

On the Further Development during Germination
of Monocotylous Embryos ; with special
Reference to their Plumular Meristem.

BY

W. EDGAR EVANS, B.Sc.,
CARNEGIE FELLOW IN BOTANY.

With Plates LIII-LIV.

INTRODUCTION.

THE germination of seeds, though by no means a neglected subject, is one which still requires much investigation. The chemistry and biology involved in the solution and absorption of perisperm and endosperm ; the further development of the embryo both during and after germination, especially from the standpoint of phylogeny ; and the effects produced on this by differences in the environment of the seed at the time of germination—for example, deep as compared with shallow sowing, the results of chemical or physical stimulation, and the like,—these are a few of the lines of research which seem most likely to repay fuller study. Moreover, it must not be thought that such problems need no more consideration because good work relating to them has been already published, for it will be found on examination that the gaps in our accurate knowledge often exceed the knowledge itself, making it even yet impossible to definitely or satisfactorily answer many an important question arising from the study of germination.

Our information regarding the relation which leaf and stem bear to one another is an excellent example of this state of affairs, for there are at the present time two schools of botanists holding diametrically opposite views on the matter. Those of the first consider the leaf an essentially lateral structure ; it has been evolved as a lateral outgrowth from the stem which bears it, while leaf-like organs which seem terminal in origin must be regarded in either of two ways—they are only apparently terminal, or they are not homologous with leaves. Botanists who uphold the second school maintain, on the other hand, that the leaf was originally terminal ; each successive leaf arose from

[Notes R.B.G., Edin., No. XXI, August 1909.]

the base of that which preceded it, while the axis was in course of time derived from their superposed basal portions. In this way, it is argued, the leaf became pushed to one side and assumed the lateral position which it occupies to-day; its development gradually became arrested, and by lagging behind the succeeding internode it now appears lateral in origin.

In the seedlings of certain monocotyledons—for instance, *Juncus*, *Sparganium*, and *Pistia*—it has been shown that the first few leaves arise one from the base of the other, and that to begin with there is no axis visible. Very soon, however, it can be seen making its appearance, at first hardly discernible, but more and more prominent as each successive leaf is produced, till finally the leaves arise upon it in quite the normal way. Just as the zoologist has found impressed on the developing mammalian embryo the shadow of its phylogeny, so do these botanists who believe in a terminal leaf see a proof of their theory in the ontogeny of such a seedling. Against this it is argued that a fuller investigation will probably show that the conditions existing in those unusual cases can be best explained as arising from a more or less complete arrest or even suppression of the axis; and since the species in which the peculiarity has been noted are chiefly hydrophytes and geophytes, it is suggested that the ecological conditions which have given rise to such types of vegetation may also tend to produce this sort of seedling structure.

At the suggestion of Professor Bayley Balfour, I began, in the autumn of 1905, a careful investigation of the structure of monocotylous seedlings at various stages during and after germination; and also of embryos taken from ripe seed. Since then the greater part of my time has been occupied by the work, and I have collected a large amount of material, much of which has now been carefully examined. The effect of deep and shallow seed-sowing upon the various organs of the young seedling, and the development of the plumular meristem, have in particular been kept in view, not only when germinating the seed, but also in the cutting and examination of sections. The object of this paper is to put on record some of the results obtained, chiefly as regards the latter question.

After some thought I have decided that the best way in which to avoid confusion is to select a small number of the plants examined, to describe these fully, and to give in each case a list of those species which, in the matter of their plumule, resemble the type chosen. An attempt has been made to so arrange the examples that they may form a series, beginning with one in which the leaves always appear lateral, and ending with as extreme a case of stem suppression—if indeed it be such—as

possible. The account of each will be complete in itself, and reference will there be made to any papers relating to the plant under discussion. A final chapter will be added in which the whole question will be reviewed and conclusions drawn; this will include a more general bibliography. I believe that in this way a clearer view of the whole subject will be secured than in any other, for, while there are very few plants whose germination is perfectly identical, there are several well-marked types of germination amongst monocotyledons, which might be lost sight of if too many examples were taken in detail.

A few remarks on the methods I have employed in germinating seed; preserving, cutting, and mounting specimens for the microscope; and in producing the photo-micrographs which illustrate the paper, may be of use to other workers.

Germination of the seed.—Seed was successfully germinated on the surface of absorbent material such as blotting-paper and cocoanut fibre. Better results were obtained, especially with certain species, by placing the seed in pots of light sandy soil, the depth of sowing being equal to the thickness of the seed. A germinating case was experimented with, but was not so successful as a properly constructed propagating house. In many instances it was found necessary to specially arrange the position of the seed, so that the young plant might develop as far as possible in one plane, otherwise good sections could not be obtained.

Fixing the specimens.—A mixture of two parts of absolute alcohol and one of glacial acetic acid proved thoroughly satisfactory, and enabled fixation to be rapidly and easily performed. The specimens were allowed to remain about twenty minutes in this reagent, were washed in 90 per cent. methyl alcohol to remove the acid, and in most cases preserved in spirit of this strength till required for embedding.

Embedding.—All specimens were embedded in paraffin in the usual way, xylol being the solvent employed. The most generally useful wax was found to be that having a melting-point of 52° C., though occasionally one melting at a higher temperature was advantageous.

Cutting the sections.—In order to obtain serial sections as easily and quickly as possible a Cambridge rocking microtome was used. The most satisfactory thickness of section was found to be 10 μ , and all sections figured are this thickness unless it be otherwise stated. The ribbon of sections in paraffin was allowed to spread upon warm water, while it was found necessary to ensure attachment to the slide by means of Meyer's albumen solution.

Staining the sections.—After having compared the results

obtained by the use of a number of stains, I am satisfied that hæmatoxylin counter-stained with Bismarck-brown is the best all-round combination. It is unsurpassed if the sections have to be photographed; while it is more restful to the eye when a large series of sections have to be examined than are most colours. I have also used hæmatoxylin along with saffranin and obtained good photographs.

Photographing the sections.—In my opinion the flame of an oil lamp is preferable as a source of illumination to more powerful methods of lighting such as acetylen and incandescent gas. For the best results the condenser should be achromatised and fitted with an iris diaphragm. Strangely enough, contrary to the published experience of most workers, I have obtained better photographs without the use of an eyepiece than with one, even when using a specially constructed projection-eyepiece and making all adjustments most carefully. Backed orthochromatic plates give in most cases the best results, and when the sections have been stained with Bismarck-brown no light-filter is necessary. In every case the magnification in diameters was carefully ascertained at the time of taking the photograph.

