Physarum variabile Rex.
Physarum viride Pers.
Spumaria alba DC.
Stemonitis ferruginea Ehreub.
Stemonitis fusca Roth.
Stemonitis splendens Rost.
Trichia affinis De Bary.
Trichia favoginea Pers.
Trichia persimilis Karst.
Tubulina fragiformis Pers.

ACRASIEAE.

Chondromyces lichenicolus Thaxter.
Myxococcus stipitatus Thaxter.
Myxococcus rubescens Thaxter.

The Germ of Pear Blight. By Lillian Snyder.

It is certainly an established fact that the well-known disease of Pear Blight, which causes such devastation among our pear, apple and quince trees, is caused by bacteria within the growing tissue of the tree. The germ which causes the disease was discovered by T. J. Burrell, and was first described and named by him in 1882.*

The germ I have isolated from the pear tree, and which I think I can say without a doubt causes the disease of the tree, I shall designate by the name used by other writers, and originated by Prof. Burrell; that is, Micrococcus amylovorus. Whether the above germ spoken of is the same as the one handled and studied by Prof. Burrell, and also by J. C. Arthur,† I leave to be gathered from the results of my experiments. Early in March, 1897, I attempted to separate the germ which causes the blight of the tree. Various methods were used, such as cutting pieces of diseased bark of pear, with a sterilized knife, and placing in bouillon; also by inserting a platinum needle between the bark and wood of diseased tissue and streaking upon agar. The latter proved the most success-

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* Eleventh Report of the Illinois Industrial University, p. 42.

† Proceedings of the Philadelphia Academy of Nat. Sciences, 1886, pp. 322-341.

Fig. 1.—Pear twig in water with the germ micrococcus amylovorus growing upon the cut surface.

ful, and by this method a germ was obtained which I carried through a number of experiments and found to be in many respects like the germ described by Dr. Arthur in his History and Biology of Pear Blight. This germ was studied and kept alive until the latter part of May, when the pear was putting forth its new shoots, the young branches being then used for inoculation. Out of about ten inoculations, at different times, not one appeared to have any effect upon the tree. I concluded I was mistaken in the germ, which resulted in a second attempt to isolate the germ from the tree.

By this time the trees were in all their foliage, thus making the disease much more readily detected than previously. The same method was used as above described as successful, and out of six or seven attempts to transfer the germ from the host upon agar, five were successful. This germ thus obtained was then used to inoculate into the young growing shoots of the tree (Bartlett pear), and every inoculation that was made caused blight of the tree. This was during the month of June, the temperature being on an average of 70° F., the atmosphere moist, rainfall 5.16 inches, being 1.34 above normal, thus favorable for the increase in growth. At the end of a week after the blight first began to appear it was not unusual to see the branches blighted 10 or 12 inches from the point of inoculation.

In inoculation only perfectly healthy pear and apple trees were used, the former taking the disease with much more readiness than the latter. The germ was taken from streak cultures upon agar, a sterilized platinum needle being used to transfer the germ to the surface of the young twigs. After the surface had been smeared over with the germ, openings were made through the bark layers at this point, enabling the germ to get well started to grow before it might be washed off by rain.

Leaves do not take the blight when inoculated. Naturally, the germ appears to be confined to the branches, the leaves only dying when their nourishment has been cut off.

GENERAL CHARACTERS OF MICROCOCCUS AMYLOVORUS.

The cells are oval, very little longer than broad, being about .59μ to .8μ wide by .89μ to 1.2μ long. Cells are colorless, very refractive and difficult to stain, resembling spores in their relation to stain. The best results in staining were obtained with carbol fuchsine. When grown

upon agar the cells are usually found single, very often in pairs and occasionally in chains of fours. When growing in bouillon or other nutritive media the germ exhibits independent movement, thus differing from the habits of the germ within the host. When sections of the diseased branches are placed under the microscope the bacteria show very little, if any, movement.

This germ appears to be aerobic, and increases in growth with an increase of temperature.

Growth upon agar was most rapid during the months of July and August, the average temperatures for these months being 73.3° F. Later in the season the germ ceased to grow with the same rapidity, although exposed to a high degree of temperature.

Spore formation was not observed, and I am inclined to believe spores are not formed.

CULTURES WITH LIQUID AND SOLID MEDIA.

Cultures with bouillon after 48 hours at 30° C. remained perfectly clear, the growth all settling to the bottom of the tube. Not in any case have I observed zoogloea formed.

In a pure corn-starch solution inoculated with the germ, the germ survived and multiplied, but the starch was not chemically changed. Two cellulose solutions* made from Swedish filter paper were used. In one glucose was used and the other sugar was not added. In these solutions I planted the germ, and at the end of a week no apparent change had taken place in either solution, but the one which did not contain sugar originally was tested, and it was found that cellulose had been changed into glucose.

Various fermentation fluids were used, but this germ does not ferment under any conditions.

The most convenient solid media for the cultivation is agar. Colonies are white, slightly opalescent, when vigorously growing raising like beads from the surface. In a streak culture the outline is slightly feathery. Agar which had not been titrated, but used as made up, slightly acid, was found to give the best results in the growth of the germ.

