Art. VII.—*Studies in the New Zealand Species of the Genus* Lycopodium. *Part II—Methods of Vegetative Reproduction.*

By the Rev. J. E. Holloway, D.Sc.

[*Read before the Philosophical Society of Canterbury, 6th December, 1916, received by Editors, 30th December, 1916, published separately, 6th July, 1917.*]

Plates VIII, IX

It is characteristic of the lower classes of plants that they show great capacity for vegetative propagation. With regard to the mosses this is a common phenomenon "In the pleurocarpous forms, the main axes gradually die away from behind, the lateral branches becoming isolated, and constituting the main axes of new plants. In probably the majority of the Musci almost any portion of the body, a piece or stem, or a leaf, will, under proper conditions, grow out into protonemal filaments, which give rise to adult shoots in the usual manner In certain species belonging to the Brymeae, multicellular gemmae are produced at the apex of the stem [and are either] modified leaves [or] are smaller and consist of but few cells [or] are borne on long stalks. On being placed under . . favourable conditions, the cells of the gemma grow out into protonema." (Vines, *Text-book of Botany*, p. 337, 1898)

Reproduction by gemmae is characteristic also of the Hepaticae "Brood-buds" have been described and figured as occurring in the Psilotaceae (*e g.*, Engler and Prantl, 1900) Also, in *Ophioglossum vulgatum*, two observers, Land (1911) and Pfeiffer (1916), have recorded that vegetative reproduction, probably by adventitious budding of older roots, is the common method of spreading.

In the genus *Lycopodium* we find a variety of methods of vegetative reproduction which are closely analogous to those cited above. The present paper is devoted to a description of the methods of vegetative propagation which I have observed in the New Zealand species of the genus *Lycopodium.*

I have not been able to gain access to Treub's *Études sur les Lycopodiacées*, in vol. viii (1890, pp 14–23) of which is his description of the root-tubercles of *Lycopodium cernuum.*

I Vegetative Propagation of the Prothallus.

Treub (1886) has described three methods of vegetative propagation which he observed in the prothalli of *L Phlegmaria* Firstly, by the progressive rotting of the older parts of the prothallus the lateral branches are set free and thereupon constitute new individuals; secondly, by the formation of small ovoid multicellular bodies ("brood-buds") from single epidermal cells of the prothallus, these becoming isolated by the rupture of their pedicels; thirdly, by the formation of thick-walled bodies consisting of only a few cells, which probably are designed to undergo a period of rest during an unfavourable season Treub compared these two latter kind of organs to the gemmae of the Hepaticae

On old or injured prothalli of *L Selago* Bruchmann (1898, pp 95–97) found adventitious shoots on which rhizoids and sexual organs were developed, and which were able to live a separate existence when isolated from the parent prothallus.

Goebel (1905) found that broken-off portions of the crown of lobes of the prothallus of *L. inundatum* developed into new prothalli. These adventitious prothalli rooted themselves to the soil with rhizoids, and produced lobes and sexual organs.

In the New Zealand species *L. Billardieri*, which is very similar to the other epiphytic species, *L. Phlegmaria*, noted above, Miss Edgerley (1915, p. 105) states that the branches on the prothalli sometimes die off from behind and form new individuals. She found " some prothalli surrounded by numerous detached branches whose rhizoids were interlaced with those of the parent prothallus."*

The long-drawn-out prothalli of *L. ramulosum* (Holloway, 1916, pp 269–71) are suggestive of the same methods of vegetative propagation—*i.e.*, by the isolation of portions of the prothallus as new individuals through the decay of the intermediate regions. I have not, however, actually observed that this has taken place. Some of those prothalli found by me showed several tubercular swellings along their length, each of which was infested by a fungus and bore a group of rhizoids. In close association with some of these swellings were groups of filamentous lobes at the base of which archegonia were developed (*ibid.*, figs. 32C and 32D, p. 269). It will thus be seen that if through injury to the prothallus or by the dying-away of an intermediate region of it a portion should become detached from the whole, that portion could still be possessed of all the essential organs for an independent existence and for the development of a young plant.

Treub (1886) was of the opinion that the majority of the prothalli of *L. Phlegmaria* found by him had been produced adventitiously from older prothalli, and that the germination of the spore was slow and the production of prothalli direct from spores consequently somewhat rare. I have shown elsewhere (1916, pp. 262–64) that in the case of the terrestrial New Zealand species the formation of prothalli from spores is by no means as uncommon as has been hitherto supposed of the Lycopodiaceae, although this may very possibly be due to the temperate climate and abundant rainfall in those parts of this country where Lycopods abound. Vegetative reproduction of the prothallus does not seem to be as common in the case of other species as it is in the epiphytic species of *Lycopodium*, but the fact that it has been noticed in certain abnormal cases in several other species shows that it may still be found to be a common method of propagation in the genus when certain conditions are present †

* The present writer has recently found the prothalli of *L. Billardieri* var. *gracile*. In some instances these showed a central more or less bulky region (on which were paraphyses and archegonia), with several branches, either long and slender or short and club-shaped, arising from it. But the majority of the prothalli found consisted of a single stout or slender branch of greater or lesser length, on which paraphyses and antheridia occurred in places, and on which also several short club-shaped vegetative branches had arisen. These latter prothalli were invariably broken off short at one or at both ends They had probably originated by being detached from older prothalli. Not a few quite short club-shaped vegetative branches (densely packed with the fungal element, &c.) broken off at one end were also dissected out. (Out of thirty or forty prothalli found, only three undoubtedly showed the original end of the prothallus intact.)

† In Part I of these Studies (Holloway, 1916, p. 274) I figured several prothalli of *L. scariosum* in which a pseudo-branching had taken place. These prothalli were of unusual size, and their peculiar form, in which the two halves of the prothallus were separated by a constriction, suggests the possibility of the separation of the parent prothallus into two or more individuals capable of separate existence.

