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B A C T E R I A L   D I S E A S E S   O F   P L A N T S

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Clyde Denney Gulick.

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T H E S I S

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B A C H E L O R   of   S C I E N C E,

in the   C O L L E G E   of   S C I E N C E,

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A.D. 1897.

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B A C T E R I A L   D I S E A S E S   O F   P L A N T S.

For over a century, diseases of plants caused by parasitic fungi, have been known to botanical investigators. The earliest work giving any knowledge of fungoid injuries was published in Italy near end of eighteenth century (Reference is made to it in Cooke's Microscopic Fungi, p.p. 48.) But the exact character of the disease was not settled until after the appearance of the Compound Microscope in eighteen hundred and thirty. The investigators were few at first, but their numbers increased until in the last decade one is not considered to have a fair knowledge of general botany until he knows the specific characters, the manner of living, the mode of reproduction, and the effect upon other plants, of these fungous parasites. But is this true of bacterial parasites? This question will be considered later in this paper.

At the beginning of the last quarter of the century, there was great controversy as to the origin and cause of many recognized diseases of plants. The most conspicuous and widely spread of these diseases was the so-called fire blight of the pear. Some attributed the cause to the freezing of the sap, others to the absence of essential elements of the soil, while others condemned the method of artificial propagation as injuring the constitutional vitality of the tree. But none of these theories were sufficient for explanation. It was Professor Burrill who first gave a satisfactory explanation for the cause of the disease. He advanced a theory and supported it by experiments, (Ill. Trustees report of 1880 p.p.62), that this was caused, "by a minute organism belonging to the lowest order of fungi, best known as bacteria". He observed the growth of the disease upon young trees and submitted the affected tissues to microscopic examination. Here he found a bacillus always present in the diseased parts. He transmitted the disease by inoculating other trees with this bacillus and found the organisms penetrating the tissues of the tree in the vicinity of the inoculation always accompanied by the characteristic death and black discoloration of the parts.

The second disease attributed to bacteria was announced the following year by Wakker "Yellow rot of hyacinths",(Bot. Cent. XIV "1883" 315). He found that this was caused by bacteria which gained entrance into the bulb before ripening and produced the disease causing the death of the plant the following season. The injury could be traced by yellow streaks within the bulb scales and upon the leaves of the growing plant. He found that the organisms followed the xylem of the fibrovascular bundles, then premeated the surrounding tissue and finally came to the surface where, when dried they were given to the air and carried to the other plants.

These were the first two diseases attributed to bacteria as a cause.

It had become generally accepted that bacteria constituted contagia of diseases among men and animals, but at this time the foremost botanical investigators did not admit that these organisms caused disease among plants. In the last edition of De Bary's Lectures upon Bacteria, dated 1886, he speaks of both of the plant diseases above mentioned, giving only a summary of Burrill's work, but he says of Wakker's hyacinth disease, "successful infection experiments and exact study of the life history of the bacterium are still wanting", (translation by Garnsay and Balfous p.p.178). He says of Bacteria in general, "according to the present state of our knowledge, parasitic bacteria are of but little importance as the contagia of plant diseases. Most of the contagia of many infectious diseases of plants belong to other groups of animals and plants, the larger number to the fungi proper".

Dr. Hartig in his Leherbuch published in 1889, gives as his opinion of these two diseases above given:- The yellow rot of the hyacinths is recognized as a bacterial disease but rather doubtfully as it is said not to attack sound, well-ripened bulbs under normal conditions; but, only when they have received wounds or been attacked by a fungus which is said to almost constantly accompany the rot. The pear blight may have been erroneously attributed to bacteria, since the fungus Nectria ditissima produces in the bark numerous little bacteria-like gonidia".

De Bary and Hartig seem to give about the same explanation for the supposed freedom of plants from the attack of these organisms. They ascribe this first to the acid reaction of the plant juices, and secondly to the peculiarity of the plant structure, especially the absence of circulatory channels for conducting nutrient fluids which serve to distribute the organisms through the plant. Hartig says:- "It is only by means of the vessels and the intercellular spaces that they can be distributed in any great numbers in the body of the plant, for in other cases they have to pass through the cellulose or woody cell walls which offer great resistance to their attack. Bacteria have hitherto been found in the tissues of plants whose cells are parenchymatous in character and possessed of very delicate cell walls as for instance, bulbs and tubers".