Many workers find it difficult to obtain sufficient density in their negatives without having recourse to intensification. The best all-round developer I am aware of is one in which the reducing agent is a mixture of metol and hydroquinone in the proportion 1 : 2; the accelerator being sodium hydrate solution to which a little citric acid is added to prevent stain.

In conclusion I desire to state that I have carried out this investigation, first, as a Carnegie research scholar, later as a Carnegie research fellow, at the Royal Botanic Garden, Edinburgh. My grateful thanks are therefore due to the Carnegie Trust for the Universities of Scotland; as well as to Professor Bayley Balfour, under whose supervision I have worked, for kind assistance in many ways, and for supplying me with seed of many plants, some of considerable rarity.

I. THE GENUS ASPARAGUS.

With the exception of *Asparagus medecoloides*, Thunb.—merely mentioned by Klebs (4) in a list of those monocotyledons having a type of germination which he terms "type 1"—the only member of this genus whose embryo and seedling have been even superficially described appears to be *A. officinalis*, Linn. This is the

more surprising since, as far back as 1809, Mirbel (1) published a wonderfully accurate account of the external features of the seedling in the latter plant; while the extreme leaf reduction and other characteristics of the numerous species might have been expected to stimulate further investigation.

I have examined six *Asparagi* other than *A. officinalis*, namely, *A. comorensis*; * *A. medecoloides*, Thunb.; *A. plumosus*, Baker; *A. trichophyllus*, Bunge; *A. tenuifolius*, Lam.; and a species, the seed of which was supplied to me as that of *A. rugulosus*, a name I have been quite unable to trace. The germination in all of these is remarkably similar, differing only in the number of kataphylls—each with a single axillary rhizome bud—which remain within the sheath of the cotyledon. The plants examined can in this respect be separated into two groups, those of the first having at the base of their primary axis one, those of the second two of these large underground buds which, when they grow, produce a part of the sympodial rhizome before forming epigeous stems. Since the retention of one kataphyll within the cotyledon sheath is characteristic of all species, and since the retention of two instead of one must be regarded only as a further specialisation along the same lines, it is no distortion of the facts to state that the various species of the genus show as close a correspondence in the features of their germination as they do in the anatomy of their mature organs.

In studying any plant-embryo and following out the changes which take place in it as it escapes from the seed and establishes its independence, it is essential that the main structural peculiarities of adult specimens be borne in mind, if the facts under observation are to be easily or thoroughly appreciated. For this reason the chief characteristics of the full-grown vegetative parts in the genus *Asparagus* may be briefly stated here, before passing on to describe in detail the type of embryo and germination which I wish also to associate with it. Of these there are two, both very strongly marked and equally worthy of mention: first, the perennation by means of a sympodial rhizome and fleshy storage-roots lying at some depth in the soil; second, the reduction of the leaves to mere scales—or in part to thorns in the case of certain scrambling species such as *A. Sprengeri*, Regel—and their replacement by cladodes (Plate LIV, Fig. 16), sometimes remarkably leaf-like as in *A. medecoloides* (C, Fig. 3). The usual presence of long, stout, almost unbranched pull-roots, though less important, should not be lost sight of.

* Though known to horticulturists for a number of years, this plant does not seem to have been specifically described, and I have been unable to discover how or when it received the name *A. comorensis*. Though, according to the Index Kewensis, it may be synonymous with *A. crispus*, Lam., it is certainly not, as has been thought, a form of *A. plumosus*, Baker. This is clear from the fact that its primary axis has only one basal kataphyll, while that of the latter species has always two.

The Embryo.

The typical *Asparagus* fruit is a berry containing a small and variable number of seeds. These are fairly large, quite close together, and when ripe are often more or less flattened on two sides owing to the pressure they exert upon each other during their development; when they escape crushing, however, they are almost spherical. On the thin, dark-coloured seed-coat there can, as a rule, be distinguished the hilum—a flat roughish area; and the micropyle, which has the appearance of a small dome-shaped protuberance. If the seed be carefully sliced open in a plane traversing both hilum and micropyle, a section will be obtained passing longitudinally through or by the side of the embryo, easily seen lying in the semi-transparent endosperm which is of extreme hardness, probably that it may resist the digestive processes of birds which feed upon the berries.

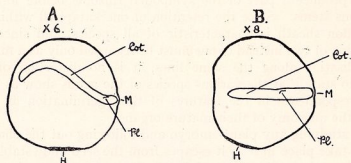


FIG. 1.—Sections through *Asparagus* seeds, passing longitudinally across the embryo. A, *Asparagus comorensis*; B, *A. medeoloides* Thunb.; M., micropyle; H., hilum; Pl., plumule; Cot., cotyledon.

The embryo may be straight as in *A. medeoloides* (B, Fig. 1) and *A. officinalis*; or somewhat curved as in *A. rugulosus* and *A. comorensis* (A, Fig. 1). It is long and narrow, reaching from the micropyle, against the dome-shaped covering of which its radicle is pressed, almost to the opposite side of the seed. In longitudinal section the extremity of the radicle shows all the structure of a normal root-apex, very well developed as compared with many other embryos (Plate LIII, Fig. 4). At a distance above this, varying in different species from $\frac{1}{2}$ th (in *A. rugulosus*) to $\frac{1}{3}$ rd (in *A. medeoloides*) of the total length of the embryo, is situated the plumular meristem, a laterally placed, rather slanting patch of tissue showing no differentiation into axis and leaf (Plate LIII, Figs. 8, 9). The remainder of the embryo forms the cotyledon (Plate LIII, Figs. 1 and 5), whose lower sheathing portion completely enfolds the plumule; the minute slit through which the shoot will emerge on germination being discernible, though not without

difficulty, at its base (Plate LIII, Figs. 2 and 7). So far as my investigations have gone, the vascular system of the embryo (Fig. 2) seems very variable in certain respects, not only in different, but also to a less degree in the same, species. Scholz (5B), referring to *A. officinalis*, mentions that three vascular-bundles traverse the cotyledon; while Miss Sargent (7) has observed in *A. officinalis* and *A. decumbens* an increase in the number of xylem strands in the hypocotyl. This is due, she states, to plumular traces which in these cases are similar to, and behave in the same way as, the cotyledonary ones during the transition to the root. In the cotyledons of those *Asparagi* which I have examined three

