THE ANATOMY OF *DIOSCOREA BELIZENSIS* LUNDELL

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A steroid-yielding wild yam from British Honduras is shown to be *Dioscorea belizensis* Lundell. The macroscopical characters of the entire plant and the microscopical characters of the underground organs are described.

IN 1941 Lundell found and described *Dioscorea belizensis* Lundell. His description was not illustrated and made only passing references to the underground organs. Lundell stated that pistillate flowers and fruits were unknown; none have since been found. A description of this species seemed desirable owing to its possible use as a commercial source of diosgenin (Blunden, 1962; Blunden and Hardman, 1963).

Habitat

D. belizensis was found growing in a five year old *Pinus caribaea* Morelet plantation at the Machaca Forest Station in British Honduras by Dr. S. S. Bampton of the Tropical Products Institute, London. The plant was only moderate in occurrence beyond the Forest Station, which is located in southern British Honduras between the Rio Grande and River Moho and about 15 miles inland from Punta Gorda.

The temperature of the region remains fairly constant throughout the year, the mean maximum varying between 23° in January and 31° in April, and the mean minimum between 14° in January and 22° in June. Apart from the two coldest months of December and January the mean maximum for 1960 varied by only 3° and the mean minimum by 4°.

The rainfall for the Machaca Forest Station is high. Of the total of 313 cm. for 1960, 201 cm. fell in the four months of June, July, August and September, and only 38 cm. in the four months from December to March.

Materials

All the plant material received from British Honduras was collected from the Machaca Forest Station by Mr. R. Waters, the Conservator of Forests, and despatched by air mail. Batches of tuber were received in October, 1960, January, February, April, May, July, August, and October, 1961. Aerial stems and leaves were received in January, February and October, 1961, and a male inflorescence was received in January, 1961. Other plants were grown in the Department of Pharmacy's hot-house at Nottingham from pieces of tuber received from British Honduras.

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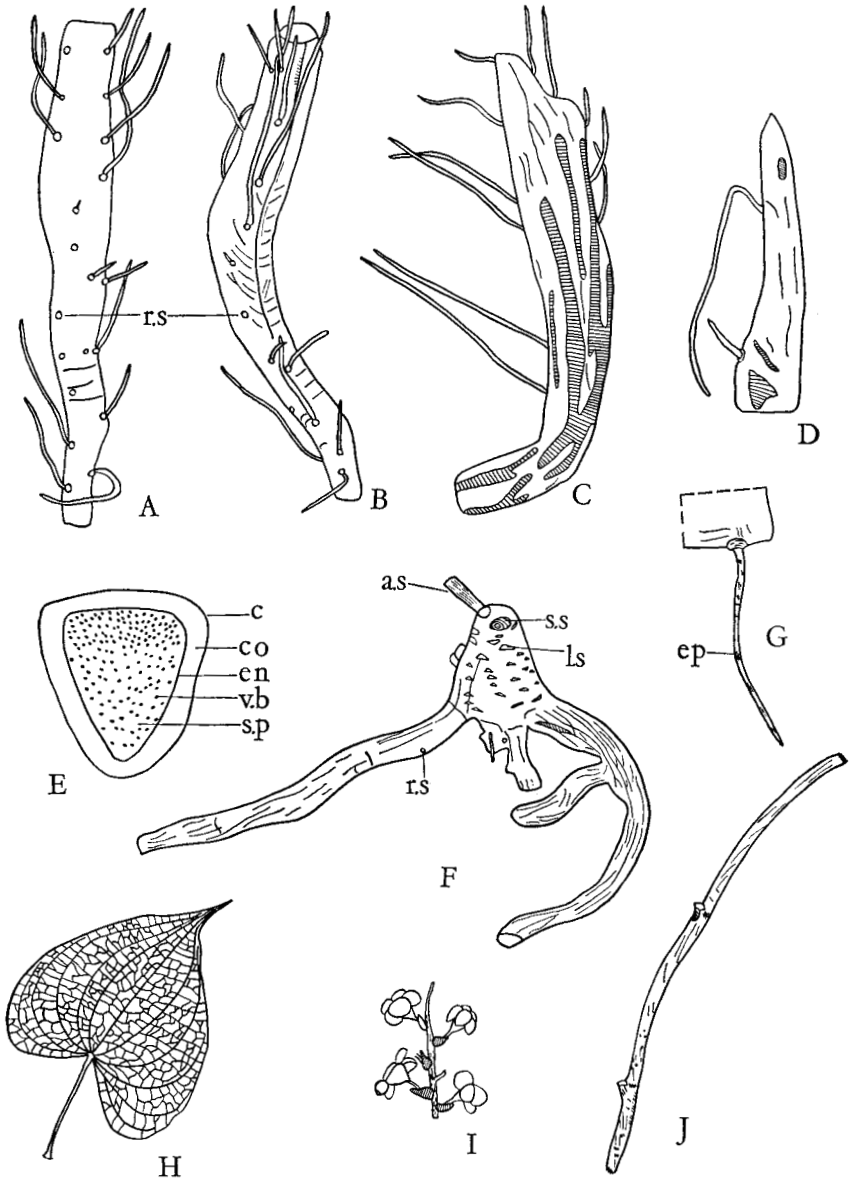

