III. *Account of a Microscopical Investigation of several Species of Pollen, with Remarks and Questions on the Structure and Use of that Part of Vegetables. By Luke Howard, Esq. of Plaistow in Essex.*

Read March 4, 1800.

IN the Spring of 1795, having much leisure, I devoted a portion of it to the examination of various minute productions of Nature in a good compound microscope. These researches would probably have terminated in present information and amusement only, but that they were accidentally turned to the pollen of flowers, respecting which, as a botanist, I found an inclination to inform myself, by comparing the several species together.

I began my observations with the Hazel-tree, *Corylus Avellana*. On a calm dry day I shook off some of the pollen from the expanded catkins upon a clean piece of writing-paper. I also gathered some of the catkins and female buds. These I viewed separately on a clear plate of glass, usually transmitting the light through them from a speculum below, and with different magnifying powers, preferring those which, without enormously enlarging the objects, gave a clear view of the structure and position of several at once. As I pursued this method with the rest I examined, I mention this to save repetition, and shall give the appearances from notes made at the time.

1. *Corylus Avellana*. Anthers furnished with transparent horn-like appendages. Pollen crumbles from the surface, and is sometimes so abundant as to fall in a visible cloud on the slightest motion

of a branch. To the naked eye it is a fine yellow powder. A few grains laid on the glass plate and viewed with the lens No. 4, some appear of an irregular angular shape, opaque, except in one or two parts, where light passing presents the appearance of a perforation; others nearly spherical, the surface divided by depressed lines into a number of convex facets. The transparency of these is such, that they reflect the image of a small object held under them, as well as a drop of liquid. On repeating the examination, the former are found to come from the most mature anthers, and to differ from the latter only as a raisin does from a grape.

A clear drop of distilled water being put on the glass, both kinds imbibe it with the avidity of a sponge; at the same time distending and spreading abroad in the water, but without any motion further than that which this expansion causes. When saturated with water they remain at the bottom, clear as the liquid itself, and all alike distended to a bulk many times greater than their original one in the dry state. They are now seen to be multilocular capsules, having septa in various directions within them, the union of which with the external membrane appears at the angles in the dry state, and at the depressed lines in the wet.

These capsules may be kept in the water for several days without any further perceptible change. When that is dried up they return to the opaque state, and the same operation may be several times repeated on them.

In exhibiting this spectacle to some friends, pure water not being just at hand, a drop of brandy was substituted for it. This gave rise to a phenomenon equally curious and unexpected. The grains expand, as in water; but in the mean time they are put into rapid motion, each grain darting from side to side with the vivacity of a swarm of gnats in the air. As they approach to complete expansion the motion dies away, and one after another sinks to the bottom.

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By a small addition of fresh brandy some few are excited a second time, but with fainter movements. Presently the liquid begins to be obscured, and in a few minutes the grains are mostly dispersed and decomposed, and the spirit, exhaling, leaves a sort of extract on the glass mixed with very minute undissolved particles; among which sometimes appear a few unbroken grains, much changed, and now resembling an empty bladder lying flat.

2. *Erica carnea*. Anthers capsular, bearing the pollen on their inner surface, and discharging it by a brisk explosion from an aperture on the side next the pistil. If the *stigma* be touched with a pin at a certain period of the inflorescence, it happens commonly that all the anthers project their pollen at once; and it may thus be collected on paper. The proper time for this is when the *stigma* is elevated a little from between the anthers. In size and structure this pollen nearly resembles the preceding, and is, in like manner, capable of imbibing water and dispersing with a rapid motion in spirit.

3. *Reseda odorata*. Mignonette.

Unripe pollen, smooth, egg-oblong, transparent, without septa? In water it expands to a sphere, and is acted upon by spirit as the preceding.

4. *Cactus flagelliformis*. Creeping Cereus.

Anthers oblong, crumbling. Pollen of a large size, compared with any others I have seen; in shape resembling a plump grain of wheat, white and diaphanous. It expands in water to a shape nearly spherical. The contact of spirit brings on a pearly opacity. The grains imbibe it slowly, and during expansion revolve on their axes with a pretty regular motion, exhibiting a spectacle no less novel than delightful. In the mean time, some minute particles are seen to be ejected, and, the motion ceasing, the transparency returns, proceeding from the surface to the centre.