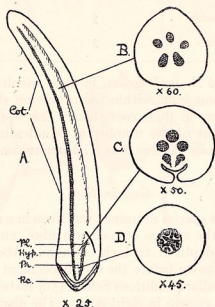


FIG. 2.—*Asparagus rugulosus*. A, Radial longitudinal section of the embryo, through the plumule. B, C, D, Transverse sections of the same through the regions indicated. In the specimen illustrated the cotyledonary xylems were not augmented on passing into the root-stele. Cot., cotyledon; Hyp., hypocotyl; Pl., plumule; Pr., primary root; Rc., root-cap.

bundles occurred only in two—*A. plumosus* and *A. trichophyllus* (Plate LIII, Fig. 1)—though not constantly in the latter plant, four being also met with. In *A. rugulosus* (Fig. 2) the number was usually four, occasionally five as illustrated; while in *A. medeoloides*, only a few specimens of which were cut transversely however, five bundles were observed. Counting the protoxylem patches in the central stele of the primary root, which, owing to the extreme shortness of the hypocotyl, forms nearly the whole of that portion of the embryo lying below the plumule, and comparing their number with that of the bundles in the cotyledon, it was found that in some cases this was the same, while in others

there was an increase (Plate LIII, Figs. 1 and 3). The numbers noted are arranged below in tabular form, but it would be rash to attempt to draw conclusions from so small a number of sections, and they are given chiefly to indicate that there is a lack of uniformity in this respect. It may be pointed out, however, that

SPECIES.	Number of Bundles in the Cotyledon.	Number of Protoxylems in the Root.
<i>A. trichophyllus</i> , Bunge .	3 or 4	5
<i>A. rugulosus</i> . . .	4 or 5	4 or 5
<i>A. medeoloides</i> , Thunb. .	5	5 or 7
<i>A. plumosus</i> , Baker . .	3	7

A. plumosus and *A. medeoloides*, both of which on germination retain two rhizome buds within the cotyledon sheath, also have most protoxylems in their root-stele. This appears, moreover, to be independent of the number of bundles in the cotyledon, since *A. medeoloides* has five, but *A. plumosus* only three.

Germination.

Seed of all the species examined was sown in a moist hot-house, one portion on the surface of cocoanut fibre, another in pots of sandy soil at a depth of an inch or more. About three weeks elapsed in most cases before the commencement of germination, though under similar conditions Scholz (5B) states that seed of *A. officinalis* began growth in eight days. The discrepancy may be accounted for by the fact that this plant is hardy, while those I experimented with require in this country protection under glass. When germination commences, that part of the cotyledon situated just above the plumule begins to elongate. The radicle is thus caused to press against the dome-shaped covering of the micropyle, and since this is less thickened than the rest of the seed-coat it gives way and falls out like a little circular lid. As soon as the developing portion of the cotyledon has lengthened sufficiently to ensure that the slit at its base, through which the shoot has later to escape, is quite free, its growth ceases, with the result that the seed remains closely attached to the seedling (C, Fig. 3). By this time the radicle has begun to elongate very rapidly; it develops into a very short hypocotyl and a stout, long-lived tap-root (Plate LIII, Fig. 15). Root hairs are not produced close to its apex, so that there is always a portion at the extremity of the root, behind the root-cap, free from hairs.

This is so from the very first, and we have here a marked difference between seedlings of the genus *Asparagus* and those of many monocotylous families, such as the *Juncaceæ* and the *Cyperaceæ*. The hairs themselves are short and wonderfully persistent, densely covering the whole of the root with the exception of this apical part. The slit in the cotyledon occupies a slanting position facing upwards owing to the curving down of the short hypocotyl and the primary root towards the soil. The margins of the slit soon grow slightly, forming a somewhat leaf-like rim to the cavity which is enclosed (A, Fig. 3), and shortly afterwards the apex of the primary axis, capped by two sheathing leaves, makes its appearance from within. A little later there

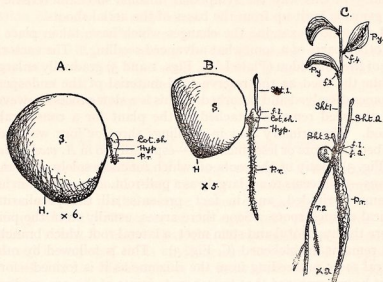


FIG. 3.—*Asparagus medeoloides*, Thunb. A, very young seedling. B, older stage. C, seedling one month old. H., hilum; S., seed; Cot.-sh., sheathing base of cotyledon; f. (1, 2, &c.), leaves of primary axis (1st, 2nd, &c.); Hyp., hypocotyl; Pr., primary root; Py., cladode; r. 2, secondary root; Sht. (1, 2, &c.), axis (primary, secondary, &c.).

also emerges a second structure, at the side of the developing stem away from the cotyledon and seed. This is the first leaf of the shoot, which invariably remains below ground at its base, partially enclosed by the cotyledon (B, Fig. 3). That this may be so the epicotyl is never elongated, even when the seeds are germinated at a depth of an inch or more below ground; in such a case one or two internodes of the basal portion of the axis become longer than usual in order to reach the surface. In two of the species examined, namely, *A. medeoloides* and *A. plumosus*, the second leaf of the shoot is also habitually retained by the side of the first, through the suppression of both the epicotyl and the internode immediately above it. These hypogeous leaves

are always sheathing kataphylls; though when two are present the second is much smaller than the first and does not protrude beyond the sheath of the cotyledon (Plate LIII, Fig. 14). In the axil of each kataphyll there is early formed a large bud which grows into the secondary stem, and this in turn produces below ground two contracted internodes bearing two sheathing kataphylls, one or both of which subtend a single axillary bud, the process being repeated indefinitely. Even in those species retaining only one kataphyll below ground at the base of their primary axis—that derived from the plumular meristem directly—there are two in the case of the secondary one and all that follow, though as a rule only the second of these has in its axil a bud. In this way the sympodial rhizome so characteristic of the genus is built up from the bases of the aerial shoots.