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*Cellulose solution was made up as follows; Peptone, 10 grams; Salt, 5 grams; Glucose, 20 grams; Pure Cellulose, 20 grams; Water, 1000 C. C.

In a stab culture in nutrient gelatine the growth spreads over the surface and along the line of inoculation, but does not penetrate the gelatine, thus remaining for several weeks without any sign of liquefaction.

Probably the most characteristic result is the growth upon pear twigs. The end twigs to a length of two or three inches were cut from the tree. These were placed in water, the upper cut surface covered with the growing germ, and the vessels containing the twigs then placed under a bell jar for 24 hours. At the end of this time about two out of every four of the twigs had a beautiful growth over the cut surface. This growth was in the form of globules, as many as three or four globules being on one twig. These bead-like colonies, being white and raised from the surface, were perfectly apparent to the unaided eye. (See Fig. 1.) This growth increases, turning yellow when old, until entirely destroyed by moulds.

Another very important characteristic of Micrococcus amylovorus is the manner of growth within the growing pear fruit. A half-sized Bartlett pear upon the tree was inoculated in about the same manner as the branches had been. The pear ceased to grow and soon began to shrivel. At the end of two weeks the pear was removed from the tree, and upon examination the whole interior was found to be composed of a soft, milky substance, which appeared under the microscope to be made up entirely of bacteria.

Half-ripe pears were taken from the tree, cut in slices, inoculated with the germ, and then placed under a bell jar. Colonies were found in 24 hours, which resembled those already described upon agar.

During the month of October cultures were taken from quinces which has been inoculated with M. amylovorus. This second germ proved to be entirely different from M. amylovorus, but alike in all respects to the germ separated from the tree in March. This germ, which I shall term No. 2, I feel convinced occurs within the tree. But what relation it bears to M. amylovorus, if any, I am not prepared to say.

Germ No. 2 when transferred to pear twigs in water grows very well, but does not survive or grow with the same vigor as M. amylovorus does when placed under the same conditions. The growth of No. 2 is not raised in bead-like colonies, but grows in a continuous mass, over the surface, and instead of being white, is transparent.

Microscopically the two germs are so near alike it is impossible to separate them. They bear about the same relation to stains and the cells are of about the same size. The arrangement of the cells of the two germs differ some, but this is not constant.

CULTURES WITH LIQUID AND SOLID MEDIA.

Cultures with bouillon after 24 hours at 30° C. are very turbid, and a thick pellicle is formed over the surface of the liquid. The germ grows well in a corn-starch solution, but the starch is not broken down. Cellulose solutions made as above stated were inoculated. At the end of two days both had fermented, thus in the one without sugar, sugar had been formed. In this one in which sugar was formed the gas production was very slow.

Germ No. 2 planted in Smith solution causes fermentation and gas forms very rapidly. Mr. A. H. Bryan, a student of Purdue University, who was so kind as to analyze the gas produced by this germ, has given the following results of his analysis:

From germ No. 2, separated from the pear tree in March, 1897:

CO_2	45.0 per cent.
O	.83 per cent.
H	26.3 per cent.
CH_4	0.00 per cent.
	73.13 per cent.
Remainder or N	26.87 per cent.

From germ No. 2, separated from the quince in October, 1897:

CO_2	50 per cent.
O	None.
CO	None.
H. by Palladium	32 per cent.
Total	82 per-cent.
Remainder or N	18 per cent.

It is presumed that the remainder in each case is nitrogen, but just what per cent. is nitrogen is not yet known.

Gelatine was inoculated while liquid, then allowed to solidify. At the end of two days colonies appeared as small white dots beneath the surface of the gelatine. In gelatine thus inoculated with M. amylovorus such colonies were not apparent.

Upon agar the only essential difference in the two germs that might be noted is that germ No. 2 grows much more rapidly than M. amylovorus does when exposed to the same temperature.

This paper has been prepared under the direction of Dr. J. C. Arthur, to whom I am very much indebted for a number of suggestions which have been of great value in my experiments.

Water Power for Botanical Apparatus. By J. C. Arthur.

In vegetable physiology a number of kinds of apparatus are required which must be run at an approximately uniform speed. Some of the most important of these pieces are used to influence the direction of growth, and as plant movements dependent upon growth are slow, the apparatus must often be kept in motion continuously from twenty-four to seventy-two hours or more.

The chief reliance where the movement is very moderate, ranging as it does for clinostats between ten to sixty minutes for one revolution, has usually been some form of clock-work, regulated by escapement or fan. For comparatively rapid movement, such as a centrifuge requires, which ranges from fifty to five hundred revolutions per minute, recourse is generally had to water or electric power. Both of these sources prove very unsatisfactory as a rule, for machines doing such light work as the physiologist requires, and especially when they must be run steadily and without interruption both day and night.

Electric power from a commercial plant usually varies greatly, and, moreover, is rarely continuous for the twenty-four hours. If the power is taken from a battery of some form of cells, the difficulty of maintaining a uniform current is almost as great, beside the annoyance of caring for the cells. The potash cells, especially those sold under the name of Edison-Lalande, have given the best satisfaction for this kind of work of any so far tried in the laboratory of Purdue University. But even these are treacherous, and quite uneven.