THE STRUCTURE OF THE PERENNIAL GROWTH OF *DISA UNIFLORA* BERG. (ORCHIDACEAE)

HONOURS SYSTEMATICS PROJECT

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ABSTRACT

The perennation of orchids is poorly understood, in particular that of the Orchidoidae. The understanding of perennation in the Orchidoidae is important because the root-stem tuberoid is used as the one character defining the Orchidoidae as a monophyletic group. The root-stem tuberoid has never been examined for variation before. This project focuses on perennial growth in the Diseae in order to study the structure and function of the root stem tuberoid in relation to other organs and to contribute to the understanding of Orchidoid phylogeny.

INTRODUCTION

Most temperate monocotyledons have evolved underground resting or perennating organs for the climatically unfavourable season (Holttum 1955). A period of underground existence may allow a plant to escape unfavourable conditions, to counter environmental uncertainty, and to build reserves for flowering episodes (Calvo 1990). This is especially evident in the temperate members of the Orchidaceae and is made possible through sympodial growth (Withner 1974). Not all temperate orchids have a resting period although they do have sympodial growth and do perennate. Nevertheless, a number of manifestations of this "resting" period are possible as laid out by Pate and Dixon (1982) but a general pattern can be distinguished. Each manifestation is a "variation on a theme" (Holttum 1955).

The research field

So far, little work has been conducted on the perennial growth strategies of the Orchidoidae. The most comprehensive study in this field was last done in 1939 by B. C. Sharman who studied the development of the sinker in *Orchis mascula* (Orchidaceae). He found that perennation arises through the formation of axillary buds found in the axils of scale leaves in the aerial component of the plant. The base of the

bud tuberizes and the whole structure is extended away from the parent plant through elongation of the base of the bud. Similar mechanisms have been described somewhat briefly by Pate and Dixon (1982) for *Pterostylis vittata* and by Dressler (1981). Calvo (1990) conducted a four year study on growth and reproduction of *Cyclopogon cranichoides* (Orchidaceae). In this study the development of the plants was monitored in terms of vegetative and reproductive growth and fruit set related to the next year's reproductive success. Calvo (1990) found that some plants "disappeared" for a period of one or more years and then reappeared with renewed reproductive vigour. He concluded that smaller plants are likely to skip above ground growth until such time as they have accumulated enough reserves to reproduce successfully.

Calvo (1990) mentions that a few terrestrial orchids have a subterranean juvenile stage that is associated with mycorrhiza (Stoutamire 1974; Wells 1981 from Calvo 1990). Salmia (1989) found that mycorrhizal infection in the cortical cells of the root assisted green and chlorophyll-free individuals of the orchid *Epipactis helleborine* in receiving nourishment. Further, he also found that the mycorrhiza may be digested by the plant and converted to starch for subsequent use. On the other hand, the fungus may attack the plant should it not be sufficiently resistant to infection (Salmia 1989).

The root-stem tuberoid

An interesting case is the so-called root stem "tuberoid" found universally in the subfamily Orchidoideae (Dressler 1981). Dressler (1981) defines the root-stem tuberoid of the Orchidoidae as largely storage roots, but with the basal portion having a sheath of root structure around a core of stem structure with an apical bud. This is the structure that survives the dormant season and in the growing season

the bud grows into a new shoot, with one of the axillary buds forming a new tuberoid, which will be the next resting phase (Dressler 1981). In the Orchidae and the Diuridae, the tuberoid has several vascular cylinders and so are polystelic, "as though several roots were grown together in one skin." (Dressler 1981:28). However, the anatomy of the tuberoid is only known from a few genera in the Orchidae (Rasmussen 1985).

The Disinae

The subfamily, Orchidoidae, are generally accepted as a monophyletic group, including all terrestrial orchids which perennate by root-stem tuberoids and which have basally attached anthers (Rasmussen 1985; Linder 1986). The root-stem tuberoid is therefore an important character. In their chapter on orchid anatomy, however, Withner *et al.* (1974) do not mention the anatomy of the root-stem tuberoid.

Within the Orchidoideae, there are two tribes, the Orchidae and the Diseae (Linder 1986). Within the Diseae there are three subtribes, the Satyriinae, the Disinae and the Coryciinae (Linder 1986). The classification of these subribes is based largely upon floral characters, the position of the anther being the most notable character. This project concentrates on the tuberoids of the Diseae, in particular those of the Disinae represented by *Disa uniflora*.

Disa uniflora occurs on Table Mountain on the Cape Peninsula and ~~again~~ also inland, on the mountain ranges that run north from the Hottentot's Holland mountains, through Franschoek and the Ceres mountains to the Cedarberg (Schelpe 1980). Unlike most of the geophytes of the south-western Cape which grow during the wet winter, flower in spring, and lie dormant during the dry summer and early autumn, *Disa uniflora* has no dormant phase (Schelpe 1980). In fact, *Disa uniflora* has its peak reproductive phase in summer from January to March. The

fact that *Disa uniflora* does not need a dormant or resting phase as in *Cyclopogon cranichoides* (Calvo 1990) in order to recuperate and accumulate reserves is interesting. A herbarium specimen (Pillans 2712, BOL) showed remains of two old flowering stems, proof that it had flowered for three consecutive seasons.

Using *Disa uniflora* as an example, the aim of this project is to understand its perennation mechanism, in the light of general patterns of perennation in monocotyledons, and to examine the variation in tuberoid anatomy within the Diseae.

Research questions

In order to understand perennation in *Disa uniflora*, several questions are addressed in this project. Firstly, what is the vegetative morphology of *Disa uniflora* like? Secondly, what strategy does *D. uniflora* adopt to form the next season's growth and which structures are involved in this strategy? Thirdly, how do the annual and perennial, structures differ? If the Orchidoidae display sympodial growth as do all the orchids (Holttum 1955), one would predict that the antomies of the annual and perennial components differ. Further, it can be predicted that the tuberoid should have the anatomy of the parent tuberoid. Fourthly, the question, where are the mycorrhiza housed and what is their function in perennation? is asked. Finally, where and what is the variation in tuberoid anatomy within the Diseae?

MATERIALS AND METHODS

Disa uniflora was collected in late summer from the Cywes' nursery as well as from wild populations in Nursery Ravine on Table Mountain.

Plants in various stages of development were fixed in FAA for at least 24 hours. For analysis of vascular arrangement

the tissue was dehydrated in a Sakura tissue processor. The dehydration run was as follows:

1. 2 * 70% Ethyl alcohol (EtOH)	8 hours each
(this is necessary to wash out FAA)	
2. 2 * 100% EtOH	8 hours each
(to dehydrate thoroughly)	
3. 2 * n-propanol	8 hours each
4. 2 * n-butanol	8 hours each
5. 1 * paraplast	12 hours
(to clear the n-butanol)	
6. 1 * paraplast	48 hours.

(to make sure that the n-butanol has been cleared and more importantly to ensure that the wax impregnates the specimen.)

The sections were then embedded in paraplast. The tissue was sectioned at 10um for most tissue analyses in a Leitz rotary microtome.

Tuberoids of *Disa uniflora* were prepared differently for analysis since their large and soft-walled cells prevented the above procedure from being effective. The tuberoids were fixed in FAA for at least 24 hours and were then sectioned at 40um-100um with a Leitz freeze microtome using Stephen's gum as a mountant. The tuberoids of *Corycium orobanchoides, Disperis villosa, Schizochiles cocilii* and *Satyrium humile* were examined from hand sections which were stained with either iodine or Sudan IV.

The sections were expanded using 1% formalin and adhered onto slides using Haupt's adhesive. They were then stained with safranin and fast green for vasculature, meristem and tissue layers. The staining procedure was executed as follows:

1. Xylene * 2	5 min each
(this step ensures that the wax is removed off the slide)	

2. Methyl cellosolve — 2 min each
(partial dehydration)
3. 96% EtOH * 2 — 2 min each
(dehydrates the specimen)
4. Safranin — 30 min (or longer)
(safranin stains the specimen for lignified tissue and nuclei.)
5. distilled water — brief rinse
(to wash off the excess stain)
6. Methylcellosolve — 1-2 min
(clears the specimen and dehydrates)
7. Fast green — 1 min (at most)
(stains for cell walls (cellulose))
8. 96% EtOH — brief rinse
(dehydrates partially)
9. n-butanol * 2 — 1-2 min each
(dehydrates completely)
10. Xylene * 2 — 5 min each
(clears, cleans and dehydrates the specimen for mounting.)

The specimens were mounted in DPX. In order to stain for myccorhiza, a Trypan Blue stain was used.

Micrographs were obtained using a Zeiss axioskop. The film used was FP4. Differential interference contrast optics were used in order to analyse vascular anatomy in the sections. Sections that could not be photographed owing to their thickness (eg. tuberoids) or size were drawn using a Camera Lucida.

OBSERVATIONS

The morphology of *Disa uniflora*

Disa uniflora consists of a perennial and annual component. The tuberoid, the roots and the underground and above ground perennial stems comprise the perennial component (fig. 1). The tuberoid may be described as ovoid or spheroid in shape. The parent tuberoid, once most of the energy

reserves have been translocated into the renewal shoot, is flaccid and dark while the new tuberoid is firm and lighter in colour than its parent. The upright perennial stem has about 8 to 10 nodes. The underground stem arises from the first node of the upright perennial stem above the tuberoid and gives rise to a new direction of development. Typical of sympodial growth, axillary buds develop in the scale leaves of the second, fourth and sixth nodes above the tuberoid (fig. 5a). A leaf forms at each node and two to three unbranched adventitious roots, which are about 0.2cm in thickness, at each internode.

An apical bud gives rise to the inflorescence stem in spring (fig 1 and fig 2). Flowering commences in January and ends sometimes as late as March (Schelpe 1960). The inflorescence stem dies back at the end of the flowering season in late summer. This then is the annual component of the plant. The annual stem is also noded, having between 6 to 10 nodes. Leaves arise from the base of the nodes and they vary in length from 50cm to 120cm.

Renewal shoot strategies

The development of the renewal shoot occurs in three different ways:

(i) Numerous tuberoidless small plants may coppice off from the second, fourth and sixth node of the rhizome above the parent tuberoid to form a cluster (fig 3). This only occurs if the tuberoid is large enough to sustain several generations of renewal shoots. Three generations of renewal shoots may be supported by one tuberoid.