II. The Isolation of Portions of Lateral Branches or of the Main Axes of the Plagiotropic Species.

This method of spreading is commonly found in all the plagiotropic species of the genus. The young prothallial plants are orthotropic in the case of all the New Zealand species whose adult plants have this plagiotropic habit of growth, but sooner or later they adopt the latter habit The main axis, which in some species is subterranean and in others above ground, is generally speaking of unlimited growth, as are also certain of the lateral branches Adventitious roots arise at intervals from the dorsal surface along both the main axis and the lateral branches The vegetative spread of such a species takes place by the dying-away of the older parts of the main stem or by the isolation of the lateral branches It is obvious that the form and habit of the plagiotropic species are adapted to this method of propagation

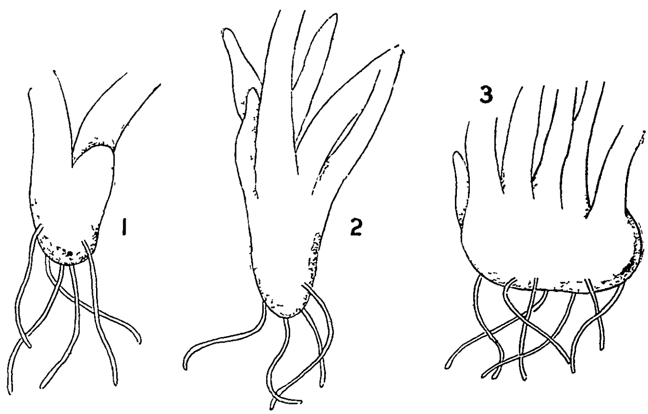
The plagiotropic species of *Lycopodium* are thus most fitted for rapid extension over new areas These are the species found in large spreads over recently disturbed areas, such as forest clearings, roadside cuttings, sluiced goldfields, &c. This method of propagation is in all probability the main one in these species, except perhaps in the initial establishment of the species in the new area. Germination of the spores of these species does readily take place where the right conditions are present, but the delicate nature of the prothallus of some of them (e g., *L. cernuum*, *L. laterale*, and *L. ramulosum*), and the unusual length of time required for the full development of the prothallus of others (e g., *L fastigiatum*, and also *L. volubile*, *L. scariosum*, and *L. densum*), are the reasons why only in exceptional localities, or during an exceptional series of seasons, are sexually produced plants firmly established. In Westland, where the rainfall is extremely high, I have frequently found in many localities colonies of young plants of the species *L fastigiatum*, *L volubile*, *L scariosum*, and *L. ramulosum* which had been sexually produced, and of which a considerable proportion had come to maturity In those parts of Canterbury and Nelson, however, where dry seasons are common I have on several occasions come across colonies of the young plants of *L fastigiatum*, *L. volubile*, and *L. scariosum* which had become dried up And on the clay gum-lands of Auckland, which are generally subject to being dried up in the summer, and where the two species *L cernuum* and *L laterale* commonly occur, it is probable that the young plants, which I have frequently found together with the prothalli, rarely come to maturity

The form and habit of the plagiotropic species are probably to be regarded as a direct adaptation to the environment. The anatomical structure of the stem will also be modified in accordance with this I have shown (1910, pp. 362–64) that the development of the parallel structure in the vascular cylinder of the developing stem of *L. volubile*, *L fastigiatum*, and *L scariosum* is the direct result of the restriction of branching to one plane We may, of course, expect to find that the plagiotropic habit of growth has been evolved in species which belong to different natural sections of the genus The fact that in the plagiotropic species there are at least two main types of stem vascular anatomy—viz , the " mixed " type of *L cernuum* (and also *L laterale*, *L. ramulosum*, and *L Drummondii*), in which the xylem and phloem elements are intermingled and present no definite structure, and the " parallel " type of *L. volubile* (and also *L scariosum*, *L. fastigiatum*, and *L. densum*), in which the vascular elements are disposed in parallel plates—is an indication that the species which conform to one type are to be more or less widely separated from those which belong to the other. The comparative study of the different species and the correlation of their characters is undoubtedly yielding indications of the different lines along which the genus has evolved, and also of the degrees of relationship existing between them: and such characters as habit of growth and stelar anatomy must also be taken into account in the construction of a natural classification of the Lycopodiaceae.

III. BULBILS ON ADULT PLANTS.

Vegetative reproduction by means of bulbils borne on the stem is well known in *L. Selago* and certain other allied species. These bulbils are stated to be modified leafy branches.

While searching for young plants of *L. cernuum* near Henderson, Auckland, some years ago, I found on a damp mossy bank a number of very young plantlets of this species which had obviously been produced vegetatively. Mature plants were also growing on the bank immediately above. It would seem that the plantlets had originated as bulbils on these older plants, but I was not able to ascertain on what part of them they had been borne. The plantlets were simply resting upon and entangled in the ends of the moss-branches, and were not in any way anchored by their rhizoids to the humus. The youngest plantlets consisted of a basal tuberous portion,

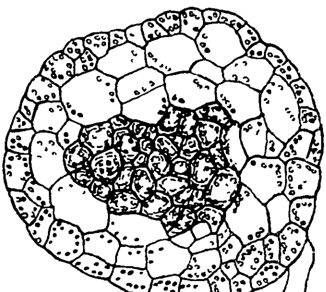


FIGS 1–3 —*Lycopodium cernuum* Bulbils from adult stems. × 20

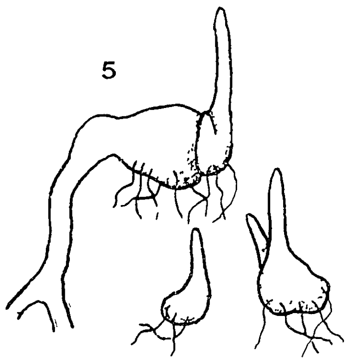
which was either round or more or less elongated, surmounted by one or two protophylls (fig 1). Many of them, which showed several protophylls, were of a drawn-up spindly form, as if the stem-apex had been initiated very early and its upward growth had been rapid, whilst the basal portion was more feebly developed (fig. 2). It is possible that these individuals may not have been detached from the parent plant till they had attained this extent of growth. In a few instances the basal portion of plantlets was extended horizontally, as is usual in the sexually developed young plants of *L. laterale* and *L ramulosum*, and as sometimes occurs also in the corresponding case of *L. cernuum* (Holloway, 1916, pp. 287–89). One or two of these plantlets bore as many as six protophylls (fig. 3) without a stem-apex having been initiated. The basal portion in all cases appeared opaque and brownish at the centre. Sections showed that this was due to a somewhat compact core of narrow cells containing much protoplasm (fig. 4) Minute starch-grains were present in abundance in the basal portion of the plantlets, especially in the epidermal cells and central cells. These stained blue with an aqueous solution of iodine No fungal hyphae were seen. The leaves were crowded with stomata.

