth of 4 m. m. The growth on the surface subsequently became whitish, and complete softening occurred in 6 days. The yellow portion of the carrot was somewhat darkened. There was no smell.

*Carrot.* (White). Abundant, whitish green, sputum-like growth, raised and wet. Outside the growth, there was a yellow to yellowish brown discoloration, especially around the vascular ring and softening had occurred to a depth of 5 m. m. In 5 days, the slice was completely softened, and the odor was pungent.

*Mangel.* In two days there was a whitish growth on surface with slight softening. In 7 days, the softening had increased; but not to the same extent as on carrot or parsnip. There was also some discoloration.

*Beet.* No growth and no discoloration.

*Sugar Beet.* In 24 hours, no softening and no growth. In 48 hours, there was a very slight growth on the surface while the softening was scarcely 1 m.m. in depth. In three days, the growth increased, was white and moist; but there was very little if any

increase in softening. No further growth took place even on slices kept for 10 to 20 days.

*Potato.* It grew with great rapidity on raw potato, in the form of a moist, creamy, yellow, spreading growth with marked softening In five days, slices 20 m.m. thick were completely softened and could be cut with a platinum needle. There was a depression in the centre and an ammoniacal smell. Nessler's reagent gave a distinct coloration to the water extract of the inoculated potato, indicating the presence of ammonia. Tincture of iodine did not color the inoculated potato blue, the starch was, therefore, destroyed.

*Celery.* In two days there was a moist whitish growth with yellowish discoloration and considerable softening. In 7 days the softening was more extensive and the discoloration brown.

*Tomato.* (ripe). After two days, there was a slight growth at seat of inoculation. In 7 days there was rotting and cracking of the skin with whitish growth extendiug from the cracks. The inside was quite soft.

*Green Tomato* behaved in the same way, but growth was somewhat quicker. The first indication of the disease was slight discoloration or premature ripening of the inoculated part followed by exudation of water and softening and later by cracking of the skin and progressive softening.

*Artichoke.* (Jerusalem). In 24 hours the surface growth was moist and dirty white in color, and there was softening beneath surface to a depth of about 7 m.m. outside the circle of growth the tuber had become red brown in color. In 48 hours the softening was deeper with pitting of the affected portion. Color around the affected portion became reddish. In 4 days the whole tuber was soft.

*Asparagus.* The upper third portion of the Asparagus stem (the edible part) was the first part to rot, presenting a water-soaked appearance. On the third day after inoculation, the middle third commenced to soften and on the fourth day, the lower third began to do the same. The pieces gradually collapsed and a dirty white skin formed on the surface.

*Horse Radish.* Softening of the tissue, even of the hardest and most woody parts, to a depth of 2 - 4 m.m. occurred in 48 hours. There was a whitish growth on the surface, gas bubbles formed, and the centre of the stem fell in. The odor on the third day was quite pronounced and very objectionable.

*Rhubarb.* The organism grew on the cut surface of rhubarb only when the petiole was well saturated with water. There was a whitish growth on the surface, and softening, especially of the tissues between the bundles. Long, slimy threads, a foot or more long, were drawn out by touching the affected portion with the platinum needle.

*Onion.* On the slices of onion, strongly acid to litmus, there was considerable growth in 24 hours. The tissue was softened and the parts affected were slightly yellow in color. In three days the growth was quite yellow, a few gas bubbles were seen on the surface, the tissues were completely softened and there was a foul, nauseating odor.

Twelve onions, of three different varieties, were inoculated; but all rotted in the manner above described.

*Morphology.* The bacillus varies considerably in length. From agar culture grown at 20°C. for 24 hours the bacilli vary from 1-3$\mu$ in length, the average is about 2$\mu$, the width 0.6$\mu$. In old (3 month) agar cultures the bacteria are shorter. In gelatine (3 days at 20° C.) the average length is 1.4$\mu$ width .5$\mu$. In beef bouillon (48 hours at 25° C.) the average length is 1.2$\mu$ and the average breadth .7$\mu$. In wort (12.2 Ball.) the bacilli are longer, averaging 4$\mu$ long and 1$\mu$ wide. The longer rods are frequently bent and will stain deeper at the poles than at the middle.

On rhubarb the bacilli are short and plump and many are ovoid in shape. They are about 1.1$\mu$ long 0.8$\mu$ wide.

In sections of diseased cabbage and cauliflower the bacilli vary greatly in length, averaging about 2$\mu$ long and 0.6$\mu$ wide.

