times in order to exclude the air and to infiltrate the wood as thoroughly as possible with the paraffin and to prevent the absorption of moisture and subsequent warping. The corn cultures of this experiment were allowed to grow for eight weeks. Shortly after they had commenced growth the wooden lid fig. 2, which was clear of all infection at first, began to show some dark colored areas on the top not only around the corn seedling but also around the opening through which the glass tube projected. This became rapidly larger around the seedling which would have dropped through the lid into the culture solution but for the presence of the "prop roots" and the rapid increase in diameter of the stem. Observing fig. 2 it will be seen that the wood was removed for a considerable area and of irregular extent and that a crack had appeared reaching to one side. The top view of the lid fig. 1, which was not used for a culture before this time, shows the exact condition of fig. 2 at the beginning of the experiment. Fig. 4 gives a view of the underside of the same lid shown in fig. 2. In this view the fungal destruction is much more apparent. Here is well illustrated the rapid removal of wood by the action of the fungus especially in long deep rifts. This is so deep in one place as to make clear the cause of the split shown in fig. 2. It is really hardly a split, in a way, but is an almost complete removal of the wood to the top. The other photograph, fig. 3 shows the under side of another such lid a few days after the fungus made its appearance. It will also be noticed from figs. 3 and 4 that the lid had been bordered by narrow strips. The lid shown in figs. 2 and 4 was only one of several used, and two of which were attacked in the way above mentioned by the fungus. The lids did not become wet from the lower surface by this culture solution. However, it is of course well known that wood of various kinds are often attacked and destroyed by such an agency. Nevertheless this brief account is unusual from the standpoint of the rapidity with which the destruction progressed under the conditions present. Having previously obtained the weight of the lids, the one shown in fig. 2 was at first 175 grs., as above stated. At the end of the experiment the lid shown in fig. 2 had lost 15 grs. in weight. Of course when available porcelain lids are preferable fitted with suitable corks and these boiled in paraffin[1]. The addition of a .05% solution of neutral potassium chromate will according to the method of Klebs[2] prevent the growth of bacteria and fungi and at the same time does not harm Algae or higher plants. The photographs of the lids shown in figs. 2, 3, and 4 indicate the extreme care that must be observed in experiments of this kind to prevent the difficulty here mentioned.

A STUDY OF POLLEN, VI

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The use of the petri-dish method as described in my previous papers on the study of pollen, have shown that this is the best method for investigation of this kind. In all cases it is advisable to use petri-dishes made from a good quality of glass with a perfectly smooth top and dishes having as little depth as possible. Only distilled water should be used in the lower half of the petri-dish to supply moisture and the previously advised precautions taken to avoid condensation. This latter can hardly be advised too strongly since when heavy condensation occurs the drop of the solution containing the pollen grains may be seriously altered. In fact, condensation may sometimes occur in various places on the

[1]Pfeffer, W. Pflanzenphysiologie 1894 Bd. 1 p. 413.
[2]Klebs, G. Untersuchungen aus dem Bot. Inst. Zu Tübingen 1886-1887 Bd. 2 p. 492.

upper lid to such an extent that the different cultures may run together when they are close to one another, especially those of the inner one of the four concentric circles. In making the four concentric circles on the upper lid, as previously described, either paraffin or beeswax may be used. A sharp pointed compass should be used to remove the paraffin or beeswax so as to insure the perfect contact of the hydrofluoric acid with the glass and to make a complete set of circles on the glass lid. A compass may be used for making the concentric circles very conveniently, if a piece of thin card-board 3 mm. square is fastened on the outside of the lid and exactly in its center, by means of paraffin in order to serve as an anchor for the stationary leg of the compass. A complete guide may be made of a circular piece of white paper having the same size as the inner diameter of the lid of the petri-dish. On this disk of white paper should be drawn a complete figure of the circles and radii in heavy lines, which will be clearly visible through the glass lid of the petri-dish and its thin external layer of paraffin or beeswax. This paper disk can be held in place on the under side of the lid of the petri-dish while drawing the concentric circles and radii on the upper side of the lid in the paraffin or beeswax by placing the lid on the inverted lower half of the petri-dish. In this way the desired figure can be quickly made. It would also be possible to have a "form" made of the exact size and kind of the figure desired, to moisten its edges with hydrofluoric acid and then gently press this figure on the outer side of the lid of the petri-dish. This, however, I have not yet tried. To make the figures on the inner side of the lid is inadvisable, since the solution may at times be inclined to follow the lines of the figure, however shallow those lines may be. The paper disk method of making the necessary figure on the petri-dish is lid quick and accurate. The "form" method, however, would be even more rapid if properly constructed. When a larger number of cultures are to be investigated at one time, wide but very low crystallizing dishes, as previously stated in my former paper, may be used to good advantage. These dishes should be provided with very thin clear glass lids in the form of circles. These lids can be quickly and cheaply obtained by removing the gelatine film with hot water from old photographic plates and then cutting the lids with a circular glass cutter. Care should be taken at all times that the conditons of temperature are favorable as well as those of moisture. The culture dishes should not be allowed to stand in the direct sunlight when the solutions have been inoculated.