FIG. 1. *Dioscorea belizensis* Lundell. A piece of tuber: A, lower surface; B, side view; C, upper surface; D, end of a tuber branch; E, transverse section of a tuber branch; F, crown of tuber; E × ¾, the rest by ¼. G, root × ½. H, leaf × ¼. I, part of male inflorescence × 2. J, piece of stem × ¼. *a.s.*, aerial stem; *c.*, cork; *co.*, cortex; *en.*, endodermis; *ep.*, epidermis; *l.s.*, leaf scar; *r.s.*, root scar; *s.p.*, stele parenchyma; *s.s.*, stem scar; *v.b.*, vascular bundle.

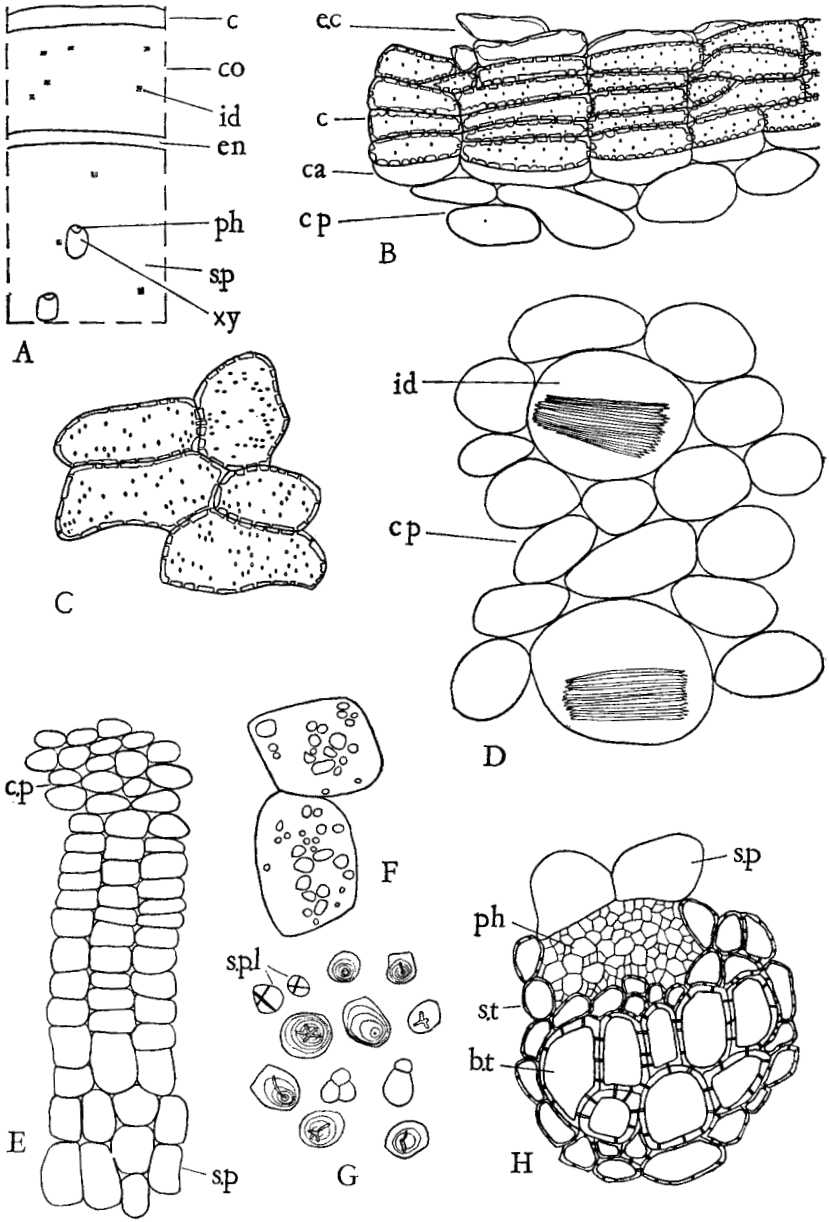

FIG. 2. Tuber of *Dioscorea belizensis* Lundell. A, transverse section, general diagram. B, transverse section of outer tissues. C, surface view of cork. D, transverse section of cortex. E, transverse section through the endodermal region. F, parenchyma cells containing starch grains. G, starch grains. H, transverse section through vascular bundle. A × 20, E × 60, the rest × 210. *b.t.*, bordered pitted tracheids; *c.*, cork; *ca.*, phellogen; *co.*, cortex; *c.p.*, cortical parenchyma; *e.c.*, exfoliating cells; *en.*, endodermal region; *id.*, idioblast; *p.*, parenchyma; *ph.*, phloem; *s.p.*, stele parenchyma; *s.p.l.*, starch under polarised light; *s.t.*, simple pitted tracheids; *xy.*, xylem.