The ends of the bacillus are always rounded, occasionally bent rods may be seen and short chains may form; but usually the bacillus occurs singly.

*Flagella.* The bacilli taken from agar cultures 24 hours old are very motile, as are also bacilli from other media, (wort, gelatine, cauliflower). The linear progression is fast and the rotary motion of the cell is quite noticeable, the rear end of the motile rod moving in a larger circle than the front.

The bacillus has peritrichous flagella, seven to thirteen in number, which stain well by Van Ermegen's method. (See Fig. 11).

*Spores.* No spores have been observed. *Involution forms* are found. Thus the bacteria may be ovoid, or long and bent, occasionally club-shaped individuals are seen.

*Stains.* The bacillus from gelatine culture stains well with gentian violet, not so well taken from agar. Carbolfuchsin gives good results, for cover glass preparations and also for sections of diseased tissues. It stains slowly with methelene blue. In three minutes the bacilli are only very faintly colored.

It does not accept Gram's Stain.

CULTURAL CHARACTERS.

*Bouillon* at 28° C. In 24 hours the culture was very turbid, no pellicle and heavy sediment. In 48 hours the turbidity increased. The sediment was heavier and flocculent masses were deposited on the sides of the tube. A ring formed at the surface. In three days a pellicle formed which settled on slight disturbances. In six days the pellicle and ring on undisturbed tubes were heavier.

Media remained turbid (4 weeks).

In cabbage bouillon with 1 per cent. of peptone the organism grew very well, and produced heavy turbidity and copious sediment in 24 hours, a slight ring formed at the surface on the fifth day, otherwise there was no change.

*Gelatine.* On plate cultures of nutrient gelatine the colonies were visible to the naked eye in 24 hours. They were punctiform and round. With $\frac{2}{3}$ objective they appeared round, homogenous, with weak refraction and entire edges. In 48 hours the surface colonies were 2 m.m. in diameter, liquefying, round, greyish white in color and with a ring in the centre composed of deposited bacilli. Under the microscope ($\frac{2}{3}$ obj.) they were round, coarsely granular, the centre was grumose, and the edges slightly ciliate. Deep colonies were considerably smaller, less than 1 m m. in diameter, round, internal structure moruloid, edges of some colonies were entire, others with effused growth. There was considerable variation.

In three days the surface colonies were from 3-5 m. m. in diameter, round, greyish color, liquefaction shallow, often with one or two concentric rings. Under the microscope the edges appeared ciliate, the centre moruloid, and the rest of the colony granular. The bacterial masses might be seen in motion.

The deep colonies were smaller with darker centre and ciliate edge, the fringe being longer and more wavy and interwoven than in the surface colonies.

In 4 days the colonies were larger in size otherwise there was no change.

In stich cultures at 20° C. there was a white growth along the line of puncture in 24 hours. Slight liquefaction at the surface, ½ m.m. in diameter, along the line of inoculation the growth was slightly heavier. In 4 days the liquefied area reached the sides of the test tube and thereafter liquefaction was stratiform.

There was often considerable difference in the rate of liquefaction, at times the whole tube might become liquefied, at other times only the half.

*Wort gelatine.* Stich cultures. The organism grew very well in this medium, with shallow pit liquefaction, followed by stratiform liquefaction to a depth of 5 m.m. in 7 days (20° C.) Growth stopped when about half the medium was liquefied.

*Whey gelatine stich cultures.* There was a crateriform depression 12 m.m. in diameter, with deposition of a flocculent mass in the centre of the pit in 24 hours. In 48 hours liquefaction had extended to the sides of the tubes and downwards to a depth of 2 m.m. at the sides and 3 m.m. in the centre. In three days the liquefied portion was 5 m.m. deep and growth ceased when 9 m.m. deep. A few gas bubbles appeared in the gelatine at some distance from the line of puncture

*Agar.* On agar plates at 28° C. colonies were not characteristic. Surface colonies spread very fast, as thin grey expansions, which varied greatly in shape. Deep colonies were dense, punctiform, round, or elliptical; in fact, there seemed every variety of shape Agar slope cultures at 28° grew very rapidly over the surface as a moist, thin, whitish growth, slightly opalescent by transmitted light. There was considerable deposit of the bacilli in the condensation water. The growth became more massive with age, otherwise there was no change.

*Carbo-hydrate agars.* Slope and shake cultures were made in agars containing 2% of the following carbo-hydrates: Glycerine, saccharose, lactose, glucose, and maltose. The media was made from Liebig's Extract of Meat, reaction neutral. Check cultures were made in agar without carbohydrates, no gas formed in these.

