THESIS,

YELLOWS OF THE PEACH,

FOR THE DEGREE OF

B. S.,

IN THE SCHOOL OF NATURAL HISTORY,

BY

Albert Carver,

1889
Introduction.

On entering the discussion of this subject, the writer will begin by saying that the information recorded in this thesis is not entirely original, but was obtained by a student under the guidance and supervision of his instructor. In pursuit of knowledge concerning the "Peach Yellows," I have found many species of bacteria, which probably take no part in producing the disease, but many of them are very curious and interesting. As it seems fit to briefly mention and describe a few of them.

It is quite apparent to the writer that he has accomplished very little in his work of investigation although much time and
labor has been involved. The growth of the organisms in the tissue is very slow, sometimes requiring several years for the tissue to be entirely killed, and this subject has now been under consideration only about six months and that during the winter.

If the specific disease had been isolated and grown on artificial media, thus inoculated with--it might not show the effect during the short period of investigation.

So as many cases as possible I have taken photo-micrographs from the negatives taken by Mr. J. H. Stratton. These prints show the actual organisms which I succeeded in isolating, but they are in every case highly magnified.

The negatives were taken with a Guibertin apochromatic objective, compensating eye piece No. 8;
and Carbutte ortho-chromatic plate: lamp light exposure 3 to 5 minutes. The organisms were in nearly every case stained with methyl violet. The magnifying power varied from 80 x to 1000 diameters.

The Photo-micrographic prints, known as blue prints, are prepared as follows: A sensitizing fluid is prepared by making a solution of citrate of iron and ammonium 1g, water 49; another solution of red prussiate of potash 10g, water 6 oz. The sensitizing fluid is ready for use by adding equal quantities of these two solutions. They, however, should not be mixed until time for using the mixture. The solution is applied to the paper by means of a thin strip of board covered with cotton flannel which has considerable nap on its surface. It is applied evenly and
smoothly, and in a few minutes it becomes dry. The paper must be kept in the dark, otherwise it will soon turn blue.

The photos-microscopical prints are then made by placing a negative in the frame, over this a sheet of sensitized paper, then a heavy cover. It is then exposed to the direct rays of the sun 3 minutes. The paper after exposure is taken from the frame and washed. It is then hung up by one corner and left to dry. If successful we have a beautiful blue print of the microbee.

**Peach Yellows.**

The first symptoms of the disease are made apparent to the eye of almost any observer by the small yellow spots, or the pale
yellow edges of the leaves. At first the spots are all small but finally assume a bright yellow color. The leaves also become more or less curled and enlarged. The twigs have a wing appearance. The woody structure is more yellow, in contrast with that of healthy trees which is nearly white.

Young trees are susceptible to the disease, but more frequently it sets in after the tree begins to bear fruit. Generally some part of the tree, as a certain branch, first shows a sickly condition. If this branch is removed, the disease, the next year becomes apparent in some other branch, usually that nearest the removed portion. The bark on the body of the tree has no longer its bright green color, but assumes a yellowish decaying look, and in a few years the tree is entirely dead.

The writer observed the slow but sure work
of a supposed disease in an orchard of peach trees near Springfield several years ago; but without the careful observations of an investigator. However many of the spectacles then presented are fresh in his memory.

Causes.

The cause of the peach tree disease has been attributed to many things, which by later investigators has been found to be erroneous. Among the earliest statements as to the cause of peach yellow was those attributing the disease to climatic influences, such as frost, floods, and drought; but they have been proven to be no more than modifying influences. Injuries by animals and insects are also among the
proved theories. Upon the same list is classed excessive cultivation, neglect of cultivation, and neglect of pruning. Also the "injuries to tap roots, propagation by buds rather than by seeds, defective drainage," etc. I think it can be quite conclusively stated that these agents may aid in the development of the yellows, but cannot cause the disease. To what then can it be attributed? The underlying cause is without doubt established as a living organism.

The spread of the yellows from one part of a bee to another, or from a diseased to a healthy bee seems to establish this fact. The great problem then is to find the specific organism causing the disease, isolate it, and establish the facts concerning it. This has been the object of the writer in his biological investigation; but how much he has accomplished in that line, if anything, time will tell for scientists.
will not long remain ignorant on this subject; although the work in itself is attended with
many difficulties.

