RESEARCH PAPERS

ANATOMICAL STUDIES IN THE GENUS *RUBUS*

PART II. THE ANATOMY OF THE LEAF OF *R. fruticosus* L.

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THE common bramble or blackberry, like the raspberry plant, has been used in medicine for many centuries. Dioscorides devotes a monograph to it in his Greek Herbal[1], stating that the leaves and tops, and the juices expressed therefrom, were used in the treatment of a wide variety of ailments; his Herbal also provides a drawing of the plant[2], with numerous prickles, illustrating compound leaves with five leaflets, flowers, fruits and the root system. In the Apuleius herbal of A.D. 600[3], there is a conventional but unmistakable drawing of the blackberry, whilst Gerard[4], whose herbal was first published in 1596, recommended both leaf and fruit as an astringent. A decoction of the leaves is recommended in Cruso's *Treasury of Easy Medicines* (1771)[5] as a fomentation for long-standing ulcers; Grieve[6] records the use of the leaves and root-bark as astringents and tonics in the treatment of dysentery and diarrhoea.

The gross morphology of the bramble leaf, like that of the raspberry, has been described in several standard botanical works[7–9], but again, only limited, and in some cases partially illustrated, histological descriptions have been published to date[11–19]; British pharmacognostical works in use at the present time contain no references to the bramble, but a survey of European works shows that blackberry leaves are more widely recognised as a medicine on the continent of Europe than are those of the raspberry.

In view of these considerations, the present investigation was undertaken to describe and depict the anatomical structure of the leaf, also to note the diagnostic characters necessary for the identification of the leaf in the broken or powdered condition and to distinguish it from the leaves of co-generic species.

MATERIAL

The literature shows considerable variation in the nomenclature of the individual species of the subgenus *RUBUS*, genus *Rubus*, family Rosaceae. In a contemporary flora[9], Warburg points out that, although Bentham[8] includes them all under one specific name, *R. fruticosus* L., the most recently available list, compiled by Watson[20], names 300 species, and even then "only widely distributed species." Warburg emphasises the difficulty of determining individual species within the subgenus—"unlike the genetically somewhat similar genus *Rosa* it does not seem possible at present to group the forms satisfactorily into a limited number of species,"

and he points out that further difficulties arise because the species appear to exhibit a more or less continuous network of forms, so that it is often doubtful to which section of the subgenus a particular species should be referred. Only a few species from each of the sections devised by Watson are described in this Flora[10].

The material used for this investigation was gathered from wild stands on Chislehurst Common, Kent, and has been assigned for the present to the section *Radulae* Focke of Watson's list. Pending a more precise identification, the specific name *R. fruticosus* is used.

Methods of Investigation

Although the leaflets are highly coloured like those of *R. idaeus*, chloral hydrate proved a satisfactory clearing agent for the examination of their epidermises, furthermore, the covering trichomes were well expanded, the spiral markings showing to advantage. Prolonged heating with this reagent accentuated the markings still further, but produced simultaneously a loss of definition of the outlines of many of the epidermal cells, similar to the effect already noted in similar preparations of the leaf of *R. idaeus*[21]. Satisfactory epidermal preparations were also obtained by heating fragments of the lamina with chlorinated lime and a little concentrated hydrochloric acid for a few minutes, subsequently mounting in 50 per cent v/v glycerol solution. Mounts made in phloroglucin and hydrochloric acid stained the covering trichomes well; the epidermis was also seen quite clearly in these mounts.

Systematic serial sections were prepared after polyethylene glycol embedding[22]; they were mounted in a solution containing glycerol 60, distilled water 30 and Delafield's Haematoxylin 10 parts by volume; the latter stained the sections to such a degree that they gave excellent contrast for camera-lucida and projection drawing.

Macerates of prickles, midrib and rachis were prepared using Schultz's maceration fluid.

Anatomical Structure

The leaf of *Rubus fruticosus*, like the leaf of *R. idaeus*, is imparipinnately compound; leaves of species of the section *Radulae* commonly possess five leaflets and the lateral, and, to a lesser extent, the basal pairs of leaflets are all stalked. Paired, adnate, subulate or linear stipules appear to arise about 3 to 5 mm. from the base of the rachis (Figs. 1, A; Fig. 6, A and Fig. 8, A).

A noteworthy sensory character for differentiation from the leaf of *R. idaeus* is that the latter has invariably a whitish underside of the leaflet lamina whereas leaflets of *R. fruticosus* are usually green or greyish-green on their undersides.

(*a*) Leaflets

Both lateral and terminal leaflets were examined, and no anatomical differences were detected between them. The following anatomical description, therefore, applies to either of these leaflets.