In glycerine agar the growth was more abundant and whiter than on plain agar. No gas in the shake culture and heaviest growth on the surface.

In saccharose agar amount of growth exceeded that on plain agar. In shake culture a few gas bubbles were present.

In lactose agar the growth exceeded that on plain and glucose agar and was more waxy looking. In shake cultures there were numerous lenticular gas bubbles. In 48 hours there was an increase in the number of gas bubbles, and the agar was rent across the tube. In three days the clear space between the rents was wider, otherwise no change.

In glucose agar the growth was about the same as on plain agar, if anything, slightly heavier. Shake cultures contained small gas bubbles all through agar. No further change after three days growth

In maltose agar growth was very abundant, moist and shiny. There was more tendency to spread. Growth exceeded that or plain agar.

In shake cultures very few gas bubbles appeared in 24 hours. In 48 hours, a few more bubbles made their appearance and no further change took place after the third day.

*Neutral red agar* (with 2 per cent. glucose at 28° C.) In 24 hours, there was no change in color, a white growth along the line of puncture and a moist white growth on the surface. A few gas bubbles were present. In 48 hours, there was no change in color but more growth. On the sixth day, the color was lilac violet and no further change occurred. (20 days.)

*Milk.* A number of milk tubes + 1.5 per cent., inoculated with 1 oese of a fresh bouillon culture and held at 25° C. shewed no change for 24 hours. In two days the milk was thicker but did not coagulate until the third day. The curd was soft and even, but thicker at the bottom of the tube. On the fourth day, the curd shrunk and on shaking, the whey separated out. The curd was flaky with a few gas bubbles in it. On the fifth day, the whey on the surface was clear and remained so. In eight days, the curd shrunk and occupied one-third the depth of the medium. No further change took place. The whey from milk cultures tested for proteolytic enzymes, by means of the caustic potash and copper sulphate test, gave a violet color indicating the presence of peptones. Another portion of whey was mixed with ammonium sulphate to precipitate the proteids, and the filtrate from this precipitate was also tested in the same way, and with the same results.

The odour of milk cultures after heating was agreeable, resembling cheese curd. No odour could be noticed in the cold cultures.

The viability of the organism in milk was as follows: Cultures, 25 days old, living; 35 days old, living; two months old, dead.

*Litmus Milk* at 25° C. In 24 hours the color compared with the control tubes was appreciably different. In 48 hours, the color was lighter, between lilac and livid (Saccardo 48 and 49), the milk was thick but not coagulated. In three days, the milk coagulated into a soft even curd with about 10 m. m. of whey on the surface and a few gas bubbles in the coagulum. Colour lilac (Saccardo 48). In four days, the curd had shrunk leaving a clear whey on the surface. The curd when shaken separated into flaky masses and gas bubbles were fairly numerous through the curd on the surface. The color of the curd at the bottom of the tube was white, the upper portion, lilac. On the fifth day, the whey was slightly tinged with color; the lower half of the curd was white and the upper half, lilac. On the eighth day, there was only a small red ring of color at the top of the curd. On the twelfth day, the lilac color again returned.

*Blood Serum* at 25° C. On blood serum good growth occurred,—first, as a moist slightly spreading growth, later becoming heavier, more opaque and opalescent by transmitted light, The condensation water was turbid. Slight liquefaction was visible on the 8th day, and in 21 days most of the sloped surface became liquid and no further change occurred. The bacilli from blood serum shewed banded and bipolar staining with carbol-fuchsin.

*Egg Media.* (Dorset's method). Good growth occurred on egg media, spreading over the entire surface. No liquefaction occurred in 24 days and the growth was not characteristic.

*Dunham's Solution* at 25° C. In Dunham's solution there was slight growth; and uniform turbidity in 24 hours, the cloudiness increased and a slight sediment formed which became flocculent in four days. Eight-day cultures gave a very slight indol re-action, while in 15-day cultures the re-action was more marked.

In Dunham's solution with Rosolic acid (in the same proportion as used by Jones), the salmon pink colour almost entirely faded in 24 hours. In 48 hours, the tubes were quite decolorised and remained so three months. Rosolic acid bouillon was decolorised in the same way. This change shewed the formation of acid.