In the fall of 1881, Mr. E. F. Smith—a special investigator of peach yellows at Washington—sent
eight diseased peach trees to Prof. T. J. Burrill of
the University of Illinois for investigation.
He had not yet made biological investigations of
the diseased trees. These trees supposed to be effected
with yellowish were sent from Dover, Delaware
by request of the agricultural department at
Washington. One of the trees was planted in a
pot in the greenhouse that fall, and began to
grow early in the next spring. The others were placed
in the garden, the roots being covered with straw,
and then earth.
The subject of this thesis is considered under three general divisions:

I. Appearance of the diseased trees.
II. Microscopical investigation of the tissue.
III. Biological studies upon the organisms found.

My work of investigating the yellows of said trees began January 17, 1887. The first work was to cut thin sections with the microtome, of healthy trees, and of diseased tissues of the trees above named. Note the difference in the sections by a microscopical examination, and if possible find the supposed parasite in the diseased tissue.

Twigs were cut from a healthy tree growing back of Prof. Orrill's residence. Small pieces from these twigs were imbeded in paraffin and thin sections made with the microtome.
In the same manner sections were made from the root and branches of the diseased tree in the greenhouse. The results of these examinations will be stated further on.

It is supposed there may be found in the diseased tissues injurious organisms which cannot be found in the undiseased. If the disease is really caused by a parasite, it should be found, and by methods described further along introduced into the healthy tree to note the results. Proof is only attained when it is ascertained that the disease follows in consequence of this inoculation. The media used in the cultivation of the organisms found, were 2 kinds. The preparation of each will here be briefly described.
I. Liquid media, or beef broth.

II. Solid media, or agar-agar, gelatin, and Irish moss.

To prepare liquid media, chop finely 1 lb. clean beef, removing as much fat as possible. This is done to best advantage when cold, then soak overnight in one bottle of cold water. After soaking squeeze all the juices from the meat. Boil gently to coagulate the albuminous substance which may then be removed from the top of the liquid. Boil the residue lively for a few minutes—until it turns white. Neutralize with NaCO₃, adding it drop by drop. Boil it gently a few minutes and filter; this leaves the liquid entirely clear, transparent and of a bright yellowish yellow color. Put the broth into small flasks and tubes closed with cotton plugs. The flasks and tubes of broth are
Their sterilization by putting them into a steamer at 90°C per hour daily for three or four successive days. Their sterilization is complete when the broth is ready for use.

The solid media, agar-agar, gelatin and Irish moss are prepared by adding to the liquid media a sufficient quantity (5-10% per cent) of Japanese seaweed glue or agar-agar, pure gelatin, or Irish moss respectively. Then completely dissolved by boiling, it is strained through a flannel cloth and sterilized in flasks and flasks as before.

The cultures were grown in an incubator at almost a constant temperature, 38°C. The organisms were in nearly every case stained with methyl violet. By so doing the microtubic are more readily observed and can be more accurately described.
Fig. I. represents a highly magnified section of a tissue from the healthy peach tree. The sections are stained with tosaX Carmine, giving the tissue a beautiful red color, and making a fine structural display. The cell walls are regular and not broken or distorted. The protoplasm and starch granules are evenly distributed throughout the cells, and not even in the slightest degree coagulated. The contents of the cell are transparent as seen in the section. In the cut, e, represents the cuticle; e, the epidermal cells, and p, parenchyma.

Fig. II. represents a section from the root of a diseased peach tree. The section was given a beautiful stain with tosaX Carmine. The cells are sometimes regular, and sometimes irregular and distorted as shown in Fig. III. They are filled with large granules of starch. Occasionally a cell is seen with only a few granules.
In other sections (Fig. 15) many of the cells contain numerous exceedingly small granules which have the appearance of organisms. They stain bright-red. The tissue here have a bright-yellow color. The presence of an organism in the tissue of the section cut has not been confirmed.