(ii) An uderground stem, or rhizome, may develop from the first node of the parent rhizome to give rise to a renewal aerial shoot (fig. 4a,b). These rhizome branches give rise to a new direction of development.

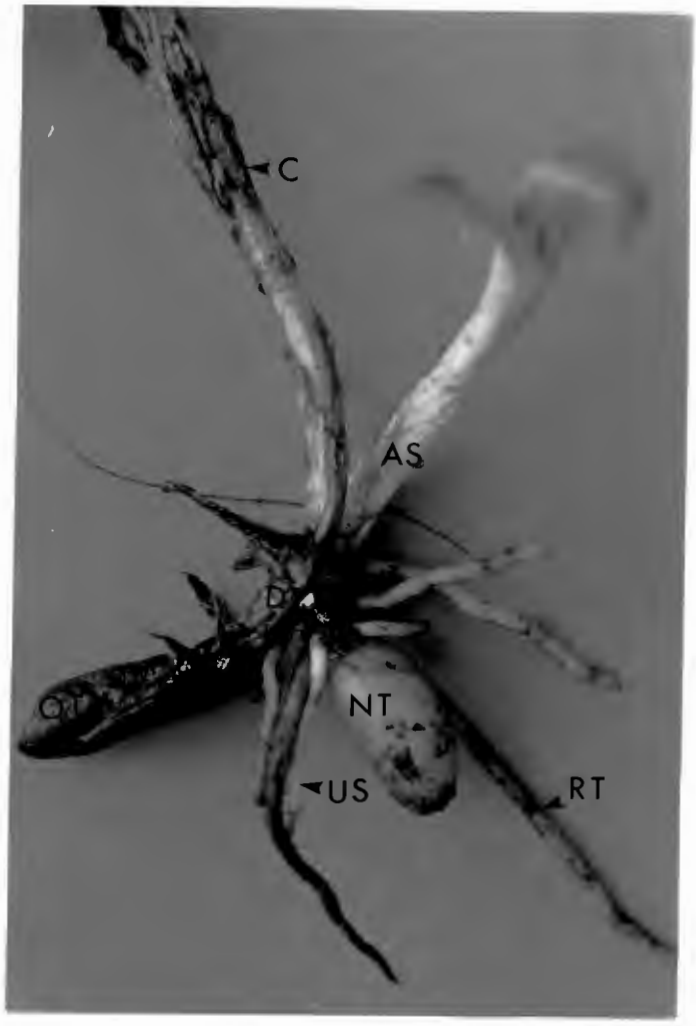

Figure 1. The gross morphology of *Disa uniflora*. The perennial component consists of the previous season's tuberoid (OT), this season's tuberoid (NT), the roots (RT), the noded above ground growth (AS), and the noded underground stem, (US). The annual component consists of the noded infloresence stem (C), all of which cannot be seen in this photograph.

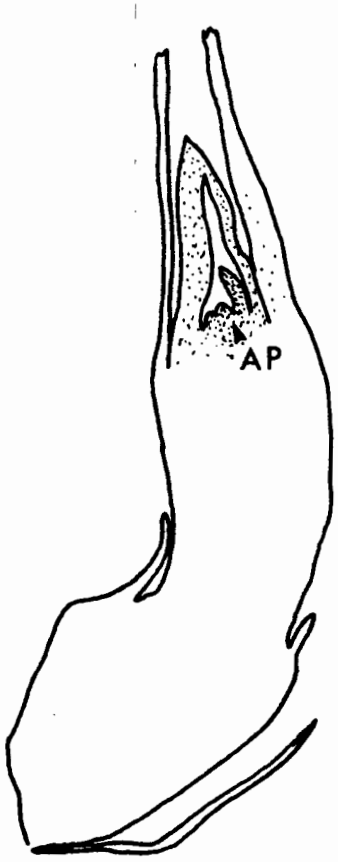

Figure 2. Longitudinal section through a renewal shoot of *Disa uniflora*. The apical bud, (AP), gives rise to the inflorescence stem in spring.

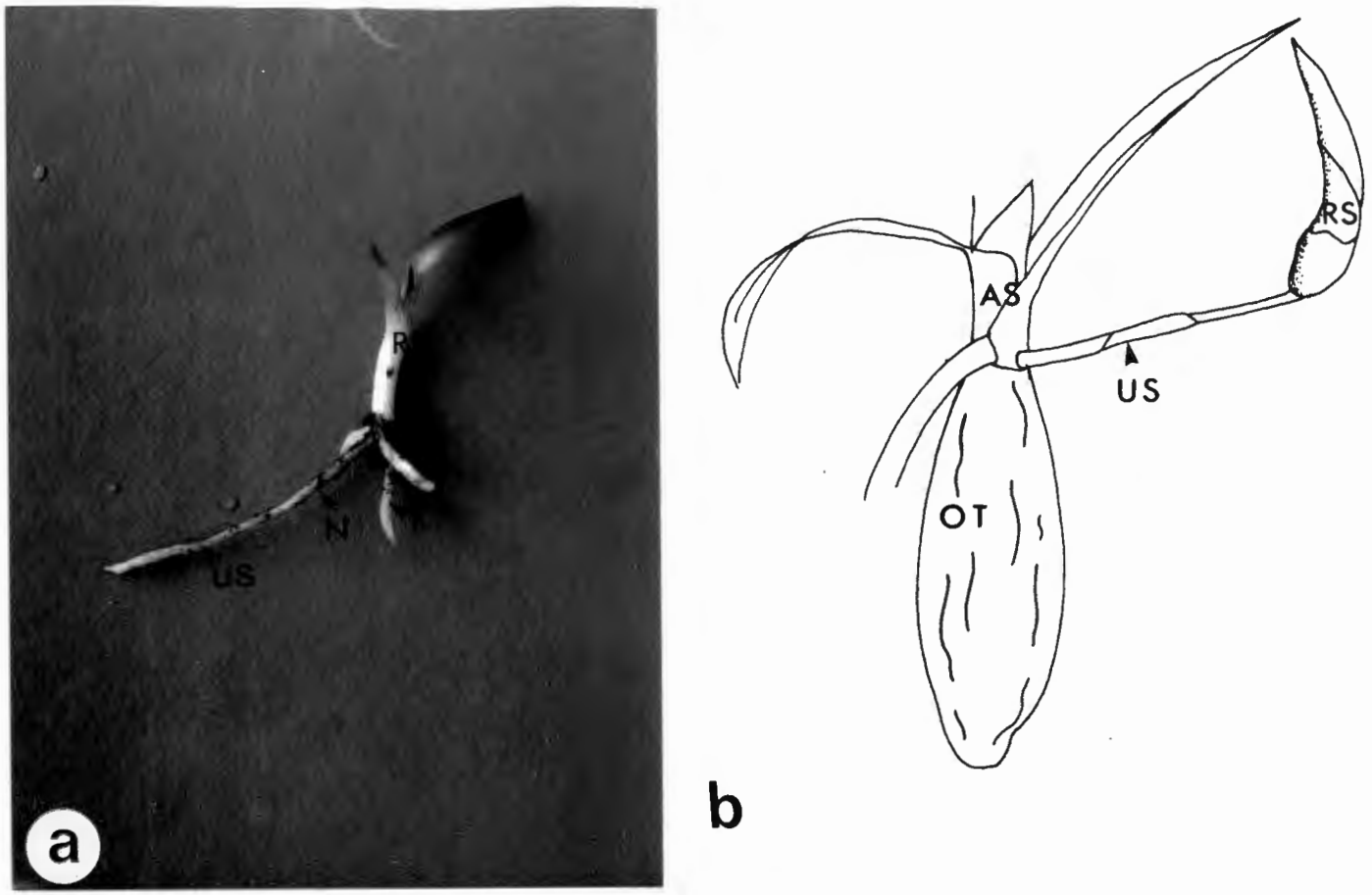

Figure 4. The development of the renewal shoot (RS), off the underground rhizome (US). (a) The underground rhizome is noded (N), and gives rise to roots (R). (b) The renewal shoot (RS) off the underground stem (US) does not possess a tuberoid. The underground stem arises from the first node above the tuberoid (OT).

Figure 3. Coppicing of the renewal shoots (R) off an old tuberoid (broken off). Three generations of renewal shoots can be seen here: the first season's growth (OP1), the second season's growth (OP2), and this season's growth (R).

(iii) The third renewal shoot strategy is the more recognised and effective one. Axillary buds develop in the axils of the second, fourth and sixth scale leaves (fig. 5a) Here the buds can be seen to possess foliar structures. Only the fourth bud develops, however, to give rise to the renewal shoot (fig. 5b). The base of the bud tuberizes and elongates to extend the bud away from the parent plant. This is why the dropper attaching the daughter and parent plant appears split along its axis (fig. 5b). Once the new shoot is established, an inflorescence stem develops from an apical bud.

The anatomy of the annual and perennial structures of *Disa uniflora*.

The anatomy of the root

The roots of *Disa uniflora* show a classical root anatomy apart from the fact that no root hairs have been found/The cells of the epidermis are dome shaped in transverse section and are either cutinized or suberized (fig 6a). Below that there is a parenchymatous cortex of large undifferentiated cells (fig 6a). The intercellular spaces between the cells of the of the cortex are conspicuous (fig 6a). No sclerenchymatous tissue is evident in the root although it is meant to be common in the roots of monocotyledons (Cutter 1971). The outermost layer of the cortex is differentiated into an exodermis (fig. 6a). The innermost layer of the cortex is differentiated into the endodermis which is very visible as a single layer of cells in *Disa uniflora* (fig 6b). These cells contain suberin in their radial and tangential walls which is typical of roots which do not undergo secondary thickening (Cutter 1971).