(*i*) *Lamina, interneural region* (Fig. 1, C and D; Fig. 2, A, B and F; Fig. 3, B and C; Fig. 5, C and D)

The UPPER EPIDERMIS is covered with a fairly thick, smooth cuticle and consists of a layer of polygonal cells having wavy, beaded anticlinal walls; they measure about* H 10 to 28 μ and Lev L and B 10 to 66 μ. Stomata are absent, but numerous prominent oval *hydathodes*, about 10 to 30 μ long and 9 to 18 μ wide are present on each of the marginal teeth (Fig. 2, A and F). *Covering trichomes* occur fairly frequently; they arise predominantly over, or in close proximity to, the veins and around the edges of the marginal teeth; a small proportion, however, arise from the interneural regions (Fig. 1, B and C; Fig. 2, C). They are unicellular, with very thick, lignified walls, tapering and acutely pointed, with heavily thickened bases commonly exhibiting fine, linear pits; they measure about 300 to 1,000 μ long and 30 to 50 μ wide at the base. The lumen is wide within the base, but narrows very sharply in most trichomes, being visible usually for a short distance only along the trichome. The bases are surrounded by about 10 to 14 radiating epidermal cells, many of which are conspicuously smaller than the normal epidermal cells and these are raised above the level of the epidermis. Double-spiral markings, extending throughout almost the whole length of the trichomes, are extremely prominent in chloral hydrate mounts and also, to a slightly lesser extent, in phloroglucin and hydrochloric acid mounts (Fig. 2, C).

The MESOPHYLL is slightly less clearly differentiated than that of *R. idaeus*; the *palisade* consists of a single layer of cells which is seen to be somewhat discontinuous in thin, transverse sections (Fig. 3, B and C). Individual cells are more or less cylindrical, but often taper slightly at the ends adjacent to the spongy mesophyll; they measure about H 14 to 30 μ, Lev 8 to 12 μ, and contain chloroplasts, about 6 μ in diameter. Scattered irregularly in the palisade are very numerous, prominent, rounded idioblasts, each containing a well-defined cluster crystal of *calcium oxalate* about 12 to 50 μ in diameter. Occasional idioblasts containing small cluster crystals of calcium oxalate, about 8 to 15 μ in diameter, also occur in the layer of the spongy mesophyll lying immediately below the palisade, but there is no well-defined crystal layer. The large, single, prismatic crystals of calcium oxalate described and figured by Gassner[22] were not observed. The spongy mesophyll consists of about 2 to 4 layers of cells, which in surface view are rounded, elongated, triangular or trabeculate and measure about H 6 to 20 μ and Lev 8 to 30 μ; they contain chloroplasts, about 3 to 8 μ in diameter; numerous air spaces occur (Fig. 3, B and C; Fig. 5, C).

The LOWER EPIDERMIS has a smooth, thin cuticle. Its cells have wavy anticlinal walls and measure about H 10 to 16 μ, Lev L and B 10 to 50 μ. *Stomata* are numerous, are of the anomocytic (ranunculaceous) type and

* The symbols H, Lev, Lev L and Lev B are suggested for the purpose of describing organs showing bilateral symmetry by Moll and Janssonius. The symbol H = height, in a direction perpendicular to the surface of the organ; Lev = in the direction of the surface of the organ; Lev L and Lev B = parallel to the surface and at the same time in a longitudinal or transverse direction respectively.

are sometimes surrounded by radiating epidermal cells; they are slightly raised above the level of the epidermis, are oval in outline and exhibit narrow but prominent ostioles; they measure about 14 to 18 μ wide and 20 to 28 μ long (Fig. 2, B).