The Plant

D. belizensis is a perennial vine with a large tuber, conical at the top and bearing a few cylindrical branches which grow horizontally about 30 cm. below the surface of the ground. In February and March, the two driest months of the year, *D. belizensis* has a resting stage, when growth is restricted to new leaves. In exceptional cases, however, as when the vine is damaged, a new shoot is stimulated into growth. The plant flowers infrequently, usually in October and November after the rainy season and preceding the two cooler months.

Macroscopical Characters

The plant has an AERIAL STEM, twining dextrorsely to heights of up to 3·7 m., which may be between 9 m. and 13·5 m. in length and up to 11·5 mm. in diameter, although it is usually less than 6 mm. Stems from British Honduras plants were woody, fibrous, tough and broke with a fibrous fracture. Younger plants grown at Nottingham under greenhouse conditions had herbaceous stems. The stems have rough surfaces, are ridged longitudinally and transversely, the transverse ridges being confined to the lower part. The thin cork separates easily from the older regions (Fig. 1, J).

THE LEAVES are alternate and often absent from the lower half of the vine. They are petiolate, the petiole being 5 to 26·5 cm. long and up to 3 mm. in diameter. The colour varies from a brownish-green in the young to a dark brown in the older leaves. The petiole has a pulvinus at each end and bears longitudinal ridges which are continuous with the main veins of the lamina. The surface is sparsely lepidote (Fig. 1, H).

The lamina is simple, broadly ovate, up to 27 cm. long and 25·5 cm. wide, margin entire, apex abruptly cuspidate, base deeply cordate with a broad open sinus, eleven veined, of which only three reach the apex of the leaf and with the outer veins branching from the penultimate ones, the veins and veinlets being impressed above and very prominent below and with reticulate venation. The upper surface is glabrous and the lower shortly villous. The lamina varies in colour from green to a dark brownish-green and has a papery texture, particularly in the older leaves.

THE FLOWER. The staminate inflorescences are axillary, solitary, up to 150 cm. long and consist of a narrow panicle of racemes up to 7·5 cm. in length. No pistillate flowers or fruits were received. The rachis and peduncle are both longitudinally ridged and somewhat woody. The surface is sparsely lepidote. The bractlets subtending the flowers are up to 2 mm. long, ovate, abruptly cuspidate and, like the flowers, are dark red in colour. The flowers are actinomorphic. The pedicels are from 1 to 2 mm. long and sparsely lepidote. The perianth has six lobes, each lobe being broadly ovate and about 2 mm. long. There are six stamens which are attached to the bases of the perianth lobes. The filaments are thick and about 0·5 mm. long. The anthers are about 0·2 mm. in length, bilocular and introrse and are not appreciably wider than the filaments. The inferior ovary is abortive, but shows a trilocular structure (Fig. 1, I).

THE TUBER constitutes the greatest part by weight of the plant, the average being 3 kg. from each plant from British Honduras. The tuber has a conical crown, with a vertical axis (Fig. 1, F). The cone varies in size from 1·5 to 7 cm. high and 1·2 to 5·5 cm. in diameter at the base. From the top of the cone grows the aerial stem (Fig. 1, F). From the base of the crown emerge from three to eight branches which run horizontally under the ground and often reach considerable lengths. Most of the tubers from British Honduras were in broken lengths, the longest was 75 cm. and the diameters vary from 0·6 to 5 cm. The horizontal branches are often bifurcated, usually near to their junction with the crown.

Near the apex of the crown there are usually one or two large depressed scars, up to 1·5 cm. in diameter, produced by previous stems. The conical crown bears several large brown triangular scale leaves up to 1·3 cm. broad (Fig. 1, F).

The older parts of the tuber including the crown are an orange-brown, the orange colour being most pronounced in the younger parts. The tuber is somewhat triangular in transverse section, the upper surface being quite frequently markedly flattened, particularly in the older lengths. The adventitious roots arise in regular longitudinal rows on the sides of the tuber; circular root scars, up to 4·5 mm. in diameter, are to be found (Fig. 1, A). The upper surface and the upper half of the sides of the tuber frequently bear marked longitudinal furrows in the cork (Fig. 1, B and C), but the lower surface and the lower half of the sides usually have a smooth surface. When the tuber has partially dried out, which sometimes occurs when received from British Honduras, very prominent longitudinal furrows are apparent.

The fresh tuber is flexible but breaks fairly easily, the resistance to breaking being mainly due to the cork; it has no marked odour and possesses a bitter, unpleasant taste. The transversely cut surface shows a thin cork, which is easily peeled from the tuber, a narrow yellowish-cream cortex, a dark band marking the endodermal region, and a large yellowish stele, in which are scattered numerous vascular bundles (Fig. 1, E).