*Synthetic Media.* In Uschinsky's medium there was turbidity with some sediment in 24 hours at 25°C. A slight pellicle formed in 48 hours, and the body of the media became more turbid with increase

of sediment. In seven days there was a thick pellicle and heavy sediment, but the body of the liquid was almost clear. In 15 days the pellicle gradually sank, the body of the liquid was pale yellow, and there was a copious sediment.

In Fermi's medium, there was slight turbidity in the upper third of the medium and a very slight sediment in 24 hours at 25°C. A thin pellicle formed in 48 hours and the top of the liquid was very cloudy. On shaking, the pellicle produced turbidity throughout the entire medium. The growth at four, seven and fifteen days resembled growth in Uschinsky's medium.

In *Lager Beer Wort*, 12.2 Ball. good growth occured, at first turbid and with considerable sediment. The liquid was several shades lighter in color, and a few gas bubbles were seen. In three days, the wort was quite clear, with heavy fine sediment and no pellicle. No further change occurred.

*Cooked Vegetables.* Generally speaking. the growth on cooked vegetables was abundant, but the softening action of the organism on the cooked vegetables was not always as marked as its action on raw vegetables; in other words the production of cystase was more marked when the organism was placed upon slices of raw vegetables.

*Potato* prepared according to Roux's method, reaction slightly acid to litmus. In 24 hours there was a moist, shiny, spreading growth distinguishable from the potato by the glistening appearance. The growth became more massive and on the drier slices the growth was more waxy looking and straw colored. No further change occurred and the potato slice was slightly softened, it could never be cut quite through with the platinum needle.

On potato cylinders prepared by immersing half the slice in water, the growth was moist and spreading. The water was at first turbid with much sediment, consisting of particles of softened potato, pure white in colour. In seven days the liquid became yellow in colour and the sediment was pure white. Gas bubbles were also present.

The immersed portion of the cylinder was softened, but in twenty days the core above the water was still firm and could not be cut with the platinum wire, a control test on raw potatoes from the same source caused complete softening of the tissues in three days.

In other tests of potatoes there were minor differences—Thus the growth would be dirty yellow, or honey yellow in colour, and the

moist and glistening appearance on some potatoes would be changed to a dull waxy looking growth.

Differences in the rate and extent of softening also occurred. In all some 60 tests were made on potatoes.

On cooked carrot at 28°C, there was a moist spreading growth with complete softening in three days.

On cooked sugar beet there was a flat, shiny, moist growth; gas bubbles were present, and the cylinder was completely softened in four days.

On cooked beet-root there was a whitish spreading growth, the beet was discoloured (brown-green), and there was a white, slightly raised moist growth, with complete softening.

On cooked onion there was a moist, dirty white growth, the onion was completely softened and fell to pieces. The odor was foul and nauseating.

*Temperature relations.* The optimum was about 30° C.; there was fast growth at 25° to 28°. Good growth occurred at 20° and at 37.5° C. the growth was better than at 20° C.

The maximum temperature was in the neighborhood of 42° C.

The minimum temperature was in the neighborhood of 5° C.

*Thermal death point.* The thermal death point was determined by Sternberg's method. The temperature of the bath during the time of exposure was varied about .25 of a degree. A temperature of 55° C. for 10 minutes was the thermal death point of the organism.

*Relation to free oxygen.* The aerobic growth was better than the anaerobic, but the organism grew in the closed arm of fermentation tubes, and in deep stich cultures.

Agar, potato, gelatine slope and litmus milk cultures were grown for eight days in a Novy jar in an atmosphere of hydrogen.

There was slight growth limited to the needle track on the agar slope; slight growth but no liquefaction of the gelatine slope culture; very slight growth on the potato; slight growth and change of colour in milk, but no coagulation. The cultures when taken out of the Novy jar grew vigorously. The bacilli from the agar culture were rather shorter, averaging about 1.5$\mu$, in length.

*Nitrate broth* at 25° C. In nitrate broth growth was better than in Dunham's solution. The media becomes turbid with first a fine and later a flocculent sediment. No pellicle formed.

The tests for nitrites on the 9th day were negative, on the fifteenth day there was a faint pink tinge with the napthylamine and sulphanilic acid test. Control tubes kept under the same conditions gave no indication of nitrites.

*Indol production.* See Dunham's solution.

*Development of odors.* The strongest and most offensive odor developed on onions, both raw and cooked. There were objectionable odors from cultures on cabbage, cauliflower, horse radish, rape and turnips.

The odor on white carrot was pungent. Milk cultures when heated give an odor of fresh curd.