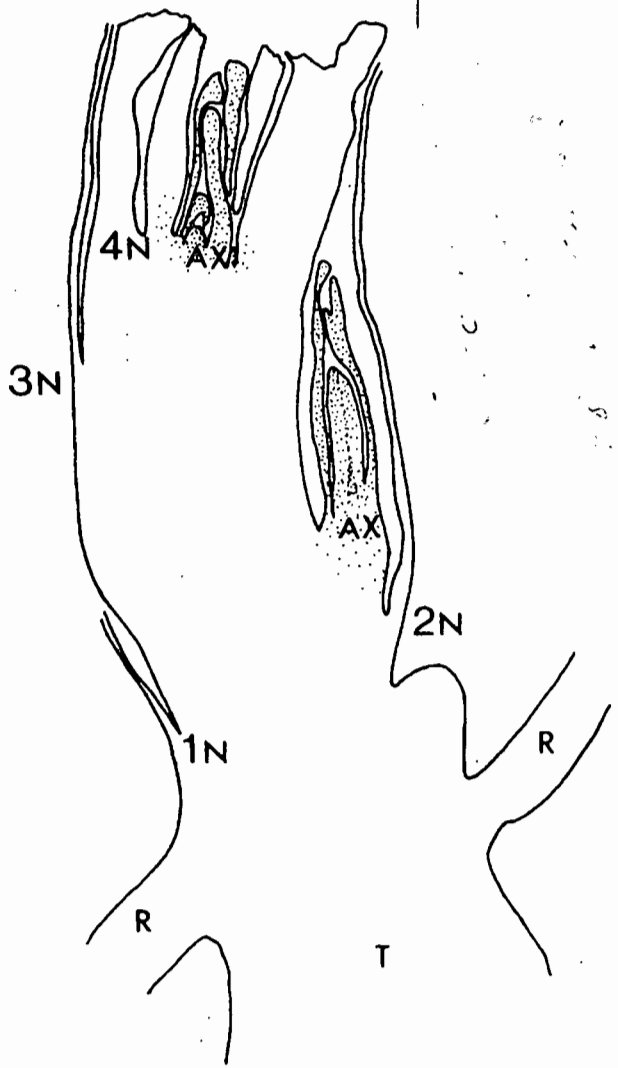

Figure 5(b). Development of the fourth axillary bud. The new shoot (NS) is suspended from the old shoot (OS) by means of a dropper (D) which appears split along its axis.

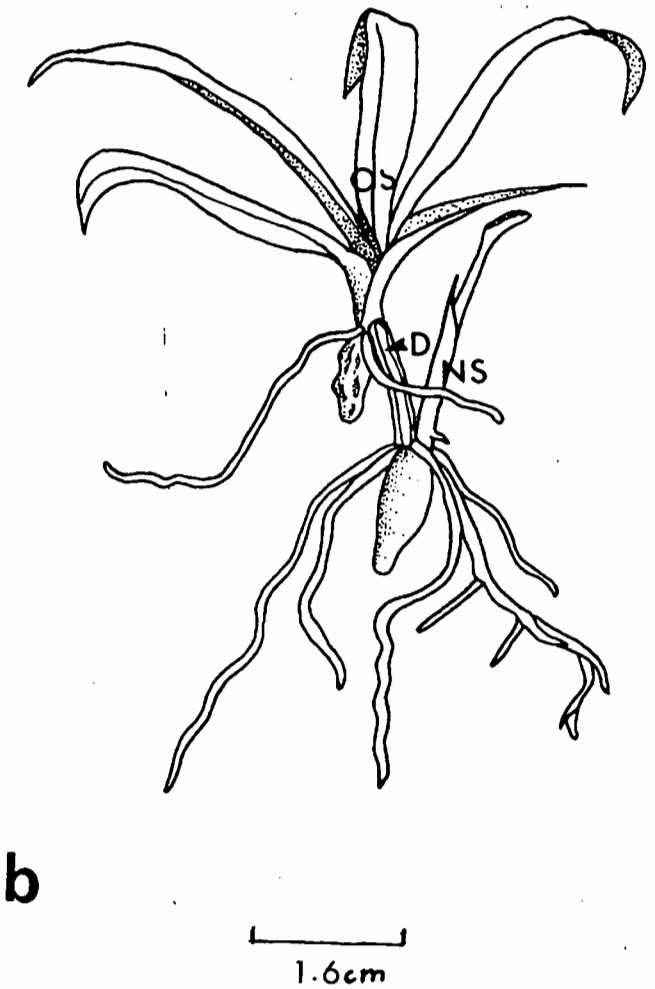

Figure 5(a). The positions of the axillary buds on the above ground stem of *D. uniflora*. Axillary buds develop within the scale leaves of the second (2N), fourth (4N) and sixth (not shown) nodes. The first (1N) and third (3N) nodes remain budless. R, roots; T, tuberoid.

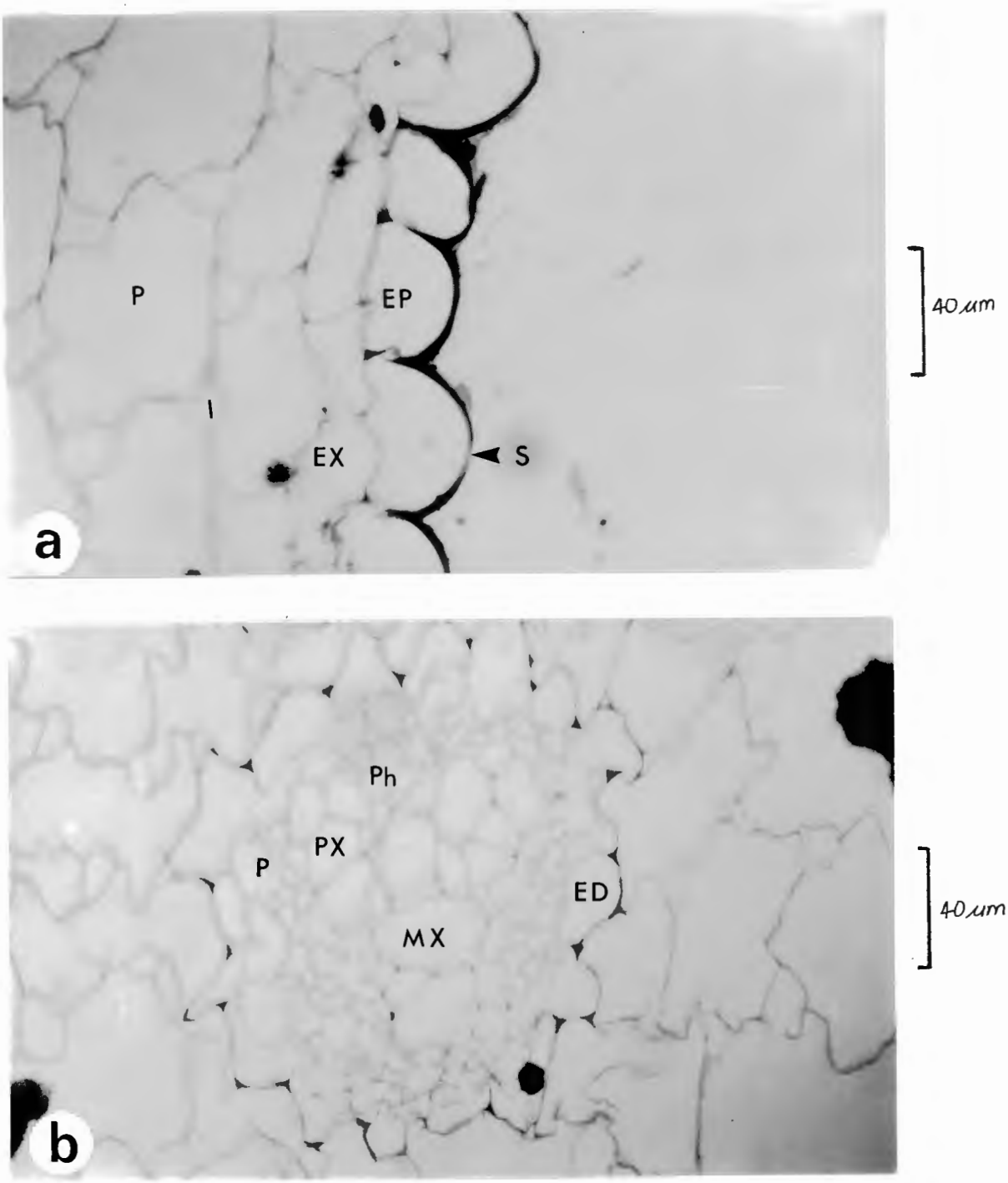

Figure 6. The anatomy of the root of *Disa uniflora*.
(a) The root epidermis. The cells of the epidermis (EP) have suberised tangential walls (S). EX, exodermis; I, intercellular space; P, parenchyma cell.
(b) The vascular anatomy of the root. ED, endodermis; MX, metaxylem; PX, protoxylem; Ph, phloem; P, pericycle.

The pericycle lies just beneath the endodermis and is a single layer of cells (fig. 6b). The vascular tissues consist of three to four triangular rays of thick-walled lignified tracheary elements, alternating with archs of thin-walled phloem. In the root of *D. uniflora* (cf. stem), the xylem and phloem do not lie on the same radius. This is typical of all root anatomy (Cutter 1971). The xylem forms a solid central core unlike the roots of many other monocots (Cutter 1971). The larger metaxylem elements can be seen in the center of the vascular bundle while the smaller protoxylem elements can be seen at the periphery of the vascular bundle. Differentiation therefore occurs centripetally from the periphery to give an exarch vascular bundle.

Mycorrhiza are found exclusively in the roots of *Disa uniflora* (fig 7). The hyphae form dense tangled masses within the cells of the cortex of the root and seem to occupy entire cells. Interestingly, they do not occur in every cell within the root, but infect isolated cells nearer the proximal end of the root. The presence of the mycorrhiza causes nuclear hypertrophy (enlargement of the nucleus). Partly digested hyphae could were evident in a few cells near the rhizomal end of the root.