THE ROOT. The roots are adventitious; they are usually less than 12 cm. but may be up to 25 cm. in length, and usually 2 to 3 mm. in diameter near their junction with the tuber (Fig. 1, G). The root is a light brown colour, with paler brownish-fawn areas where the epidermis has exfoliated; the cortex is brittle and easily separates from the inner tissues exposing the endodermis and giving rise to dark brown areas. Along the length of the roots, particularly those from British Honduras, there are transverse cracks in the cortex. The root has a dry powdery texture, breaks easily with a porous fracture, does not possess any marked odour and is tasteless. The transversely cut surface shows a thin epidermis, a wide brownish-fawn lignified cortex, a dark brown lignified endodermis and a brownish-fawn stele, which except for the phloem, is lignified; the vascular bundles have the normal monocotyledonous arrangement (Fig. 4, A).

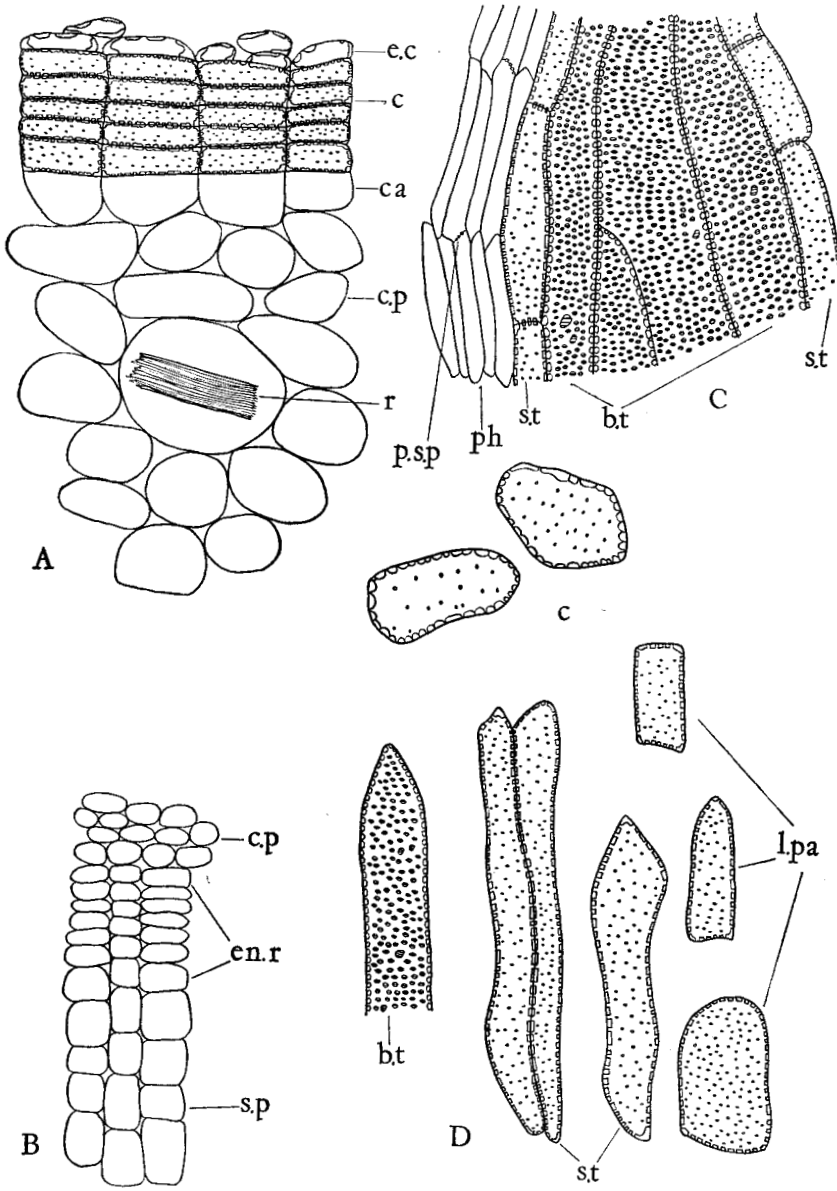

FIG. 3. Tuber of *Dioscorea belizensis* Lundell. Longitudinal sections through: A, the outer tissues; B, the endodermal region; C, vascular bundle. D, lignified cells isolated by maceration. B × 60, the rest × 210. *b.t.*, bordered pitted tracheid *c.*, cork; *ca.*, phellogen; *c.p.*, cortical parenchyma; *e.c.*, exfoliating cells; *en.r.*, endodermal region; *l.p.*, lignified parenchyma; *ph.*, phloem; *p.sp.*, sieve plate; *r.*, raphide of calcium oxalate; *s.p.*, stele parenchyma; *s.t.*, simple pitted tracheids.