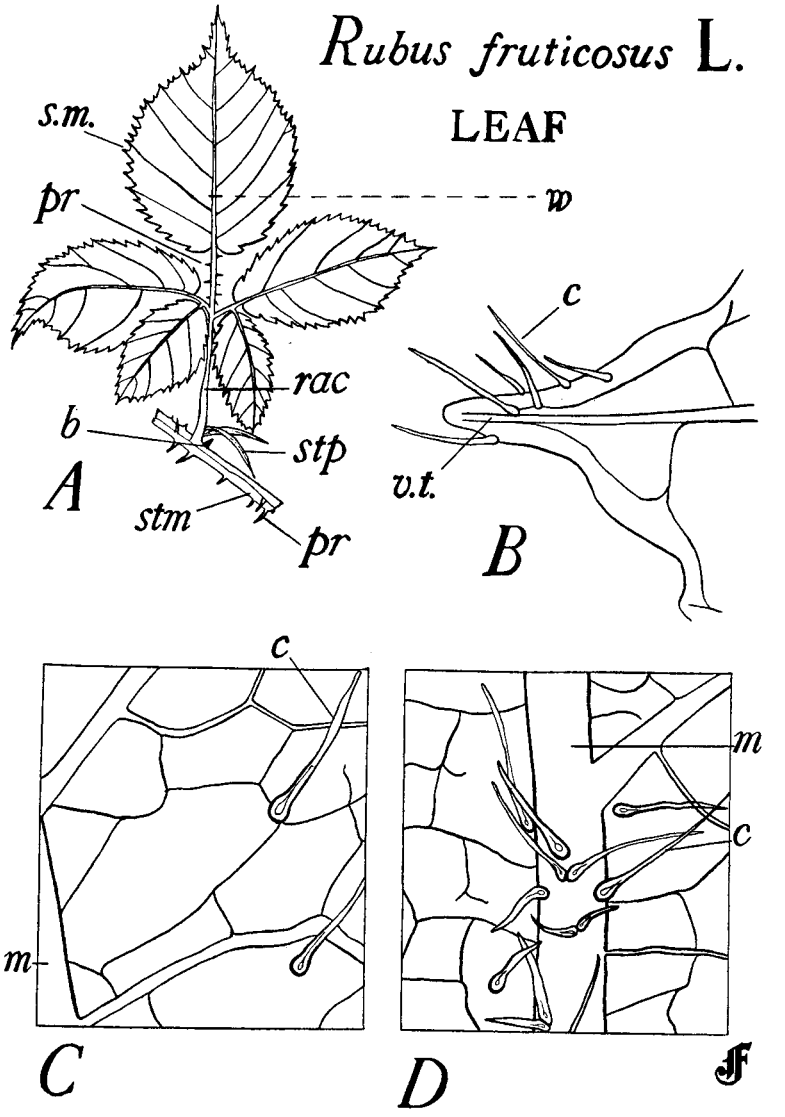

FIG. 1. Leaf of *Rubus fruticosus* L. *A*, complete, compound leaf with terminal, two lateral and two basal leaflets and paired stipules adnate to the rachis. *B*, marginal tooth of leaflet. *C* and *D*, upper and lower surfaces of the lamina to show distribution of covering trichomes. *A*, x $\frac{1}{3}$; *B*, *C* and *D*, x 33. *b*, bud; *c*, covering trichome; *m*, midrib; *pr*, prickle; *rac*, rachis; *s.m.*, serrate margin; *stm*, stem; *stp*. stipule; *v.t.*, vein termination; *w*, position at which transverse section illustrated by Fig. 3, *A* was made.