The perennial stem

The perennial upright stem (rhizome) shows a polystelic structure since there are numerous vascular bundles scattered throughout the cortex (fig. 8). The tracheary elements are large, angular, well lignified and spirally thickened. The phloem cells are much smaller than that of the xylem and are irregularly-shaped. The phloem elements occur towards the peripheral end of the transverse section, forming a cap, while the xylem elements occur towards the

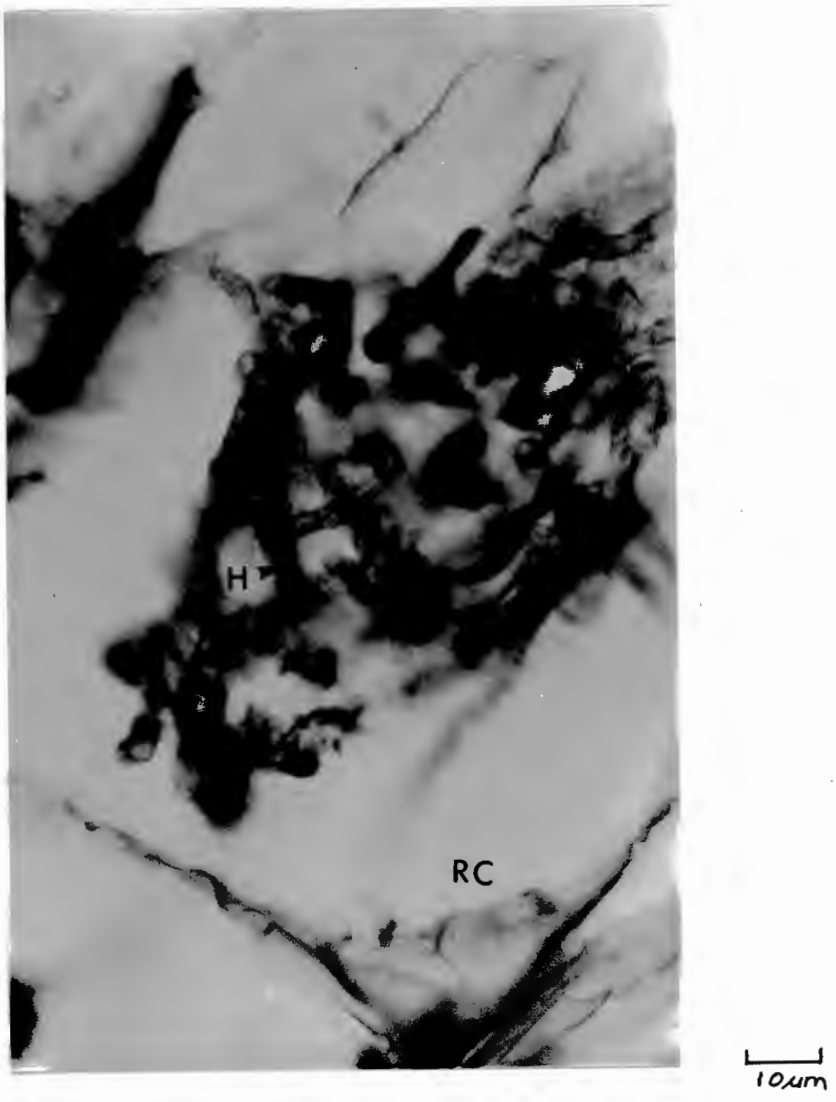

Figure 7. Mycorrhizal infection of the root of *D. uniflora*. H, hypha; RC, cortical cell of the root. Nuclear hypertrophy is not evident in this figure.

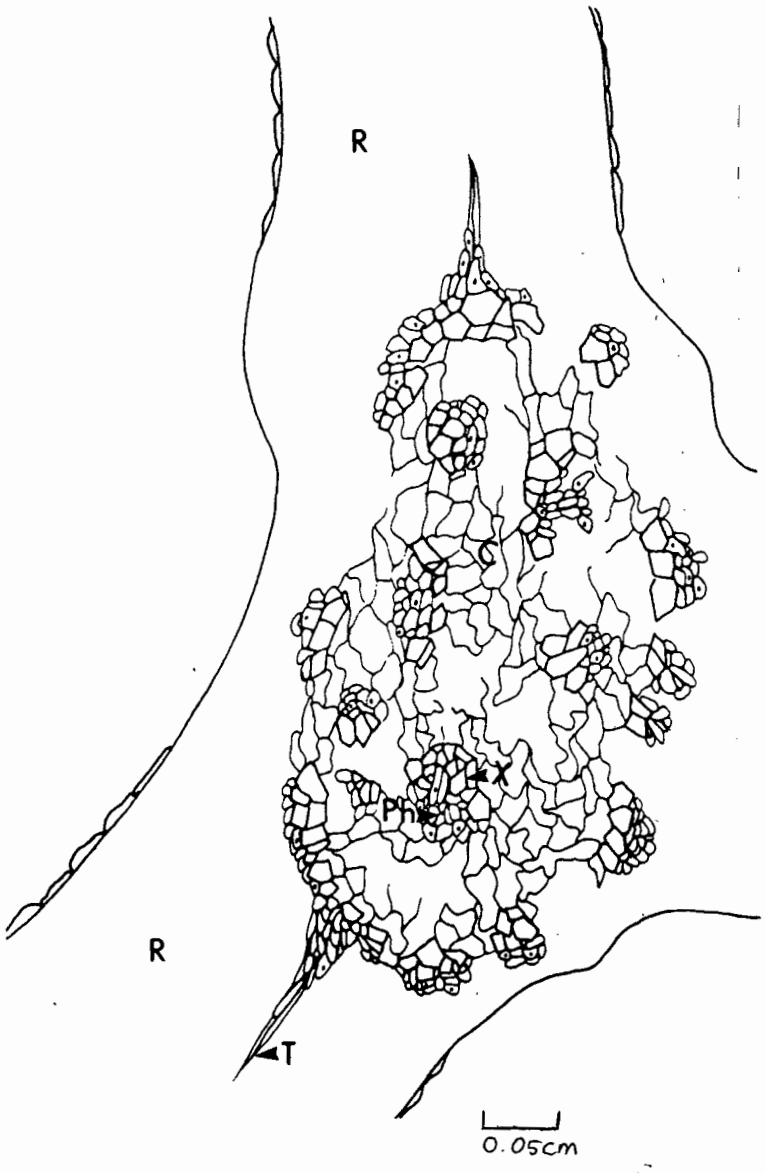

Figure 8. The vascular anatomy of the above ground perennial stem. X, xylem; Ph, phloem; R, root; T, root vascular trace; C, cortical cells.

inside. There seems to be no distinct pericycle although it is possible that a pericycle does exist. There is also no evidence of sclerenchyma tissue associated with the vascular bundles. Unfortunately, no data of the epidermis could be obtained since the branching of roots off the rhizome obscured its surface.

Lateral buds are found in the scale leaves of the second, fourth and sixth nodes of the perennial stem as mentioned above (fig. 5a). An apical bud is also evident in young shoots (fig. 2). However, this is discussed above with relevance to renewal shoot growth.

Oil bodies seem to be ubiquitous in the cortical cells of young shoots and in the daughter tuberoid (fig 9a), but no evidence of them can be found in the older shoots. Crystals called styloids also occur in the cortical cells of the young stem shoots (fig 9b). Styloids are rod-like crystals which, in *Disa uniflora*, form conglomerates to create the appearance of fairly large crystals.

Infection of the erect perennial stem by mycorrhizal hyphae is non-existent.

The underground perennial stem

The underground perennial stem differs little in its anatomy from that of the erect perennial stem (fig. 10a). The vascular tissue is markedly similar to that of the erect stem in that it is polystelic, the discrete vascular bundles lying in a ring about the ground tissue (fig. 10a). The tracheary elements appear as angular lignified cells which, once again, occur on the inner side of the bundles while the the phloem cells form a cap on the peripheral side of the bundles. No sclerenchyma sheath is evident nor a

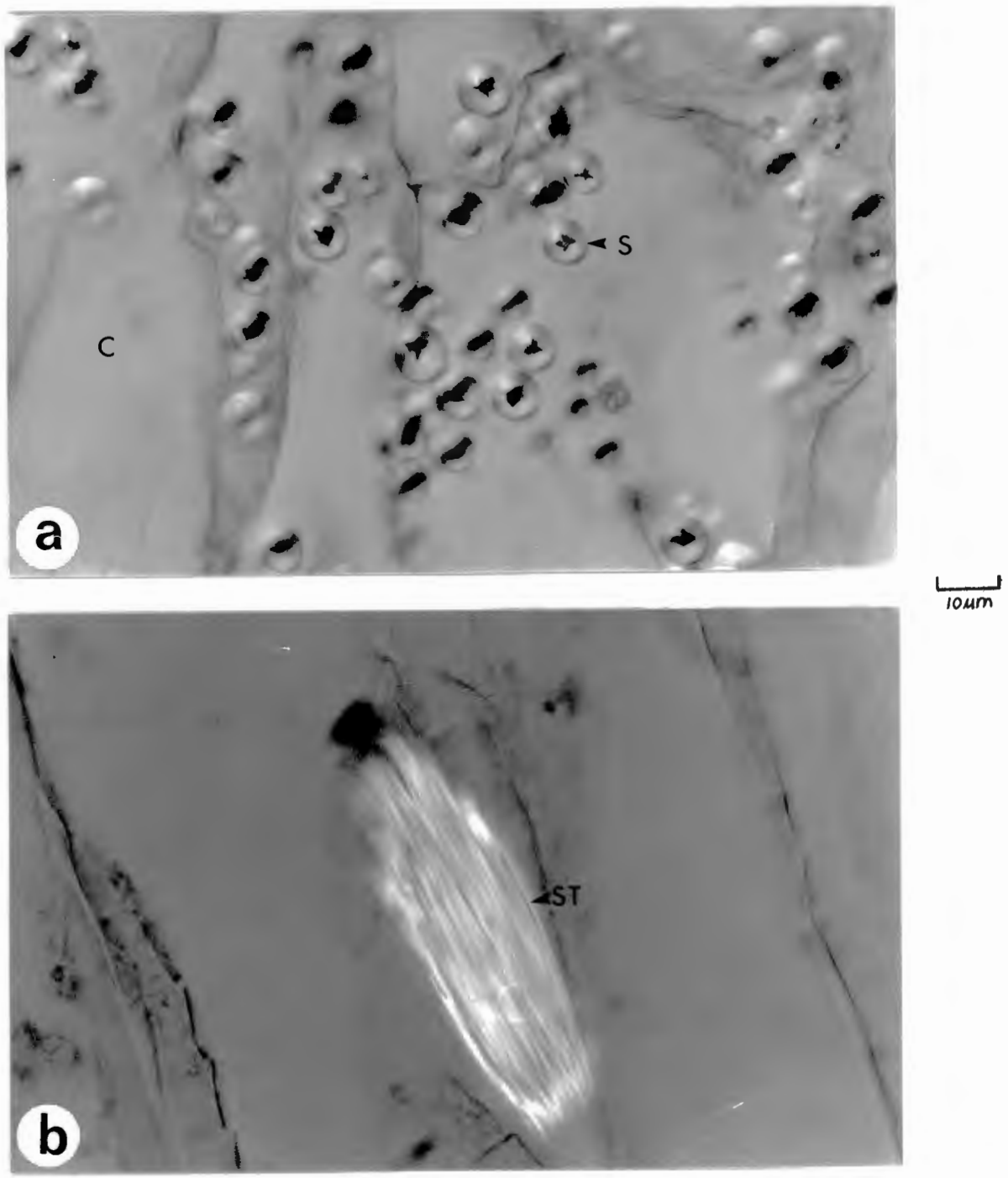

Figure 9. Storage and defence compounds in the renewal shoot of *D. uniflora*. (a) Starch or oil bodies are found in the cortical cells (C) of the young shoots. (b) Styloids (ST) are found mostly in the epidermal cells of the tuberoids.