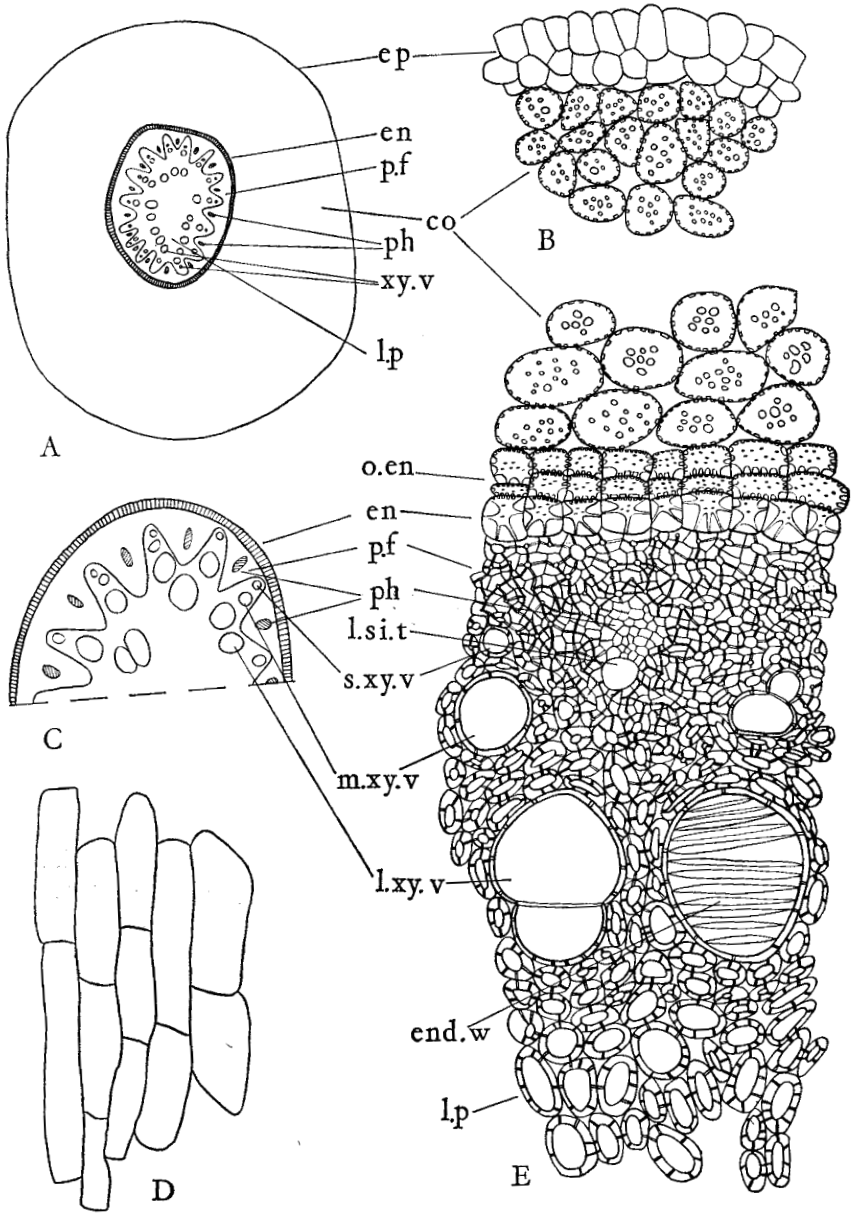

FIG. 4. Root of *Dioscorea belizensis* Lundell. A, transverse section, general diagram. B, transverse section through outer tissues. C, transverse section of stele, general diagram. D, surface view of epidermis. E, transverse section of inner tissues. A × 28, C × 52, the rest × 210. *co.*, cortex; *en.*, endodermis; *end.w.*, end wall of vessel; *ep.*, epidermis; *l.p.*, lignified parenchyma; *l.si.t.*, large sieve tube; *l.xy.v.*, large xylem vessel; *m.xy.v.*, medium xylem vessel; *o.en.*, outer endodermis; *p.f.*, pericyclic fibres; *ph.*, phloem; *s.xy.v.*, small xylem vessel (protoxylem); *xy.v.*, xylem vessel.

Microscopical Characters

THE TUBER. The outer protective layer of the tuber is composed of a number of very regularly arranged rows of lignified cork cells (Figs. 2, B and 3, A); usually between four and nine, but there may be as many as thirty-five. Of these rows only up to thirteen appear to be completely functional, the outer layers being in the process of breaking down and exfoliating; outside the cork, there is usually a layer of broken-down, brown coloured cells, which are often non-lignified (Figs. 2, B and 3, A).