IV Root-tubercles.

Treub (1887) has described root-tubercles in *L cernuum* thus · "The root-tips change into propagative organs of a remarkable form These root gemmae or bulbs produce on germinating young plants very much like those which come forth from prothalli." This method of vegetative reproduction is found in several species of *Ophioglossum*. Land (1911) thoroughly investigated colonies of *O. vulgatum* in a Mexican locality, in which many young plants were seen to be present. He noticed that the plants were generally in groups of three to ten, usually radiating from a large plant When the root-system of these groups was laid bare it was found that nearly all the plants of a group were connected, and that the smaller plants were produced by adventitious budding of the roots of the larger



4



5

FIG 4 —*Lycopodium cernuum* Transverse section of basal region of such a plantlet as that shown in fig 2 × 100

FIG 5 —*Lycopodium ramulosum* Root-tubercle with attached and detached plantlets × 6

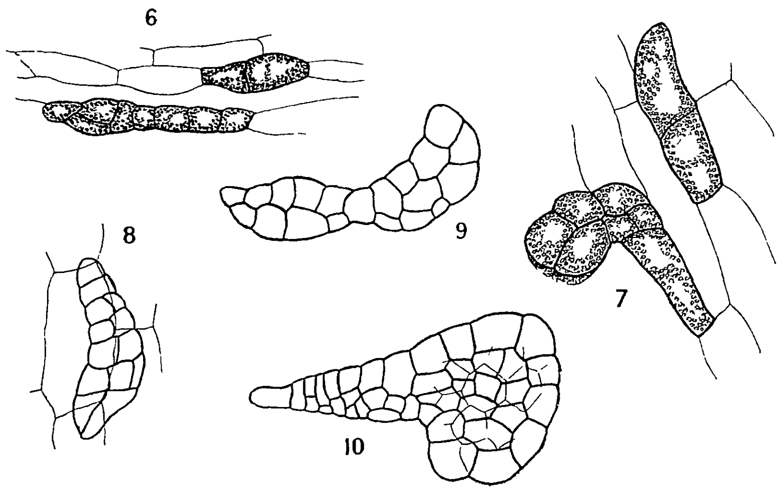
plants Pfeiffer (1916) found the prothalli of *O vulgatum* in a wet locality near Chicago At the end of her paper she remarks that probably reproduction of this species by vegetative spread is by far the more common method, but scattered among plants so produced are the far less numerous specimens arising after gametophyte production has occurred

I have found root-tubercles in both *L cernuum* and *L ramulosum*. In the former of these species instances were observed in which the actual tip of the root had turned into a swollen tuber, as described by Treub Again, in the case of the adventitious plantlets of the same species described in Section III of this paper, two instances were seen in which a tuberous swelling occurred immediately behind the apex of the root of a plantlet—*i.e.*, the root-tip itself was not concerned with the process but grew on in the ordinary way. I was not able to collect sufficient material to enable me to trace the development of the root-tubercle of this species into the young plant, but there is no doubt that the tubercles observed were instances of the same process which Treub has described. In *L. ramulosum* I have found many instances in which the root-tip was swollen to form a somewhat egg-shaped tuber. On one occasion I dissected out from each other several pieces of old roots, one of these pieces bearing a secondary root whose tip had swollen to form a tuber, whilst entangled amongst the roots were two very young plantlets, one with two protophylls and the other with one. I have no doubt that these latter had originated as root-tubercles. In several other instances a detached root was found whose end was swollen to form a large massive irregularly-shaped tuber (*e.g.*, fig. 5), at the growing end of which a secondary tuber was attached surmounted by one or more developing protophylls. Both tubers were always green in colour, and both bore rhizoids. In the case of the plant shown in fig. 5 two other young green plantlets were disentangled from the rhizoids of the tuber. They had probably been budded off from it. Plate VIII, fig. 3, is a photograph of a root-tubercle of *L ramulosum*.

V GEMMAE PRODUCED FROM CORTICAL CELLS OF OLD ROOTS.

In dissecting out young plants and prothalli of *L. ramulosum* from mossy turves obtained from a clay roadside cutting near the Mikonui River, Westland, I discovered a large number of instances of very young green plantlets attached along old isolated rootlets of this species. The older of these plantlets did not seem to be very firmly attached to the roots, but could be more or less easily brushed off with a camel's-hair brush in the process of cleaning. The youngest plantlets, however, were more firmly attached. A large number of these old isolated rootlets were found in the material dissected, and adventitiously produced plantlets were also in great abundance. In no case was a prothallus found attached to a plantlet, and only two isolated prothalli altogether were here found. Each plantlet consisted of a basal tuberous portion, the central region of which was opaque and brownish owing to the dense protoplasmic contents of the centrally placed cells, surmounted by one or more developing protophylls. Thus these plantlets were in general structure closely similar to all other young plants of this species, whether vegetatively or sexually produced, of the same degree of development The original end of the basal tuberous region, however, was generally irregularly shaped, and in older plantlets the whole tuber spindly and even sausage-shaped, in this respect these plants being distinguishable from sexually produced plantlets. The characteristic form of these adventitiously produced plants corresponded closely with what I had previously noticed in the case of a number of very young plantlets of the same species found in material obtained from quite a different locality. I had there dissected out a good many plantlets of this form, and had been struck by the fact that none of them were attached to prothalli. I have no doubt now that they had been produced adventitiously from roots in the manner described in this section. It would seem, then, that this method of vegetative reproduction is not uncommon in this species. Whether or not it is due to an unusual season, or to some other such cause, I was not able definitely to determine. It is to be noted that none of the more fully grown plantlets gave any evidence of that extended horizontal habit of growth of the basal tuber with the postponement of stem-formation such as is so characteristic a feature in the sexually produced plants of this species. The development of the stem-apex is early, so that the plant becomes somewhat spindly in form Abundant starch commonly occurred in the plants both in the tuberous region and in the rhizoids