Let us now examine the changes which have taken place in the other parts of a somewhat advanced seedling. The suctorial end of the cotyledon (Plate LIII, Figs. 1 and 5) gradually enlarges within the seed as the reserve food-material of the endosperm becomes dissolved and absorbed. This is a slow process however, and the seed remains attached to the plant for a considerable period. The primary root in seedlings three or four weeks old has become more or less thickened—especially so in *A. medeoloides* (C, Fig. 3), many of the roots of which function solely as storage organs—and seems to act largely as a pull-root. It never branches, becomes wrinkled, and in fact presents all the peculiarities typical of such roots. Soon there arises, usually from the point where the hypocotyl and stem meet, a lateral root which branches and remains unthickened (C, Fig. 3). This is followed by other lateral roots proceeding from the rhizome as it is formed—for it must be remembered that two or more joints of this are produced each year—some of which develop in the same way as the primary root. The hypocotyl (Plate LIII, Fig. 15) remains short during the whole of its life, and, as I have already said, I have never, under any circumstances, seen one which had elongated. The stem apex in older seedlings is always protected by two or more scale-leaves, which later on are left behind, but not until others have been produced to take their place. At first they appear opposite in pairs; then, as the internodes lengthen, they assume their true position, alternating in a $\frac{1}{2}$ spiral. The first few scale-leaves of the stem—not including the basal kataphylls—sometimes have apparently no axillary buds, for example *A. comorensis* (Plate LIV, Fig. 16); but in other cases, such as *A. medeoloides*, may subtend lateral aerial branches or even cladodes (C, Fig. 3). In a longitudinal section of the stem apex in the latter plant (Plate LIII, Fig. 6) it is interesting to note how the development of the large leaf-like cladodes is almost as rapid as that of the

leaves in whose axils they arise ; while the vascular traces run out not into the leaves but into the cladodes.

There yet remains to be mentioned a matter of some interest and no little importance. Owing to the increasing girth of the seedling the sheathing base of the cotyledon becomes pushed to one side of the lower extremity of the stem ; while opposite it, and at the same level, arise the one or two kataphylls, now no longer enfolded by it (C, Fig. 3). *A. officinalis*, the only species previously described, has only one of these, and Henslow (8) and others have thought that it represented a second cotyledon, delayed in development and reduced in size. Following out this idea still further, it has been suggested that monocotyledons have been derived from a dictotylous ancestor by the suppression of one of the cotyledons. A more detailed account of the changes which take place in the plumular meristem during germination will be necessary before discussing this question. I may say, however, that while it seems quite likely that the ancestor of all present-day angiosperms was a dicotylous type, the above attempt to prove this will not stand examination.

The Development of the Plumular Meristem.

After the examination of a large number of monocotylous embryos I have found that, knowing the relative development of stem and leaf in the mature plant, one can guess with fair accuracy what the structure of the embryonic plumule will be. In other words, I believe that the characteristics of the adult produced in response to its environment, have tended to appear ever earlier in the life-history of the individual, till now they are found impressed even on the embryo, shut up though it be within the seed. Moreover, I am inclined for this reason to doubt the utility of searching for evidence regarding a plant's phylogeny in this region at least of its embryo. Applying what has been said to the genus *Asparagus*, let us consider what might be expected in the plumular meristem, and then observe how this agrees with its actual structure, as described later on. We are dealing with plants whose leaves are reduced to mere scales, while very much specialised branches function in their stead ; the stem has in fact become dominant over the leaf. It would therefore be natural to infer that the formation of leaf-primordia might possibly not yet have taken place in the embryo, and that in its subsequent growth the greater part of the plumule would be devoted to the formation of axis.

Though in the case of many monocotyledons the plumule of an embryo taken from a ripe seed will be found to consist of one or more leaf rudiments arising from an axial part, in the

genus *Asparagus* it is quite undifferentiated. It would be incorrect to apply the term "bud" to the simple dome-shaped meristematic patch which here constitutes this region. It is laterally placed near the lower end of the embryo, and slants downwards and outwards so that it may almost be said to face the slit in the base of the cotyledon through which the structures derived from it are later on to find an exit (Plate LIII, Figs. 8 and 9). On the commencement of germination there is developed from a portion of its outer and lower margin, constituting less than a quarter of the entire meristem, a small swelling, which grows slowly larger and finally forms the first leaf of the primary axis (Plate LIII, Figs. 9-11). As previously mentioned, this is a kataphyll, and remains permanently in the position in which it comes into existence, directly opposite the cotyledon; while very soon a

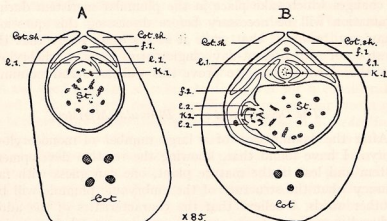


FIG. 4.—Transverse sections through the base of the primary axis in *Asparagi*, having respectively one and two basal kataphylls. A, *A. rugulosus*. B, *A. plumosus*, Baker. Cot., cotyledon; Cot.sh., sheathing base of cotyledon; f. (1, 2), kataphyll (1st, 2nd); k. (1, 2), rhizome-bud (of 1st, 2nd kataphyll); l. (1, 2), leaf of 1st, 2nd rhizome bud; St., primary axis.

remarkably large rhizome bud is produced in its axil (Plate LIII, Fig. 12). This bud takes part in the construction of the main underground sympodial rhizome. In those species which have two kataphylls at the base of the primary stem, the second of these arises in precisely the same way as the first, and almost simultaneously. It is, however, always smaller than the latter in the two species in which I have observed it; especially so in *A. medeoloides*, where it might easily be mistaken for a leaf belonging to the large rhizome bud which it also subtends, were it not for the evidence afforded by its position and earlier origin than the bud which appears to bear it (Plate LIII, Fig. 14). This second kataphyll is placed at right angles to the plane in which both the cotyledon and the first kataphyll lie, as shown in the accompanying outline drawing (Fig. 4).

The remainder of the plumule grows upwards and forms the first epigeous axis, upon which there soon appear the scale-leaves characteristic of such shoots (Plate LIII, Figs. 12 and 13). These scale-leaves arise close to the apex, two or more being always present as a protecting cap upon it (Plate LIII, Fig. 6). At first they appear almost opposite, but as the stem elongates they become separated by relatively long internodes, and assume their normal position in $\frac{1}{2}$ spiral. They differ from the basal kataphylls in not being amplexicaul and in having much smaller axillary buds which often remain undeveloped in the lower part of the shoot, but in the upper produce lateral branches bearing cladodes, or even cladodes directly, but never shoots resembling the parent axis (Plate LIV, Fig. 16).

The opinion of certain writers already alluded to, that the single sheathing kataphyll, which had been observed in advanced seedlings of *A. officinalis*, should be regarded as a second cotyledon because it arose at the base of the primary axis immediately opposite and at the same level as the cotyledon, falls to the ground for two reasons. Firstly, as has been shown, it is only after the commencement of germination that the development of this leaf takes place; there is absolutely no trace of it before that time. It cannot, to my mind, be regarded as the homologue of an organ belonging essentially to the embryonic phase of the plant's life, especially when what has been already stated regarding the influence of adult structure upon the state of differentiation of the plumule before germination is recalled. Secondly, since we now know that some *Asparagi* have two quite similar kataphylls occupying side by side the same position as the single one of *A. officinalis* does; and since there is no difference whatever in their mode of origin and subsequent behaviour, we must give up the claims of both, or be prepared to gift certain species with no less than three cotyledons.

