Methods Employed in Uredineal Culture Work.

By Frank D. Kern.

The first researches which proved a positive relationship between the different fruit-forms of the Uredinales not only played an important roie in the classification of these fungi, but invested a further study with much interest. A flowering plant which would produce even two separate and distinct sorts of fruit would indeed be a curiosity, and yet these parasites exhibit from one to four kinds of fruiting bodies, and many of them, seemingly to vary their existence, possess the power of living upon two entirely unlike hosts. Further attempts at classification, as well as all economic efforts to control the pests have demonstrated a necessity for a more intimate knowledge of the life-history of these parasitic plants. The connection between the different stages in a life cycle is best shown by means of cultures, and the scientific importance of these inoculation experiments can not be overestimated.

Among the immediate advantages to be gained is the connecting of unattached aecia with their later stages, and to ascertain the range of hosts. Some rusts are doubtless restricted to single species of hosts, both for their aecial and telial forms, but since cultures have shown that some heteroecious species may have their aecial hosts belonging even to different families of plants, it is evident that exact relationships can be established only by morphological characters and field observations, affirmed by artificial cultures. In the autoecious species it is sometimes impossible to tell how many spore forms there may be. Such a specimen can not be placed in its proper species on account of the close resemblance of some isolated spore forms. Cultures offer a ready solution to this problem.

Although the various processes in the cultivation of the rusts are comparatively simple, so little has ever been said regarding the apparatus and methods employed that a more detailed account does not seem out of place.

The spring months are the period when most of the work must be done, as this is the normal germinating period for the resting spores, and

is also the active growing season for the host plants. Any sort of spores can be employed for the experiments and the methods vary accordingly. A smaller portion of the work, with urediniospores and teliospores which germinate at once, may be carried on through the summer months and early autumn.

The best success has been attained through the sowing of teliospores which give rise to pycnia and aecia in turn. All grass and sedge rusts furnish telial material, and since they are, with a single exception, known to be heteroecious, any collection affords culture material. Teliospores are usually resting spores and normally retain their viability through the winter. Collections made in the fall and kept in a warm, dry room during the winter usually fail to germinate. The freezing temperature of the outdoor atmosphere does not seem to be detrimental, and some plan to prevent the specimens from thoroughly drying is a necessity. Cloth packets are to be preferred to paper, as they do not take up moisture so rapidly and allow a better circulation of air. These packets may be hung out of doors, or an unheated shed without a floor seems to furnish good conditions. The material put up in this manner may be sprayed occasionally in the fall and winter, but an effort must be made to keep them in a uniform condition. Collections made in the early spring after they have wintered over in the field usually show vigorous germination. Late spring collections are of less value, as the most vigorous are liable to have grown in the field. Spores collected as early as July and August have been brought to germination and have been sown with success, but October and November collections survive through the winter better. In the spring the packets are brought into a warm room some little time before conditions outside are favorable for growth, and after a few days of warmth and moisture some of the spores will show signs of growth. The packets may be sprayed and thrown together in heaps, but they must be spread out and aired and caution taken to prevent molds from starting. The material in germinating condition is carefully separated from that not yet ready to germinate.

If negative results are to be given any weight the spores must be tested just before a sowing is made to ascertain if they are in germinating condition. Teliospores of Pucciniaceous species are tested by means of a hanging drop culture. In a space of twenty-four hours viable spores push out germinating tubes which are readily made out with the

microscope under the ordinary high power. Melampsoraceous species are best tested in a moist chamber, such as a Petri dish, without being removed from the host. Growth can be detected by the unaided eye, or with a hand lens, by means of the light yellow sporidia which cover the sori, making them appear pulverulent instead of waxy. Teliospores of such species as the Coleosporiums germinate as soon as mature, which is in the fall of the year. They are sown when fresh and if suspended over a slide in a moist chamber, the sporidia drop upon it, and their germination can in turn be observed with the aid of the microscope.