The old roots on which these plantlets were borne were frequently seen to be green, and even vividly green, in isolated patches, especially in the neighbourhood of the attached plants. Where a young plant was torn away from a root by brushing, a gaping hole was left in the latter, showing that the plant had taken its origin in the interior of the root and had had to emerge through a split in its external tissues. It was observed that at the point where a young plant was attached a group of rhizoids was present, and these rhizoids were found to be developed not from the plant but from the epidermal cells of the root. Plantlets at a somewhat later stage, and those detached from the parent roots, showed rhizoids on their basal portions. Isolated plantlets in a few instances were seen to show fragments of torn parenchymatous tissue attached to the basal region at one point or other. This would probably represent the remains of the cortical tissues

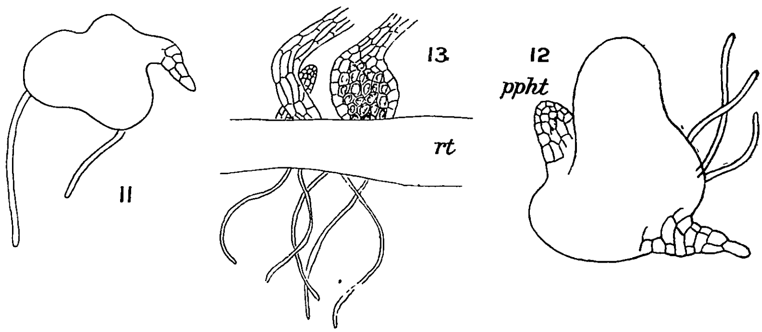


FIGS 6–10 —*Lycopodium ramulosum* Gemmae developed from cortical cells of old isolated rootlets Figs 6 and 10 × 100 ; figs 7, 8, and 9 × 120

of the parent root. The first-formed end of the tuber of some of the plantlets was of irregular form, the superficial cells growing out, or one or more haustoria-like prolongations of the tuber being developed Probably this is to be put in connection with the nutrition of the developing plant from the root-tissues.

It commonly was the case that several plantlets in different stages of development were borne at the same spot on the root (fig. 13, and Plate VIII, fig. 2) In one instance I noticed, before dissecting out a certain piece of mossy turf, that three groups of protophylls were showing above the surface of the moss at distances of about a quarter of an inch from each other, and all in a straight line These groups were found to be connected by an old root In each group were well-grown young plants, as well as a large number of plantlets of all ages, some separate and others attached to the old root or to its various branches All the individuals in these three groups had probably arisen adventitiously

By dissecting under the microscope portions of old roots which showed green patches in the cortical tissues I was able to obtain a good series of developing plantlets. Certain individual cells of the cortex, which are similar in appearance to the other cortical cells except that they contain abundant protoplasm and chloroplasts, divide transversely (figs. 6 and 7). In some cases transverse division is continued so that the original cell becomes a filament several cells in length (fig. 6). In most cases, however, longitudinal walls appear early in the cells of the filament, so that it begins at its growing end to take on the form of a cell-mass (figs. 6–10). The growing end soon becomes globular in shape and bends away more or less at right angles from the original direction of the initial and other cortical cells, evidently turning towards the upper surface of the root (figs. 7 and 10). The globular growing end of the young plant becomes the basal tuberous region of the plantlet of a later stage of development. In some cases it grows very regularly to form an egg-shaped cell-mass, the summit of which is continued as the first protophyll (fig. 13). Again, in other cases the tuber grows very irregularly before the first protophyll appears (figs. 11 and 12).



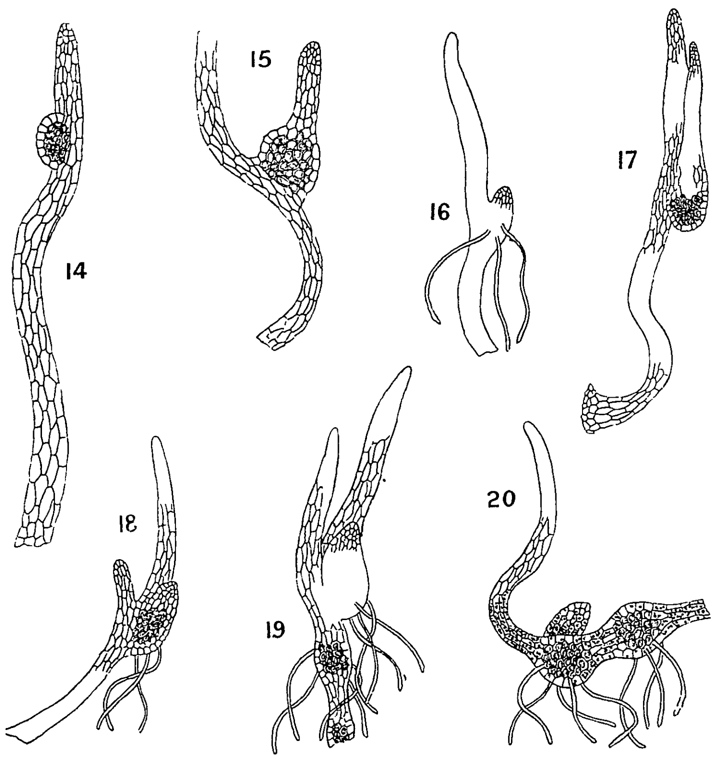
FIGS 11, 12 —*Lycopodium ramulosum.* Gemmae developed from cortical cells of old isolated rootlets × 45

FIG 13 —*Lycopodium ramulosum* Group of adventitious plantlets in different stages of development attached to old rootlets × 12

About the stage of development attained by the plantlet shown in fig. 11 rhizoids first make their appearance on the tuber. From the first initiation of the young plant the adjacent cells of the root give rise to a group of rhizoids, showing that the developing plant is in its first stages nourished from the cells of the parent root. As the plantlet increases in size it bursts the outer tissue of the root, and the first protophyll is then quickly developed (Plate VIII, fig. 1) The first-formed tapering portion may clearly be seen on the developing plantlets (figs. 11 and 12), and may sometimes still be distinguished at the base of plants which show as many as three or four full-sized protophylls. As the plant grows, the tuberous basal portion becomes elongated in most cases by the swelling of the lower extremity of the protophyll till it assumes a sausage-like shape, but the original end of the tuber retains its first-formed somewhat irregular form. As has been noted above, I have observed one or two plants in which the young developing tuber had grown very irregularly so as to form one or more distinct haustoria-like protuberances. There can be no doubt that these penetrated the cortical tissues of the parent root and functioned as absorbing surfaces.