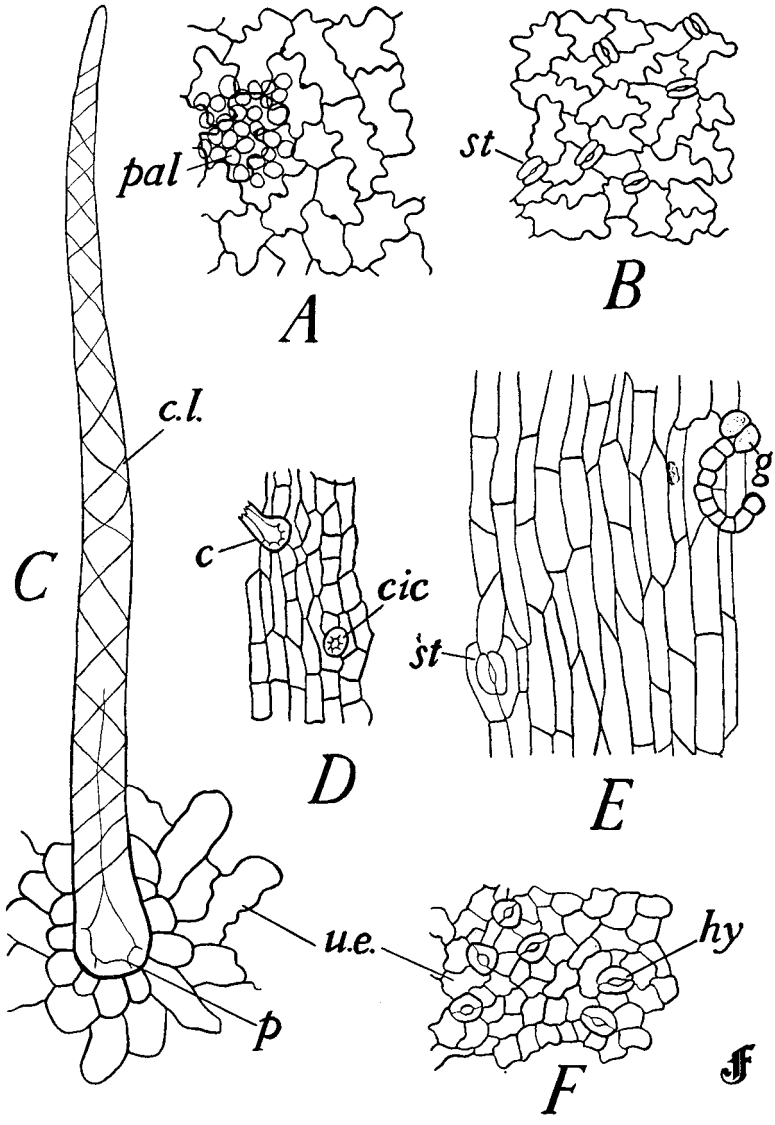

FIG. 2. Leaflet of *Rubus fruticosus* L. *A*, upper epidermis of leaflet. *B*, lower epidermis of leaflet. *C*, covering trichome on upper epidermis of lamina. *D*, upper epidermis of midrib. *E*, lower epidermis of midrib. *F*, upper epidermis of marginal tooth, showing hydathodes. All x 200. *c*, covering trichome; *cic*, cicatrix; *c.l.*, crossed-line effect; *g*, glandular trichome; *hy*, hydathode; *p*, pit; *pal*, palisade; *st*, stoma; *u.e.*, upper epidermis.

Covering trichomes occur frequently, and especially over the minute veinlets rather than on the interneural epidermis (Fig. 1, D); they are similar in character to those on the upper epidermis and frequently are also surrounded at the base by raised epidermal cells. The two- and

four-membered trichomes described by other authors[11,12] were not observed, but a few two-celled trichomes of this kind occur on the lower epidermis of the midrib—*vide infra.*

The lamina has a serrate margin, individual teeth being acutely pointed. The minute ends of the secondary and tertiary veins extend to within about 50 μ of the teeth apices and terminate in a few, very small spiral elements. Two fine veinlets, one on either side, converge towards the central veinlet and unite with it about 0·3 mm. from the tip of the tooth (Fig. 1, A and B).

(*ii*) *Midrib* (Fig. 2, D and E; Fig. 3, A and D; Fig. 4, A and B; Fig. 5, A, B and D).

The midrib has typically dicotyledonous structure; no significant variation, other than a gradual increase in amount of pericyclic fibre and of reticulated cells, was noted in transverse sections cut serially from apex to base.

The UPPER EPIDERMIS consists of a single layer of elongated, well-cuticularised, straight-walled cells measuring about H 14 to 25 μ, Lev B 7 to 15 μ and Lev L 15 to 60 μ; stomata are absent (Fig. 2, D; Fig. 3, D; Fig. 4, B). *Covering trichomes* are present in moderate numbers and are similar in character to those of the upper interneural epidermis. Frequent *glandular trichomes* about 80 to 180 μ long occur on the upper epidermis of both midrib and secondary veins; they comprise a biseriate, at times, multiseriate or, rarely, uniseriate multicellular stalk, about 3 to 7 cells long, frequently with coloured contents, and a subspherical, multicellular, glandular head about 45 to 70 μ in diameter (Fig. 5, D (1)).

The LOWER EPIDERMIS consists of strongly cuticularised, longitudinally elongated, straight-walled cells, measuring about H 10 to 20 μ, Lev B 7 to 20 μ and Lev L 42 to 120 μ; occasional stomata are present (Fig. 2, E).

Covering trichomes arise frequently; the majority are similar in structure to those of the upper interneural epidermis, but occasionally, and more particularly in the upper half of the leaflet, two-celled covering trichomes, similar to those described by other authors[11,12], occur (Fig. 1, D; Fig. 5, D (2 and 3)). Numerous *glandular trichomes* arise; they are frequently bent over at right angles with a multicellular uniseriate stalk of about 6 to 10 cells and a two- to four-celled head about 20 to 40 μ in diameter. More rarely they may somewhat resemble the glandular trichomes of the upper midrib epidermis in having a biseriate stalk (Fig. 2, E; Fig. 5, D (4)).

Laterally compressed, curved or elongated-conical *prickles* occur frequently on the lower surface of the midrib. They are about 0·5 to 2 mm. high and 80 to 600 μ long at the base, and consist of strongly lignified, fibre-like sclerotic cells, about 45 to 220 μ long and 6 to 22 μ wide, their walls being either smooth and thick, or thin with occasional small oblique or transverse pits. Towards the apex of the prickle the cells have pointed, interlocking ends, narrow lumens and thick walls (Fig. 1, A; Fig. 5, A and B; Fig. 6, A, C, D and F).

The CORTEX contains a few rows of supporting hypodermal *collenchyma* beneath both surfaces of the midrib, that towards the lower surface being greater in extent. These cells are heavily thickened, particularly in the angles, and measure about L 8 to 90 μ, R and T 6 to 24 μ; chloroplasts

are present, measuring about 6 μ in diameter. The interior of the cortex is of *parenchyma* which is often, however, slightly collenchymatous, the cells being sometimes slightly thickened at the corners and occasionally exhibiting small pits in their walls; individual cells measure about L 30 to 130 μ, R and T 10 to 60 μ.