For indoor experiments, small but vigorous growing potted plants are used as trial hosts. Since the pots must be handled, it is desirable to select plants with as small roots as possible and still have them maintain their vigor. The tops are placed under bell-jars when the spores are sown, and in order to have them cover more readily all extra foliage is carefully pruned away. A few young and vigorous leaves are all that is required.

The manner of applying the spores to the plants differs slightly according to the kind of spores. If they are aeciospores the leaves bearing the aecia are suspended over the portions to be infected, in such a manner that as the spores fall from the cups they will light upon the desired area. In all cases the host plant is first sprayed, the parts which will not wet being rubbed with the fingers until water will adhere. The spores do not need to be placed in water, in fact they should not be. Teliospores readily begin the germination process in water but seldom form their sporidia there. A moist surface and a saturated atmosphere are necessary factors for the germination of all kinds of spores. Urediniospores and teliospores are removed with a knife or scalpel blade, care being taken to apply the edge to the sorus in such a manner as to loosen the spore by breaking the pedicle, leaving the cell-wall uninjured. If certain areas to which the spores are applied be marked by pieces of thread the watch, which must be maintained for the first sign of infection, will be greatly facilitated.

To secure reliable results, it must be positively made out that a plant is free from infection when a sowing is made. Wild or native plants brought in from the field or garden should remain in the greenhouse a period of eight or ten days so as to preclude a possibility of outside infection.

After the application of the spores the whole plant is covered with a bell-jar and set in a shaded place for a period of three days. The bell-jar prevents rapid evaporation, thereby securing the necessary condition of moisture during the germination time of the spores. A certain amount of warmth is desirable, but during the whole period the plants should be screened from the direct rays of the sun. The bell-jars are temporarily removed each day to allow a change of air and are sprayed on the inside with an atomizer before being replaced. After the second day the plants can be sprayed, but previous to this there is danger of washing away the spores before there has been an opportunity for infection. On the third day the bell-jar is removed and the plant changed to a location where there is more light, in order that growth may be more normal and observation made easier.

A label, bearing the date of the sowing and the name of the species of rust, is a very valuable aid to the observer of results. If the infection is a successful one the first signs are usually noticeable in five to ten days, although some species require fifteen days or even longer. Ordinary Puccinia and Uromyces species, such as the grass and sedge rusts, usually develop pycnia in six or eight days, the aecia following about an equal length of time. Some species, having only teliospores, show signs of infection in four or five days by means of yellow spots, requiring twelve to fourteen days to develop spores. Uredinia sometimes do not follow the sowing of aeciospores for a period of fifteen days or more, while they will reproduce themselves in five or six. The Gymnosporangiums show pycnia in a few days and the mycelium may keep on producing pycnia for a considerable period, but a month or two passes before the aecia appear. Many of the species which produce their aecia on the evergreens germinate their teliospores in the fall, but there will be no visible sign until the aecia develop the next spring, as the pycnia are very inconspicuous.

The procedure throughout is a simple one. No sterilization is necessary, only care and cleanliness. The bell-jars are ready for use a second time after a thorough washing. All organic matter should be removed so as to avoid the starting of molds. No bits of rusted material can be left on the pots or shelves near the plants without the liability of a stray infection. As soon as a developing spore form becomes mature it should be removed for the herbarium or separated from the plants not yet showing infection. In all cases where it is the object to test the range of a

species it is always wise to carry a controlled experiment with as nearly similar conditions as possible. A success in one case and a failure in the other can then usually be relied on as representing the actual state of affairs. If a plant fails to show any results within the reasonable time it is best not to use it again until a sufficient number of days of grace have passed, as there is always a possibility of a belated infection.

It will be seen that care in execution and accuracy of observation are the main features in this work, costly apparatus not being required, and it is hoped that this brief description of the operations may be of service to those interested in this modern method of studying and classifying this group of fungi.