method of obtaining the zygospores of *Cunninghamella* by contrasting in cultures different strains of this species. By a sufficient accumulation of material from different sources, one might expect eventually to obtain the two sexual strains and, by their synthesis, the zygospores as well of any form in which the sexes are separated on different mycelia.

Undoubtedly a careful search below the sporangial growth would show that zygospores are more common in spontaneous cultures than is usually supposed. In searching for forms of the mucors, the writer had occasion to make cultures on sterilized paste and on bread of various substances among which the shells of different kinds of nuts were thus investigated. It may not have been a mere accident that in all the several instances in which shells of almond nuts were used, zygospores were produced, but failed to appear when shells from other kinds of nuts were employed. These almond nuts were obtained from different places in the neighborhood of Boston, though they all probably came from the same source originally. The writer would be glad to learn if others have a similar experience with the shells of this species from other localities.

In making cultures, the bread should be raised above the bottom of the culture dish by some convenient object, otherwise the bread is likely to become soggy and attacked by bacteria. Layers of moistened filter paper on the sides and bottom of the dish, which should be covered, ensure a proper moist condition in the surrounding air. Those desiring to have absolutely pure cultures may first sterilize the bread dry and then allow it to soak up a sufficient amount of sterilized water to give it a spongy consistency, after which it may be sterilized with steam for about five minutes. Prolonged sterilization as well as too much water in the bread makes it soggy and less advantageous for cultures. Sterilization is not necessary, however, for class work. Zygospores when present are likely to form in dense masses between the layers of moist filter paper lining the culture dish or in the folds of crumpled pieces of filter paper placed in the culture as traps for their capture. Sporangia form where the air is dryer, and the habit of growth can be readily studied from the filter paper in the upper parts of the culture. The individual stolons can be more easily distinguished if darkened paper be employed. When zygospores are once obtained, mass transfers of the mycelium may be made to new cultures and thus the *Penicillium,* which is a usual weed in spontaneous cultures, may be eliminated. A culture producing zygospores may be dried with its substratum and used from time to time as 'seed' whenever zygospores are needed. Zygosporic cultures of the 'Harvard Strain' have thus been kept running for nearly ten years. The sporangiospores of *Rhizopus* are comparatively short lived, however, and generally do not retain their vitality for more than a year.

For methods of separating out the two sexual strains from a zygosporic culture, which is often a tedious process, one may refer to the writer's detailed experiments with this species already cited.

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Results from Moore's Method of Shipping Bacteria on Cotton.

In Science of March 23, Messrs. Kellerman and Beckwith have called attention to the statement in Bulletin 270 of the New York Agricultural Experiment Station that [certain[1]] 'cultures of nodule-forming bacteria dried upon cotton were worthless for practical purposes and that the failure of such cultures was inherent in the method of their preparation.' At the same time they presented some excellent data upon the effect of drying legume bacteria under various conditions. Their most interesting experiment consisted in placing a culture "on cotton half of which was placed in a sterile Petri dish, to make drying very slow, half was dried rapidly and kept

[1] An important word omitted by Messrs. Kellerman and Beckwith in summarizing the statements contained in Bulletin 270.

over calcium chloride. After 25 days the cotton in the Petri dish was sterile; the cotton from the desiccator was a pure culture in good condition, containing numberless organisms." They also found that cultures properly dried and then exposed to moist air died within a few days.

The experiment with cotton in the Petri dish was evidently intended as a repetition of similar experiments given in Bulletin 270 in which inoculated cotton placed in Petri dishes became practically sterile within a few days. The analytical results obtained at the two laboratories were practically identical and it may now be considered fairly established that legume bacteria placed upon cotton according to Moore's method do not survive slow drying. The exact limits of the exposure which they will survive remains to be established.

It should be kept in mind that the present discussion is restricted to certain packages of inoculated cotton which were offered to the public last season and to the methods by which these packages were produced. The point which should be made clear is which of the portions of cotton in the experiment of Messrs. Kellerman and Beckwith most nearly represents these packages. The eighteen cultures discussed in Bulletin 270 were purchased in the market and were the product of a single commercial laboratory. They were repeatedly examined, six of them being tested in each of five different laboratories, and were found to contain extremely few or no living specimens of the desired germ. Their worthlessness for practical purposes was accordingly settled beyond question. Eight other packages of inoculated cotton put up by the same firm but obtained through other channels were examined with similar results.

The method of preparation of these cultures, as stated by the manager of the company to the writer, was to dip the rolls of absorbent cotton into the culture fluid and suspend them in a room until air dry. The cotton was then cut into squares and shipped in pasteboard boxes. Judging from the fact that the germs were practically all dead in the packages examined by us, either the cotton dried slowly or the germs were killed by being exposed to moist air. The chemicals accompanying the cultures uniformly showed the presence of absorbed moisture.

