

cannot be the region of gravitational perception. Where such a temporary root-cap is removed at first, the root perceives the gravitational stimulus just the same, so the root-cap in this case perceives no stimulus. The root-cap can also be removed mechanically and the root perceives the geotropic stimulus just the same. The same holds true of roots which normally possess a permanent root-cap, for in some cases such permanent root-caps may be removed by proper care, without great injury to the root tip. Nevertheless, here also the root-apex perceives the geotropic stimulus and responds in the normal way. The experiments of Piccard do not prove the points under consideration here and his investigations are open to question. In addition, the idea that the cotyledon apex is instrumental in geotropic perception in some of the Gramineae needs further study.

The masses of tissue above mentioned could be removed from the roots without damage if care was exercised. The root-tip then perceived the gravitational stimulus in the usual way and with normal speed and conduction. But as long as the mass of tissue remained on the root no response to gravity was possible. The roots with these masses of tissue were subjected to the same experiments described in my former paper on this subject and such as Czapek carried out with glass-caps. The rate of growth in the roots encased by these masses of tissue, as well as those roots that were enclosed in the glass-caps, was nearly as rapid as in roots of the controls which were entirely free. The danger of injury that the glass-cap method often occasions was eliminated by the masses of tissue which in some cases were present. Although these masses of tissue were of accidental occurrence, they nevertheless furnished a natural method of proving in perhaps even a better way what has been successfully demonstrated by the use of glass-caps, namely, that the apex of the root is the region of perception of the stimulus of gravity.

STUDIES ON POLLEN, IV.

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As this investigation of the pollen of various plants has progressed, new and improved methods and apparatus have been devised and used. The large number of plants studied and the many cultures made from each required more elaborate and rapid methods and for the sake of accuracy, arrangements which would provide uniform conditions. The old method of using paper cells, while effective, was soon discarded for the glass ring cell. These rings were conveniently attached to slides by means of paraffin. They had the advantages over the first form, that they could be conveniently and individually darkened or illuminated for certain investigations; would prevent desiccation; were somewhat more rapid and were less liable to accident. These glass ring cultures, however, involved more expense and were liable to the same lack of uni-

formity of growth conditions as the paper cells, unless special provisions were made. The gas chamber method made possible various experiments not possible by either of the two methods just mentioned and also was a convenient way of studying by transfer of cover glass cultures to the gas chamber used, a large number of cultures in rather rapid succession. But these methods in all cases involved a large amount of work for each culture made, as well as a considerable expenditure of time and occasionally, uncertainty as regards uniformity of conditions.

The difficulties here mentioned were conveniently and effectively removed by using properly constructed petri dishes about 10 cm. in diameter and 12 mm. deep. In the bottom of this dish was placed a layer of distilled water about 2 mm. deep. Near the periphery of the lid and on the outside was placed at regular intervals the designations of the ten solutions used. These were distilled or tap water, and sugar in the following percentage: 1, 5, 10, 15, 20, 30, 40, 50, and 60. The designations of the solutions on the outside may be made with pencil; or very quickly and conveniently with hydrofluoric acid.

These designations should be made in very small, thin letters so as not to interfere with the observation of the specimens. The lids of most petri dishes are too thick for observation with ordinary high powers, but are thin enough for observation and counts with many of the so-called low powers of the microscope, which are ample for much of the study. The lids of some petri dishes were bored with holes one cm. in diameter and a cover glass used on these. The risk of breakage in so doing even with proper machinery is great, but considerable success was experienced. Better than this a large thin sheet cover glass for the entire dish may be used in place of the usual lid. This will then allow the use of ordinary high powers for more minute study. This lid should be provided with checks to prevent movement on the dish. In case such a large thin cover glass is not at hand and where observation with high powers is desirable, very thin cover glasses may be placed on each of the ten cultures on the lower side of the ordinary lid when this is inverted. This will thus allow direct observation of each culture with high powers when the lid is removed from its base and inverted on the stage of the microscope and for longer or shorter periods of time according to the arrangements for moisture. When such study is completed the lid may again be placed in the usual position on its base. Unless great care is used a troublesome feature of this study is condensation. This can be prevented by keeping all the pieces of apparatus at the same temperature. To do this the glass-ware, tools and other things used should be placed in a thermostat at the desired temperature long enough to acquire a constant temperature before the experiment is commenced. A Molisch freezing-box in which warm water of the desired temperature is used is convenient for the microscopical observation when properly arranged. A constant temperature room is also very convenient in this study if properly adjusted. One of the most convenient and accurate heat controlling arrangements for the cultures on the stage of the microscope is an electric cell similar to one I have constructed and described elsewhere.

The petri dish method has proved to be the most convenient of all the methods thus far adopted. It is more rapid and has the additional advantage of placing a whole series of cultures, aside from the nature of the solutions used, under precisely the same conditions. I have investigated to date the pollen from 705 different species of plants both wild and cultivated. Both tap and distilled water were used and in addition nine different solutions of sugar above mentioned. The longevity and the time required for germination were determined. It is necessary to make fresh solutions of sugar at frequent intervals. The pollen was mounted so that some of the grains were submerged and others were well moistened. In these two cases some of the submerged pollen at times did not grow, whereas the pollen on the surface or just moist did grow.

The pollen of eleven of the 705 plants studied produced two or more tubes. The pollen which germinated most quickly was that of *Scabiosa atropurpurea* and the next were *Asphodeline lutea* and *Tradescantia virginica*. The largest pollen grains were those of *Mirabilis Jalapa* which were .25 mm. in diameter, while among the smallest were those of *Myosotis Scarpoides* which measured on the average .003 mm. in diameter. The pollen grains of *Nelumbo lutea* were also extremely small and were no larger than the very narrow pollen-tube which they produced. In number, the variation is very great. In *Mirabilis Jalapa* there are on the average only about 32 pollen grains in each anther cavity, while in others, as in certain representatives of the Boraginaceae, there are many thousands in each anther cavity. Thus *Borago officinalis* may have as many as 60,000 in an anther cavity according to Kerner. The pollen-tubes of *Scabiosa atropurpurea* were four in number and very short. Other pollen-grains such as those of *Verbena fruticosa* merely commence to germinate in certain solutions. Part of the pollen of one plant, *Vaccinium stamineum*, germinated in some cases in the anther cavity. Other pollen-tubes, such as those of *Tritonia crocosmaeflora*, *Sinningia speciosa*, and *Torenia asiatica*, germinate readily in sugar solutions and in certain strengths grow so rapidly that the advancing tube may be seen with the microscope when only moderate magnification is used. In addition *Tritonia* may, as Strasburger points out, occasionally form cross-walls in the pollen tube. What occurs in this respect when the pollen of *Tritonia* germinates on its own stigma opens up a point for inquiry. In sixteen of the 705 plants experimented with, 85 or more pollen grains per hundred germinated in certain of the solutions used. The pollen of *Tradescantia virginica* grown in the shade showed better growth than the pollen of this plant when it was grown in direct sunlight.