VI. Bulbils on Detached Leaves.

In the same material in which the vegetatively produced plants described in the last section were found I discovered a large number of young plantlets of the same species (*L. ramulosum*) developing on detached leaves I could not ascertain for certain whether these leaves had become detached from mature plants or from young sexually produced plants of the previous season. The material dissected contained many old detached roots and rootlets. A somewhat dry year had intervened since the previous season, and it is possible that a colony of young plants, or perhaps a mat of adult

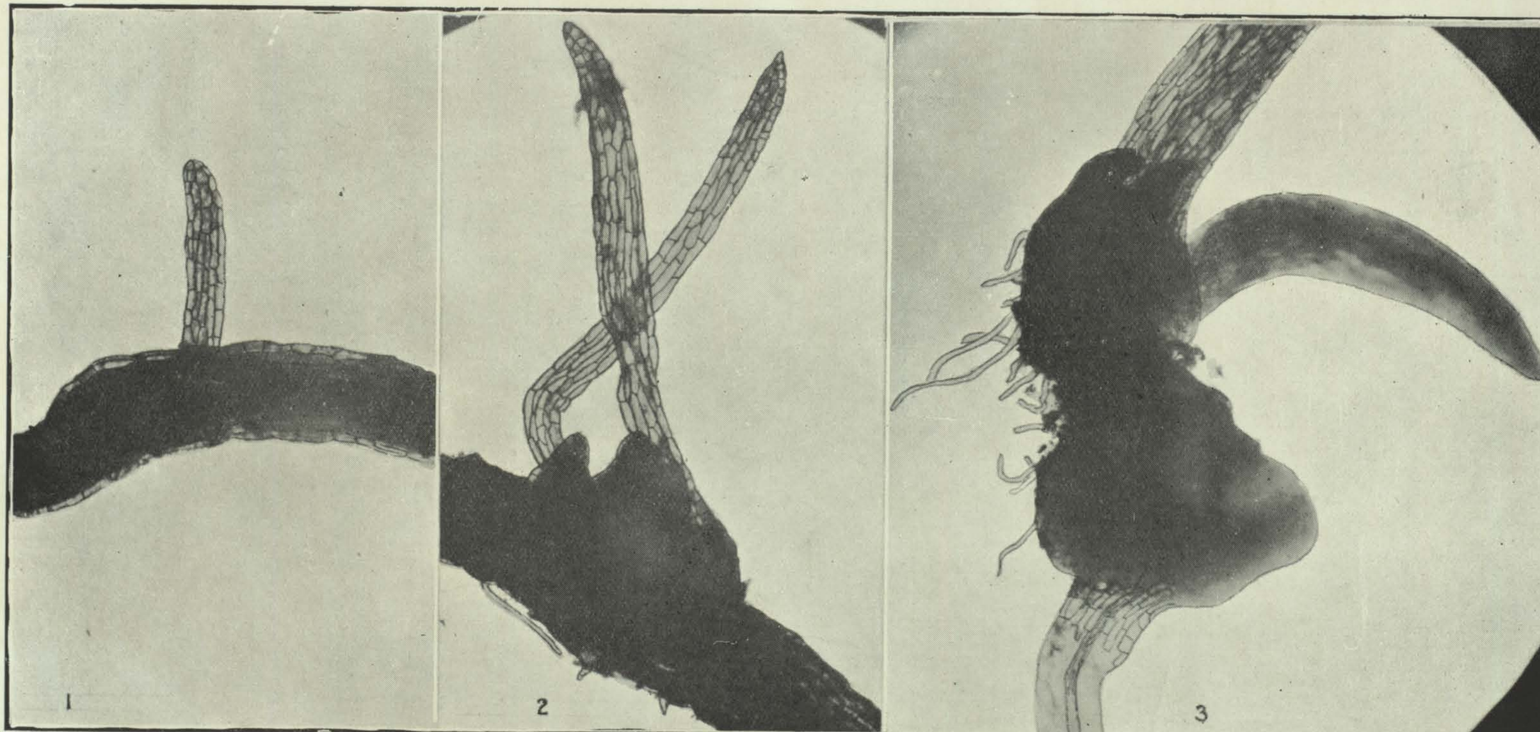


Figs 14–18 —*Lycopodium ramulosum* Adventitious plantlets developed as buds from detached leaves Figs 14 and 15 × 12, figs 16–18 × 10

Figs 19, 20 —*Lycopodium ramulosum* Detached leaves bearing daughter plantlets, and showing bulbous swellings in their lower portions × 10