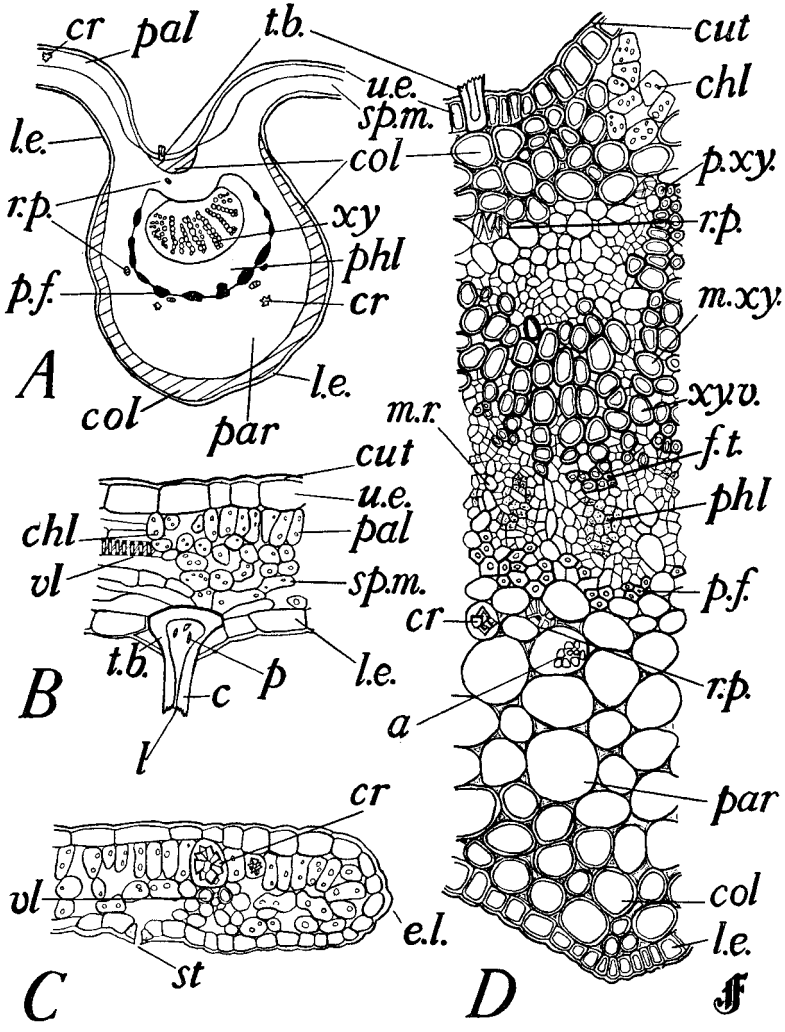

FIG. 3. Leaflet of *Rubus fruticosus* L. *A*, transverse section of midrib of terminal leaflet, cut at the position *w* (see Fig. 1, *A*). *B*, transverse section of lamina, interneural region. *C*, as *B*, but at the edge of the lamina. *D*, central region of Fig. *A*. *A*, x 55; *B-D*, x 200. *a*, starch; *c*, covering trichome; *chl*, chloroplast; *col*, collenchyma, *cr*, cluster crystal of calcium oxalate; *cut*, cuticle; *e.l.*, edge of lamina; *f.t.*, fibre-like tracheid; *l*, lumen; *l.e.*, lower epidermis; *m.r.*, medullary ray; *m.xy.*, metaxylem; *p*, pit; *pal*, palisade; *par*, parenchyma; *p.f.*, pericyclic fibre; *phl*, phloem; *p.xy.*, protoxylem; *r.p.*, reticulate parenchyma; *sp.m.*, spongy mesophyll; *st*, stoma; *t.b.*, trichome base; *u.e.*, upper epidermis; *vl*, veinlet; *xy*, xylem; *xy.v.*, xylem vessel.