Some even of the earliest investigators, such as Mirbel (1) and Braun (2), seem to have thought that the study of *Asparagus* seedlings helped to settle that vexed question, the homology of the cotyledon. If the secondary and all subsequent axes bore two sheathing basal scale-leaves, while the primary one—at the base of which is attached the cotyledon—had but one, did not this go to support the view that the cotyledon was the first leaf of the stem below which it arose? It would thus correspond to the first of the pair of leaves of the succeeding axes, and could be called quite correctly a "seed-leaf." This idea is of course no longer tenable when it is known that in such species as *A. plumosus* and *A. medeoloides* the shoot derived from the plumule, as well as those which follow possess a similar pair of these kataphylls.

THE GENERA *RUSCUS*, *DANAE*, *SEMELE*.

The development of the embryo being so characteristic, and the features of germination so constant in the six species of *Asparagus* studied, it was decided to make an examination of those genera—*Ruscus*, *Danae*, and *Semele*—which in Engler's "Pflanzen-familien" are placed, along with *Asparagus*, in the sub-division *Asparagæ* of the family *Liliaceæ*. Like the latter genus they are characterised by the reduction of the leaves to minute colourless scales, by the presence of remarkable cladodes, and

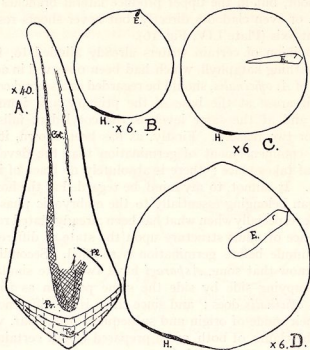


FIG. 5.—A, Longitudinal section passing through the plumule of an embryo of *Ruscus aculeatus*, Linn. B, Section of seed of *Smilax herbacea*, Linn., passing through the embryo. C, Similar section of *Ruscus aculeatus* seed. D, The same of *Danae Laurus*, Medic. Cot., cotyledon; E., embryo; H., hilum; Pl., plumule; Pr., primary root; Re., root-cap.

by the possession of a hypogeous sympodial rhizome. One would thus expect to find little or no difference between the anatomy of their embryos and young seedlings and those of *Asparagus*. There are, however, certain modifications in structure observable which are worthy of note, and I shall chiefly confine myself here to a short account of these. Through the kindness of Professor Bayley Balfour I obtained good seed of the following plants:—*Ruscus aculeatus*, Linn.; *R. Hypophyllum*, Linn.; *Danae Laurus*, Medic.; and *Semele androgyna*, Kunth. These were germinated, sectioned, and

examined in the same way as was done in the case of *Asparagus*.

The fruit and seed in these genera are like those of *Asparagus*, except that the seed-coat is commonly of a paler colour. Both micropyle and hilum are visible; they occupy the same positions and have the same appearance as in that genus. The embryo is shorter and broader (A, Fig. 5) but its orientation is quite similar (C, D, Fig. 5). Its various parts, including the plumule (Plate LIV, Figs. 19 and 24), closely resemble those of *Asparagus*, only that the number of vascular bundles in the cotyledon is greater (Plate LIV, Fig. 17), while the tissues of the primary root are perhaps slightly less clearly differentiated (Plate LIV, Fig. 18).

In all three genera the features of germination are remarkably alike, so much so that it will be unnecessary to fully describe and illustrate more than one; for this purpose *Ruscus aculeatus* has been chosen. Observed from the exterior, the only differ-

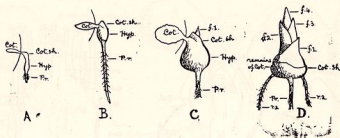


FIG. 6.—*Polygonatum latifolium*, Desf. Seedlings at various stages of development, showing the growth of the tuberous hypocotyl, &c. (All $\times 3$.) Cot., cotyledon; Cot. sh., sheathing base of cotyledon; f. (1, 2, &c.), kataphyll (1st, 2nd, &c.); Hyp., hypocotyl; Pr., primary root; r. 2, secondary root.

ence is that the primary axis does not make its appearance first as in *Asparagus*, but is preceded by several kataphylls which cover it over, and have much of the appearance shown in the case of the genus *Polygonatum* (D, Fig. 6); later on it rapidly elongates, leaving these leaves below-ground at its base. I have not followed the growth of seedlings to maturity and so cannot say exactly how many of these basal kataphylls—separated only by short internodes—are normally produced, but I have before me a section of *Semele androgyna* in which no less than eight are shown, each with a very large axillary bud. In *Ruscus* a smaller number seems to be typical, and the first apparently subtends no bud; while here, as in the other two genera, both the epicotyl and the internodes between the kataphylls elongate a little, especially in deep-sown seedlings (Plate LIV, Fig. 23). In longitudinal sections of the young seedling it is seen that the greater part of the plumular meristem is devoted to the formation of leaves, and that these sheath the axis more completely than in

Asparagus. The sheathing bases may be relatively large as in *Ruscus* (Plate LIV, Fig. 21), or smaller as in *Semele* (Plate LIV, Fig. 25); but they are only produced when the leaf has become well developed, being represented before then by a narrow band of meristematic tissue passing round the axis outside and at the base of the succeeding leaf, and distinguishable from a leaf-primordium by the absence of the well-marked vascular bundle which passes out into the latter (Plate LIV, Fig. 22). With these exceptions, the rest of the *Aspargeæ* differ but little from the genus *Asparagus*.

THE GENUS *POLYGONATUM*.

I have included a few drawings of *Polygonatum latifolium*, Desf., to illustrate an interesting modification in the development of the seedling, which I think further investigation will prove common to the genus. The structure of the seed and embryo is similar to that of *Asparagus medeoloides*

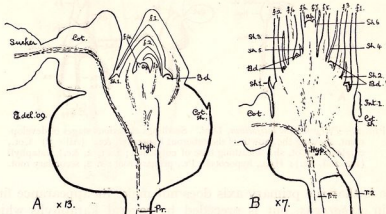


FIG. 7.—*Polygonatum latifolium*, Desf. Longitudinal sections of seedlings through the plumular region. A, a young; B, an advanced seedling. Cot., cotyledon; Cot. sh., sheathing base of cotyledon; Ap., shoot apex; Bd., rhizome buds in the axils of the kataphylls; f. (1, 2, &c.), kataphyll (1st, 2nd, &c.); Hyp., hypocotyl; Pr., primary root; r.2, secondary root; sh. (1, 2, &c.), sheathing base of leaf (1st, 2nd, &c.); Int. (1), internode (1st).