Slide No. 94 is a mount from a culture in broth inoculated with a bit of the tissue from a diseased peach tree. A small particle of the tissue was taken in sterilized forceps and passed through the alcohol flame to sterilize the surface. It was then dropped into the tube of beef broth and kept in an incubator at a temperature of 38°C. On the fourth day a thick film of pellicle covered the surface of the liquid, which adhered to the walls of the tube. The surface of the pellicle was
wavy and reticulated, cast of the names pure white. Liquid below the particle quite clear.

Fig. 20 shows the bacilli after a growth of three days. Spores present but not shown in the micrograph. Bacilli stained with methyl violet. They are seen in long and short rods. Length 2\(\frac{1}{2}\)\(\mu\) - 6\(\frac{1}{2}\)\(\mu\), width 1\(\frac{1}{2}\)\(\mu\). Spore numerous, single, or in chains of two or three. All about the same size. 1\(\frac{1}{2}\) - 2\(\frac{1}{2}\)\(\mu\) (micro).

Slide No. 98. Mounted from a culture in broth, which was inoculated from the tissues of the diseased peach pit, as were all the following. Growth three days old, liquid cloudy with many fluorescent particles suspended throughout the medium. Bacillus, as all the following, stained with methyl violet. Straight cylindrical rods, slightly rounded at the ends. Length varies considerably, but average length is about 4\(\frac{1}{2}\)\(\mu\), width - 1\(\frac{1}{2}\)\(\mu\).
Spores numerous and in long chains. Edge and end.
Chi-remains of the cell walls deeply stained. Length
2.5 μ, width 1.4 μ. Fig. 21 is a micro-graph showing
indistinctly the bacilli and spores.

Slide No. 106, from a growth on Drick moss.
On the fourth day it covered about 1/4 the surface of
the medium. Proceeding irregular, amoeba-like, Surface
articulated. Bacillus not yet developed. Sporae numerous
lying side by side or in chains. Remains of the old
cell adhering to the ends of the spores and deeply stained.
Length 1.5 - 1.6 μ, width 1.2 - 1.4 μ.

Slide No. 107, from a culture grown on Drick moss.
Bacilli very small and numerous, cylindrical, needle-
shaped organisms. Deeply stained. Length -1.3 - 2.4 μ, width -

Slide 107. Bacilli and spores present. Bacilli
straight cylindrical rods, length occasionally four times the width.

Stated in Fig. 22. Spores present. Length 2.0 - 2.4 μ.
Slide No. 184, from a culture grown in broth. Pure growth of a Bacillus. Long needle-shaped rods. Length $2\frac{3}{4} - 3\frac{1}{2} \mu$, width $\frac{4}{4} \mu$. Shown in Fig. XX.

Slide No. 113, shows an organism found in a peach in supposed to be healthy. Bacilli and spores present. Bacilli straight cylistrical rods. Some of the bacilli with truncate ends, others slightly rounded. Sometimes connected in long chains, straight or serpentine. Bacilli various in length, some very long, others short. Spores thicker than the rod. Length $2\frac{1}{2} \mu$, width $\frac{4}{4} \mu$.

Slide No. 114. A very small Bacillus. Short, and appearing in chains. Ends rounded. Length $2\frac{4}{4} \mu$, width $\frac{4}{4} \mu$. Propagating by division. Deeply stained. Shown in Fig. IX.

Slide No. 119, examined on the 7th day. Liquid colored red. A long, fine thread-like mass filling filling a greater portion of the fluid. Pellicle formed on the surface but having a tendency to sink.
A long straight-cylindrical Bacillus, with truncate ends. Organisms few in number.

Slide No. 120. The culture examined on the seventh day showed a thick pellicle, partially sunken beneath the surface of the liquid; but adhering to the walls of the tube. A long growth suspended from the bottom and center of the pellicle. Under the microscope there were found a very long and continuous growth having very short joints, which after one or two days growth became broken up into individuals. The threads are about \( \frac{1}{100} \) in. in thickness. Stained deeply, seen in Fig. XV.

Slide No. 131, is a mount from a culture on solid medium which was inoculated from another growth on broth. Bacillus and spores present. Bacillus deeply stained. Straight cylindrical rods, ends slightly rounded. Length very various. Width \( \frac{1}{100} - \frac{1}{50} \) in. Spores oval or oblong.
Slide No. 132 shows a very thread-like Bacillus, multiplying by division only. Straight-cylindrical rods. Length various, but many times the diameter. Not deeply stained.