Since this commercial method of preparing cultures included both slow drying and shipment in a package which exposed the cotton to moist air and the germs were actually killed by this treatment, it is plain that it is the cotton in the Petri dish in the experiments by Kellerman and Beckwith which fairly represents the commercial packages.

A careful study of the facts leads to the conclusion that the criticisms justified by our results with commercial cultures are equally applicable to the methods of the Bureau of Plant Industry up to the season of 1905 at least. Until a few months since, the member of the Bureau of Plant Industry then in charge of the legume-bacteria investigation was also in close touch with this commercial company. It was accordingly to be expected that the methods employed in both laboratories would be practically identical and any important criticism against the methods employed in, or the product of, one laboratory would apply to the other. For this reason an examination of the methods employed, and the packages of inoculated cotton put out, by the Bureau of Plant Industry during the past two seasons is both interesting and instructive.

The earliest official description of the bureau's method of preparing the cultures on cotton is given in Letters Patent No. 755,519, dated March 22, 1904, and signed by G. T. Moore. These state that 'absorbent cotton or other equivalent material is dipped into the water containing the organisms or the water containing the organisms is sprinkled upon the cotton or other material and the same thoroughly air dried in a chamber free from dust or contamination by mold.' A more recent and detailed description of the method is given by L. P. Sprague in a thesis presented to the faculty of the University of Vermont on 'The Fixation of Nitrogen by Leguminous Plants,' dated May 1, 1905. Mr. Sprague served as an assistant to Dr. Moore in the Bureau of Plant Industry and gives the following detailed description of the method there employed. "Absorbent cotton of the

best grade, which has been sterilized by heating, without unrolling, in the dry oven about four hours at 150° C., is unrolled and placed in a tin pail, the pail having been sterilized by rinsing in boiling water, and the culture poured over it. The cotton is thoroughly saturated with the culture solution, by pressing and squeezing it with the hands. It is then hung up in the culture room, where it is nearly free from foreign organisms, and allowed to dry, *drying taking place in twenty-four to forty-eight hours.* It is then stored in pasteboard boxes in the laboratory until ready for use. Other methods for inoculating and drying have been attempted, but thus far they have been unsuccessful."

In a recent repetition of our experiments in Petri dishes as detailed in Bulletin 270 it was found that the inoculated cotton under such circumstances becomes air dry in approximately twenty-four hours. Accordingly it is seen that the method of preparation employed by the Bureau of Plant Industry not only is practically identical with that employed by the commercial company above described, but also the rate of drying is practically the same as that in our Petri dish experiments in which the germs placed upon the cotton died promptly.

Our own examinations of the bureau cotton is limited to six packages. While the number of packages is small, it is significant that from none of these packages did we succeed in isolating a single specimen of *P. radicicola* although some of the packages were repeatedly examined. Similar laboratory tests of a number of packages of bureau cotton were made at the agricultural experiment stations in Delaware and North Carolina with similar negative results. The results of pot and field tests at the agricultural experiment stations in Pennsylvania, Oklahoma, Georgia, Maine, New York (Cornell) and Wisconsin as published fail to show any well-marked results from the use of inoculated cotton furnished by the bureau. Two other stations, Michigan and Virginia, have kindly furnished us with a summary of like unpublished tests which are also negative.

Although such may exist it is a surprising fact that thus far we have not learned of a single experiment conducted at one of the many state agricultural experiment stations where the inoculated cotton put out by the Bureau of Plant Industry has given good results.

The marked exception to this wide record of failure is furnished by Bulletin 71, Bureau of Plant Industry. When considering the favorable reports there presented we are forced to conclude that an explanation is to be looked for largely in the psychological, rather than in the biological, realm.

When we consider the methods of preparation, storage and shipment employed by the Bureau of Plant Industry as described by Sprague in connection with the data upon the effect of slow drying and moist air as given by Messrs. Kellerman and Beckwith, an utter failure of the bureau's cultures was the only result which could be logically expected.

H. A. Harding.

New York Agricultural Experiment Station, May 2, 1906.

QUOTATIONS.

ZOOLOGICAL GARDENS AND SCIENTIFIC RESEARCH.

Dr. Gustav Loisel, who is a professor of zoology in the Sorbonne, is making persistent efforts to have the menagerie in the Jardin des Plantes adapted to the needs of experimental science. He would have it so transformed as to become a school of zoological research without at the same time ceasing to be a place of entertainment for the people. Such a plan has been partly carried out in Bronx Park, so that certain fauna are permitted to live and breed almost as if they were in 'the wild.' The experimental stations in this country where marine animals and plants may be studied have proved of the highest value to science; and the laboratory in connection with the Naples Aquarium has long been a favorite resort for naturalists. Two of Dr. Loisel's suggestions are not likely to meet with popular approval. One is to do away altogether with the monkey house, which he says is infected with tuberculosis, and the other is to diminish the number of the more formidable wild animals to make room for beasts whose habits