These were the views advanced a decade ago, but later investigators with improved microscopes and new methods of working, have proved the association of the germs with the progress of the disease, and by the aid of artificial cultures and plate separations, have been able to isolate the organism and from pure cultures to produce the disease by inoculations. From the artificially effected tissue the same germ has been recovered.

Not only has the first work of Burrill and Wakker been corroborated, but many plant diseases have been traced to parasitic bacteria as the active agent of the injuries.

Among the recent workers who have added to our knowledge in this line of investigations, besides these already given, we may mention Arthur, Waite, Halsted, Bolley, Kellerman, Galoway, Smith and Russell in America, besides a few investigators in Europe.

Among questions discussed by later workers in the general subject, the one that seems most important is, "Has vegetable cell juices a germicidal effect"?

It is known that the blood serum of animals has the peroperty of destroying bacteria, thus rendering the animal immune to the action of the organism. It was at first supposed that the fluids of plants possessed a similar property. The carefully performed experiments of Russell, (Bacteria in their relation to vegetable tissue. Thesis, Johns Hopkins University, 1892), with different plant juices obtained under aseptic conditions and inoculated with pure cultures, showed that no such germicidal properties existed. He also tried juices obtained under sterile conditions, by root pressure from lima beans and Pelargoniums. These gave a similar result and he came to the following conclusion, "Vegetable cell juices, aside from their acid reaction, are entirely powerless against bacteria, and do not possess any germicidal properties like the blood serum of animals".

The former view that plants are free from the attack of bacteria, because of the acid reaction of their tissues, is further shaken by the fact that some bacteria are adapted for growth in an acid medium. It has been also disturbed by the discovery that the juices of different parts of plants react differently as to acid tests. It may be said that plant fluids are commonly near the neutral line and are continually changing in order to carry on the life processes. Also many bacteria are alkali producers and may be able, if they once gain a foothold, to change an acid medium to one less acid or even alkaline, better adapted for their growth. In this last consideration three things are at least certain; (1) It will not do to assume that all parts of a plant are acid because some part shows an acid reaction; (2) It cannot be stated that any given micro-organism will thrive only in an alkaline media until this fact has been determined by direct experiment; (3) Many bacteria, if they are started, are able to change slowly an unsuitable acid media into one more alkaline and better adapted to their use.

The distribution of the organisms in the affected tissue will be considered later under spread of the disease. Although the study of the bacterial diseases of plants has not since been considered to any great extent until recently, yet in the last five years many parasitic bacterial diseases have been worked out. Generally these injuries are found first in the locality where they are the most destructive; but after attention has been directed to them, they are often found to be widely distributed. Some are, however, confined to a certain part of one country; while others are universally scattered wherever the host is grown. Some are confined to a certain region though the host is grown as extensively elsewhere, e.g.

Table II — Bacterial Plant Diseases.

Specific Name of Disease	Name of Disease	Host Plant Affected by natural infection	Organs of Plant Affected	Mode of Inoculation	Bibliography
Bacillus amylovorus (Burrill) De Toni.	Pear blight Fire blight.	Pear (Pyrus communis) Apple (P. malus) Crab-apple (P. sinensis) English haw Shadbush.	Flower clusters, young fruit, more succulent woody tissue	Insect bites, stings, etc.	Burrill: 3d. Ill. Rep. Agric. 1890. Amer. Nat. VIII, 1883. Indus. Univ. Rep. 1880. Arthur: 4th, 5th, 6th. N.Y. Agric. Exp. Rep.
Bacillus sorghi. Burrill.	Sorghum blight.	Sorghum vulgare a) Saccharine var. b. Non- " " S. halapense	Leaf-sheaths and leaves, roots	Stomata and roots	Burrill:- 8th Rep. Soc. Prom. Ag. Sc. 1887. Kellerman Kan. Rp. 1887. Bul. No. 5 Dec 1888.
Bacillus zeae. Burrill.	Corn blight.	Zea mays.	Roots and stem. leaves and sheath.	Stomata	Burrill: 30. Ill. 1890. Ill. Bul. No. 6 1889.

Table III – Bacterial Plant Diseases.