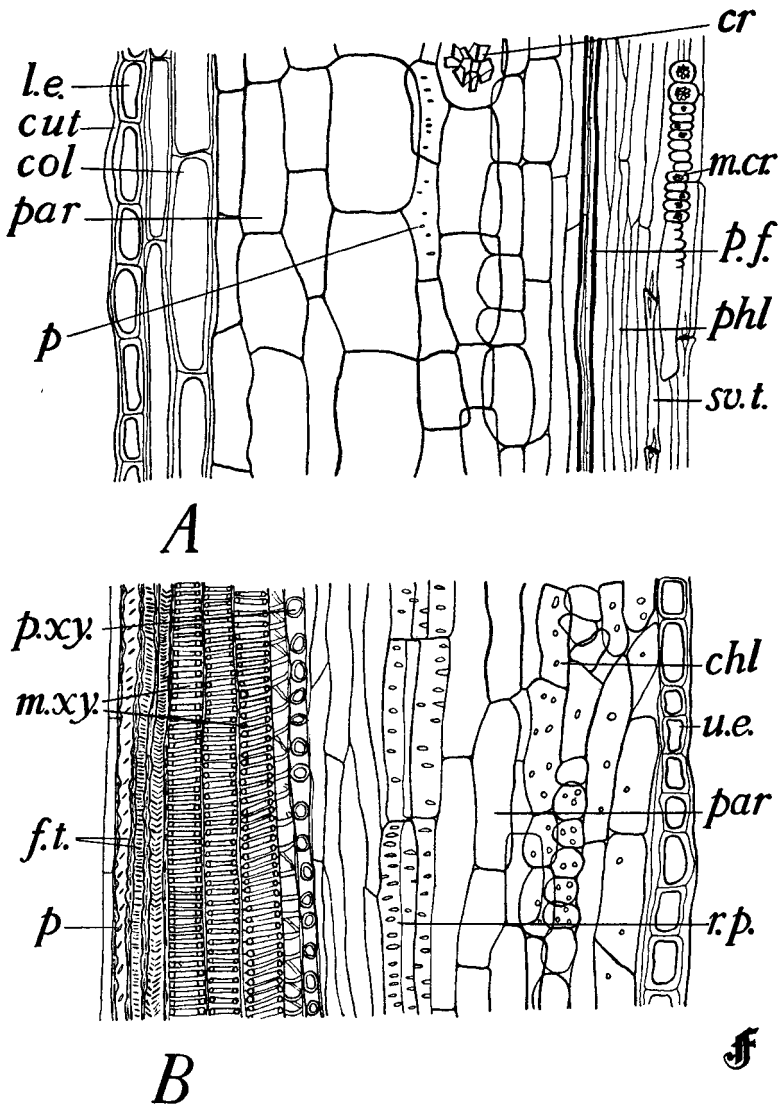

FIG. 4. Leaflet of *Rubus fruticosus* L. *A* and *B*, radial longitudinal section of midrib of terminal leaflet, cut at approximately the position *w* (see Fig. 1, *A*). Both, x 200. *chl*, chloroplast; *col*, collenchyma; *cr*, cluster crystal of calcium oxalate; *cut*, cuticle; *f.t.*, fibre-like tracheid; *l.e.*, lower epidermis; *m.cr.*, micro-cluster of calcium oxalate; *m.xy.*, metaxylem; *p*, pit; *par*, parenchyma; *p.f.*, pericyclic fibre; *phl*, phloem; *p.xy.*, protoxylem; *r.p.*, reticulate parenchyma; *sv.t.*, sieve tube; *u.e.*, upper epidermis.

Idioblasts occur frequently in this tissue; they are of two kinds. Those of the first type contain cluster crystals of *calcium oxalate* about 10 to 35 μ in diameter. The second type occurs in the inner part of this tissue and consists of slightly lignified, *reticulated cells* which are elongated

longitudinally and rounded or oval in transverse section; they measure about L 40 to 190 μ and T 18 to 36 μ, with very slightly lignified reticulate bands of thickening traversing them (Fig. 3, A and D; Fig. 4, A and B; Fig. 5, A).

Many cells of the innermost layer(s) of the cortical parenchyma are frequently lignified; in some specimens the innermost layers are non-lignified, but contain minute starch grains 2 to 7 μ in diameter; some of the cortical cells adjacent to this layer also contain similar starch grains. Owing to this irregular distribution of starch, an endodermis (or starch sheath) is not so clearly defined as it is in many other leaves, such as those of *Digitalis purpurea* and *Datura stramonium*. Engard[23] has said that the endodermis is absent from the genus *Rubus*, but it is possible to regard the innermost layer or starch-bearing cortical cells as constituting an endodermis, especially as this layer is continued over the upper side of the meristele (Fig. 3, D).

The MERISTELE is crescent-shaped in transverse section and well defined.

The PHLOEM consists of strands of sieve-tissue and small-celled parenchyma, alternating with medullary rays. The *sieve-tubes* are small; individual segments being about 100 μ long and about 3 to 7 μ in diameter, with transverse or oblique sieve-plates (Fig. 4, A). The medullary rays are clearly defined and are usually one or two cells wide (Fig. 3, D).

The XYLEM is well-developed and the conducting elements are radially arranged. The protoxylem consists of lignified, annularly and sometimes spirally thickened *tracheids* about 6 to 10 μ in diameter. The greater part of the metaxylem consists of lignified spiral, reticulate and sometimes pitted *vessels* about 14 to 26 μ in diameter; that part adjacent to the cambium consists of *fibre-like tracheids* about 100 to 600 μ long and 4 to 10 μ wide (Fig. 3, A and D; Fig. 4, B; Fig. 5, A).

In longitudinal sections, files of micro-clusters of *calcium oxalate* crystals about 3 to 8 μ in diameter are frequently seen in the parenchyma of the meristele (Fig. 4, A).

The *lateral veins* exhibit similar anatomy to that of the midrib, all features progressively diminishing towards the margin.