(B, Fig. 1), while the general characteristics of germination are very like those found in *Ruscus*, *Danae*, and *Semele*. The interesting point, however, is that the hypocotyl—which in these plants remains almost undeveloped—becomes in this case enlarged into a tuberous structure, and thus helps in the formation of the first joint of the thick, fleshy, sympodial rhizome typical of the genus *Polygonatum* (Fig. 6). The growth of the plumule resembles that of *Ruscus*, but the epicotylar and few succeeding internodes are rather more elongated and fleshy; while the kataphylls are

a little more prominent. Some five of these leaves are produced upon this shortened underground part of the primary axis, which then elongates to form an aerial shoot, leaving them at its base (Fig. 7). In the axil of each lies a large bud; that of the fifth continues the growth of the main rhizome, while those of the preceding four—if they develop further—give rise to its lateral branches.

THE GENUS SMILAX.

Two species of *Smilax* were examined—*S. herbacea*, Linn., and a plant of uncertain identity. In these the seed and its germination were indistinguishable, exhibiting the following peculiarities as compared with *Asparagus*. The micropylar region of the seed forms a small protruding point, in which

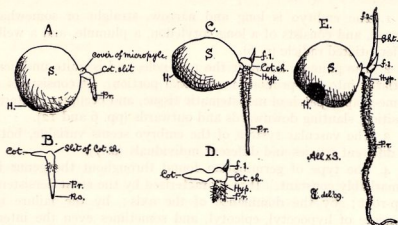


FIG. 8.—*Smilax*, Sp. Seedlings in various stages. Cot., cotyledon; Cot. sh., sheathing base of cotyledon; f. (1, 2), leaf of primary axis (1st, 2nd); H., hilum; Hyp., hypocotyl; Pr., primary root; S., seed; Sht., primary axis; Rc., root-cap.

lies the extremely small embryo (B, Fig. 5). I have been unable to detect any differentiation in these embryos, several of which have been cut and examined, though this may be due to the difficulty of correctly orientating so small a structure when embedding it. I am, however, inclined to believe that, even in "ripe" seed, the embryo is at a very early stage in its development, and remains so during its resting period within the seed. The cotyledon of quite young seedlings is so very much larger than that region of the minute embryo that I think it probable that between sowing and actual germination—as this term is usually applied—the embryo undergoes further growth, as has been observed in the case of *Juncus* and other plants.

It will be seen that the appearance of seedlings at various stages (Fig. 8) at once recalls the genus *Asparagus*. As one

would expect in plants, such as *Smilax*, with large leaves and long climbing stems, both are well represented in the plumule of a young seedling (Plate LIV, Fig. 26). The first leaf—a kataphyll—is rapidly developed and appears from the sheath of the cotyledon before the primary axis (C, Fig. 8), which soon follows (E, Fig. 8). This is the only basal scale-leaf produced, and subtends a very large rhizome bud exactly like that of *Asparagus* (Plate LIV, Fig. 27). Moreover, both the hypocotyl and the epicotyl remain, as in that genus, quite unelongated.

SUMMARY.

Asparagus (p. 4).

1. The embryo is long and narrow, straight or somewhat curved, and consists of a long cotyledon, a plumule, and a well-differentiated radicle (p. 6).

2. The plumule lies near the root-pole, and is quite undifferentiated into leaf-primordia and axial portion. It consists of a dome-shaped patch of meristematic tissue, and occupies a lateral position, slanting downwards and outwards (pp. 6 and 12).

3. The vascular system of the embryo seems variable, both in different species and different individuals (p. 7).

4. The type of germination found throughout the genus is remarkably constant. It is characterised by the stout persistent tap-root; by the dominance of the axis; by the failure to elongate of hypocotyl, epicotyl, and sometimes even the internode above the latter; and by the presence of hypogeous kataphylls at the bases of the shoots, in whose axils large rhizome buds arise (p. 8).

5. The mass of the plumular meristem gives rise to axis, the basal kataphylls being derived from a small portion at its outer margin (p. 11).

6. Each kataphyll subtends a single rhizome bud (pp. 10 and 12).

7. The species examined can be divided into two groups—those having at the base of their primary axis one, and those having two kataphylls (pp. 5, 9, and 12).

8. The apex of the shoot is always protected by two or more scale-leaves. In its upper part the cladodes arising in the axils of the leaves develop almost as rapidly as the leaves themselves (pp. 10 and 13).

9. The elongation and branching of the sympodial rhizome is brought about by the rhizome buds (p. 10).

10. The primary root acts as a pull-root, and sometimes as a storage-root (p. 10).

11. The statement that the first kataphyll represents a second cotyledon is disproved by the fact that it only makes its appearance after germination, and that some species have two (p. 13).

12. For the second of the above reasons it also follows that the anatomy of *Asparagus* seedlings does not support the view that the cotyledon is a leaf (p. 13).

Ruscus, *Danae*, and *Semele* (p. 14).

13. The embryo is shorter and broader but in all other respects resembles that of *Asparagus* (p. 15).

14. The germination is almost identical in all three genera, and differs from that of *Asparagus* in the larger number of more sheathing basal kataphylls; in the less dominant axis; and in the slight elongation of the epicotyl and succeeding hypogeous internodes of the primary axis (p. 15).

Polygonatum (p. 16).

15. The germination much resembles that of *Ruscus*; the most interesting deviation is the part played by the tuberous hypocotyl in the formation of the fleshy rhizome (p. 16).

Smilax (p. 17).

16. In ripe seed the embryo is very minute and apparently undifferentiated (p. 17).

17. The germination very closely resembles that of *Asparagus*; axis and leaf are, however, about equally developed (p. 17).

PREVIOUS PUBLICATIONS RELATING TO THE GERMINATION OF ASPARAGUS.

(Literature cited in chronological order.)