Specific Name of Disease	Name of Disease	Host Plant Affected by Natural Infection	Organs Affected	Mode of Inoculation	Bibliography.
Bacillus hyacinthi Wakker	Yellows of hyacinths	Hyacinthus Orientalis.	Xylem portion of fibro-vas. bundles. bulbs		Wakker: Bot. Cent. XIV, 1883, 315: Arch. Neerland XXIII 1888
Bacillus hyacinthi septicus. Heinz.	Hyacinth rot.	Hyacinthus sp, sp. Allium Cepa.	Leaves, flowers and bulb.	Injuries	Heinz: Cent. f. Bakt. 1889. Bd IV, S, 535.
B. oleae-tuberculosis Savastano. (B. oleae) Trev.	Tuberculosis.	Olea Europea.	Cambium, bast of old, new wood.	Stomata, lenticels.	Savastano: Ann. d. R. Scuola Sup. d'Agri. Cavoru Monograph '84
B. Vuillemini Trev.	Tumors of Aleppo pine	Pinus halapensis	Mature wood cambium Phlo.	Insect wounds	Prillieux: Monograph. 1890
Bacillus avenae Galloway	Blight on oats	Avena sativa	young plants	Stomata Spraying	Galloway: Bot. Gaz. XV. 228. Amer. Assoc. 1890.
Micrococcus of carnation Arthur[?]	carnation blight.	carnation pinks, other varieties.	Leaves	External application	Arthur: A.A.A. S. Aug. 1890. Ind. Exp. Bul. 1890

Table IIIV — Bacterial Plant Diseases.

Specific Name of Disease	Name of Disease	Host Plant Affected by natural infections	Organs Affected	Mode of Inoculation	Bibliography
Bacillus of surface scab of Potato Bolley	Superficial potato scab.	Solanum tuberosum	Tubers (young) Stems, leaves young sprouts	Lenticels	Bolley: Ag. Sc. IV nos. 9 and 10. A.A.A.S. 1890, 334-5:
Bacteria of wet rot. Burrill.	Wet rot of potato	Potato (S. tuberosum)	Tuber, possibly leaves.		Burrill: Proc. Soc. Prom. Ag. Sc. 1890, 21.
Bacteria of wet rot. Kramer.	wet rot.	Potato	Tubers	Lenticels wounds	Kramer: Oest. land Cent. II 11.
Bacillus of beet rot. disease. Arthur and Golden	Sugar beet rot disease	Beta vulgaris. (saccharine var.)	Parenchyma of root and leaves.	unknown	Arthur and Golden, Ind. Bul. no 39, 1892, Exp. Stat. vol IIII, no 12.
Bacteriosis of Fodder beets Kramer	Sugar beet disease	Beta vulgaris L.	Parenchyma of root and leaves.		Kramer: Oest. land cent. II, 2, pp. 30-36 3 pp. 40-41 (1891).

Table IV. — Plant Disease Probably of Bacterial Origin.

Name of Disease	Host Plants	Characteristics	Bibliography.
Geranium blight.	Pelargonium. Geranium cult. Potatoes.	A complete disorganization by a blackening shrivel appearance of cuttings.	Galloway: Journal of Myc. VII, 114. Prillieux: C.R. Acad. CXI
Cucumber rot. Tomato blight.	Melons, squashes, tomatoes, potatoes	Rapid decay of stem, leaves and fruit, potatoes, tubers.	Halsted: Miss. Exp. Bull no. 14. Jan. 1892
Root rot of vegetables.	Salsify, egg plant, Potato, onion, tomato Apple.	A slimy and very offensive decomposition of root crops, from bottom upwards.	Halsted: N.J. Exp. Rep. no. 11. 351. Garden and Forest Nov 26 '90.
Cabbage rot	General rot of heads	Cabbage and turnips.	Garman: Amer. Assoc. Coll. Exp. 1891.
"Sereh" disease	Sugar cane	General depletion on account of stopping of vessels with a gummy slime.	Janse: Ref. in Cent. f. Bakt XII, 641. Kruger: Ber. d. Vers. Stat. f. Zucker, 1890

Table V. — Plant Diseases Probably of Bacterial origin.

Name of Disease	Host plant	Characteristics	Bibliography
Bact. gummis Comes.	figs, grapes, mulberry, tomatoes cabbage, beets.	Affects starch cells, and fills vessels with gummy exudate.	Sorauer: Zts. f. Pflanz. III. Comes: Crittogam. Agraria.
Celery blight.	celery	Leaves and stems. watery.	Halsted: N.J. Bull 21. 1892.
White Slimy Flux. Ludw.	Oaks, willows, ash, poplars.	Subcortical tissue	Ludwig: Cent. f. Bakt 1891, 10.
Brown Slimy Flux. microc. dendroporthos Ludw.	Fruit trees, shade trees, elms poplars, birches.	Wood and bark. Yellowish brown slimy exudate, from wood layers, finally destroys bark of tree.	Ludwig: Zeitschr. d. med. Krypt. 1891.

pear blight, unknown in Europe, occurs in United States and Canada. The hyacinth disease is found in both the old and new continents, while the peach yellows, recognized as the worst enemy of the peach growers, is found only through United States and Canada. Again the wet rot of the potato is generally considered as a disease of the warmer climates, but it is found wherever potatoes are grown.