The individual cork cells are straight-walled and usually five or six sided in surface view (Fig. 2, C), in transverse and longitudinal section they appear rectangular (Figs. 2, B and 3, A), individual cells being elongated tangentially, and some also elongated longitudinally. The walls are thick, lignified and cuticularised and have frequent simple pits. The cells measure T = 28–**60**–**114**–123 μ, L = 24–**36**–**63**–81 μ and R = 10–**12**–**23**–32 μ.

Under certain conditions a second phellogen arises deep in the cortex, producing lignified cells to the outside. The parenchymatous cortical cells thus cut off then gradually become pigmented and eventually are exfoliated along with the outer cork layer. Cork layers are produced also to cover cut or damaged surfaces of the tuber.

The cortex consists of cellulose-walled parenchymatous cells, oval in both longitudinal and transverse section (Figs. 2, D and 3, A), of varying dimensions: T = 35–**60**–**120**–162 μ, L = 42–**54**–**110**–145 μ and R = 15–**30**–**60**–80 μ.

Idioblasts containing raphides of calcium oxalate are found in the cortex and stele, but occur more frequently in the cortex. Although they have the same shape as the surrounding cells the idioblasts are much larger with dimensions: T = 87–**105**–**147**–168 μ, L = 84–**100**–**155**–180 μ and R = 65–**75**–**105**–138 μ. The calcium oxalate raphides measure: T = 60–**68**–**96**–135 μ and width 20–**30**–**45**–48 μ. These are surrounded by material which is stained red by corallin-soda and orange-brown with iodine solution suggesting mucilage.

No endodermis is distinguishable, but there is a region which appears darker than the surrounding layers and contains less starch than the cortical cells or the tissues of the stele. In this endodermal region the parenchyma consists of several layers of regularly arranged, straight-walled, rectangular cells which measure: T = 48–**63**–**114**–135 μ, L = 60–**100**–**140**–165 μ, and R = 18–**30**–**70**–108 μ. Towards the centre of the stele the cells show a gradual radial elongation, as opposed to the tangential elongation of the cortical cells (Figs. 2, E and 3, B).

The ground tissue of the stele consists of radially elongated parenchyma; the cell dimensions are: T = 45–**70**–**110**–144 μ, L = 75–**90**–**140**–156 μ. Radially most of the cells are in excess of 100 μ and are up to 230 μ towards the centre of the tuber.

Starch grains (Fig. 2, F and G) are found in the parenchyma of both the cortex, where they are few, and stele, where they are abundant. Most of the grains are simple and a few 2- to 3-compound; individual granules have blunt angles or are more or less rounded with an eccentric

hilum which appears as a circular spot or frequently as a simple curved or multiple cleft. Striations are often clearly visible and individual grains are up to 35 μ in diameter, although about 40 per cent are less than 10 μ.

No protein bodies or fat crystals were seen in sections of the tuber, but sections were very strongly stained with tincture of Alkanna, picric acid, and Millon's reagent particularly those from the cortical region.

Scattered throughout the stele are numerous collateral vascular bundles The phloem consists of longitudinally elongated, cellulose-walled sieve tubes, the sieve areas occurring in the end walls of the cells (Fig. 3, C). The xylem consists of a central core of long, wide-diameter tracheids, having thick lignified cell walls with numerous bordered pits having very marked extended pit apertures. The outer zone of the xylem consists of lignified parenchyma and tracheids, both of which have relatively thick, lignified walls with simple pits. This zone contains cells which show gradual changes from wide diameter lignified parenchyma to narrow diameter lignified tracheids (Figs. 2, H, 3, C and D). The cell dimensions of the outer zone of the xylem cover a wide range and there is a gradual transition between lignified parenchyma and the tracheids; the dimensions are: L = 60–**90**–**270**–480 μ, and width 9–**24**–**57**–78 μ. The central zone of tracheids with bordered pits have thicker walls than the simple pitted tracheids; they also have a wider diameter, i.e. 21–**48**–**87**–111 μ and a much greater length, being almost invariably in excess of 500 μ.

THE ROOT. The epidermis of the root together with one to four subjacent rows have yellowish-brown, suberised walls. The individual cells are roughly hexagonal to rectangular in transverse section (Fig. 4, B), but they are elongated longitudinally (Figs. 4, D and 5, A) measuring L = 39–**75**–**162**–220 μ, R = T = 8–**21**–**39**–51 μ. The epidermis is fragile and on many of the roots received from British Honduras was absent or present in a broken-down form.

The cortex is a wide band and accounts for about two-thirds of the root diameter. The individual cells are circular or oval in transverse section, but are more elongated longitudinally, this being most pronounced in the outer layers of the cortex (Fig. 5, A); L = 21–**33**–**75**–228 μ, T = 18–**36**–**60**–72 μ, and R = 18–**30**–**48**–63 μ. The cells have relatively thin, slightly lignified cell walls with very large simple pits (Figs. 4, B, 5, A and B).