1. MIRBEL. Observations sur la germination de l'oignon et de l'asperge; lues à la classe des sciences de l'Institut, le 13 février 1809. Ann. de Mus. d'Hist. Nat. de Paris, xiii. (1809) p. 160.
2. BRAUN, A. Betrachtungen über die Erscheinung der Verjungerung in der Natur. Prorectorate address issued to a small circle, Freiburg 1/B, May 1850. Published in book form, Leipsig, 1851. English translation by A. Henfrey, London, 1853.
3. IRMISCH, THILO. Beiträge zur vergleichenden Morphologie der Pflanzen, Abth. iii. 1856.
4. KLEBS, GEORG. Beiträge zur Morphologie und Biologie der Keimung; dated 20th November 1884. Tübingen Untersuchungen, Band I., Leipsig, 1881-85.
- 5A. SCHOLZ, EDUARD. Morphologie der Smilaceen mit besondere Berücksichtigung ihres Sprosswechsels und der Anatomie der Vegetationsorgane. 23 Jahresber. des nied.-österr. Landes-Realgymnasiums zu Stockerau. 1888.

- 5B. SCHOLZ, EDUARD. Entwicklungsgeschicht und Anatomie von *Asparagus officinalis*. Festschr. zum 50 Jahresber. des Schottenfelder k. k. Staats-Realsch. vii. Bez. Vienna, 1901.
6. ČELAKOVSKÝ, L. J. Ueber die Kladodien der Asparageen. Abh. (Rozpravy) d. böhm. Akad. d. Wiss. 1893.
7. SARGANT, ETHEL. A Theory of the Origin of Monocotyledons founded on the Structure of their Seedlings. Ann. Bot. xvii. (1903.)
8. HENSLOW, G. The Heredity of Acquired Characters in Plants. London, 1908.

EXPLANATION OF PLATES, LIII AND LIV.

Illustrating W. Edgar Evans' paper "On the Further Development during Germination of Monocotylous Embryos."

EXPLANATION OF THE ABBREVIATIONS USED IN LETTERING THE FIGURES.

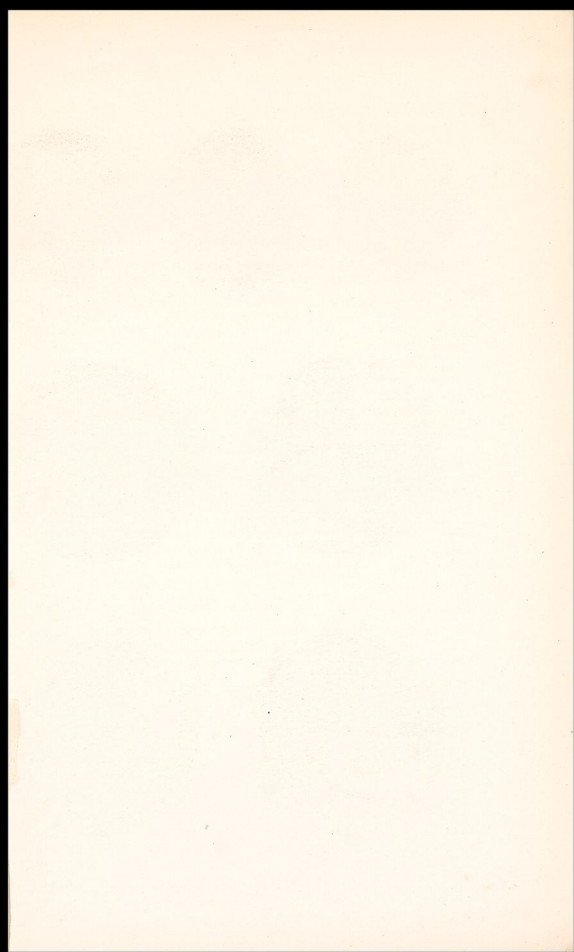
ap. = apex of primary axis.	pl. = plumule.
cot. = cotyledon.	pler. = plerome.
cot. sh. = sheathing base of cotyledon.	pr. = primary root.
der. = dermatogen.	py. = cladode.
f. (1, 2, &c.) = leaf of primary axis (1st, 2nd, &c.).	rc. = root-cap.
hyp. = hypocotyl.	sh. (1, 2, &c.) = sheathing base of leaf of primary axis (1st, 2nd, &c.).
int. (1) = internode of axis (1st) = epicotyl	sht. (1, 2, &c.) = axis (primary, secondary, &c.).
l. (1) = leaf of secondary axis (1st).	
per. = periblem.	

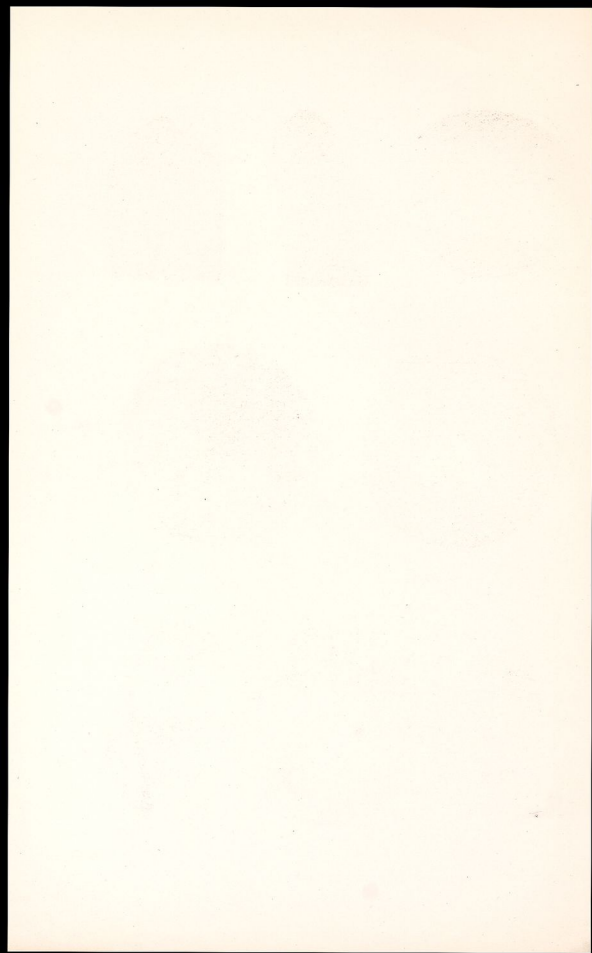
Plate LIII.