Sections of fresh material mounted in ferric chloride solution exhibit a dark greenish-black colouration due to tannin, most particularly in the phloem of the meristele and the mesophyll of the lamina and, to a lesser degree, in the medullary rays and stelar parenchyma. The cortical parenchyma and collenchyma of the midrib show a weak reaction, but the epidermises of the lamina are not affected.

(*b*) RACHIS

The rachis is about 4 to 10 cm. long, and 1 to 3 mm. wide; prickles are present throughout the length of the lateral and adaxial surfaces (Fig. 1, A; Fig. 6, A). Transverse sections show a shallow groove on the adaxial side in certain places, but elsewhere it is almost terete. The vascular system is more complicated than that of the rachis of *R. idaeus*; the drawings (Fig. 6, B to J) are representative of many hundreds of transverse sections cut serially from the base of the terminal leaflet to the bottom of

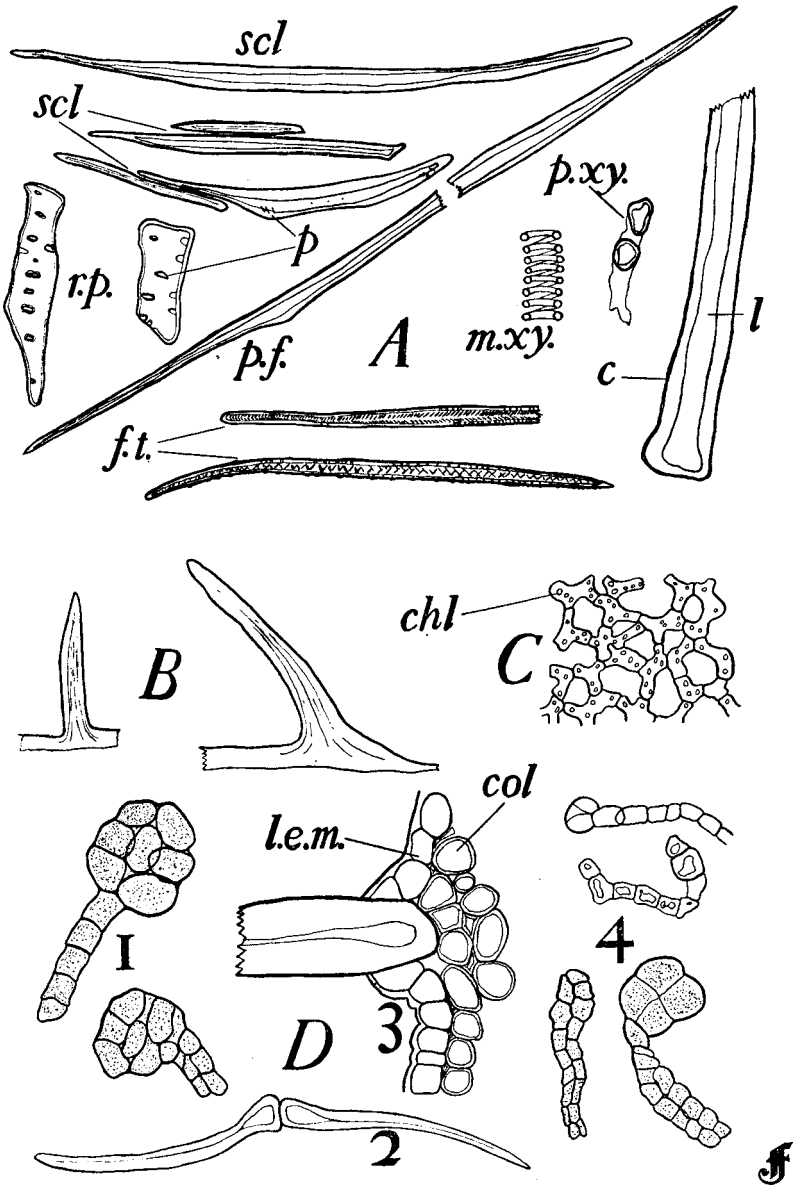

FIG. 5. Leaflet of *Rubus fruticosus* L. *A*, isolated elements obtained by maceration. *B*, prickles. *C*, spongy mesophyll in surface view. *D*, trichomes: 1, glandular trichomes from upper epidermis of midrib; 2, bicellular covering trichome from lower surface of midrib; 3, base of large covering trichome from lower surface of midrib; 4, glandular trichomes from lower surface of midrib. *A*, *C* and *D*, x 200; *B*, x 25. *c*, covering trichome (or fragment of); *chl*, chloroplast; *col*, collenchyma; *f.t.*, fibre-like tracheid; *l*, lumen; *l.e.m.*, lower epidermis of midrib; *m.xy.*, fragment of spiral vessel of the metaxylem; *p*, pit; *p.f.*, pericyclic fibre; *p.xy.*, fragment of annular tracheid of the protoxylem; *r.p.*, reticulate parenchyma; *scl*, elongated sclereids from the prickles.