If a pistil be separated from the expanded flower with grains of pollen adhering to it, the latter will be found already expanded to a spheroid. Cover the whole with a drop of spirit on the glass, the pistil is not affected in any way, but some of the grains quit it and revolve on their axes. When these are exhausted, the addition of more spirit excites others: after a few minutes, some of those first excited begin to put out a small thread, which gradually elongating, the grain diminishes in proportion, until it is entirely drawn out into a vermicular fibre, which again is presently dissipated into particles too minute to be seen in the now opake medium. The liquor from the tube of the pistil, and a solution of sugar in water, were also found to produce this evolution in very mature grains from the anther. It also sometimes takes place in the twinkling of an eye, so as to be scarcely traced in some few grains out of a number put into spirit at once. Those which have been some time in contact with the pistil are always most sluggish in their evolution.

5. *Carex acuta.*

Authers oblong, crumbling. Pollen angular, opake, with apparent perforations. It dilates in spirit and remains stationary, but ejects numerous minute particles in rapid succession from its surface. When it has become transparent it appears as if filled with seeds.

The preceding may serve for examples of these phenomena; but there was not one among the various species I examined, which did not exhibit them in a greater or less degree. Yet various other parts of the flower, immersed in spirit under the microscope, did not betray the smallest vestige of them.

The proper spirit for this purpose seems to be a mixture of one part pure spirit of wine with two of water. A stronger spirit, or even spirit of wine alone, may sometimes be required when we operate upon a pollen which has, by any means, become previously

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saturated with moisture, (or has lost, by keeping, a part of its irritability?) but it does not enter the dry grains so readily as water alone.

I have since subjected pollen to examination in several of the most stimulant oily and saline liquids, but have not been able to perceive that any of them had a similar effect on it.

It is proper here to remark that the utmost care is requisite to prevent accidental mixtures of the subjects or menstrua in these experiments, which might greatly embarrass and mislead the observer. Separate pieces of clear glass for the several kinds, and separate pointed glass tubes to convey the liquids, will therefore be requisite. It will be proper attentively to examine the pollen dry, as well as the liquids, before they are used, in order to be satisfied of the absence of animalcules and other extraneous matters which might be suspected to influence the appearances.

I do not pretend to say that the above-related experiments were absolutely free from optical deception; but I may venture to affirm, from frequent repetition of them, that, when tried with due precaution, they will scarcely ever be found to fail of producing the appearances related.

Engagements of superior importance at present prevent, and may long continue to prevent, my pushing these inquiries much further. It is for this reason, and because I should take a pleasure in seeing it done by some person more capable of executing them with due exactness, and drawing proper inferences from them, that I am willing to make them known in their present imperfect state. For the assistance of those who may incline to prosecute the subject, I shall now state the amount of the present discovery, and the hints for further investigation which have occurred to me.

Should it be found, on repeating and extending these observations,

tions, that the pollen of vegetables is in all cases similarly acted upon by water and by spirit of wine, it will follow,—

1. That each grain of pollen in the anther is an organic body, variously constructed in various species, and containing

a Vessels or pores capable of imbibing water, of distending thereby and contracting again when it quits them; in which particulars they resemble sponge, &c.

b A parenchyma, consisting of some substance (of greater specific gravity than water, and insoluble therein), which is emitted with a greater or less degree of force when the stimulus of alcohol is applied to the absorbent vessels. This substance is either in part soluble in alcohol, or the grains contain

c An essential oil or resin, to which they owe their colour and odour.

2. That there exists in the grains of pollen, in a very eminent degree, ~~that property~~ of vegetables called irritability, which they are capable of retaining for a certain time after separation from the anther.

3. That alcohol is the proper stimulus by which this irritability may be excited, and the texture of the pollen in some manner developed in consequence thereof. I prefer this method of accounting for the appearances that take place when the pollen is immersed in spirit to another that might be supposed on chemical principles, being assured, ~~that any one~~ who has once inspected the process will be satisfied that ~~something more than~~ mere solution or chemical decomposition takes place therein, and that the vital principle of the pollen is the chief agent.

The liquor from the tube of the pistil and the solution of sugar were, indeed, found to bring on the evolution of the pollen of *Cactus flagel.* in a slower manner than spirit; but when we consider how
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speedily such matters pass into the vinous state, it seems possible that both of these might contain alcohol. Yet, it is also possible that something common to this latter substance, with the saccharine matter it is producible from, may be the real exciting cause.

The existence of absorbent vessels in the pollen is proved by the change of form, increased transparency, and great distention produced by the water. It is remarkable, that complete saturation usually brings the grains near to a spherical shape, however remote from it their original one.