*Production of hydrogen sulphide.* Strips of filter paper moistened with lead acetate were suspended over bouillon and potato cultures. In both cases the paper turned black indicating the production of hydrogen sulphide.

*Production of acid.* Acid was produced in all sugar media, in milk, in Dunham's solution, and in bouillon.

*Production of alkali.* Ammonia was produced in potato cultures in considerable amount, it could be detected by the smell, as well as more exactly by Nessler's reagent. Cultures on several other vegetables (turnips, carrots, beets) also gave the Nessler reaction.

*Relation of growth to acid and alkali.* Various quantities of normal soduim hydrate and normal hyrochloric acid were added to neutral broth. The following results were from 48-hour old cultures kept at 28° C.

| | | | | | |
|---|---|---|---|---|---|
| Neutral broth. | | | | | Turbid and considerable sediment. |
| Alkaline broth | + | 10 | c.c. of normal | NaOH per litre: | Same as neutral tubes |
| " | " | " | " | " | " " |
| " | " | 20 | " | " | " " |
| " | " | 30 | " | " | Turbid and slight sediment. |
| " | " | 40 | " | " | Very slight turbidity. |
| " | " | 50 | " | " | Quite clear, no growth. |
| Acid broth | + | 10 | c.c. of normal | HCl per litre: | Turbidity greater than in control. |
| " | " | 20 | " | " | Same as neutral tubes. |
| " | " | 30 | " | " | Slight turbidity. |
| " | " | 40 | " | " | " " |
| " | " | 50 | " | " | Very slight growth. |

*Effect of sunlight.* Cover glass preparation made from 24-hour old bouillon culture and exposed to direct sunlight gave the following results:—

| | | | |
|---|---|---|---|
| 15 minutes | + / + | 30 minutes | + / + |
| 45 " | + / + | 1 hour | + / — |
| 1.15 hours | + / — | 1.30 hours | — / — |
| 1.45 " | — / — | 2 " | — / — / + |

$\frac{+}{-}$ Living on some cover glasses but dead on others; + living; — dead.

Agar plates inoculated with 1 oese of a fresh bouillon culture and exposed to direct sunlight gave the following results:

Control plates not exposed 1,200-2,000 colonies per plate.

| | | |
|---|---|---|
| Plates exposed 15 minutes | centre, 2-5 | colonies per plate. |
| | edge, 220-400 | " " |
| " " 30 " | centre, 0-10 | " " |
| | edge, 130-200 | " " |
| " " 45 " | centre, 0-1 | " " |
| | edge, 10-60 | " " |
| " " 1 hour | centre, 0 | " " |
| | edge, 10-60 | " " |
| " " 1.30 hours | 0 | " " |
| " " 2 " | 0 | " " |

The plates were exposed in the afternoon between 2 and 4 p.m. in the month of October. Temperature of the plates, 30° C. Latitude, 43°, 30.'

*Resistance of the organism to desiccation.* For determining the resistence to dessication cover glass preparations were made from a 24-hour old bouillon culture and exposed to the light in a room for various periods of time. Under these conditions the bacillus was killed after two days exposure.

*Growth in Fermentation Tubes.* The foundation medium was composed of 1 per cent. peptone, .25 per cent. Nahrstoff Heyden, and 5 per cent. salt, with the reaction carefully neutralised to phenolphta-

lein, 2 per cent, of the following sugars, saccharose, lactose, glucose was added to the above medium, and the tubes sterilized at 100° C. on three successive days.

*Saccharose bouillon.* Both arms of the tube became cloudy, considerable sediment formed but no pellicle. Reaction after 10 day's growth + 1.8 per cent.

*Lactose bouillon.* After 24 hours both arms of the tube became cloudy, the closed one with less turbidity, there was some sediment but no pellicle or gas. After 48 hours, the amount of sediment increased and 1 per cent. of gas formed, subsequently the closed arm became clear, but there was no increase of gas. Reaction after 10 day's growth, + 1.43 per cent.

*Glucose bouillon.* There was more growth in this medium than in the others, 0.5 per cent. of gas collected on the 2nd day, with no subsequent increase. Sediment very copious. Reaction after 10 day's growth, + 1. 8 per cent.

*Enzymes.* Proteolytic enzymes, cytase, and diastase are produced by the organism. Evidence as to the formation of these enzymes is afforded by the following experiments.

*Proteolytic Enzymes.* These enzymes are produced in small quantities. Gelatin is slowly liquefied, blood serum even more slowly, milk is partially peptonized.