The endodermis consists of a single layer of deep brown, longitudinally elongated cells. To the outside, are one or two layers of cells which have a somewhat similar form to those of the endodermis, but the cells are not usually so large and have less thickened walls (Figs. 4, E and 5, B). The endodermal cells (Fig. 4, E) measure: L = 70–**100**–**186**–270 μ, R and T = 14–**24**–**36**–45 μ; they have thick, simple pitted walls, the inner and radial walls being considerably more thickened than the outer walls (Figs. 5. B and 6, D).

Within the endodermis is a pericycle composed mainly of yellow, strongly lignified fibres, with a few yellow stone cells; this layer forms a band within the endodermis and extends between the different xylem

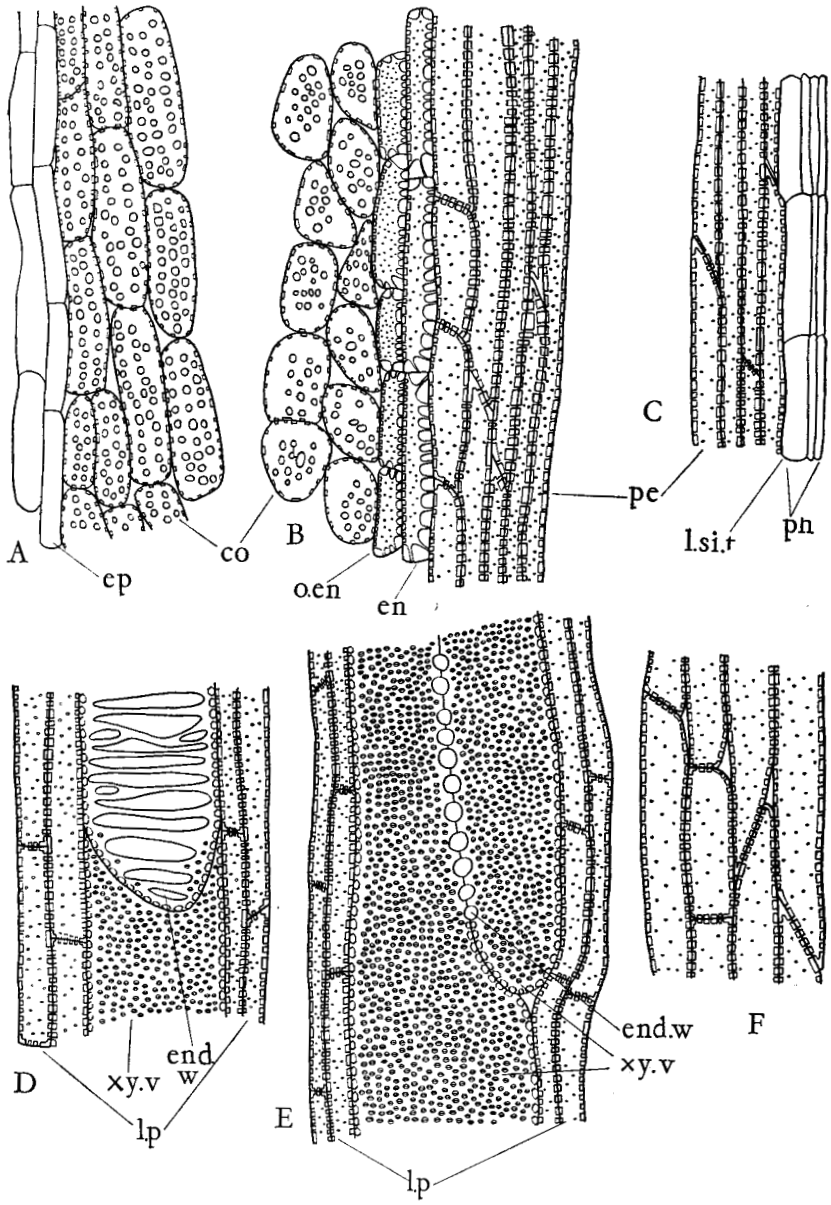

FIG. 5. Root of *Dioscorea belizensis* Lundell. Longitudinal sections: A, outer tissues. B, endodermal region. C, phloem tissue. D and E, xylem areas. F, lignified parenchyma. All × 210. *co.*, cortex; *en.*, endodermis; *end.w.*, end wall; *ep.*, epidermis; *l.p.*, lignified parenchyma; *l.si.t.*, large sieve tube; *o.en.*, outer endodermis; *pe.*, pericycle; *ph.*, phloem; *xy.v.*, xylem vessel.

