NOTE ON THE PRODUCTION OF FRUITING BODIES OF COPRINUS MICACEUS IN CULTURE[1]

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During 1927 and 1928, the writer made some studies of the sex reactions of the common glistening ink cap fungus, *Coprinus micaceus* (Bull.) Fr. In this work fruiting bodies were produced in pure cultures started from spores. The methods employed differ in some respects from those used by other investigators of the sex reactions of the higher Basidiomycetes and, with the results, may prove of interest to many in the botanical field.

Fruiting bodies of *Coprinus micaceus* were found growing abundantly in several locations within a few city blocks of the Ohio State University campus and thus a constant source of supply was assured throughout most of the summer. This fungus is often found in lawns, where shade trees have been removed and the roots left in the soil. Although not specific for Cottonwood (*Populus deltoides*) it was found growing commonly from the decaying roots of this species. Spore prints were made, by the following method, from fruiting bodies which had reached maturity but had not started to deliquesce. Portions of lamellae a few millimeters in area were removed with a small pair of forceps and placed on a microscope slide. Another slide, which had been previously washed, flamed and cooled was gently pressed to the upper surface of the lamella and a spore print resulted. These slides were stored in slide boxes until they were needed for further work. This was usually within a week of the time when the prints were made. After some practice, spore prints could be obtained in which the four spores from individual basidia retained their original position with respect to each other. These spores were later picked off the slides with a sharp needle as described below. Some experience was found necessary in selecting fruiting bodies in which the spores were in the proper stage of development to make spore isolation by this method possible. If too young, the spores were light colored and so delicate that they were mashed when touched with the isolation needle. If the fruiting

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bodies were old, the spores were covered by the slime resulting from the deliquescence of the lamellae and adhered to the slide rather than to the needle. When of the proper age the spores were black in color, the spore print was dry, and the spores did not adhere in masses. At this stage individual spores adhered to the point of the isolation needle rather than to the slide and were easily removed.

For the reception of isolated spores, Petri dishes were prepared by cementing four ring cells in the bottom of each with beeswax. The covers were then placed on the dishes, which were sterilized in a hot air oven for one hour at 80 degrees centigrade. Square cover glasses, of dimensions which would cover the ring cells, were cleaned, flamed and cooled. In the center of each glass, a drop of dung agar was placed with a loop needle. The cover glasses with the agar drops on the lower surfaces were then placed over the ring cells in the Petri dishes. The dung agar used in this work was prepared by stirring up 300 gms. of fresh horse dung in 1 liter of water and filtering off the resultant liquid through cotton. To this was added 18 gms. of agar and the mixture heated until the agar dissolved. Where the medium was unusually opaque it was clarified with egg albumen. This is essentially the same medium as used by Newton (1926).

Working under a 16 mm. objective of the microscope, individual spores were picked up from the spore print slides with a sharp darning needle which had first been passed through several thicknesses of clean linen handkerchief. The 16 mm. objective gives ample working space between the lower face of the objective and the spore print slide. When a given spore was selected for transfer, all surrounding spores were first swept away with the needle. The needle was then cleared of spores by passing through the handkerchief several times and the desired spore touched with the needle, to which it adhered. When the spore had been picked up, the needle was thrust into a drop of agar on the lower surface of a cover glass, and the cover glass returned to its ring cell. The Petri dish, without cover, was then placed under the microscope and the drop examined through the cover glass to verify the spore transfer and also to make certain that no other spores had been transferred with the one desired. Since the spores were black and the medium clear amber in color, this could be easily done. In cases where the four spores of a single basidium were isolated,

these were placed separately in the four ring cells of one Petri dish. The spores were then examined daily until germination was observed, when the agar drops containing the germinated spores were transferred with a sterile needle to separate tubes of dung agar. By this method of spore isolation, which follows mainly that described by Hanna (1924), some 120 spores were isolated. Of these, 35 germinated and developed monosporous mycelia in the tubes to which they had been transferred.

These monosporous cultures were planted together in pairs on dung agar in plates. After a period of about one week, the mycelium in the plates was examined for the presence of clamp connections which is considered by most investigators to be evidence that hyphal fusion has occurred. In all, 79 of these pairings of monosporous mycelia were made and clamp connections were found to be present in abundance in 39 of the plates, while in 40, no clamp connections could be found. If the presence of clamp connections is an indication that hyphal fusion has occurred and is consequently a good criterion of the diploid condition of the mycelium resulting from certain of the pairings of monosporous mycelia, then monosporous cultures of *Coprinus micaceus* are unisexual rather than bisexual, since only those monosporous mycelia resulting from spores of opposite sex were capable of hyphal fusion. The unisexual nature of monosporous cultures of this species had previously been reported by Vandendries (1926) although this was unknown to the writer at the time.

An attempt was made to grow typical fruiting bodies of this fungus from mycelium which bore clamp connections. About one inch of sand was placed in the bottom of each of four small beakers and on this was placed one-half inch of a mixture of decayed cottonwood and horse dung. The beakers were then sterilized for one hour at 15 pounds pressure and, after cooling, agar from those Petri dishes which contained mycelium having clamp connections was placed on the mixture. The beakers were covered with cheese cloth and placed under one of the greenhouse benches. The cultures were watered with an atomizer through holes in the cheesecloth covers, care being taken not to saturate the soil. In about one month the first small "buttons" of the fruiting bodies appeared in two of the beakers. These later grew above the soil and expanded in the usual manner.

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