Fresh milk serum sterilized by filtration was inoculated with a culture of the bacillus, and the medium held at 25 degrees C., for 10 days. At the end of this time a portion tested for peptones gave the biuret reaction. The proteid bodies except peptones in the larger portions were precipitated with ammonium sulphate and the filtrate treated with caustic potash solution and copper sulphate gave a violet color indicating the presence of peptones.

*Diastase.* Diastase is produced in small quantities in ordinary bouillon. Equal parts of sugar free starch paste and thymol were mixed with a 10-days old bouillon culture and left at 25 degree C., for 12-24 hours. A test of the filtrate of this mixture with Fehling's solution showed small traces of sugar to be present.

The organism when grown on potato also destroyed starch. Slices of raw potato inoculated with the organism did not give any coloration when treated with iodine, which indicated the destruction of the starch.

*Cytase.* The greatest interest in this organism is its power of destroying the cell walls of various plants. The quick spreading nature of the rot shows that the cell-wall-destroying-enzyme must be elaborated in considerable amounts.

This enzyme was isolated in the following manner:—

Sound potatoes were peeled and pieces cut out of the centre with sterilized knives. These pieces were scorched over the naked flame of a Bunsen burner and then dropped into wide mouth sterilized flasks containing 50-200 c. c. of sterilized distilled water.

This operation, although carefully carried out in a chamber washed with corrosive sublimate, was not always successful as a number of the flasks became contaminated with foreign organism: however, some flasks were obtained which contained nothing but *B. oleraceae*, and these, after incubation at 25 degree C. for 10 days, were emptied into a fine sterilized cloth and the juice pressed out.

This juice was then filtered through absorbent cotton and treated with four times its bulk of 94 per cent. alcohol, which gave a fine cloudy precipitate. The mixture was frequently shaken and was left at room temperature for 24 hours. After the final shaking the precipitate was allowed to sediment for 12 hours when the supernatant liquid was siphoned off, and the sediment collected on a hard filter paper. washed with alcohol, dried and then a hole was made in the filter and the precipitate washed off into a sterile flask with sterilized distilled water. This solution was then forced through a Pasteur-Chamberland filter, collected in a sterile flask and 5 c. c. portions of the liquid filled into sterilized test tubes. The liquid was clear with a yellowish tinge and was quite sterile. (Incubation of tubes at 25° C.)

Twenty test tubes were thus obtained and 8 of them were treated as follows:—

4 were heated to a temperature of 65 degrees C. for 10 minutes.

4 were heated to a temperature of 212 degrees C. and then cooled.

Small slices of potato and white turnip were then cut with sterile knives and introduced into the tubes which were placed in a thermostat at 20 degrees C. At the end of 24-36 hours the tubes were carefully examined and those that showed bacterial contamination were put aside. The small pieces of tissue were fished out with a sterile spatula and placed on a slide, a cover glass placed on top and the preparation examined under microscope. The sections of turnips and potato in the boiled and heated tubes were unchanged, they were

firm, the cell walls unaltered with sharp outlines, and about 2 3-5$\mu$ in width, The tissues in the unheated tubes were very soft, much swollen, and in some cases quite disintegrated. The cell walls were much enlarged, some striated and from 5-8$\mu$ in thickness.

This experiment shows that *B. oleraceae*, secretes a cytase which has a very powerful action on the cell wall and particularly on the middle lamella, and that this enzyme is killed by a temperature of 65 degrees C. for 10 minutes.

### Conditions Affecting the Spread of the Disease.

1. *Metereological Conditions*. The weather of July, August, and part of September was very favourable for the growth and spread of both fungus and bacterial diseases. In Ontario, the rust on cereal crops was very bad. Many newspapers spoke of the grain "being blasted in a single night."

The Toronto Metereological Register shows that July and August, 1901, were warmer and rather moister than the average; in the month of August when the cauliflower disease was noticed, the average humidity was 86, and the rainfall 3.67 inches. The temperature also was high. Very many mornings in July and August, the dew was so heavy that, in spite of great heat and cloudless sky, one could get quite wet when walking through the rows of cauliflowers in the afternoon. An examination of these plants in the field showed that the base of the plant, or the juncture of the petioles of the leaves with the stem, contained considerable water and in most cases particles of soil and if the organism exists in the soil, which is probably the case, it would be in a favourable situation to cause infection.