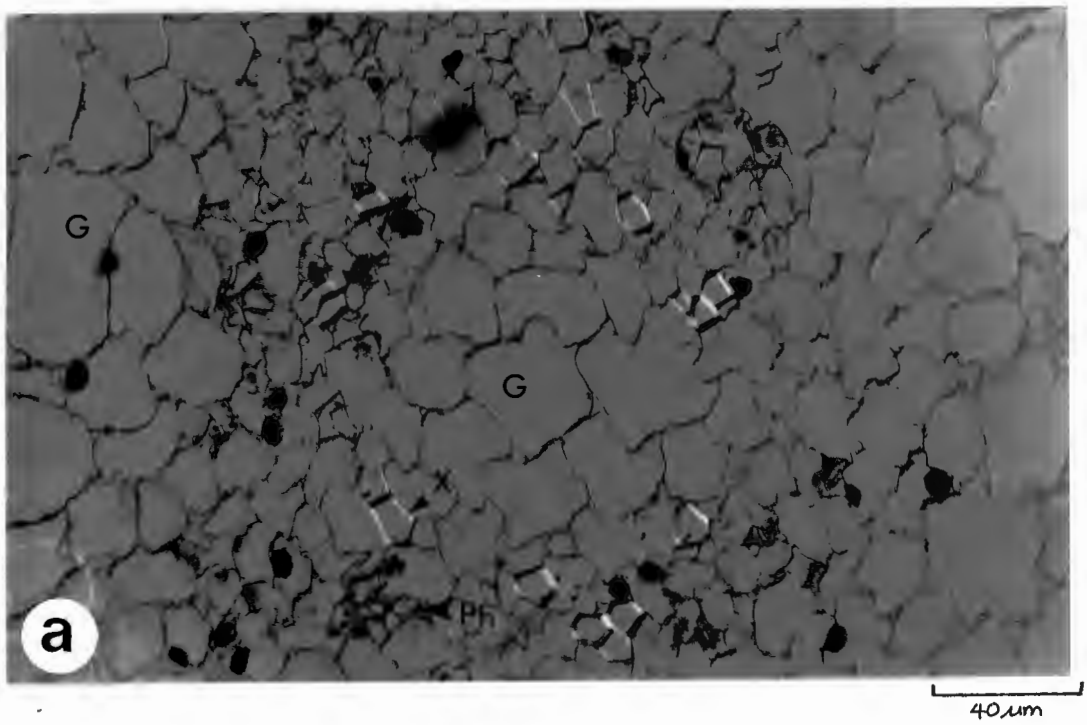

Figure 1Ø(a). The vascular anatomy of the underground perennial stem. X, lignified xylem; Ph, phloem; G, ground tissue.

clearly defined pericycle. Further, unlike the erect stem, the oil or starch bodies do not occur in the cortex of the underground stem. The epidermis consists of one layer of elongated cells in transverse section which are not suberized.

The tip of the underground rhizome is a highly meristematic region, as is the tip of the erect rhizome (fig. 10b). Three buds, one of which will give rise to the new shoot, can be seen in the section of the tip of the underground rhizome. Further, the axillary bud iniation can also be seen in figure 10c.

The dropper

The dropper shares many features in common with the perennial stems (fig. 11a). Once again, the center of the vascular bundle does not consist of a solid core of vascular tracheary elements but rather has its vascular bundles arranged in a circle around the periphery of the stele with the small phloem cells towards the periphery and the xylem elements towards the inner edge. It does, however share one feature in common with the root. The cells of the epidermis are dome-shaped in transverse section as in the root (fig. 11b).

The annual stem

The annual stem shows the most differentiation of all. The epidermis consists of one layer of cells bearing papillae on their tangential walls (fig 12a). Stomata and guard cells are situated in the epidermis (fig 12b). The stem cortex consists of thin-walled parenchyma tissue. Several vascular bundles can be found in this region close to the epidermis. Below this region there is a layer of elongated cells which seem to form a pericycle. The central steles are surrounded by a single sclerenchyma ring which can be seen as stained green or red (fig 12b and 12c). There are two alternating

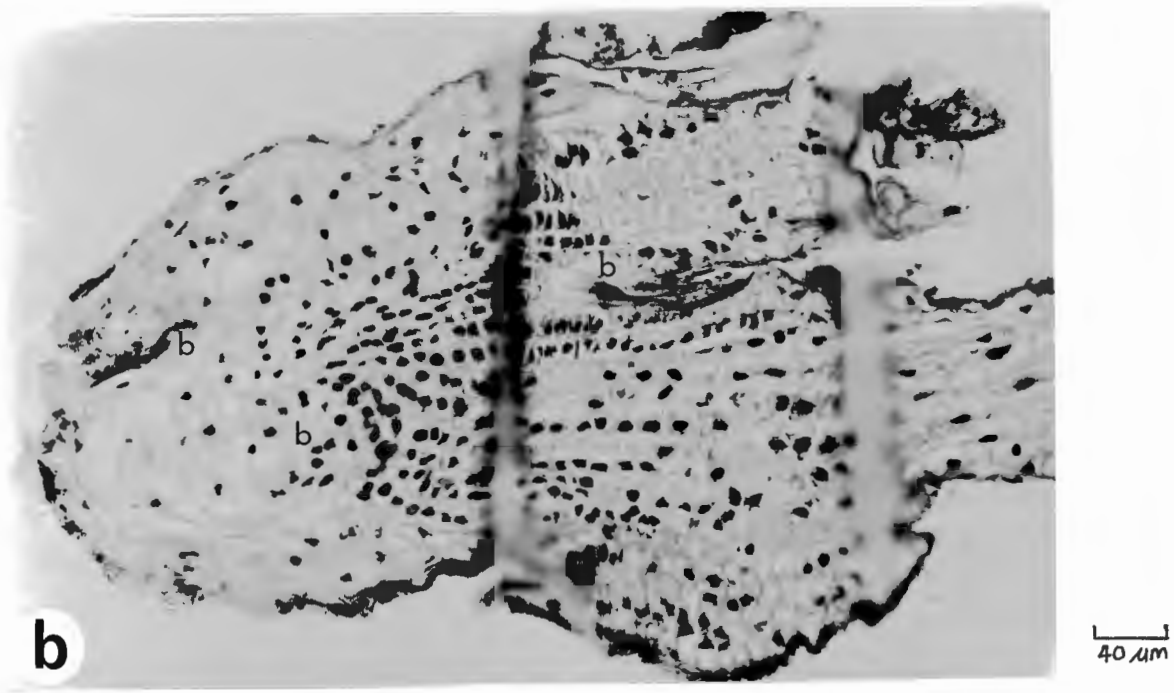

Figure 1Ø (b). The meristematic tip of the underground stem. Three buds (b), one possibly apical, can be seen.

What is the orientation of this?

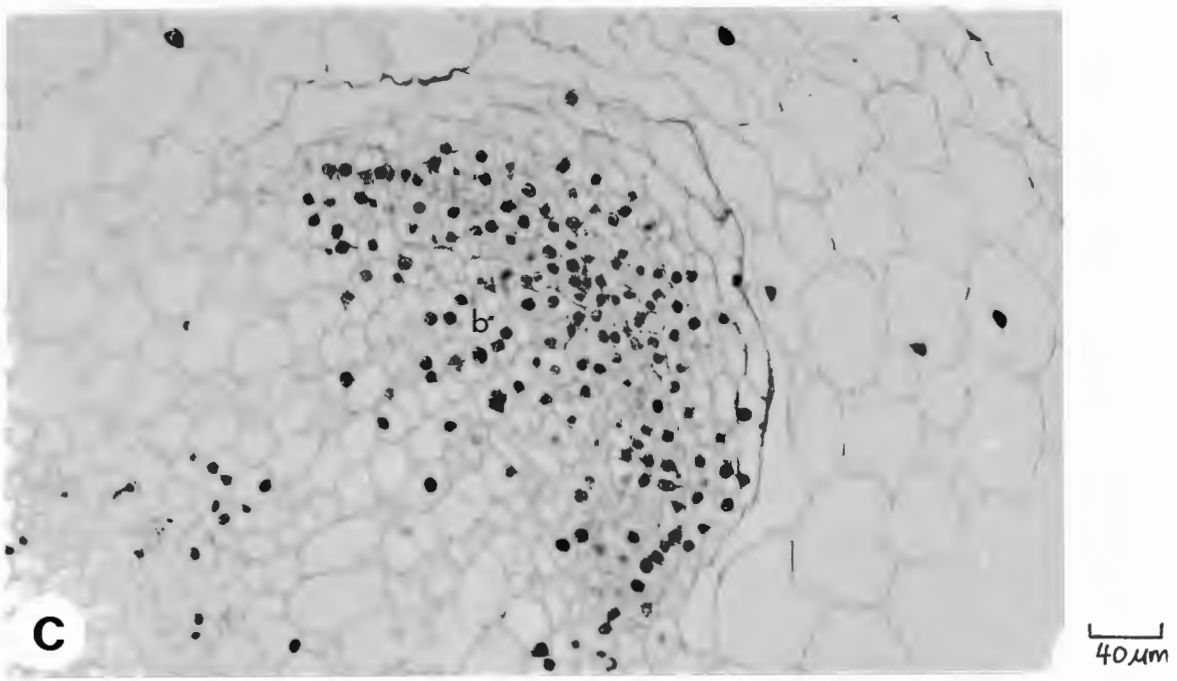

Figure 1Ø(c). Cross section showing the axillary bud initiation (b) in the underground stem.

