Effect of Environment on the Hypocotyl in the Genus Luzula.

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With Plate XXII.

I have thought it best to divide the contents of this short paper into two parts; the bulk of the observations and descriptions forming the first of these, and the second containing a few remarks on the minute anatomy of the hypocotyl. My best thanks are due to Prof. Bayley Balfour, under whose supervision the work has been carried out at the Royal Botanic Garden, Edinburgh, for the facilities he has at all times allowed me, and for help in many other ways.

Part I.

On comparing some seedlings of *Luzula purpurea*, Link., germinated this spring with a series collected from a sowing made last year, I was struck by a marked difference in the appearance of the seedlings in the two gatherings. In both cases the seed had not been separated from the capsule, or from the flower perianth enclosing it; and it was owing to this fact that my attention was at first arrested. The seedlings germinated last year corresponded to the description given by Laurent (1): the green cotyledon bearing on its apex the seed, along with the first foliage leaves formed a rosette, while the primary root arose almost directly below this, owing to the extreme shortness—amounting almost to absence—of the hypocotyl. The remains of the perianth and capsule, where present, were in this way situated immediately below the point of origin of the foliage leaves, the primary root having bored its way through these relics of the parent plant in its elongation (Fig. 2). On the other hand, the seedlings germinated this spring, while corresponding in other respects with the foregoing description, showed the remains of capsule and perianth as though carried downwards for some considerable distance by the primary root in its growth (Fig. 1). How was this diffeience to be accounted for?

Upon closer observation it was noticable, in the case of the seedlings in which the remains of the flower parts were situated some distance from the leaves, that what I had at first taken to be a portion of the primary root lying above the remains of these flower parts—which had previously surrounded the seed—was thicker and more transparent than the undoubted primary roots of the seedlings in the other series, and differed in the same respects from what I had thought to be its continuation beyond the perianth segments investing it. The probability of its being an elongation of the short hypocotyl described by Laurent (1) then occurred to me; and a consideration of the type of germination which, according to him, is common to all the Juncaceæ (and which I had already observed myself in the case of *Juncus glaucus*, Sibth.), as well as other points to be mentioned later, convinced me that this was the truth. A short account of those features of this germination which bear upon the present case may help to make my point clearer.

Owing to the elongation of the cotyledonary axis on growth commencing, the embryonic primary root is thrust outwards through the micropyle of the seed. The cotyledon continues still to elongate, bringing the root pole into contact with surrounding material, and later, when the plantlet has become firmly attached to the rooting-medium, growing upwards as the first green leaf of the plant, and carrying on its apex the seed. Immediately the rudimentary root comes into touch with any surface, a collar of long root hairs appears at its upper limit, which serve to anchor the young plant and allow the primary root the leverage necessary for the burrowing operations it has soon to commence. This fixation of the starting-point of the root before its elongation is what I wish to emphasise, because, if we imagine a seed to germinate thus within the remains of the perianth and capsule, it seems clear that they cannot be carried downward by the root, since they are firmly held by the fixing hairs, as had undoubtedly happened in the case of the seedlings collected last year.

No trace of a piliferous layer can be seen throughout the whole length of this structure—in some cases more than half an inch in length—which separates the leaves from the flower parts which had enveloped the seeds, in this year's seedlings. Moreover, lateral roots, when developed upon it later, arise in acropetal succession.

On seeking an explanation for the difference in growth of the two lots of seedlings, I at once remembered that, while last year I had sown all the seeds I was observing on the surface of moist fibre or other material, this year, owing to certain failures in germination, I had had all my seeds sown in the ordinary way, below the surface, in pots of sandy soil. Fortunately, the whole of the seedlings which interested me had not been removed from the pot in which they were growing, and on examination it was found that the seeds had been sown exactly as far below the soil surface as the hypocotyl had elongated. That this was so, was made very clear by the fact that, though some seeds had been considerably nearer the surface than others, the hypocotyl had only elongated sufficiently to bring the plumule above ground. In other words, the elongation was just sufficient to raise the embryonic shoot to the surface of the earth.