The number of plant diseases already worked out is quite large; but owing to the fact that many of the descriptions are meagre and little notice has been taken of them, it is difficult to make a complete list. Nothing of this kind has been attempted since Russell's paper, (Russell:- Thesis; Bacteria in their relation to plants, John Hopkins), published in 1892, in which he gives a list of thirteen established bacterial disease and nine probable ones. In the table there are some additions to those given by Russell.

Bacterial diseases of plants show in almost all cases a peculiar progress of growth. When the injury is upon the outer tender tissue of a limb or twig, the progress of the disease can be noticed, as each day the line between the healthy and affected tissue moves outward and gradually encroaches upon healthy and sound cells; yet in many instances the germs have been found beyond this line of diseased tissue, showing that the destruction of the part was caused by the action of the organisms.

The question that is demanding an answer now is: How do bacteria gain entrance into the tissues of the plant?

The epidermal tissues of the plants are much more resistant to external influence than the inner parenchy matous cells. This is due in the younger parts of plants to the resistance of the outer epidermal wall with its deposit of cutin. This cutinous coat of the young parts gives place in the older ones to a thicker and more resistant corky layer. The protective epidermal layer is not continuous over the entire plant, but is pierced by numerous openings, the stomata which give direct communication to the outside world with the interior of the plant. At first nothing appears to hinder bacteria from entering by these openings; because it is known that certain species of fungi gain admittance in this way. Some species of bacteria adapted for parasitic existence do gain entrance in this way, e.g. bacillus sorghi and bacillus zea, Burrill, and Bacillus avenae, Galloway.

Those not gaining entrance in this way appear to demand some nutrient material or an artificial breaking of the epidermis in order that they may be able to gain a foothold, e.g. Waite in his experiments with pear blight found that the spraying of the flowers with the organisms suspended in a liquid was sufficient for infection. It is probable that the nectar of the flower furnished a medium for thegrowth of the germs and they were able to multiply sufficiently or to produce a ferment so that they are capable of penetrating the tissues. Or when they are in contact with young non-cuticularized surfaces, the entrance may take place without such aid. In Galloway's experiments with the oat disease, he found that the young plants were infected by spraying; but that the older plants failed to take the disease in this way. This may argue that the stomata do not provide a means of entrance as the older plants are provided with these structures as well as the younger ones. But in this disease the older plants are not usually affected, which fact may better explain the result.

Some insist that bacteria enter through the root hairs in case of the oat and sugar cane plants. This may be possible, but without extreme care in the test experiments it would be difficult to distinguish in such fine organs whether the bacteria were inside of the root hairs or simply upon the surface. Yet they are found within in the knots and root-tubercules of Leguminosae and some other plants.

<u>How are bacteria able to spread through the tissues of the plant?</u>

If this is not due in the action of the germs themselves, then it must be by one of two ways: by diffusion, or by the transpiration current. If diffusion is the method, a fluid is necessary which is continuous throughout the plant. Against this are the cellulose walls of the cells acting as a filter to all solid particles.

As it is now held that the transpiration stream passes upward by attraction in the cell wall itself, we can hardly conceive of it carrying solid particles unless they possess some kind of a power of penetrating the cell walls. The bacterial plant diseases do not spread their infective material all through the plant as do those which develope in animals. We might conceive of them being able to do this if the transpiration stream functioned similar to the blood of animals in carrying infection. But we cannot compare the transpiration stream in this way to the circulation of the blood of animals.

It is possible that the lumina of the vascular tissue furnished the least resistance to the spread of the disease. In the case of Wakker's disease of hyacinths, bacteria were found in the fibro-vascular vessels, not only occupying the cavity of the air cells but also attacking the surrounding tissues and converting them into a gumy exudate. Through these cells the disease can be transmitted quite rapidly.

As in the case of the brownish rot of lima beans, in which the bacteria are found in the vascular tissues, a laboratory note may show the rapid spread of the organism. March 4th, a lima bean with four leaves was inoculated by puncturing the petiole of one of the lower leaves, about two and one half inches from base of leaf. March 8th, a brown discoloration was observed about the point of inoculation. Three days later the leaf blade showed brown spots about three inches from the point of infection. By sectioning the petiole at the base of the leaf, the vessels were found to contain numerous germs.