- FIG. 1. Transverse section through cotyledon of embryo of *Asparagus trichophyllus*, Bunge, showing three vascular bundles . . . x75
- FIG. 2. Trans. sect. through plumule of embryo of *A. trichophyllus*, showing plumule and sheathing base of cotyledon . . . x75
- FIG. 3. Trans. sect. through radicle of embryo of *A. trichophyllus*, showing 5 protoxylem patches in the central stele . . . x75
- FIG. 4. Longitudinal sect. through root-apex of embryo of *A. rugulosus*, showing the tissues . . . x110
- FIG. 5. Long. sect. through cotyledon of embryo of *A. rugulosus* . . . x60
- FIG. 6. Long. sect. through stem-apex of *A. medeoloides*, Thunb., showing scale-leaves and cladodes . . . x58
- FIG. 7. Exterior view of slit in base of cotyledon of embryo of *A. trichophyllus* . . . x145
- FIG. 8. Tangential long. sect. through plumule of embryo of *A. trichophyllus* . . . x145
- FIG. 9. Radial long. sect. through plumule of embryo of *A. rugulosus* . . . x145
- FIG. 10. Rad. long. sect. through plumule of very young seedling of *A. rugulosus*, showing appearance of the 1st kataphyll . . . 145
- FIG. 11. Rad. long. sect. through plumule of slightly older seedling of *A. rugulosus* . . . x145
- FIG. 12. Rad. long. sect. through seedling of *A. rugulosus*, showing axis, and 1st kataphyll with axillary rhizome bud . . . x54
- FIG. 13. Rad. long. sect. through seedling of *A. plumosus*, Baker, showing axis, kataphyll, and rhizome bud . . . x26
- FIG. 14. Long. sect. through base of primary axis of *A. medeoloides* seedling, showing 1st and 2nd kataphylls and their axillary rhizome buds . . . x50
- FIG. 15. Rad. long. sect. through young seedling of *A. rugulosus*, showing cotyledon, plumule, hypocotyl, and primary root . . . x30

Plate LIV.

- FIG. 16. Seedling plant of *A. comorensis*, showing cladodes (Py) and scale-leaves (1, 2, &c.) . . . x1
- FIG. 17. Long. sect. through cotyledon of seedling of *Ruscus aculeatus*, Linn., showing vascular bundles . . . x30
- FIG. 18. Long. sect. through radicle of embryo of *R. aculeatus*, showing the tissues . . . x60









16.

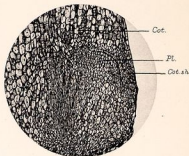


17.

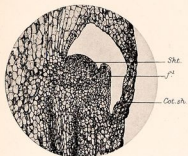


18.

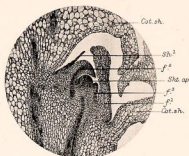
W. E. Agar Evans, photo.



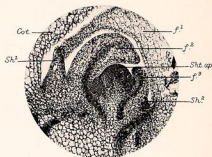
19.



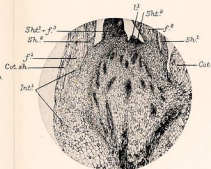
20.



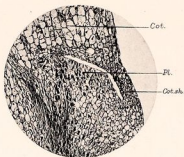
21.



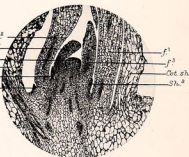
22.



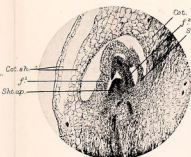
23.



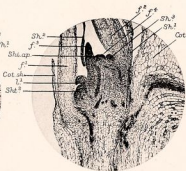
24.



25.

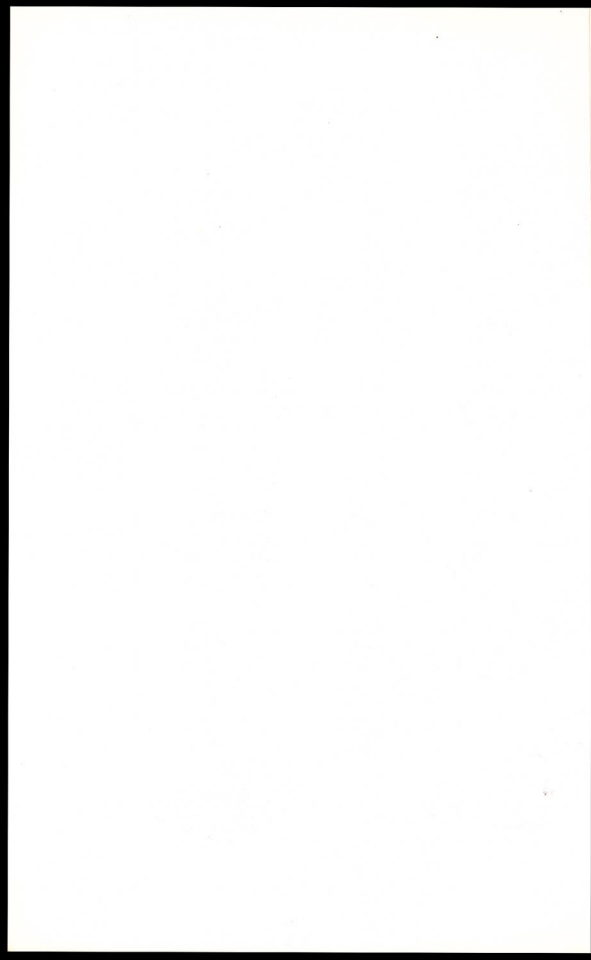


26.



27.

Emb. coll.



- FIG. 19. Rad. long. sect. through plumule of *R. aculeatus* embryo . . . ×80
 FIG. 20. Rad. long. sect. through plumule of young seedling of *R. aculeatus*,
 showing the appearance of the 1st kataphyll . . . ×48
 FIG. 21. Rad. long. sect. through plumule of more advanced seedling of *R.*
aculeatus, showing 1st, 2nd, and 3rd kataphylls . . . ×27
 FIG. 22. Rad. long. sect. through plumule of seedling of *R. aculeatus*, showing
 vascular bundles passing into the kataphylls . . . ×50
 FIG. 23. Long. sect. through seedling of *R. aculeatus*, showing elongation of
 epicotyl and formation of rhizome bud in axil of 2nd kataphyll.
 This section does not pass through the centre of the primary
 axis, part of which along with the 3rd kataphyll are seen at sht.
 +f.3 . . . ×21
 FIG. 24. Rad. long. sect. through plumule of embryo of *Danae Laurus*,
 Medic. . . ×80
 FIG. 25. Rad. long. sect. through seedling of *Semele androgyna*, Kunth. . . ×44
 FIG. 26. Rad. long. sect. through plumule of young seedling of *Smilax* sp.,
 showing leaves and axis . . . ×46
 FIG. 27. Rad. long. sect. through seedling of *Smilax* sp., showing basal kataphyll
 with axillary rhizome bud, axis with leaf, &c. . . ×33