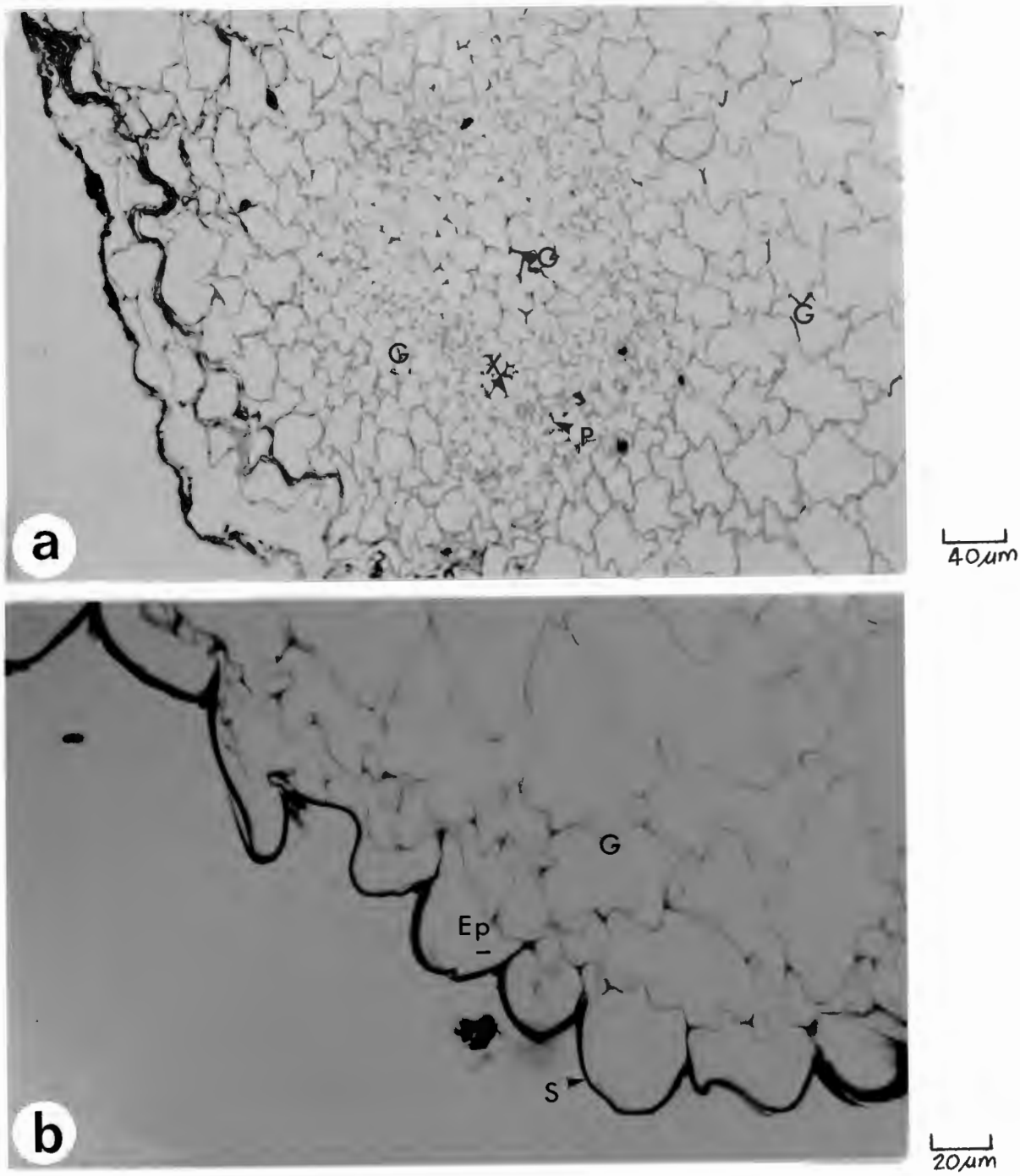

Figure 11. The anatomy of the dropper. (a) Vascular anatomy. X, xylem; P, phloem; G, ground tissue. (b) Wall anatomy. Dome-shaped epidermal cells (Ep); S, suberised tangential walls of the epidermis; G, ground tissue.

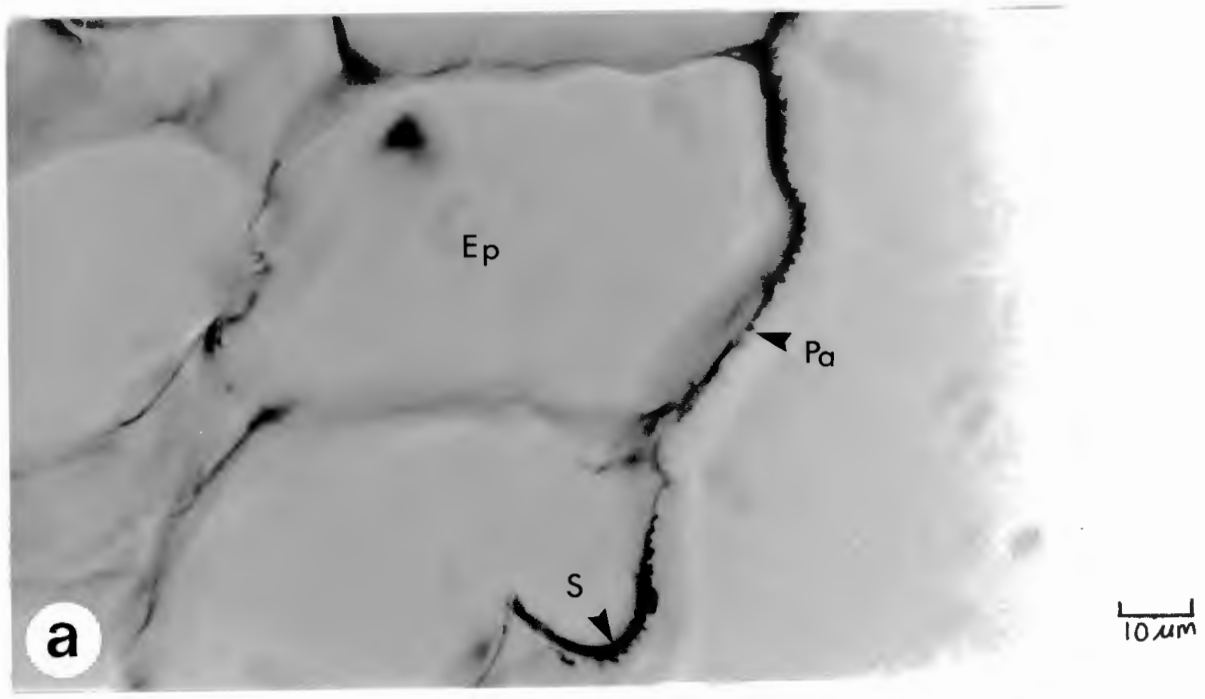

Figure 12(a). The epidermis of the annual stem. Epidermal cells (Ep) have suberised walls (S) and papillae on their surfaces (Pa). 1.0 (

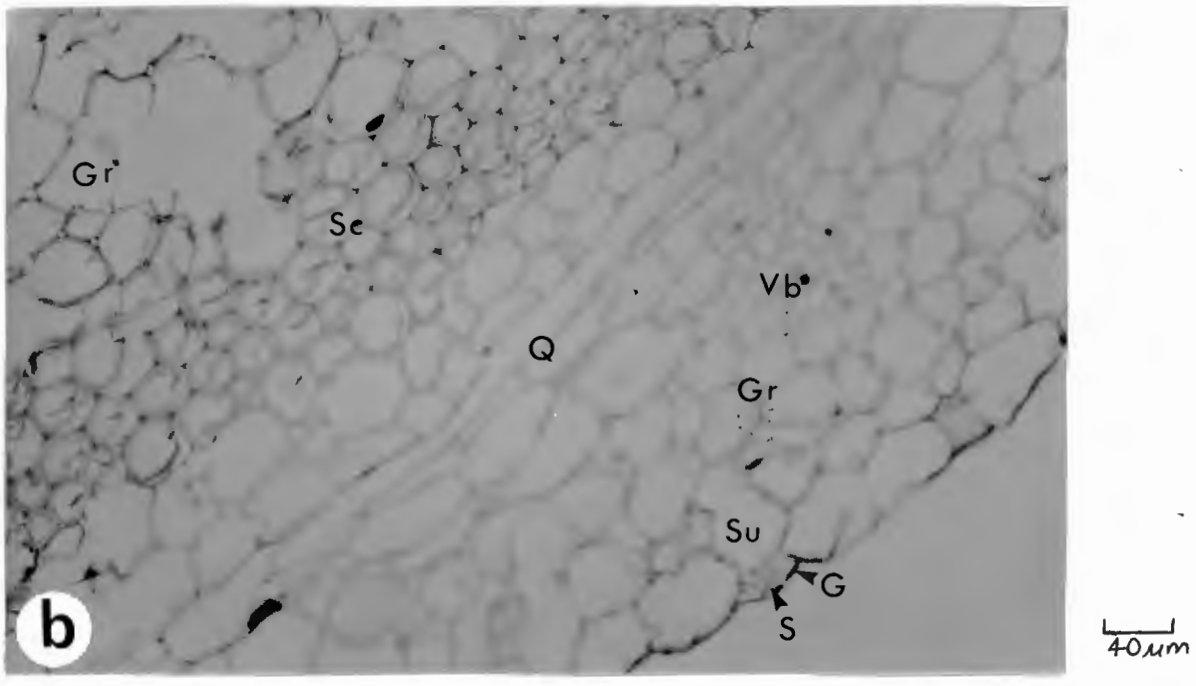

Figure 12(b). The structure of the inflorescence stem wall. S, stomata; G, guard cells; Su, substomatal cavity; Gr, ground tissue; Vb, vascular bundle in the outer cortex; Q, pericycle; Sc, sclerenchyma ring.

