NOTES.

ON THE GERMINATION OF THE POLLEN-GRAIN AND THE NUTRITION OF THE POLLEN-TUBE [1].—The germination of the pollen-grain, leading to the protrusion of a pollen-tube, suggests, at once a process analogous to the formation of the prothallium of the Vascular Cryptogams. Its peculiar situation and the fact that it has to make its way, unlike the latter, through a mass of tissue, suggest however certain peculiarities attending its nutrition. The absence of chlorophyll and the probable richness of the environment in various elaborated materials makes it probable that the progress of the tube downwards through the style is attended by a process of intra- or extra-cellular digestion, depending on the occurrence and activity of enzymes.

It has been shown by various observers that pollen-grains allowed to grow in solutions of cane-sugar speedily bring about the appearance of a reducing sugar. Even when germination has been inhibited by antiseptics, the same inversion of the cane-sugar has been observed to take place. Certain grains again when cultivated in weak starch-paste have been shown to liquify it, with formation of maltose.

The research which forms the subject of the present paper was directed first to the preparation and identification of digestive enzymes from pollen, and to the variations in their amount which attended the progress of the pollen-tube through the tissue of the style.

Both diastase and invertase were found to exist in various pollens; some containing both, some only one of the two. To extract them the pollen was collected from dehiscing stamens, bruised in an agate mortar, and extracted with various solvents, usually 5 per cent. of NaCl. As an antiseptic during the digestions, ·2 per cent. KCy was found most serviceable.

[1] Abstract of a paper read before the Royal Society, February 8, 1894; see also Annals of Botany, Vol. v. 1891.

The ground pollen after a few hours' exposure to the solvent was filtered off and the filtrate tested by allowing it to remain in contact with cane-sugar in solution or starch-paste of 1 per cent. strength for some hours. The reducing sugar was estimated by titration with Fehling's solution. Details of the various experiments made are given at some length in the paper.

Diastase was by this method prepared from the pollen of *Lilium*, *Helleborus*, *Helianthus*, *Gladiolus*, *Anemone*, *Antirrhinum*, *Tropaeolum*, *Pelargonium*, *Crocus*, *Brownea*, *Alnus*, *Tulipa*, and *Clivia*. It is very widely distributed, very few pollens examined yielding no result. Invertase was found in the pollen of *Helleborus*, *Narcissus*, *Richardia*, *Lilium*, and *Zamia*.

During the germination of the pollen the quantity of both enzymes was found to be considerably increased; in some cases four or five-fold. The difficulty of extraction was greater in the case of the ungerminated grain, the thin-walled pollen-tube yielding it to the solvent fairly easily. The facility of extraction was shown, however, not to be the explanation of the greater activity of the latter extract, but a definite increase in amount was made evident, the increase being generally greater when the tubes were grown in a nutritive fluid than when they were cultivated in distilled water.

In one case the increase was found to be preceded by an initial diminution, which lasted only till the tube was about four or five times the length of the diameter of the pollen-grain.

When the power of germination of the grain was becoming feeble, which usually took place about three weeks after collection, the amount of enzyme contained in the pollen was very considerably diminished.

Further experiments were carried out to trace the changes in the contents of the grains and tubes as the germination proceeded, and to see what was taking place in the cells of the style during the same period.

The contents of the pollen-tube were generally granular, the protoplasm showing streaming movements. Besides the granularity of the latter, however, larger refringent granules were observed towards or actually at the tip of the tube, which were being continuously or intermittently extruded into the culture-fluid. In one case (in *Narcissus*) this extension was observed to take place through a pore with well-defined lips at the tip of the tube. The granules so extruded

were remarkably like those noticed by Marshall Ward, as put out of the hyphae of *Botrytis*, and were probably, as in the latter case, the enzyme itself. The extrusion of the contents of the pollen-tube by certain definite pores has already been described by Van Tieghem.

When pollen-grains were treated with a strong solution of chloral hydrate in which a little iodine had been dissolved, they were found to contain in many cases large quantities of starch in very minute grains. Other experiments, of somewhat complicated nature, showed that many contained, either with or without starch, various quantities of cane-sugar, glucose, and maltose. During germination the starch-grains were found to pass in a sort of streaming motion down the tube, and to be digested as they went. When the tubes were of some considerable length, iodine coloured the granules blue close to the pollen-grain, violet and purple further down the tube, and nearly red close to the tip, indicating the gradual hydrolysis of the starch and the coincident formation of dextrin.

The style of the Lily was the one chiefly examined for evidence of the disposition of nutritive material in this organ. It was found to have a very definite relation to the progress of the pollen-tube. The style of the Lily contains a canal continuous with the cavity of the ovary and opening outwards at the surface of the stigma. This canal shows a very delicate epithelial lining, the cells being somewhat papillate. There are three fibro-vascular bundles running up it, placed symmetrically.

The cells of the epithelium and of three or four rows of the loose conducting tissue immediately underlying it were found to be the great seat of the storage of starch. In some styles they were quite full, turning almost black when treated with iodine. The soft tissue round the bundles was also full of it, indicating a definite deposition in the style of starch originally formed elsewhere. The starch did not reach quite to the stigma, but stopped short a little below it. The conclusion apparently led to by a consideration of the disposition of the starch in the grain and in the style is that in both it serves as reserve nutritive material, the grain on germinating using up first its own reserves by intra-cellular digestion and then being fed by the starch of the style which is digested in large measure by the diastase excreted from the tip of the tube. The initial diminution of diastase, already mentioned, occurs while the intra-cellular digestion of the store in the pollen is proceeding, the subsequent increase being connected with

a continuous excretion into the tissue of the style to act upon the reserves deposited there.

In addition to this excreted diastase, in certain cases the style itself secretes the same enzyme, the quantity being greatest while the style is young and diminishing after fertilization has been effected.

Besides starch, styles of various plants were found to contain cane-sugar, maltose, and possibly glucose.

The nutrition of the tube is consequently a process in which both the grain itself and the tissue through which it grows take a part; both contain reserve materials and enzymes, though the latter are much more abundant in the pollen than in the styles.

The absorption of nutritive material by the tube in most cultures is followed by an increase in the amount of reserve material deposited there as starch. In some cases (as *Zamia*) the resting grain contains neither starch nor diastase. On its absorbing sugar-solution, however, starch makes its appearance, and later, diastase can be detected.

The formation of the enzymes is therefore largely helped by the absorption of nutriment, which seems to stimulate the pollen-grain to produce them.

This investigation was carried on in the Jodrell Laboratory of the Royal Gardens, Kew.

J. REYNOLDS GREEN, London.

BOTANICAL NOTES, No. 6: ON THE EXTRA-FLORAL NECTARIES OF ALEURITES.—It seemed possible that an examination of the relations which laticiferous tubes bear to the nectaries of a plant, might throw some light on the vexed question as to whether the tubes conduct carbohydrates or not[1]. I selected for observation a plant of *Aleurites cordata* (Euphorbiaceae) which was growing in the gardens at Whampoa.

Aleurites cordata has large, long-stalked, palmati-lobed leaves. The large veins terminate in the angles between the lobes, and at the end of each vein stands a sessile nectary. In addition, erect, stalked nectaries are situated on the petiole at its point of junction with the lamina.

Some of the leaves of this plant are not lobed, but are entire and

[1] See papers by Dr. Scott, and by myself (with literature). Annals of Botany, Vol. iii. 1889.