The pollen of nearly 750 different species of plants, both wild and cultivated, have been investigated to date. A large number of these plants have been obtained from other localities than Monroe County, Indiana. In all more than 7,500 cultures have been studied. This does not include the extra cultures which have been made of most of the pollens of the plants investigated for the purpose of verification and in order to ascertain their behavior at different times. This would greatly increase the number of cultures made. In all of the cultures some of the pollen grains were submerged or floating in the culture solution and others were merely resting on the glass surface which was moistened with the desired solution. The solutions were kept at least 48 hours in order to allow ample time for germination. The Compositae of all the forms investigated to date had small pollen grains, and generally showed feeble germinating qualities in all the different percents of the sugar solutions. *Centaurea Cyanus* showed the largest number of germinating grains per hundred of any of the Compositae studied. This plant showed that 40 pollen grains in 100 grew in a 15 percent solution of cane sugar, but it required 24 hours for germination to begin. *Achillea Millefolium* showed

only one pollen grain that germinated and that was in a 50 percent solution of cane sugar. The same is true for *Aster Shortii* but the germination in this case was in a 30 percent solution of cane sugar. More than 60 species, both wild and cultivated, of the Compositae have been investigated in this study up to the present time.

Some variation has been found in the number of tubes sometimes produced. Most pollen grains, as would be expected, produce but one tube. But deviations from this has been observed in the case of *Malva crispa* which produces many tubes. I have found to date in this study the pollen grains of ten different species, some of which responded differently in the different solutions of cane sugar. These are as follows: *Symplocarpus fœtidus* pollen grains germinated in water to the extent of 98 in 100. Of these six pollen grains had two tubes. In the other solutions of cane sugar only one tube was produced to a grain in this plant, and the number of germinations varied greatly. *Medicago lupulina* produced three tubes out of 19 germinations in 40 percent cane sugar and two tubes out of 12 germinations in 60 percent. In the other percents of cane sugar only one tube was formed to the grain and the number of germinations varied considerably. *Amaryllis Belladonna* germinated sixteen grains in water, six of which produced two tubes. *Dipsacus sylvestris* showed three germinations in water all of which had three tubes and 12 germinations in a one percent solution of cane sugar three of which had three tubes. *Scabiosa atropurpurea* produced, as previously noted in this study, 96 pollen grains in 100 that germinated at once in water each having four short tubes. *Eschscholzia Californica* germinated 35 grains with two tubes in a 30 percent solution and 49 grains with two tubes in a 40 percent of cane sugar. *Fraxinus americana* showed 10 germinations in a 20 percent solution of cane sugar, five of which produced two tubes. *Arum Dracunculus* produced 17 germinations in a 40 percent solution of cane sugar, 15 of which had two tubes. *Vaccineum stamineum* often produced germinations in the anther and at times formed from one to four tubes to the pollen grain, while *Vaccineum virgatum* at times formed four tubes to the pollen grain.

In some plants the growth of the pollen was perfect so far as the number of germinations per hundred was concerned, although the percents of the cane sugar which produced these were different in some cases. For example in *Plantago lanceolata* and *Caragana arborescens* every one of 100 pollen grains germinated in 30 percent cane sugar, and the same was true of *Carex prasina*, except in this plant it was 40 percent cane sugar which produced the growth. In *Staphylea trifolia* 99 germinated in 1 percent cane sugar and 98 pollen grains grew in water in both this plant and in *Symplocarpus foetidus*, while 97 pollen grains of *Lilium longiflorum* germinated in water in each 100 grains. In a 5 percent solution of cane sugar 98 pollen grains in 100 of *Podophyllum peltatum* germinated. In a group of 100 grains of *Pinus sylvestris* all germinated in a 15 percent solution, while *Primula obconica* produced 82 germinations in 100 grains in a 20 percent solution of cane sugar. Representatives of the Cruciferae generally showed poor germinating qualities with the exception of *Dentaria laciniata*. *Amsonia Tabernaemontana* was obtained at New Harmony, Indiana, from low ground. The pollen of this vigorous specimen produced 50 germinating pollen grains in 100 in a 15 percent solution of cane sugar. This specimen was transplanted to Bloomington, Indiana, on rather high ground. In this new location, which was unfavorable to good growth, it produced weak specimens, with smaller flowers and less vigorous pollen as only 17 grains germinated in a 15 percent solution of sugar.