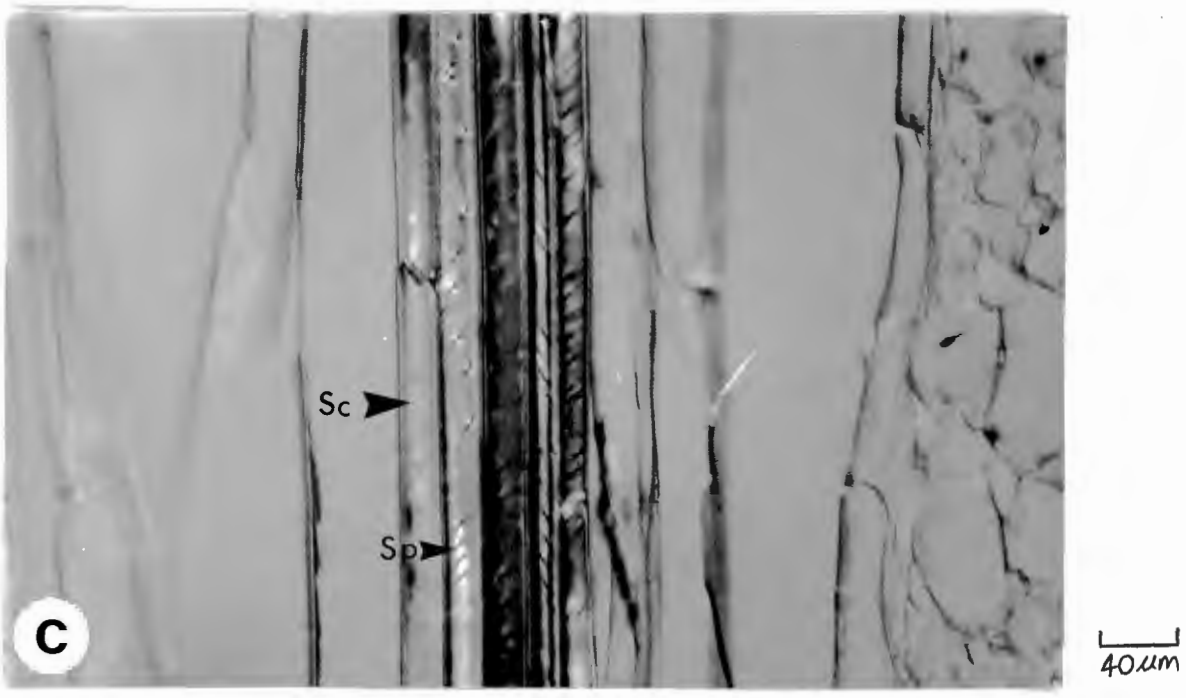

Figure 12(c). Sclerenchyma ring (Sc) in longitudinal section. Spiral thickening (Sp) can be seen in the walls of the sclerified cells.

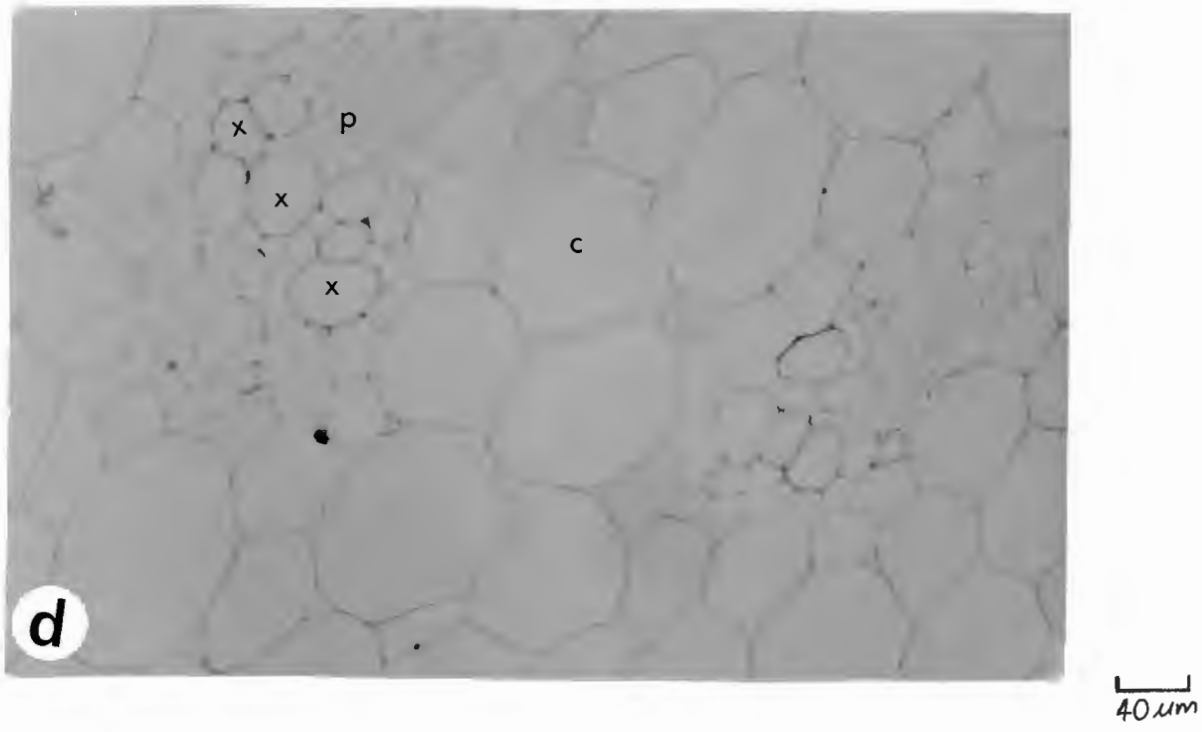

Figure 12(d). Vascular anatomy of the annual stem. The xylem (X) forms a Y-shape and the phloem (p) forming a cap on the peripheral edge.

rings of vascular bundles with spirally tickened walls within the sclerenchyma ring again surrounding a core of soft-walled cells (fig 12d). The xylem elements form the classical Y-shape typical of stem anatomy, with the metaxylem forming the stem and the protoxylem forming the arms.

The tuberoid anatomy of the orchidoids

1. The subtribe Disinae

The epidermis of the tuberoid of *Disa uniflora* consists of three layers of cells which are slightly elongated in transverse section (fig. 13). The tangential walls are suberised and give rise to small epidermal hairs (not evident in figure 13 owing to scale). The tuberoid also has a curiously undifferentiated vascular system. It seems to be monostelic. There is one central vascualar cylinder where the xylem elements which are spirally thickened and weakly lignified, almost seem to form a ring although a few elements are distinctly apart from the rest. The xylem ring is bounded by a phloem ring of smaller cells. The two layers of cells surrounding the stele may constitute a pericycle or larger phloem cells and contain no oil or starch bodies or glucomannans.

Bounded by the vascular tissues is a parenchymatous core of large isodiametric cells. These cells are similar to the cells of the cortex lying just outside the stele. This ground tissue houses many small oil bodies as well as crystals called glucomannans. Glucomannans occur in the large vacuolated regions of the tuberoid cortex but are absent from the two or three layers of epidermis. They appear as granular networks and have a papillate surface in

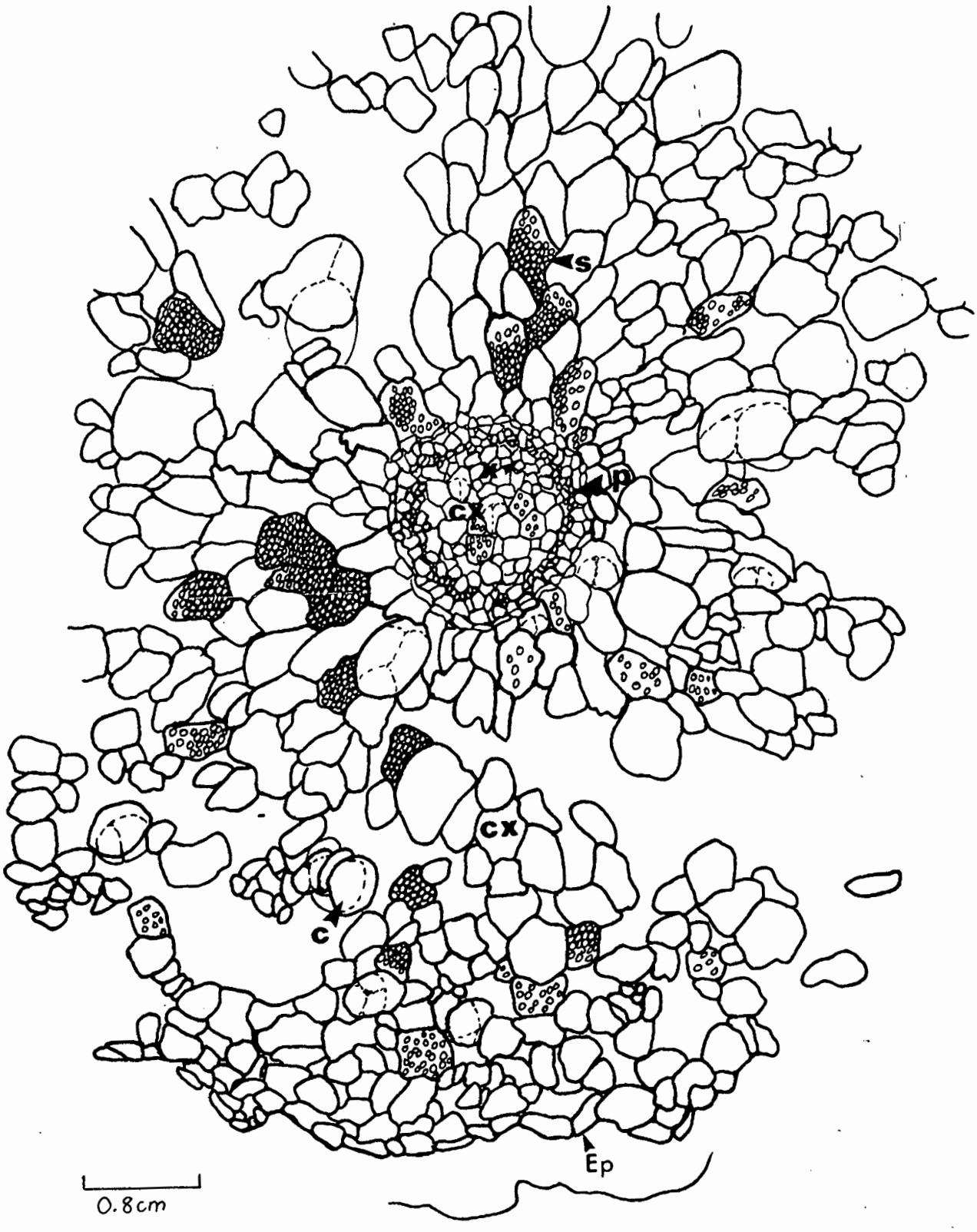

Figure 13. The anatomy of the tuberoid. X, xylem; P, phloem; CX, cortical cells; S, storage bodies; C, glucomannan crystals; Ep, suberised epidermal cells.

Disa unflora. These crystals do not occupy the cortical cells. Rather, they form between cells within the intercellular spaces. The oil bodies on the other hand, are housed within the cells themselves.

Interestingly, there is no mycorrhizal infection in the tuberoid of *Disa uniflora*.