The warm weather, combined with excessive moisture, both of the soil and the exterior of the plant, and the fact that transpiration would be checked by this condition, and consequently the plant-cells themselves would be full of sap, undoubtedly played an important part in the spread of the rot amongst the cauliflowers and turnips. In short, we can state that the atmospheric conditions were ideal for vigorous bacterial growth, and that these metereological conditions have considerable influence on the ease with which the bacillus penetrates the plant.

2. *Rankness of Growth*. The weather conditions above mentioned, and the plentiful use of manure by market gardeners, favor very quick, rank growth. The plants most affected were large, heavy, and

with many leaves shading the surrounding soil, thus conserving moisture and promoting quick growth.

3. *Abundance of Insect Pests.* The disease is chiefly spread by means of infection from wounds, and under field conditions these are usually produced by insects, especially the cabbage worm (*Pieris brassicae*) which was very numerous upon cabbage and cauliflower leaves. A careful examination of over 100 plants showed that one or more larvae were present on each plant. Slugs also do considerable damage to these plants, and obviously smear themselves with a number of soil organisms, and as I have already mentioned, the *Bacillus olearaceae* is probably a soil organism.

Ants and other insects swarm around turnips, eating the rotted pulp, and no doubt serve to carry the germs to other plants.

4. *Injury from Planting, Cultivation or Wind.* Leaves of turnips are frequently bruised or injured during cultivation, by either hand or horse hoes. Cauliflowers may be injured during planting out, and the infecting organism brought into contact with the broken surface. In cases of very rank growth, a heavy wind may cause leaves to be broken off, and thus afford bacteria a chance to penetrate into the plant tissues. Many gardeners trim their cauliflowers on the field, and when these are infected they carry the disease on to another season. The same ground is often used year after year for the same crops, a dangerous procedure when disease is present, as it is likely to make the trouble endemic.

5. *Susceptibility of Varieties.* According to the limitations placed upon the meaning of "resistance" and "immunity" in plants by Russell, we shall define resistance as the "inherent power of the vegetable organism to withstand the action of bacteria in general;" and immunity as "the ability of the organism to repel the attacks of a germ which produces a pathological condition in a closely allied form."

We find that white turnips and cauliflowers are very susceptible to inoculations of *Bacillus oleraceae*, whether carried out in the laboratory, or met with under field conditions. Our laboratory experiments were all carried out on the Greystone variety of white turnips, which, under field conditions, seems to have some immunity; but which readily succumbs to artificial inoculations. We have kept careful record of the amount of disease present among the different varieties tested on our trial grounds.

Bacteriological examination of the disease present in the different varieties showed that we were working with the same bacterial disease. The amount of disease present is shown in the following list of varieties :—

*Immune*: Jersey Navel.

*Less than 5 per cent. Rotted*: Greystone Improved, Purple Top Mammoth, Early American Purple Top, Red Top Strap Leaf, White Flat Dutch Strap Leaf, White Egg, White Lily, Warly La Crosse, Red Top White Globe, Rennie's Selected White Globe, White Top Strap Leaf, Hunter's Purple Top Globe.

*Between 5 and 15 per cent. Rotted*: White Stone, Cow Horn, Yellow Stone, Green Barrel, Lutton's Imperial Green Globe, White Six Weeks, Milk Globe, Orange Sweet, Long Tankard, Sutton's Favorite P. T. Yellow Hybrid, Sutton's Perfection Green Top Hybrid, Yellow Finland, Large White Norfolk, Sutton's Purple Top Scotch.

*Between 15 and 30 per cent. Rotted*: Early Purple Top Murrich, Pomeranian White Globe, Red Globe Norfolk, Purple Top Hybrid, Jersey Lily, Early White Model, Extra Early Milan.

*Between 30 and 50 per cent. Rotted*: Orange Jelly, Imperial Green Globe, All Gold, Yellow Globe.

*Between 50 and 65 per cent, Rotted*: Yellow Aberdeen Green Top, Yellow Aberdeen Purple Top.

Fig. 1. A healthy cauliflower plant: uninoculated and grown under the same conditions as the inoculated plants.

Fig. 2. Cauliflower plant inoculated from a pure culture of *B.oleraceae* by means of a single needle prick at the base of the petiole. At the end of six days. Note the fallen leaf, wilted appearance of the leaves on the left side and the blackened stem above the fallen leaf.

Fig. 3. Cauliflower plant inoculated from a pure culture of *B.oleraceae* by means of a single needle prick at the base of a petiole. Shows the rotting of the flower. Ten days from inoculation.