Slide No. 133. A very large bacillus. Propagating by division. Rods straight or curved, with truncate ends. Sometimes connected in long chains. Length may be several times the width. Deeply stained. No spores observed. Width - 1.4 μ. This organism is undoubtedly an "impurity" from the atmosphere and has nothing whatever to do with the disease of the peach tree. It is one of the most common microbes and is present almost everywhere - Bacillus figmantis.

Slide No. 134, shown by the Micrograph (Fig. XI). The culture is a long continuous chain of a thread-like Bacillus. When young the plants are almost imperceptible, but with age the individuals become.
separated. The length varies from very short to many times the thickness. Organisms deeply stained. No spores present. Thickness 44 µm.

Slide No. 138 is a mount from tube No. 136, which was inoculated with a portion of the tissues of the bark of the diseased peach tree growing in the greenhouse. The tissues were taken from the branch about 2 in. below the canalized limb, and was about 2 in. long 7/16 in. wide. The surface of the tissue was sterilized by passing it twice or thrice through the alcohol flame. Then two incisions were made with a sterilized scalpel about 4 in. apart, broken open, and some of the underlying tissues were taken out with sterilized forceps and introduced into beef broth. This process was supposed to prevent the introduction of any microbe except that in the tissues.

A microscopic examination of the liquid in the
next day after inoculation, showed numerous short, cylindrical Bacilli. Mostly straight rods as shown in Fig. XIX, but sometimes slightly curved. Propagation by division. Ends truncate when division first takes place but soon becoming rounded. Deeply stained. Length 13-15 μ, width 1/4 - 1/4 μ.

Slide No. 147. Growth one day old. A short, straight, or curved Bacillus. Deeply stained. Length 13 - 2½ μ, width 1/4 μ. Bacilli shown in the Micro-graph (Fig. VII). Some of the mounts from the culture showed numerous spores, small and elliptical. Length 1 1/3 μ, width 1/4 μ.

Fig. X is a Micro-graph taken from slide No. 153. A very small micrococcus, which occur 3-6 in pairs.

Slide No. 242. A culture from a roll tube. The growth of the bacteria in the roll culture was very characteristic. It soon covered the whole surface of the medium, as a white film. Under the microscope it revealed a short...
Bacillus, in some cases having an oval form, deeply stained and multiplying by division. No spore formation observed. Length 22.55 µ (Fig. VIII).

Slide No. 232. Enzyme culture, containing a large and small Bacillus, both deeply stained. Large Bacillus, in length many times the thickness, shown in Fig. V. Width 154.4 µ. Small Bacillus oval, or oblong. Length 131 µ, width 174.1 µ, not seen in the Micrograph.

Slide No. 241. A short oval Bacillus. Propagating by division. Organisms for a short time after division perfectly round, but becoming oval or elongated later. Length 131 µ, width. Undoubtedly the same organism shown in Fig. IX, but at a younger stage of development.

Fig. XIX is a micrograph of a slide No. 172. It shows a pure culture of a Bacillus. Propagation by division, endo-luminate, and connected in long chains.

Fig. XXI is a micrograph from slide No. 183. This organism is by far the most important since it is supposed to have some close connection with the peach yellows. It was in three different cases isolated from the diseased peach trees and grown on artificial media. The organisms resemble very much those causing "pear blight" (Fig. XIX), but the former are more elongated than the "blight." Four young healthy peach trees were inoculated March 25, 1889, with this organism, three of them by introducing the organisms beneath the bark one by pouring water containing the microbes into the earth about the tree. Up to the present time, April 30, no sign of the disease is made apparent by the young trees. The young trees were planted in the greenhouses and had begun to leave out their...
inoculation was made.

Fig. X shows, also, a very small Bacteria which was isolated from the root of the diseased peach tree.

Fig. XVI and XVII show two different kinds of micrococci which were isolated from the diseased peach trees in the biological laboratory, but by another person.

Many other slides were mounted, examined and described, but were found to be the same organisms which had been previously described and were therefore omitted.