Being anxious to ascertain the subsequent fate of the hypocotyl, a number of seedlings were kept growing for a time and examined at intervals. It was found that very soon lateral roots began to appear, arising from the central vascular cylinder, clearly visible in the hypocotyl. The first of these usually appeared about the same time as the first foliage leaf began to emerge from the sheath of the cotyledon. It was situated, as a rule, in the lower half of the hypocotyl, very often quite close to the primary root. One by one other similar lateral roots arose above the first, each stronger and thicker than that preceeding it; till finally, when about the third foliage leaf had appeared, several lateral roots arose from the base of the leafy rosette (Figs. 4-7). Very soon quite a bunch of these roots had been formed, and the hypocotyl, with the roots it bore, began to shrivel. Within three months from the date of germination, there only remained an almost indistinguishable brown thread, in the midst of the functional roots, to mark its former position. It is interesting to note that the cotyledon was at that time still quite green, and as yet showed no signs of decay; it remained an active assimilating organ for a considerable period after the total disappearance of the hypocotyl.

My next step was to ascertain the environmental conditions under which the species grew, in case this might throw some light on the use of such an expansion of the embryonal axis to the plant.

Luzula purpurea, Link., is a native of the Canary Islands and Madeira, and according to Buchenau (2) its habitat is in shady, moist places in rock clefts in wooded valleys; he also states that it is the only known annual species of the genus. The surroundings of the plant would therefore appear to be very similar to those in which such of our British luzulas as *L. maxima*, DC., and *L. vernalis*, DC., are usually met with. For this reason it seemed likely that, in these species also, a similar development might be found to occur. I was, however, only able to obtain a small supply of seeds of *L. maxima*, DC., and of *L. campestris*, DC. Some of the seed was in each case sown upon the surface, some under about a quarter of an inch, of soil. In both cases the same results were observed as in the case of *L. purpurea*, Link., already described, with the exception that the germination below ground was much poorer in the case of the two British species. The experience of Mr. L. Stewart, at present Foreman of the Glass Department in the Royal Botanic Garden here, agrees with mine in this respect, for he informs me that *L. purpurea*, Link., is more easily raised from seed sown below the soil than is any other species of *Luzula* he has knowledge of.

I had now satisfied myself that the case of *L. purpurea*, Link., was no exception to the general rule, and that the behaviour of its hypocotyl was not caused by the fact that it was an annual, but must have to do with the environment which the genus, as a whole, tends to favour. The seeds of a plant growing in a shady, wooded situation, where the soil is very loose and the leaf-fall great, must tend to become more or less buried before germination—which is often long delayed amongst luzulas—commences. A means of bringing the leaves of the seedling above the material covering the seed would give the young plant a much more favourable start in its life.

Apparently, under ordinary conditions, the internodes of the stem itself take no part in the work performed by the hypocotyl, but remain quite as short as in a surface-grown seedling. The possibility of their elongating if necessary is, however, hinted at by the fact that one of my seedlings of *L. maxima*, DC., germinated below ground, showed the first internode of the shoot elongated as well as the hypocotyl (Fig. 3). Why this seedling should have developed in this exceptional way I am unable to explain, as the seed was no more deeply covered by the soil than any of the others, and the internode between the base of the cotyledon and the first foliage leaf projected entirely above the earth. According to Sorauer (3), two internodes (apparently the so-called mesocotyl and the first internode of the stem) take part in bringing to the vicinity of the surface the leaves of too deeply sown rye seedlings. In surface-sown plants these two internodes remain contracted and closely superposed.

An experiment was made to test the effect upon the hypocotyl of lack of sufficient light at the time of germination. A surface sowing was made of seed of *L. campestris*, DC., and the pots of soil placed in almost complete darkness. The seed germinated readily, and, as I had expected, the hypocotyl became elongated in the same way as before, though to a rather less extent. A longitudinal section through the median plane of one of these seedlings is shown in Fig. 8, the drawing being, unfortunately, in part diagramatic, owing to the large cortical cells having become much crushed in the process of embedding in paraffin. It is probable, then, that lack of light is the chief stimulus in causing the elongation of the hypocotyl in seedlings of *Luzula*, which would hardly ever be so deeply buried in the soil as the rye seedlings mentioned by Sorauer (3), who attributed the elongation in their case to a need for better aeration.

I have not been able to observe any phenomena such as those described above in the genus *Juncus*.

PART II.