In case of the pear blight, Russell gives a laboratory note, (Russell: Bacteria in their relation to plants. Thesis, John Hopkins, "1892", p.p. 32), that a seedling inoculated by a puncture of the stem showed after five days a local discoloration of tissue about inoculation point. Two days later the stem showed that the disease had progressed fully six inches from point of inoculation, as shown by the blackening of the tissue. The presence of the bacteria was also demonstrated, microscopically, fully an inch beyond the blackened tissue, showing that after the germ had gained a foothold, the spread of the disease was quite rapid.

Bolley finds the disease causing the surface scab of the potato, (Bolley: Agricultural Science 1V 284), imbedded in the protoplasm of the cell. In this case the cell membranes were seen to be actually eroded by the bacillus.

It seems that certain germs which are adapted to this parasitic existence possess an ability to set up fermentation which dissolves the cellulose or at least acts upon the cell wall in some manner so that it is easily broken down by the organism. It may be the case that those which do not destroy the cell wall, produce a ferment which renders it possible for them to pass through the wall without causing a permanent rupture. Certain species of Ustilagineae are known to possess the power of penetrating the cell wall which after the hypha has passed through, closes so that no opening is apparent. This is a conceivable penetrating method in the case of so small an organism as a bacillus.

It is not generally true that we have so rapid a spread of the injuries as cited above, as pear blight and the rot of lima beans show. Many of the diseases are restricted to less than two or three inches of destruction in the tissue about the point of infection, as the mulberry disease is limited to a small blotch the first year. So the spread of baterial injuries is commonly rather slow.

<u>The local effect of bacteria upon the tissues of the plant.</u>

A general statement with a few examples is all that can be given here, as different germs produce dissimilar results upon the same host, as well as upon different hosts. Bacteria exert a local influence upon the tissue in which they live.

It has been given before that the pear blight germ causes externally a blackening of the tissue. Internally it destroys the starch grains and stops the activity of the cells.

In the hyacinth disease as already given the organisms completely break down the cells of the xylem of the fibro-vascular bundles into a gumy exudate. Externally the disease can be traced by the yellow discoloration of the tissue.

In the mulberry, the cells seem penetrated by the organisms, the starch disintegrates and the tissue ceases to grow. At first, the diseased portion can be found by a black or dark brown blotch, and the following year by a pit in the bark the size of the affected portion of the tissue of the previous season. There is a retardation of growth.

It is probable that there is augmentation of growth, by bacteria, in the production of tubercules and root knots.

In conclusion concerning the effects of bacteria it may be said, (1) they may destroy the functions of the cell; (2) they may completely break down the tissue; (3) they may retard growth; (4) they may augment growth.

Method of Experiment.

All cultures were obtained first from diseased tissue of effected plants. The bark was well washed in one per cent aqueous solution of corrosive sublimate; then in water; then in alcohol and the alcohol was burned off, thus killing all germs upon the outside. If the tissue was too thin for heating with the alcohol, it was simply washed and then passed through flame of a bunsen burner once or twice. The outer bark was taken off with a heated knife and sections taken for microscopical examination and for broth cultures.

If the organisms were not obtained pure at first, they were separated by means of gelatin plate cultures. After which the germs were grown upon the different media to note mode of growth and effect upon media.

After a culture was found to be pure, inoculation experiments were begun. In all inoculations of plants young shoots were used, so as to obtain the best conditions for growth. The inoculations were made by puncturing the epidermis with sterile needle or the point of a heated scapel, and by applying a drop of a 12-24 hour liquid culture by means of a loop platinum wire, or any object which could be sterilized by passing it through a flame and was easily inserted into the wound.

In these cases in which the symptoms of the diseased condition developed after such inoculation it is safe to say that the organism was present; but a negative result can not argue that the germ was not present, but perhaps that the surrounding conditions were not favorable to its growth. Most of the inoculation experiments were commenced during the winter term in the conservatory part of the vegetable physiological laboratory and it was difficult to regulate the temperature for the best results. The temperature fell too low, many times, for an ordinary green house. With these conditions negative results are of no benefit, while positive results show all the more.