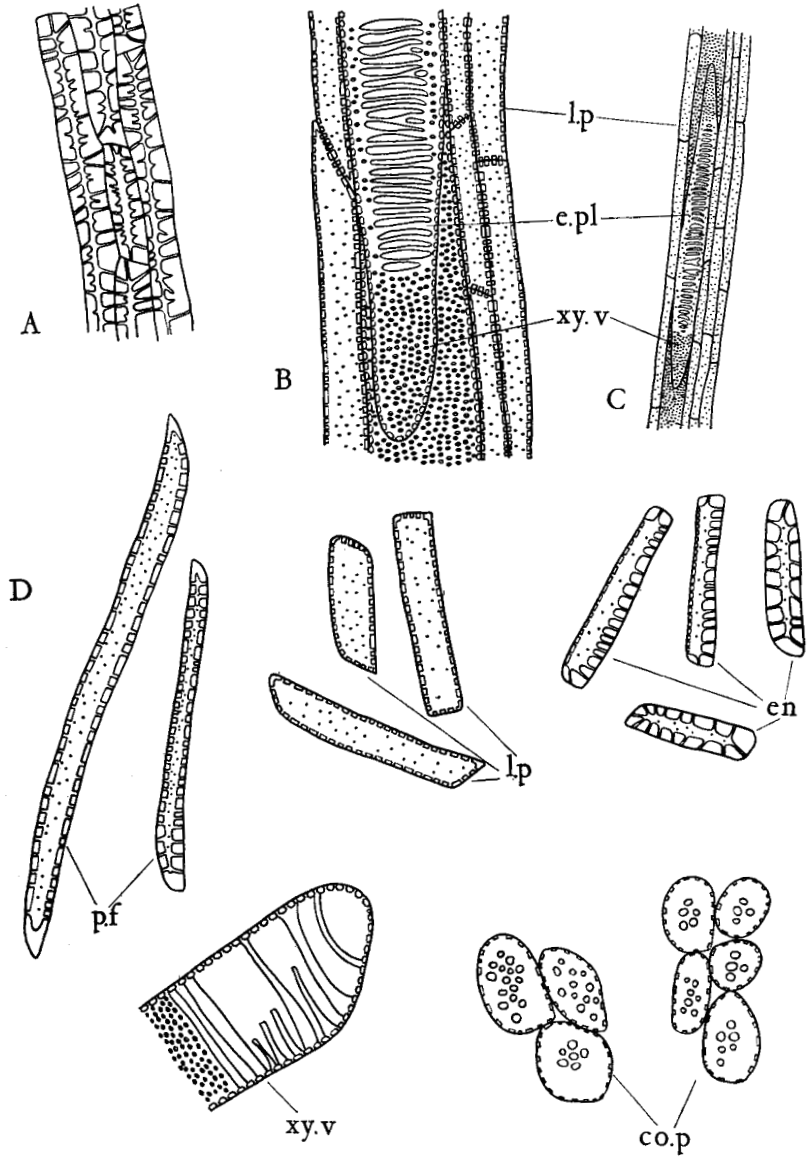

FIG. 6. Root of *Dioscorea belizensis* Lundell. A, surface view of endodermis. B, longitudinal section of xylem. C, longitudinal section of xylem showing entire end plate. D, lignified cells isolated by maceration. C × 60, the rest × 210. *co.p.*, cortical parenchyma; *en.*, endodermis; *e.pl.*, end plate; *l.p.*, lignified parenchyma; *p.f.*, pericyclic fibres; *xy.v.*, xylem vessel.

strands (Fig. 4, C). The cells are polygonal in transverse section, and are usually much elongated longitudinally (Figs. 4, E, 5, B and 6, D). The pericyclic cells measure: T and R = 9–**12**–**21**–30 μ and L = 39 to at least 1,200 μ; they have thick, strongly lignified, simple-pitted walls, the lumen being occasionally very narrow.

Between the xylem strands and enclosed by the pericyclic fibres are the phloem groups (Fig. 4, C). The individual phloem areas are small and consist of a large sieve tube of diameter from 15 to 36 μ, to the outside of which is a group of sieve-tubes of very small diameter; the cells are longitudinally elongated and have relatively thin walls (Figs. 4, C and 5, C).

The xylem is composed of nine to fifteen radiating strands each of which has from two to four vessels, which in transverse section appear circular or slightly oval (Fig. 4, C and E). The vessels fall into one of three diameter ranges; the inner vessels are the widest, R = 45–**105**–**144**–159 μ, whilst the protoxylem vessels have the least diameter R = 18–42 μ; the vessels in between have dimensions of R = 33–**42**–**75**–99 μ (Fig. 4, C). The vessels have thick, lignified walls with numerous bordered pits which have extended pit apertures. The vessels taper at both ends and the end plates have large openings which are continuous with similar openings in the end plates of the overlapping vessels (Figs. 5, D and E, and 6, B, C and D).

The pith consists of cells with thick, lignified, yellow walls containing simple pits. Transversely the cells appear oval due to slight radial elongation, but longitudinally they are much elongated (Figs. 4, E, 5, F and 6, D); they closely resemble the cells of the pericycle, although those of the pith usually have a wider cell diameter and a shorter cell length than the pericyclic cells; L = 36–850 μ, R = 10–**21**–**30**–48 μ and T = 10–**14**–**20**–45 μ.

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