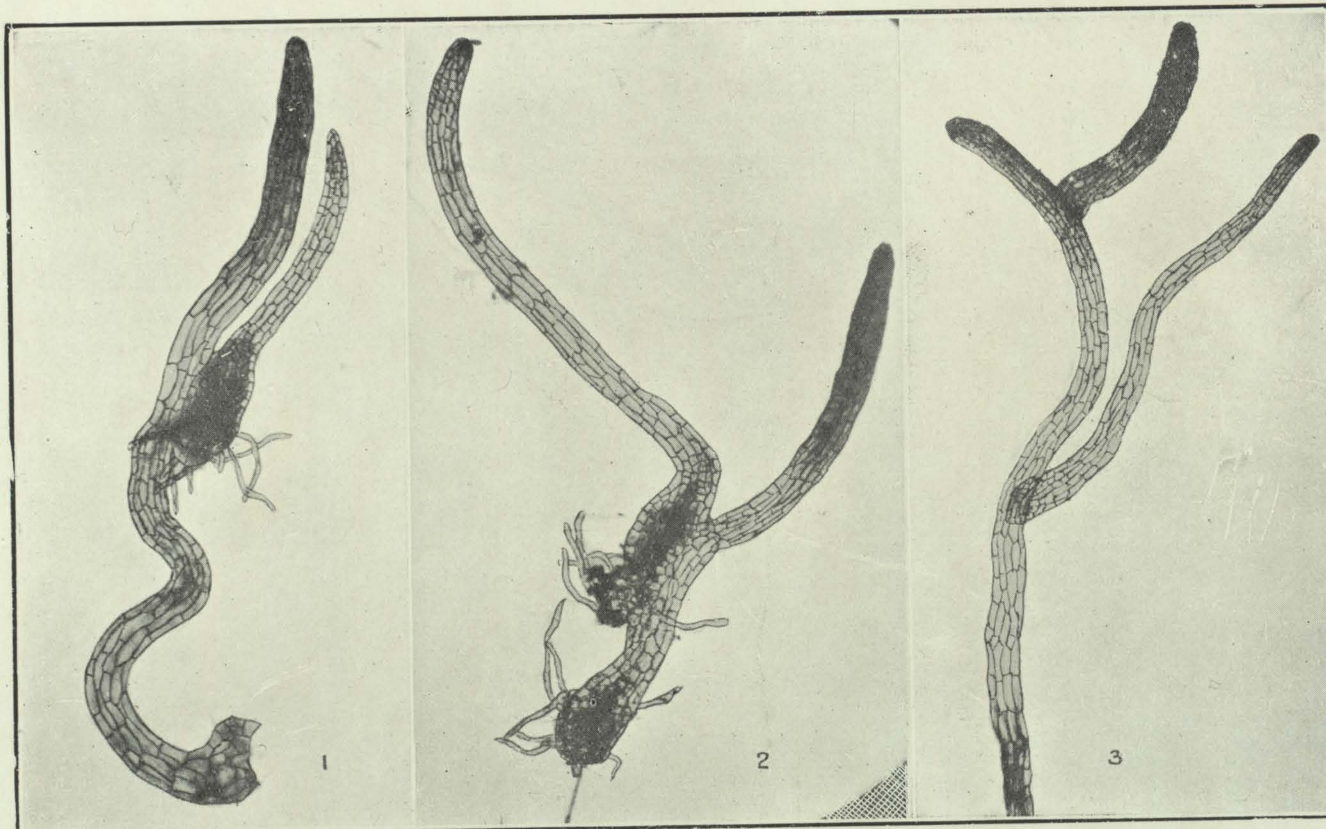
plants, had gradually died off, and that the conditions had stimulated the adventitious budding of their detached roots and leaves An adventitious bud on a leaf shows first as a small roundish green cushion of meristematic tissue (fig 14), which has originated probably from one or more epidermal cells This cushion develops into a roundish or egg-shaped cell-mass, which gradually elongates, and on which at an early stage rhizoids arise (fig 16) The attachment of the young bud to the parent plant is clear from fig. 15,

Face p. 88.]



Lycopodium ramulosum.

FIG. 1.—Adventitious plantlet arising from cortical cells of an old detached root. × 28.
FIG. 2.—Adventitious plantlets arising from cortical cells of an old detached root. × 28.
FIG. 3.—Adventitious plantlet arising from tuber at the end of an old detached root. × 22.



Lycopodium ramulosum.

FIG. 1.—Adventitious plantlet arising from an old detached leaf. × 28.
FIG. 2.—Adventitious plantlet arising from leaf of a second adventitious plantlet × 28.
FIG. 3.—A detached leaf which has branched twice. × 22.

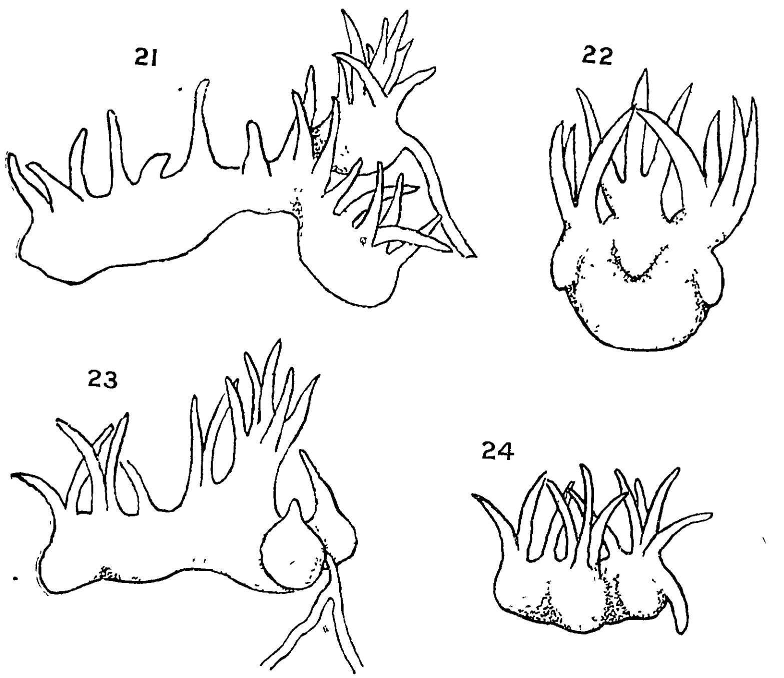
which shows that the main tissue of the leaf at the point of attachment is undisturbed. The bud from the first is vividly green, and is usually crowded with minute starch-grains. Its central cells appear brownish and opaque, owing to their dense protoplasmic contents. The first protophyll arises simply by the continued growth of the buds (figs. 15 and 18, and Plate IX, fig. 1). A second protophyll and then a third arises from the first-formed bulbous portion alongside the first (fig. 19). By this time the young plant has become well attached to the underlying humus by means of its rhizoids, and the parent leaf is beginning to rot away. In one instance a plantlet which had originated adventitiously from the cortex of an old detached root, to which it was still attached, had developed a very young bud near the apex of its single protophyll. Both protophyll and bud were packed with starch-grains, and a single rhizoid had developed about half-way up the protophyll from one of its epidermal cells. Plate IX, fig. 2, shows a well-grown bulbil developed upon a second adventitiously produced plantlet. A second example of a rhizoid being formed on an old leaf which bore a young adventitious plant was also observed, the leaf in this case being detached

