This raises the question as to the effect of the composition of the soil solution upon the amount of easily freezable water in plant tissue. Possibly we shall be able to present results of experiments dealing with this question at a later date. It seems that the amount of water that readily freezes in the roots, stems, leaves, fruits, and seeds of plants and the factors that affect the freezing should be of general interest, at least to the physiologist, and it is probable that a knowledge of it would be valuable especially where the changes in the concentration of the cell contents of plants as well as winter injury are being investigated.

The difficulty encountered in causing tissues to solidify at the higher temperatures, especially when small amounts are used in the determinations, raises some important questions relative to winter injury of plants grown in different soils. It is possible and probable that some soils do not solidify, although the temperature may go appreciably below the freezing point. It is very easy to cause a sandy soil to solidify without much supercooling; with clay it is more difficult; while it is far more difficult in case of muck or peat. Instances have been observed where fruit trees growing in sandy soils have been severely injured by low temperature, while those growing in adjacent soils largely escaped. It is true, however, that sandy soils are more responsive to air changes than are the finer textured ones.—M. M. McCool and C. E. Millar, *Michigan Agricultural College, East Lansing, Mich.*

ISOLATING SINGLE SPORES

(WITH ONE FIGURE)

A new method of isolating single spores has been devised, which differs from other methods in common use in the substitution of a mechanical method of marking the location of the spores in the poured plates for the usual procedure of marking with ink-dots under the microscope. A cylinder of brass about the length of the ordinary 1.9 mm. objective is turned in the form shown in fig. 1, one end being provided with a thread like that of the objectives of the microscope to be used, and the other turned down and the end hollowed out so as to form a tube of the size desired.

This device is then screwed into the revolving nosepiece of the microscope in place of one of the objectives. The cover is now removed from the Petri dish containing the poured plates, and the spores are located under the microscope. When a spore is located with the objective, the tip of the marker is sterilized by flaming it with a gas burner or alcohol lamp, the nosepiece is rotated so as to bring the marker

over the spore in the place of the objective, and the marker is lowered so as to cut out a disk of agar inclosing the spore. The spore may then be examined again with the objective to see that it is really included in the agar disk, after which the disk is lifted from the Petri dish with a flattened platinum wire and placed in a culture tube. This method has the advantage of allowing a rapid and accurate location of spores and of guaranteeing that only a single spore is transferred. The disadvantages of exposing the culture to the air for rather long intervals is not a great one, because contaminations need not occur if proper precautions are taken. For such work the ringed tip of the marker may have a diameter of about 5 mm.

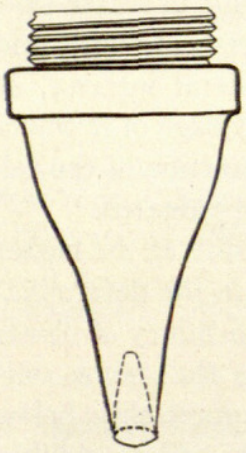

FIG. 1

A modification of this method has been found of great use in the selection of spores of *Pestalozzia*. In this case it was necessary, not only to locate single spores accurately, but also to measure the spores when located, so that the longest and the shortest might be taken. Finally it was found that this could be done by making dilution cultures, and then spreading the agar of the tube containing the desired dilution in a thin film on sterile glass slides. These slides could then be examined under the microscope, the spores measured, and those of the size desired cut out by the marking device, and finally the disks containing the spores could be transferred to culture tubes.

The agar for making the dilution cultures needs to be very carefully filtered so as to be as transparent as possible. For the work with *Pestalozzia* a 1 per cent solution of Liebig's beef extract with 3 per cent agar was used and found satisfactory. The nutrient solution, of course, must be of such a nature as to be suited to the fungus in question. The agar film on the slides is formed by pouring two or three drops of agar on the slide and then spreading them over the entire surface with a sterile needle. Too thin a film cannot be lifted from the slide; too thick a film will not allow the high power objective to be used, which is necessary with measurements of very small spores, so that some practice is needed to secure good results. I am indebted to Miss BACHMANN[3] for the idea of using agar films on slides, although films formed according to her method are too thin for this purpose. In this selection work a marker with a tip 1.5 mm. in diameter was found most useful.—CARL D. LARUE, *Kisaran, Asahan, Sumatra.*

[3] BACHMANN, FREDA M., Amer. Jour. Bot. 5:32–35. 1918.