A pear limb was obtained from the experiment station orchard which showed the characteristic blackening of the bark. The broth cultures having been made under aseptic conditions, the organism was obtained unaccompanied by others. It was an oval bacillus, but little longer than broad, giving in broth cultures an abundant growth with a very thin pelicle in a temperature of 35 deg. C. for two days. On agor and gelatin it gives at first a thin white irredescent growth spreading over the surface, then later it turns to a grayish color. In a short stab agor culture it grows out in small nodules along the stab. On steamed potatoes it gives a pearlly white growth raised one sixteenth of an inch in thickness. Several crab apples were inoculated and after thirty-six days the organism was obtained from the tissue of the apple by means of plate cultures. The tissue of the apple was at first darkened about the inoculation point, and began to decay. Inoculation upon seedlings potted and grown in the conservatory were unsuccessful.

This organism does not stain well with the common stains, but takes readily carbonized fuchsin.

The disease known as "yellows" of the peach tree exists upon the University grounds in trees inoculated four years ago. Material was obtained from one of these diseased trees and cultures were made as above described with successful results.

In beef broth these organisms produce a large amount of sediment and a thin gray pellicle. Two organisms were isolated, differing in size and mode of growth. One was an oval bacillus about one-third longer than broad, rapidly liquifying gelatin, giving a gray growth upon both agar and gelatin; the other was from three to six times as long as broad, united in chains, slowly liquifying gelatin, giving at first a white then an amber growth upon gelatin and agar. Inoculation experiments upon potted plants were unsuccessful.

A curious condition was noted late in fall in an immature (Bovista) puff ball. It bore a dark brown watery blotch upon one side. Observing it for some twenty minutes it was seen that the colored portion had spread. Then the rate was noticed by placing a pin at the edge of the blotch; at the end of one hour the coloring had spread one and a half inches past the pin; and at the end of four hours had covered the entire ball. A very small micrococcus was found. It gave a characteristic green color in beef broth, upon gelatin, and upon agar. It stained readily with the common stains. This discoloration may have been due to the ripening of the fungus, but it would have been an interesting investigation to follow further, which want of suitable material prevented.

Some raspberries received from Ohio showed a black decay of the growing ends of the branches, also blackened portions along the main stem. This same condition was found in a patch of raspberries owned by Mr. Tucker at 1011 West Church Street Champaign. The disease attacks the inner bark of the old wood and the tender tissues of the growing twigs. A germ was isolated from the affected tissue and proved to be a bacillus from one and a half to three times as long as broad, rounded at the ends, appearing in pairs, but not connected in chains. It stains readily with methyl blue requiring about 11 minutes. In beef broth it produces a thin pellicle and a heavy yellow precipitate. On gelatin it grows in a raised yellow mass not spreading. On agar the growth is thin, yellowish and spreading. The plants potted for inoculation experiments did not grow well, so no attempts to produce the disease were made.

Potatoes affected with a wet rot, giving off a foul odor, were found in a box of potatoes in a cellar. The skin of those not so badly affected showed no signs of the disturbance, but the tissue beneath was soft and watery, yielding readily to light pressure. Potatoes more affected showed a wrinkling of the skin and a general shriveling. A little of the solid tissue just beneath this watery mass placed in a drop of distilled water upon a slide and examined with a microscope, showed a swarming mass of slender bacilli. Two organisms were isolated; one liquifying gelatin more readily than the other. Each produced a thin white pellicle in beef broth and a thin white growth upon gelatin and agar. On steamed potato they gave a characteristic raised brown growth. The organism which liquifies gelatin more slowly is a little shorter and thicker than the other. These germs take a clear stain with methyl blue, but not so well with gentian violet.

From a rot of cabbage was isolated a small bacillus which stains with the blue and violet stains. In beef broth it gives a heavy pellicle and a granular precipitate. On gelatine the growth is thin and white and does not liquify; on agar it is thin, white and shining by reflected light and green by transmitted light.

The disease was found upon the leaves and stalk of broom-corn. This was noticed by the characteristic red-brown coloring which was seen in a number of places upon the plant. From the affected tissue was isolated a thick bacillus about twice as long as broad, sometimes united in a chain of four.

In beef broth it gives a thin white pellicle and a pinkish sediment, on gelatine it produces a thin translucent growth which liquifies the medium quite rapidly; on agar it gives a thin almost transparent growth, spreading over the surface of the medium. It stains well with the methyl blue.

Disease upon Mulberry.