The description of the minute anatomy of seedlings of *Luzula* given by Laurent (1), in his excellent and exhaustive treatise on the development of the Juncaceæ, is that of surface germinated plants. He shows the hypocotyl as extremely short, and speaks of the almost immediate change from root arrangement (alternating) to cotyledonary arrangement (superposed) of the xylem and phloem in the vascular system. I was anxious to ascertain the structure found in the elongated hypocotyl, in case the transition should prove more gradual; and I also determined to examine seedlings at a later stage than those observed by Laurent, so that they might have had time to develop sufficiently to give some idea of the passage from hypocotylar to shoot arrangement. Unfortunately the results of this quest—which proved much more difficult than I expected—were very unsatisfactory. Sections were cut both by hand and in the microtome, the specimens being embedded in paraffin in the latter case, using xylol as the solvent for the wax. The comparatively large size of the thin walled, almost empty cortical cells made it impossible to prevent them from being crushed during the process of infiltration; while hand cut sections were not sufficiently fine for the purpose, and the tissues had not enough strength to resist the razor well when unembedded. Other difficulties were the numerous lateral roots which disturbed the vascular arrangement at short intervals; and the very imperfect differentiation of xylem and phloem due partly to the youth of the seedlings, partly to the transitory nature of the hypocotyl. First, then, I shall describe a transverse section through the hypocotyl, cut about midway between its extremities; and shall afterwards explain, as far as I have been able to make them out, the changes which take place on passing downwards or upwards from this point.

In such a central section we find a vascular core, completely surrounded by a very well marked endodermis, and representing about one-third of the diameter of the hypocotyl. The tissues to the exterior of the endodermis, forming the cortex, are composed of large, thin-walled cells, almost devoid of contents, thus giving a very transparent appearance to the axis when examined as a whole. The cells of the outermost layer of this cortex do not show the thickening of their exterior walls so often seen in epidermal cells, and are in fact quite similar to those situated further within the tissues; no root-hairs or other outgrowths are anywhere produced by them. Within the endodermis an arrangement, quite like that of a root, is observable, and in all but one of the sections examined a diarch symmetry was seen (Fig. 10). The exception was a seedling of *L. campestris*, DC., where a faintly developed third xylem and phloem were noticed (Fig. 9).

In passing downwards from the section described no difference is visible, and the vascular system passes into the primary root, apparently without any alteration except a distinct narrowing of its area just as it reaches the region where the collar of fixing hairs arises.

Passing upwards, we find that the xylem and phloem become more and more difficult to determine, but continue to occupy the same relative positions so long as they can be distinguished (Fig. 15). The endodermis remains very obvious, and no other change is seen till quite close to the upper limit of the hypocotyl. Then, quite suddenly, there become evolved from the mass within the endodermis three separate bundles, one larger than the other two (Fig. 14). Next, the marked endodermis disappears from the side of the stele next the largest of these bundles, while the other two become somewhat oval in outline (Fig. 13). This point marks the upper limit of the hypocotyl, for immediately above it we find the largest bundle passing to the side into the cotyledon, now seen (Fig. 12) partly attached to and partly sheathing the shoot, into which the two oval bundles have passed, each dividing into two in doing so, and giving rise to four bundles lying near its centre. A little higher still, where the cotyledon has become quite separated from the shoot, we find these four bundles more separated from one another (Fig. 11).

It would thus appear that the structure of the stele in the elongated hypocotyl of *Luzula* is typically that of a diarch root throughout almost the whole of its course. Many similar hypocotyls have been described, especially amongst Dicotyledones by Hill (4), Tansley and Thomas (5), and others; while Miss Sargant (6) has pointed out that amongst Monocotyledones the transitional region between stem and root is always of extreme shortness, in her experience rarely reaching a length of

3 mm. even in arborescent forms, and being often much shorter than the external limits of the hypocotyl. My hope of finding a more gradual passage from root to shoot arrangement was thus not realised.

SUMMARY.

1. Under natural conditions the hypocotyl in the genus *Luzula* is actively concerned in raising the plumule of young seedlings to the surface of the soil, if the seeds have become buried. The fact that this elongation has not been noted in previous papers on the subject is probably due to the method usually employed in such observations—germination upon the surface of moist material such as cotton, blotting paper, &c. Under such conditions the hypocotyl remains unelongated.