The parent leaf is generally greenish in its upper portion but colourless below, and is always obviously broken off at the lower extremity. The leaves shown in figs 19 and 20 had each developed two swollen areas in their lower portions. These bulbous places on the leaves were of much the same appearance as the first-formed swollen portion of the developing bud, the central cells being brownish and opaque owing to their abundant protoplasmic contents. A group of rhizoids was present on each of these swollen areas In many cases it was noticed that the rhizoids arising from the developing buds, and also the cells of the lower portion of their protophylls, and especially the cells of the basal bulbous regions, were crowded with starch-grains Some of the parent leaves also, including the two in which swollen areas had formed, showed the presence of starch in their lower portions A single instance of a branched leaf was found (Plate IX, fig 3). Two branches had arisen upon the parent leaf, but on neither of these were rhizoids present, nor were their bases swollen. They had evidently developed somewhat differently from a bulbil.

Fungal hyphae do not seem to be present in the cells either of the parent leaf or parent root of the plantlets The transverse section of the base of an adventitiously produced bulbil of *L. cernuum*, shown in fig. 4, is closely similar to sections of plantlets of *L. ramulosum* which had developed from detached roots and from leaves. The central cells of the lower bulbous portion of the plantlet contains abundant protoplasm and also numerous minute starch-grains As the plantlet develops, these cells become narrow and elongated, and seem to function both as a storage and as a conducting tissue. A distinct epidermal layer of small-sized cells is differentiated from which rhizoids arise, whilst the cells in the zone which lies between the epidermal layer and the centrally placed cells gradually assume in the developing plantlet a large size, and are then for the most part empty. It is clear that the core of slightly elongated parenchymatous cells in the young plantlets is not to be regarded as the primordium of the permanent stem vascular cylinder. That, of course, arises, as in the case of the sexually produced plants of *L. cernuum*, *L. laterale*, and *L. ramulosum*, in the first place by the extension of the protophyll-strands down into the tissues of the plant, and their aggregation there, and secondly by the development of a plerome from the meristematic tissue at the stem-apex.

VII. Vegetative Reproduction of the Protocormous Rhizome.

In the two New Zealand species *L. laterale* and *L. ramulosum* the "protocorm" of the young sexually produced plant elongates sideways and attains a considerable size before a stem-apex is differentiated on its dorsal side. This protocormous rhizome plays a much larger and more important part than does the simple protocorm of *L. cernuum*. It constitutes the plant-body for a whole season, and may even branch. It appears also that in both *L. laterale* and *L. ramulosum* it is able to reproduce itself vegetatively. I found one branched protocorm of the former species, on each of the branches of which a stem-axis had been initiated (Holloway, 1916, fig. 67).



Figs 21, 22.—*Lycopodium ramulosum* Branched protocormous rhizomes with two or more stem-axes × 6

Figs 23, 24.—*Lycopodium ramulosum* Protocormous rhizomes with bulbils attached × 6

In Part I of these present Studies I briefly described one young plant of *L. ramulosum* at the growing end of whose protocormous rhizome two young bulbils were developing (*ibid*, p. 285). Since then I have found eight to ten additional instances of the vegetative reproduction of the rhizome of this latter species From the material mentioned above as having been obtained near the Mikonui River, Westland, I dissected out several young plants whose rhizome was branched In these instances the rhizome was generally an old and abnormally large one The growing end had given rise to two more or less equal branches, which were green in colour and bore a number of young protophylls, whilst the old first-formed portion was brown and surmounted by only a few old brown and decayed protophylls. Fig. 21 shows one such plant, in which the two growing ends were swollen and slightly globular in form, each being marked off from the rest of the rhizome by a slight constriction. There is no doubt that both these branches would sooner or later become independent individuals.

At least two other instances were observed in which two swollen and vividly green branches were present on the end of the rhizome. Three plantlets with branched rhizomes were found on which two, and in one case three, separate stems had arisen. It would seem that the original rhizome persists intact well over one season, although its empty cells show that it no longer functions as an absorbent or storage organ before by its decay it sets free the daughter plants It may be noted here that in almost all young developing plants of this species, as also in *L. laterale*, the protocormous rhizome remains attached to the base of the plant-stem till the latter attains a length of an inch or more, although it probably ceases to function soon after the development of the first root. Fig. 22 shows the end view of the rhizome of a young plant of *L. ramulosum* on whose branching end three stems had arisen. It will be seen that the formation of the first root had just been initiated at the base of each of the three stems, the three roots having been developed simultaneously.

As well as the vegetative reproduction of the protocormous rhizome of *L ramulosum* by means of the isolation of daughter branches, reproduction sometimes takes place by the formation of bulbils on the end or on some other part of the rhizome. A figure of a rhizome with two such bulbils was given in Part I of these studies (Holloway, 1916, p. 269), and is reproduced in the present paper (fig. 23) In another instance quite a small and comparatively young unbranched rhizome was found on which were borne two such bulbils (fig. 24).. These bulbils are very simple swollen globular protuberances of the tissues of the rhizome. They are vivid green in colour, and are surmounted by one or two young green protophylls. The colour of the bulbils is in striking contrast to the opaque yellow appearance of the parent rhizome. Not a few instances of a group of very young plants, consisting of from two to seven individuals in different stages of development, were found, where there was every appearance of their having arisen as bulbils from an old original rhizome. In several of these groups an old broken-down rhizome was in close proximity to the young plantlets. Although many of these plantlets were exceedingly small and young, in no case was a prothallus present, this fact indicating that they had arisen by vegetative reproduction It was noticed that these detached adventitious plantlets almost invariably consisted each of a basal globular mass of opaque brownish-looking tissues with one or two semi-decayed protophylls, whilst at some point or other on the plantlet there was a vividly green area which was obviously the growing-point of the plantlet. In some cases this green area was in the form of a bluntly rounded protuberance of the tuberous portion of the plant, and in others it was a single protophyll or a very young developing protophyll In connection with this it may be noted that a possible interpretation of the detached leaves bearing adventitious buds, described above in Section VI, is that they had become detached from an old rhizome, or from a bulbil produced vegetatively from such a rhizome The fact that these bulbils become brownish in colour after being detached from the parent rhizome, and that their growth becomes localized in one spot, seems to indicate that their development is arrested for a time, and that they act as resting bodies till the external conditions are suitable for their further development and for the initiation of a stem-axis.