A disease effecting the growing bark and young wood of the limbs of the mulberry can be identified by the black blotches upon the young and by sunken pits upon the older limbs. By removing the outer bark at the edge of one of these blotches under aseptic conditions, and putting a portion of the under bark into a tube of broth one obtains a light growth with a very thin, reticulated pellicle, at a temperature of 35 deg. C. for twenty-four hours. Examining a drop of this liquid, it is found to be filled with small oval bacilli which stain lightly with methyl blue, but take the gentian violet readily. On gelatine it produces a very thin translucent growth which liquifies the medium after the second day. On agar it grows very thin and transparent. Attempts to grow it upon steamed potato were unseccessful. In March about a half a dozen limbs were cut from the tree and the cut ends were placed in water in the conservatory, the water being changed every day. In about two weeks the leaf buds began to open and put forth the leaves. As soon as the leaves became large enough they were inoculated with a liquid culture of the germ. The inoculations were made in the petiole of the leaf by puncturing the epidermis with the point a scapel and inserting a drop of the culture by means of a looped platinum wire. The puncture was closed with vaseline to prevent contamination and evaporation. The tissue showed a darkened spot at point of inoculation after eight days. The enlargement of the discolored area progressed very slowly. After twenty-five days from time of inoculating, the petiole was taken off, and a culture was made from the affected tissue. A portion of the diseased part was placed at once in absolute alcohol to harden and to prevent the organisms from washing out. Then it was placed in Xylol and subsequently imbedded in parafine, sectioned, mounted upon a slide, stained in gentian violet and examined. The deposits of the stain made it difficult to distinguish the organisms; but along with these, sections frest cut in water were examined and the germ was found within the cells containing little starch, as tests with iodine showed, or had remaining only some contracted protoplasm.

Experiments for methods of infection were tried upon the leaves of the cut limbs and upon those of the tree in early spring. One half of upper surface of leaves was covered with a liquid culture of the organism and a half of the lower surface of leaves was similarly treated. Of the five leaves tried in the conservatory, three were smeared on the upper surface and two on the under surface. The two latter and one of the former showed brown spots in four days. There were twenty leaves upon the tree, tested for stamatic infection, ten tests were upon the upper surface and ten others upon the lower surface. Care was taken to select leaves of about the same age for each set of experiments, but for different trials, leaves of varying ages were chosen; five young and five older leaves for each set. It was found that only three out of the ten upon the upper surface showed any signs of disease within ten days, and these were of the younger leaves; while eight of the under surface tests showed the brown spots within five days. Other tests were started but were unsuccessful on account of unfavorable weather. There remained no time for further experimentation.

Disease upon Lima Beans.

This disease was first noticed on some bean plants in the conservatory, grown for embryological investigations. It was noticed that stems showed a moist, brown decay below the cotyledons. In the first case this decay showed on the surface for about three inches. When first noticed the first pair ofleaves had commenced to dry around the margins. These were cut off and a section examined in sterilized water. At first nothing was found, but upon examining a small piece of the tissue of the stalk there were found a number of small bacteria. There was no difficulty in finding them in sections of the wrinkled petioles. After the beans had commenced to die from the effects of this malady the stem was cut off and cultures were made from it under as aseptic conditions as possible. It was found that the injuries had progressed up the interior of the stem which showed no signs exteriorly above the attachment of the cotyledons except a slightly shrunken appearance. The whole interior of the stem for about seven inches was a mass of moist brown rot. The organism isolated is a short thick bacillus with round ends appearing connected only in twos, no chains. It stains well with gentian violet, in four minutes. In beef broth cultures, it produces a very thin pelicle sometimes restricted to a thin ring around tube at surface of medium, also a light granular precipitate. On gelatin the growth first appears very thin and grayish, then it becomes thicker and yellowish brown, slowly liquifying the medium after the second or third day; on agar it grows thick and of a dark brown color; on steamed potato it is of a thin dark growth. Inoculation experiments were tried both by infecting with a liquid culture and also by inserting a small piece of diseased tissue. It was seen that the disease could be produced by either of these methods anywhere along the stem of a bean plant from four to six weeks old. After each artificial inoculation cultures were made from the affected tissue and the germ was isolated again and identified with that first found. Reinoculation were made and the same germ was attained a second time. Sections were made of the diseased tissue, imbedded in parafine. These sections were cut from the healthy side of stems toward diseased portion in such a way as to prevent , if possible, the knife from carrying the germs into the healthy part. After staining in gentian violet and mounting in balsam, it was found that the cavities of the fibro-vascular bundles were filled with these organisms, and in places the adjoining tissue was broken down. The germs were also found imbedded in the protoplasm of adjoining cells, not yet broken down. All these affected cells belonged to the xylum portion of the bundle.