2. Seedlings grown at the surface, but in darkness, show a similarly elongated hypocotyl, the stimulus to elongation being probably lack of sufficient light.

3. The amount of elongation always corresponds exactly to the depth of the sowing of the seed.

4. Normally the epicotyl takes no part in the elongation, remaining quite unelongated when the hypocotyl may be as much as $\frac{1}{2}$ in. long.

5. The primary root and the hypocotyl are very short-lived, being early replaced by secondary roots arising just below the leaves.

6. The stele of the elongated hypocotyl shows typical symmetry of a diarch root.

7. I have not observed similar phenomena in the genus *Juncus*.

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EXPLANATION OF FIGURES IN PLATE XXII.

Illustrating Mr. W. Edgar Evans' paper on "The Effect of Environment on the Hypocotyl in the genus *Luzula*."

FIG. 1. Seedling of *Luzula purpurea*, Link., from sowing made below the soil surface, showing elongation of the hypocotyl. . . . . × 16.

FIG. 2. Seedling of *L. purpurea* from surface sowing. . . . . . × 16.

FIG. 3. Seedling of *L. maxima*, DC., from sowing covered by soil, showing the elongation of both hypocotyl and first internode of the shoot. . × 16.

FIGS. 4–7. Stages in the growth of *L. purpurea* seedlings. . . . . n. s.

FIG. 8. Longitudinal section through hypocotyl and plumule in a seedling of *L. campestris*, DC. (slightly diagramatic). . . × about 400.

FIG. 9. Transverse section through the hypocotylar stele of *L. campestris*, about its middle point, showing triarch arrangement (unusual). Highly magnified.

FIG. 10. Transverse section through the hypocotylar stele of *L. purpurea*, about its middle point, showing diarch arrangement (normal). Highly magnified.

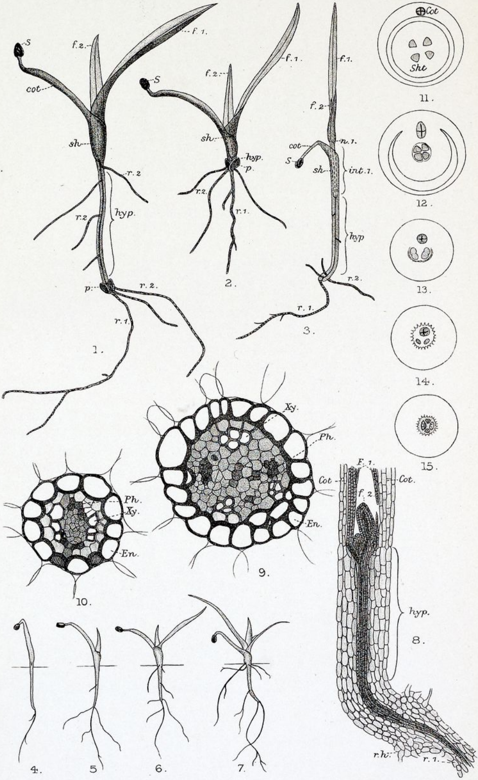
FIG. 11. Diagram of arrangement of vascular bundles at a point above the junction of shoot and cotyledon. (Trans. Sect. *L. purpurea*).

FIG. 12. Diagram of arrangement of vascular bundles at the point of junction of shoot and cotyledon. (T. S. *L. purpurea*).

FIGS. 13–15. Diagrams showing stages in the passage from the arrangement of the bundles shown in Fig. 12 to that found throughout the greater length of the hypocotyl. (T. S. hypocotyl, *L. purpurea*).

EXPLANATION OF THE ABBREVIATIONS USED IN LETTERING THE FIGURES.

cot. = cotyledon.
en. = endodermis.
f. (1, 2, &c.) = foliage leaf (1st, 2nd, &c.)
hyp. = hypocotyl.
int. (1.) = internode of shoot (1st).
n. (1.) = node of shoot (1st).
p. = remains of perianth and capsule.
ph. = phloem.
r. (1, 2,) = root, (primary, secondary).
r.h. = region of fixing root hairs.
s. = seed.
sh. = sheath of cotyledon.
sht. = shoot axis.
xy. = xylem.



W. Edgar Evans del. Huth lith et imp.

EVANS — HYPOCOTYL OF LUZULA.