2. The subtribe Coryciinae

The tuberoid of *Corycium orobanchoides* is monostelic, possessing a stele very similar to that of *Disa uniflora* although the phloem cells are indiscrete owing to extensive crystalisation. The most notable feature of *C. orobanchoides* is that the cells of the cortex are densely packed with starch or oil bodies. Many styloids are located within and near the epidermis. The epidermal cells give rise to very thin-walled and long epidermal hairs.

Disperis villosa shows weak polystely. It has one large stele and two to three smaller steles, all quite central within the tuberoid. The xylem elements are much larger than those of *Disa uniflora* and *Corycium orobanchoides*, almost forming a ring within the vascular bundle. The core of the large stele is filled with ground tissue which houses oil or starch bodies. The glucomannans occur towards the periphery of the cortex. The cortical cells near the three steles are also densely packed with the oil or starch storage bodies. A few styloids are evident in the tuberoid.

3. The subtribe Satyriinae

Satyrium humile is strongly polystelic. The small vascular bundles are evenly distributed in the ground tissue. The xylem elements are small, lignified and spirally thickened. The phloem is not very distinguishable. Glucomannans are

ubiquitous in *Satyrium humile,* occurring throughout the cortex. The starch or oil storing bodies are much smaller than those found in the rest of the orchidoids mentioned in this project. Styloids occur in the epidermis.

4. The Orchidae (subtribe Orchidinae)
Schizochiles cecilii shows a remarkable degree of polystely, strongly resembling the perennial stem of *Disa uniflora.* The vascular bundles are arranged in a circle towards the periphery of the cortex with the xylem on the inside and the phloem forming a cap on the outer edge of the bundle. The distribution of crystals and oil is even throughout the cortex and the epidermis is once again an area where many styloids can be found.

In all the subtribes mentioned above, no evidence of mycorrhiza was found in one of them. Table 1. below summarises the distribution of types of tuberoid steles within the Orchidoidae.

Table 1. The distribution of polystely and monostely within a few members of the Orchidoidae

Tribe	Subtribe	Species	Type of stely
Orchidae	Orchidinae	*Schizochiles cecilii*	strongly polystelic
Diseae	Disinae	*Disa uniflora*	monostelic
	Coryciinae	*Corycium orobanchoides*	monostelic
		Disperis villosa	weakly polystelic
	Satyriinae	*Satyrium humile*	strongly polystelic

DISCUSSION

Perennial growth in Disa uniflora

Growth in *Disa uniflora* is sympodial since successive growth is made possible by lateral buds on the rhizome. The ways in which renewal growth is brought about, are variations of a general pattern of sympodial growth. In orchid stems, the sympodial habit is ancestral and the monopodial habit is derived (Holttum 1955). The sympodial habit is primitively characterised by successive growths, each originating from the base of the preceding one (Holtumm 1955). The initial portion of the new growth, with its shortened internodes may form a rhizome or corm at the base of the new growth, or it may develop directly into an upright shoot with leaves and determinate growth (Withner *et al* 1974). This pattern is simple but has led to some misconceptions which can be seen in Rasmussen's (1985) treatment of the tuberoid. Rasmussen (1985) mentions that the tuberoid has an apical bud which in the next season will grow into a new shoot, with one of the axillary buds forming a new tuberoid. In simple terms, this would mean that the tuberoid itself is a modification of the rhizome, having indeterminate growth. From the findings of this study and of that of Sharman (1939), this allegation is untrue. The true rhizomes, which are both aerial and underground, are clearly noded and both possess axillary buds. It is in fact the perennial stem of *Disa uniflora*, not the tuberoid as has been suggested by Dressler (1981), which possesses the apical bud to give rise to the annual stem. The tuberoid forms <u>after</u> the axillary bud elongates off the aerial rhizome - it is merely an extension of the rhizome. The differences between the annual and perennial stem are clear, although both have a stem anatomy.

The annual stem differs from the rhizomatous stem in a number of ways. Firstly, the epidermis is interrupted by stomata and guard cells which is not found in the rhizome anatomy. Secondly, the annual stem has a sclerenchyma ring

which surrounds the stele. The aerial rhizome has neither sclerenchyma ring nor a pericycle surrounding the steles. The vascular bundles are many as in the annual stem but are less differentiated, not having the classical Y-shaped xylem formation of the annual stem.

The fact that mycorrhiza are absent from the all other organs except the root, implies that the roots are the only organs involved in the uptake of nutrients. This would suggest that the tuberoid has the sole function of storage. Glucomannans seem to be an important carbohydrate storage product. The smaller storage bodies always referred to must therefore be oil and not starch. The presence of the styloids in the epidermis of *Disa uniflora*, *Corycium orobanchoides*, *Satyrium humile* and *Disperis villosa* might be important in defense against fungal atttack. Salmia (1989) mentions that mycorrhiza may become detrimental to the plant. Styloids are also evident in the new rhizome. It would seem that the roots are the only organs in which infection is allowed.

Why does *Disa uniflora* not have a resting period? The reason why storage organs and perennation evolved, was to survive the dry season in temperate environments (Holttum 1955). *Disa uniflora* occurs within a temperate environment but is restricted moist habitats, such as on rock faces next to waterfalls or in high altitude areas which receive mist in summer (Schelpe 1980). It can therefore afford to flower in the driest seasons of the year without any severe cost. However, if *Disa uniflora* does not need a resting period, why does it perennate? Firstly, sympodial growth in orchids is a structural constraint. Sympodial growth is in fact an ancestral feature in orchids and monopodial growth derived (Withner *et al.* 1974). Withner *et al.* (1974) suggest that a simple underground rhizome which gives rise directly to new shoots is probably the most primitive vegetative habit. If

this is true then *Disa uniflora* carries an ancestral trait - an example of canalization. The underground rhizome buds off from the base of the aerial rhizome and gives rise directly to a new shoot without a tuberoid.

Perennation in *Disa uniflora* may also be an ecological aptation. The moss and detritus substrate in which *Disa uniflora* stands, is known to become leached (Schelpe 1980). Perennation is therefore helpful in exploring new substrates. Perhaps this is why it is possible for some tuberoids to sustain at least three generations of renewal shoots to form a dense cluster. Once a highly favourable unleached site has been reached, perhaps sufficient reserves (oil or starch) are accumulated within the tuberoid for extensive renewal growth. In such a case it is affordable to allow all the lateral buds of the second, fourth and sixth nodes to develop, instead of only one from the fourth node. This explains why one ramet of *Disa uniflora* may from such a dense cluster.

The affinity of the root-stem tuberoid

The name and definition of the root-stem tuberoid are, according to the findings of this study, incorrect. The confusion here probably arose from the fact that the process of perennation in orchids with tuberoids was poorly understood as is highlighted above.

The tuberoid is clearly made up of rhizomatous stem tissue. This is supported by the fact that in the tuberoid of *D. uniflora* the vascular bundles do not form a core within the tuberoid as in the root, but are arranged in a ring around the stele, as are the more discrete bundles of the two types of rhizomes and the dropper. The tuberoid has what might be a pericycle surrounding the stele. According to Withner *et al* (1974), pericycles are not uncommon in rhizomatous tissue. The only evidence to suggest that there may be any

root tissue in the tuberoid is the fact that the epidermal cells give rise to epidermal hairs which could possibly be root hairs. However, the actual roots of *D. uniflora* do not have epidermal hairs and therefore the hairs cannot be inferred as an homology with the root.

Any departure of the tuberoid from typical rhizome anatomy can probably be explained by the variation in tuberoid anatomy within the three subtribes of the Diseae.

Variation in tuberoid anatomy within the Orchidoideae

The source of variation in the tuberoid anatomy within the orchidoids stems mainly from the types of steles. Linder (1986) gives a cladogram showing the possible phylogeny of the subtribes of the Orchidoidae using floral characters. The results of this study show that tuberoid anatomy is an important character within this subfamily.

It is difficult to draw conclusions about the distribution of types of steles from so few representatives from each subtribe, but some speculation can be made. If the subtribe Orchidinae is taken as an outgroup for the Diseae, then polystely in tuberoids is an ancestral character and monostely is derived. The Satyriinae show strong polystely as in the Orchidinae and therefore were the first subtribe within the Diseae to evolve. The Coryciinae show both weak polystely and monostely and must therefore have been next to evolve. Last to branch off were the Disinae which have only monostelic tuberoids.

The reason for this trend away from polystely may be because the tuberoid is not a branching structure since it is only an extension of stem tissue. For this reason, vascular tissue is not needed on the periphery of the tuberoid in order to give rise to traces. Further, the tuberoid evolved as a storage structure and therefore polystely may have

disappeared within the Diseae to conserve and maximise storage space. Within the Coryciinae, the monostelic *Corycium orobanchoides* has its cortical cells densely packed with storage bodies and crystalization is extensive. The polystelic *Disperis villosa* also has its cortical cells densely packed with storage bodies but glucomannans only occur towards the periphery of the cortex.

That polystely may be ancestral in the tuberoids of the Orchidoidae further supports the fact that the tuberoid is comprised of stem tissue since polystely is typical of the stem structure. The tuberoid anatomy of *Schizochiles cecilii* is remarkably similar to the annual stem of *Disa uniflora*. Any peculiarities of the more derived taxa, therefore can be attributed to modification of the basic stem structure in order to carry out the function of storage.

CONCLUSION

The confusion about the root-stem tuberoid arose because the perennial mechanism was poorly understood. Because of this, there was a lack of distinction between the perennial and aerial stems. It was therefore not realised that the perennial stem is in fact a modification of the ancestral rhizome, having several axillary buds and one apical bud. This confusion also led to a misconception about the root-stem tuberoid which was alleged to have apical and axillary buds (Dressler 1981). What this study has clearly shown is that perennial growth in *Disa uniflora* fits in with the general pattern of sympodial growth typical of monocotyledonous plants - that the tuberoid is not a mixture of root and stem tissue, but rather a further modified extension of the rhizome. A stem tuber would therefore be a better term for this structure since tubers are stem storage structures (Dressler 1981)

Disa uniflora does not have a resting stage as do so many other monocotyledonous plants of temperate regions. The resting period has become defunct in *Disa uniflora* since it only occupies habitats which are moist throughout the year. Perennation is still advantageous, however, since it enables the ramet to explore new fertile soil.

This study also suggests that the tuberoid is an important character in elucidating the phylogeny of the Diseae. This is based on the fact that the stem tuberoid is ancestrally polystelic in the Orchidinae, a trait which tends towards monostely within the Diseae.

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