Experiments were tried by smearing the leaves with a liquid culture. If the young leaves are smeared just after they open, they die within two days, but older leaves show no signs of infection if not punctured, but the least little perforation of the epidermis is followed by the discoloration due to the disease. Several leaves were tried, over which a spider had spun its web, and they readily showed signs of infection.

The disease therefore is produced by a bacillus invading the xylem portion of the vascular bundles, which obtains entrance by ruptures through the epiderman covering.

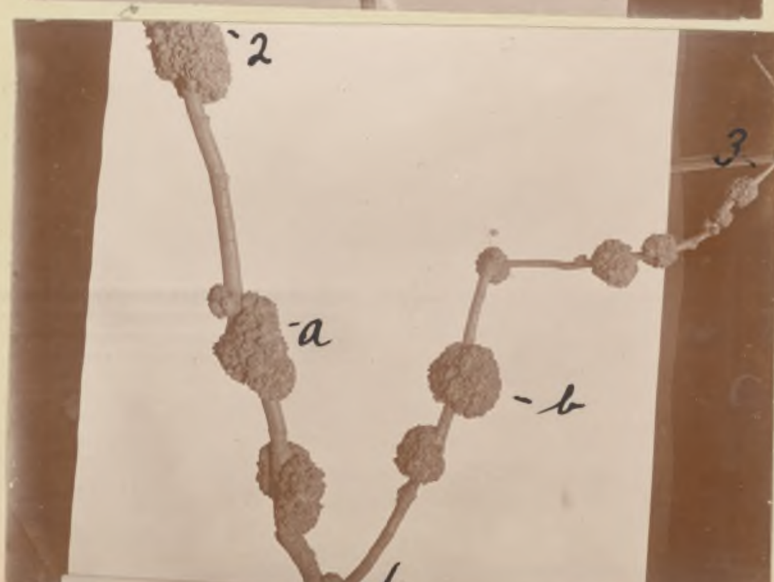
There is often seen upon the limbs of various oaks, hickory and other forest trees, very conspicuous rough hard knots or woody excrescencies, sometimes encircling the branch and at other times protruding from one side. These enlargements vary from a fraction of an inch to a foot or more in diameter, and much longer ones, usually called burls on trunks, appear to be of the same character. The bark of these knots is similar to that of the limbs upon which they are borne, except that it is rougher with deeper cracks and fissures. In cutting a cross section through the knot and limb (see plate) one notices that the red spongy outer bark of the former is quite thick compared with that upon the limb. The uneven growth of the wood in the different parts is transversed without regularity by black decayed portions, apparently starting from the center. From these decayed spots and from the growing bark in early spring was isolated a Micrococcus, which in beef broth gave a characteristic slimy sediment and a very thin pellicle, sometimes only a thin ring around tube at surface of liquid. On gelatine it gives a thicky gray growth which liquifies themedium after a few days. On agar it is similar to that on gelatine, but is not confined to the place of inoculation. It spreads over entire surface. In fresh sections, cut in water, of the bark, these small organisms could be seen coming from the tissues, but could not be identified in the cells. Upon the outside of the bark of these knots was seen a fungus which sent its mycelium into the tissue, but could not be traced to the growing cells. Nests or bunches of the tissue of this fungus was found among the cells of the outer bark in the ridges which appear upon the outside of the knot. It is a question as to which of these two organisms, presuming that it is one or the other, produces the augmented growth. If the fungus is traced to the growing cells it may be considered the active agent, or if the bacteria are found in the living tissue they may act as the instigators of the rapid cellular multiplication.

Peculiar growths of similar character were examined

Fig IIII.

Fig III.

Fig II



upon the limbs of certain apple trees and they have also been found upon the roots of such trees, not due to plant lice. It is probable that the cause of these knotty formations is the same that produces those described upon the forest trees.

Description of Plate.

Fig. I. This is a branch from a large limb of an affected tree, the actual distance from 1 to 2 is three feet and from 1 to 3 is four feet.

Fig. II. This branch is from the same limb as that of figure 1. The distance from 1 to 2 is three feet six inches. Knot "A" is fourteen inches long and six inches in diameter.

Fig. III. "A" is a longitudinal section of knot "A" figure I, six inches long and three and a half inches broad.

"a'" spongy outer bark.

"b'" black rotten streaks.

"B" surface view of knot "b" figure II.

"C" cross section of knot "b" figure I. "a" outer bark, "b" black decayed portions of interior wood.