Fig. 4. Cauliflower plant inoculated by placing a piece of softened tissue, taken from the interior of an affected inoculated petiole, on the surface of the healthy flower. The flower is reduced to a pulpy, black mass. Five days from time of inoculation.

Fig. 5. A white turnip plant inoculated at the crown from a pure culture of *B.oleraceae* by means of two needle punctures. The photograph shows the plant nine days after inoculation.

Fig. 6. A turnip plant cut in half in order to show the extent of the rotting process, six days after inoculation with *B. oleraceae* by one needle puncture.

Fig. 7. A turnip plant cut in two in order to show the almost complete rotting. Ten days after inoculation with *B.oleraceae*, one needle puncture at the crown.

Fig. 8. The edible portion of a cauliflower cut in two with a sterilized knife and inoculated with a pure culture of *B. oleraceae* by means of a single needle prick in the centre of the flower. Note the blackened portion which was softened to a considerable depth and also the water drops upon the blackened area.

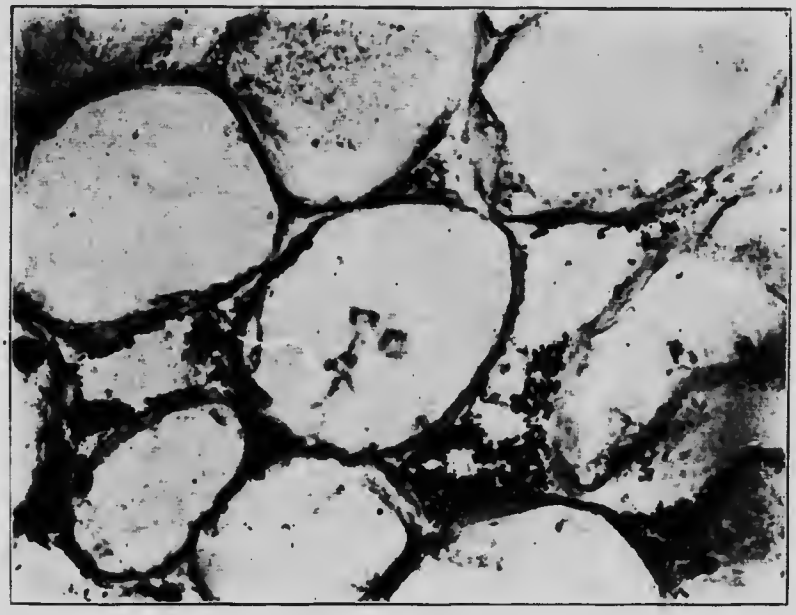

Fig. 9. Cross section of part of the petiole of a diseased cauliflower inoculated with a pure culture of *B. oleraceae*. Note the bacteria in the intercellular spaces and their penetration along the middle lamella.

Fig. 10. Cross section of the petiole of a cauliflower plant inoculated with a pure culture of *B. oleraceae*. At a later stage than Fig. 9, showing the almost complete collapse of the tissues, the enlarging and softening of the cell walls and the great increase in the number of bacteria.

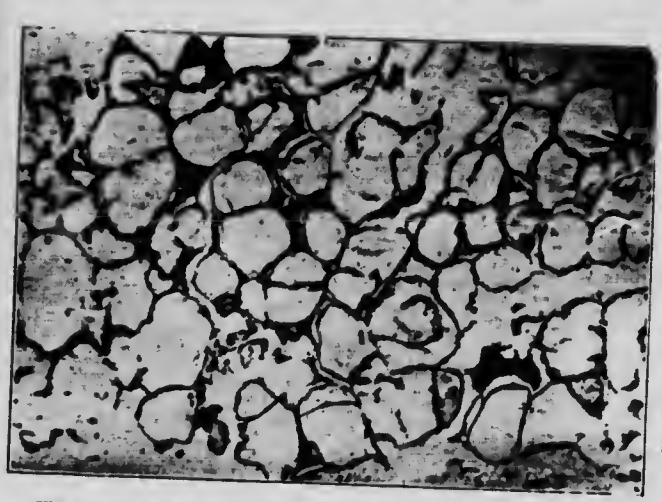

Fig. 11. Cross section of a piece of turnip. This was taken from a plant inoculated with a pure culture of *B.oleraceae*. Note the disorganization of the cells and the large numbers of bacteria in the intercellular spaces.

Fig. 12. *B.oleraceae*. The flagella stained by Van Ermegen's method. The bacteria were taken from an agar culture 18 hours old.