It seems necessary to suppose the parenchyma for the following reasons. Something is evidently given out to the spirit before the dispersion of the grain commences. In some cases this is visible in minute particles moving about in the drop; in others it is discoverable by the tinge on the dried space, and by the striæ which appear when more spirit is added. Now, if the grains consisted merely of the vegetable fibre formed into vesicles or cells, their texture would no more be destroyed by spirit than by water, and the penetration of the water would produce the same motions as that of the spirit. But if we suppose that, in proportion as the spirit penetrates the several parts of these curious capsules, some transparent substance is forcibly expelled from them; the various motions into which they are thrown will be easily explained by the recoil of the grain in the opposite direction. It will hence appear why the pollen of 1. which seems to consist of many separate cells, is driven alternately in all directions by their successive discharge, and why that of 4. which is a long tube rolled up, and probably with but one orifice, is thrown into a rotatory motion. The opacity of this species during the discharge may be attributed to the evacuation of this canal, and the returning transparency to the entrance of the spirit into it from the absorbent vessels, or at the orifice. I do not remember to have

have seen a single bubble of air escape from the grains of pollen in the whole course of my observations. As their texture was in many cases quite destroyed, if it had been porous, as that of dry wood, &c. air must have appeared. I therefore conclude them perfectly solid in the dry state.

I think it possible that the prosecution of these inquiries by means of the microscope, may throw some light on the obscure subject of vegetable reproduction; may teach us why the anther is almost always exposed to the air for some time previous to the discharge of the pollen, and this even in aquatic plants; as also what is the office of that saccharine liquor with which the stigma is furnished, and of which such a store is sometimes provided in the nectary. The very manner in which the impregnation takes place may possibly be learned by attentive observation.

The similarity of the unfolded pollen of No. 4. to the form of the plant it comes from, might furnish matter for speculation; but I decline this, believing that experiment and careful observation must always precede sound theory.

Reflecting on some of the properties of pollen in which it bears a resemblance to starch, I was led to examine that also in a similar manner, and was not disappointed to find its structure the same. Starch consists of homogeneous grains or capsules shaped like No. 3. capable of imbibing water with increase of bulk and transparency, and of returning to their original state on parting with it. They are also dispersed, with more or less of motion, in spirit; but in this particular different specimens were found to vary, which may be attributed to difference in age or soundness. If a little wheat flour be mixed with water and spread on the glass, these grains appear in great abundance, mixed with fibrous matter. Other kinds of grain afforded the same result, with a difference in the form of
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the capsules. As the vegetable fæculum, which consists entirely of this kind of matter separated from the soluble and fibrous part, has been long considered as the same substance, though obtainable from different parts of vegetables, I extended the inquiry to tuberous roots, and obtained a further confirmation of the identity of pollen and fæculum. A potatoe seems to be almost nothing else but an assemblage of grains of fæculum, with their interstices occupied by the juice. If this root be boiled or baked until it becomes mealy, the juice will no longer be found; and we might be at a loss to know what was become of it, if the microscope did not show that it has entered into the grains of fæculum, which are thereby greatly distended, as is, indeed, evident to the naked eye. The vital principle is thus destroyed; for these bloated grains will not move in spirits, but give out a tincture to it like other dead matter. By this means, and the loss of solidity, they are prepared for more easy decomposition in the stomach.

Starch is absolutely insoluble in water. If water containing it be made to boil, it becomes a jelly. I do not apprehend that a true solution takes place even in this case. It appears that the same effect is produced on the grains by the heat as by spirit of wine. They are dispersed into very minute particles; and the surface being thus multiplied, a greater degree of attraction takes place between the starch and the water, and the former remains suspended.

It appears to me to be worthy of future inquiry,

1. In what parts of vegetables in general the pollen or fæculum is to be found.

2. In what respects that which is secreted on the anther differs from that which is contained in the root, seed, or sap. In the leaf, petal, bulb, fibre of the root, or other parts already brought to perfection, I am inclined, from some observation, to think it will not be met with.

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3. Whether the germ or embryo of the seed, previous to the impregnation, contains it.

4. In what manner the pollen of plants in general will be acted upon by the liquor from the nectary, when exposed to it in circumstances similar to those of Exp. 4.

5. And lastly, to investigate the varieties in form and structure of the different species of pollen; and to examine how far they agree or differ in the several species of each genus, and genera of each natural order.

IV. *Observa-*