Conclusions.

Vegetative reproduction is a common phenomenon in the Lycopodiaceae, as it is well known to be also in the Mosses, and it may take place in a great variety of ways. It is noteworthy that the swollen tuberous basal region termed the "protocorm" by Treub in the case of the sexually produced plants of *L. cernuum* is present also in the adventitious plantlets of *L. cernuum*, and in all the plantlets of *L laterale* and *L. ramulosum* whether produced sexually or adventitiously. In no case in any of these three species have I ever noticed the presence of fungal hyphae in the cells of the "protocorm," although Goebel (1905, p. 233) says that a fungus infection occurs in the root tubers of *L cernuum* and in certain swellings on the stem of *L. inundatum*, and that it there appears to promote an increase of plastic material. In all the cases of protocormous swelling examined by me I have never been able to discover more than abundant protoplasm and starch-grains, and have concluded that the swelling acts mainly as a storage region, although it must be added rhizoids are always developed early on the tubers, and in some cases also these rhizoids show the presence of starch-grains. A swollen area comparable to the "protocorm" occurs sometimes on detached leaves of *L ramulosum*, in conjunction with the formation on the leaf of an adventitious bud, and also the plantlets which are developed adventitiously from cortical cells in the roots of the same species begin as "protocorms" more or less regularly formed. Plantlets formed vegetatively as outgrowths from the protocormous rhizome of the sexually produced plantlets of *L ramulosum* also begin as tubers. It may not be out of place also to add to this list of vegetatively produced "protocorms" the annually produced tuber in *Phylloglossum* which is the first stage in the development of the new plant Here also the tuber acts as a storage tissue

It is instructive to compare the Lycopod "protocorm" or bulbil with the flat or sometimes solid gemma of the Hepaticae, and with the filamentous protonema which in the Musci precedes the formation of vegetatively produced plantlets. They may be compared in the light of being a response made by the plants belonging to those different classes to certain external conditions Klebs' well-known experiments in the Algae and Fungi have shown that sexual or vegetative reproduction may be respectively induced by varying the conditions of light, water, and food under which the plants are growing This may well be a natural phenomenon also in the case of the Hepatics and Mosses, or even of the Lycopodiaceae and other pteridophytes It is possible that in the Lycopodiums the vegetative tuber or "protocorm" is to be regarded as a resting body designed to withstand a dry season. If this can be accepted as a possible explanation in the case of the vegetatively produced plantlets, there is a strong suggestion that the same interpretation should be applied to the case of the first-formed "protocorm" in the sexually produced plants of the species of the *cernuum* type The tuber of the vegetatively produced plant is similar in appearance and function to that of the sexually produced plant, and the origin from it of the protophylls, stem-axis, and first root is also identical Thus the study of the protocormous tuber in the three New Zealand species *L cernuum*, *L ramulosum*, and *L. laterale* suggests that in all its forms it is to be regarded as a vegetative adaptation characteristic of certain sections of the Lycopodiaceae, rather than as the persisting rudiment of a highly primitive organ.

LITERATURE CONSULTED.

BRUCHMANN, H., 1898. *Uber die Prothallien und die Keimpflanzen mehrerer europaischer Lycopodien.* Gotha.

EDGERLEY, Miss K. V., 1915. The Prothallia of Three New Zealand Lycopods, *Trans. N.Z. Inst.*, vol. 47, pp 94–111.

ENGLER, A., and PRANTL, K., 1900. *Naturliche Pflanzen-Familien*, Teil 1, Abteilung iv, Psilotaceae (Solms).

GOEBEL, K, 1905. *Organography*, pt. ii, pp 213–14 (Engl. ed.)

HOLLOWAY, J. E, 1910 A Comparative Study of the Anatomy of Six New Zealand Species of *Lycopodium*, *Trans N.Z. Inst.*, vol. 42, pp. 356–70.

—— 1916 Studies in the New Zealand Species of the Genus *Lycopodium*, Part I, *Trans. N.Z. Inst.*, vol. 48, pp. 253–303.

LAND, W. J. G, 1911. A Protocorm of *Ophioglossum*, *Bot. Gaz.*, vol. 52, No 6, pp. 478–79.

PFEIFFER, NORMA E., 1916. The Prothallia of *Ophioglossum vulgatum*, *Bot. Gaz*, vol. 61, No. 6, pp. 518–22.

TREUB, M., 1886. Prothallus of *L. Phlegmaria*, *Ann. Jard. d. Bot. Buit.* v (Review by F. O. Bower, *Nature*, vol. 34, 1886, pp. 145–46).

—— 1887. Some Words on the Life-history of Lycopods, *Ann. of Bot.*, vol. 1, pp. 119–23.

APPENDIX.

While this paper was being printed, Chamberlain's paper on "Prothallia and Sporelings of Three New Zealand Species of *Lycopodium*" (*Bot. Gaz.*, vol 63, No. 1, 1917, pp 51–65) came to hand. In it he gives a short account of preserved specimens of the prothalli of *L. laterale*, *L. scariosum*, and *L. volubile*, and also describes the stele of the sporeling of the two last-named species. In Part III of these studies I hope to give an account of the internal structure of the prothalli of seven New Zealand species which were described in Part I, and will compare my results with Chamberlain's observations. In the course of his paper Chamberlain draws attention to the fact that those who have hitherto been at work upon the Lycopodiums have devoted the greater part of their attention to the prothallus and to the adult plant, and that the vascular structure of the sporeling has received little notice There is no doubt that this has been largely true, although the understanding of the various types of the Lycopodium stele can only come, as Chamberlain remarks, through the comparative study of their ontogenetic development in complexity I hope to supplement my observations on the vascular structure of the sporelings of eight New Zealand species given in Part I of these studies by a more detailed account in a further contribution, now partially completed. This will include also an account of the vascular structure of the adventitious plantlets described in the present paper, and will deal as well with the young plants of one or two other New Zealand species found by me.