THE AGAR-AGAR AND PARAFFIN METHOD FOR IMBEDDING PLANT TISSUES.

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In the Journal of Applied Microscopy and Laboratory Methods 6: 2591–2, 1903, the writer gave an account of a method for killing and imbedding plant tissues in a hot solution of agar-agar. While this method is applicable for most histological work, sections cannot always be obtained as thin as are sometimes desired. Recently a method for imbedding and sectioning plant tissues in paraffin after they had been killed and imbedded in a hot agar-agar solution was tried.

The following are some of the sections made by the agar-agar and paraffin method: Sections of leaf of date palm; sections of leaf of Ficus elastica; sections of stem of Begonia; sections of stem of Equisetum arvense; sections of leaf of beech; sections of a Uromyces on Sparganium eurycarpum; sections of a Phyllachora on Panicum; sections of a rust on Scirpus.

The tissues were first killed and imbedded in a 2 per cent and 5 per cent solutions of agar-agar and then imbedded in paraffin in the usual way.

The 2 per cent solution of agar-agar can be made as follows: Take 10 grams of agar-agar to 500 c. c. of distilled water and boil for two hours. An ordinary oat-meal cooker can be used for boiling this mixture. Filter the agar-agar through a cheese cloth into a glass jar before it is allowed to cool and add formalin in the proportion of one part of formalin to nine parts by volume of the agar-agar.

The 5 per cent solution is made in the same way as the 2 per cent solution, only 25 grams of agar-agar to 500 c. c. of distilled water are taken. Formalin should be added in the same manner and proportion as in the 2 per cent solution. Large quantities of the agar-agar solutions can be prepared and preserved in air tight vessels to prevent evaporation.

The tissues were first put into the 2 per cent agar-agar solution. Put a small quantity of the 2 per cent agar-agar into a test tube or small wide mouth bottle and place with contents into a vessel of boiling water until the agar-agar is melted. After the agar-agar is melted it should be kept at a temperature of 70° C. The tissues are placed directly into the hot 2 per cent solution for two hours. Then they are transferred into the 5 per cent solution, which has been melted in the same manner as the 2 per cent solution and allowed to remain for an hour or more. The tissues are imbedded in the 5 per cent agar-agar. Take a small wooden block or a plate of glass and with a camel's hair brush put a layer of the hot agar-agar on one end of the

block, let it cool for a few seconds and place one of the pieces of material on the block and cover with more agar-agar. Allow it to cool for a few minutes, when it is removed from the block and placed in 70 per cent alcohol and passed thro the different grades of alcohol to paraffin and imbedded. The tissues should remain for two or more hours in each of the different grades of alcohol.

No albumen fixative is necessary to attach the sections to the slides and the sections can be stained as any other paraffin sections. Delafield's haemotoxylin and Safranin and gentian violet are favorable stains. The agar-agar surrounding the sections stains in Delafield's haemotoxylin but it takes only a slight stain in Safranin and gentian violet.

It seems that this method will be very valuable for sectioning tissues that would be easily torn by the ordinary paraffin method, and especially applicable in the study of rusts and other parasitic fungi. The layer of agar-agar around the tissues becomes very tough when passed thro the alcohols and forms a firm medium which prevents the tissue from being torn when sectioned.

The Phyllachora mentioned above, was dried and kept in the herbarium. The material was firmly pressed and thoroughly dry and in spite of these facts, the perithecia were sectioned without any injury and the hyphae could be seen in the adjacent tissues of the leaf. The Uromyces was collected in October, 1904, and the tissues of the leaf were entirely dead. The sections showed the delicate teleutosorus and spores in fine condition. The parts sectioned were cut into small pieces and placed in hot water at about 70° C. for an hour and then transfered to a 10 per cent solution of hydro-fluoric acid for twelve hours to remove the silicon which would otherwise interfere with the sectioning. The material was washed and imbedded in the manner already described. The stem of Equisetum was also herbarium material and was treated in the same manner as the Phyllachora and Uromyces. The sections obtained were in good condition for such material. The beech leaf was from alcoholic material and the sections showed the different parts of the leaf in excellent form. This method can be used to the best advantage where a histological study of the plant tissue is desired.

It is much shorter than the oridnary paraffin method as the aqueous solutions of agar-agar penetrate the tissues without any preliminary dehydration. Serial sections were cut as thin as 10μ.

A few scale insects found on a palm were also imbedded and sectioned and fairly good sections were obtained. This method will perhaps be useful in the study of insects.