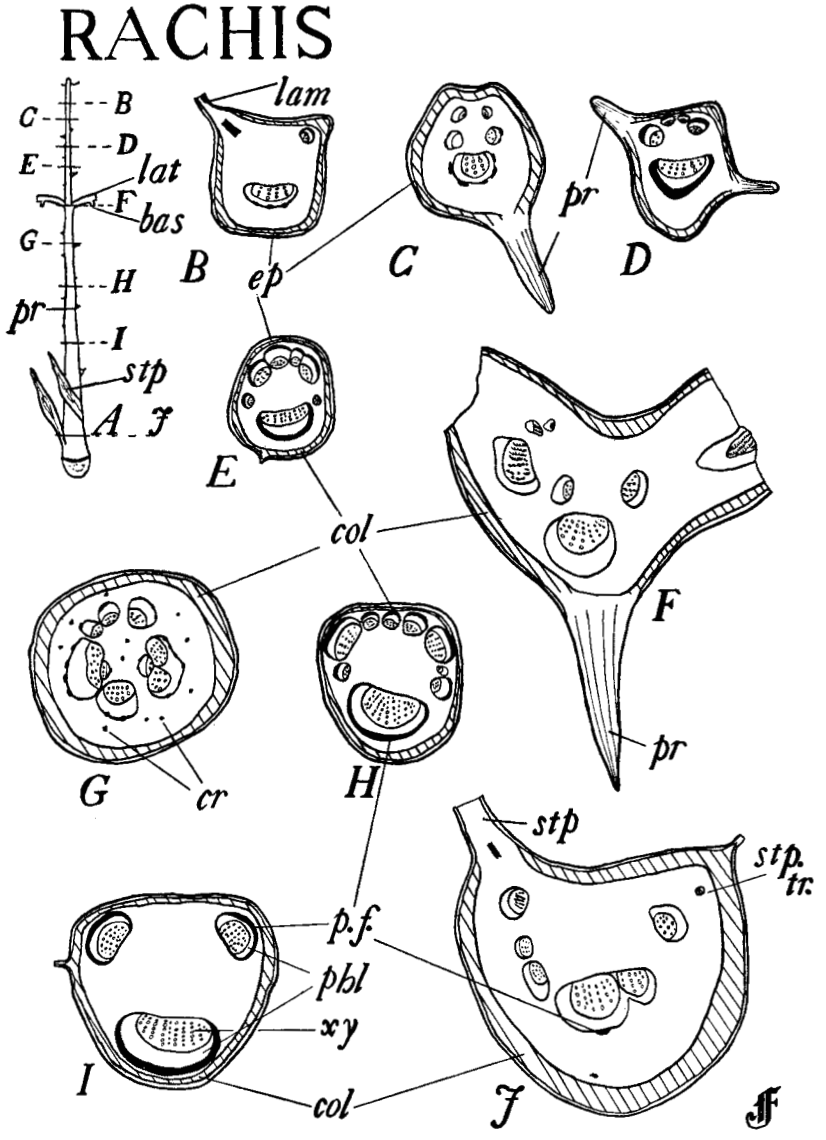

FIG. 6. Rachis of *Rubus fruticosus* L. *A*, rachis, denuded of leaflets; *B-J*, transverse sections cut at the positions indicated in Fig. *A*. *A*, x ½; *B-J*, x 14. *bas*, point of attachment of basal leaflet; *col*, collenchyma; *cr*, crystal of calcium oxalate; *ep*, epidermis; *lam*, lamina; *lat*, point of attachment of lateral leaflet; *p.f.*, pericyclic fibres; *phl*, phloem; *pr*, prickle; *stp*, stipule; *stp. tr.*, stipule trace; *xy*, xylem.

the rachis. The central, crescent-shaped meristele remains prominent throughout the length of the rachis, but the courses of the small fibro-vascular bundles on the adaxial side vary considerably; moreover, slight variations in the courses of these bundles have been observed in different

leaves. In the leaf figured, the lowest pair of lateral veins of the lamina of the terminal leaflet become the first two lateral traces of the rachis (Fig. 6, B); two more traces are abstricted from the meristele a little way further down (Fig. 6, C). Below this, the four traces, after some simple variations in their courses, become symmetrically arranged on the adaxial side (Fig. 6, D); still further down, but before the junction with the lateral leaflet traces, the meristele abstricts two further traces, whilst the other four unite to form a virtual arc of fibrovascular tissue on the adaxial side (Fig. 6, E). This arc and the two abstricted traces become gradually modified into two separate bundles just before the lateral traces enter the rachis (Fig. 6, F). Fibres are absent in the region of this junction, but below it fibres are present again and the smaller traces eventually form an almost continuous arc of fibrovascular tissue on the adaxial side (Fig. 6, H). However, a short way above the bases of the stipules and rachis, the arc divides and the section has the appearance shown in Figure 6, I. The stipule traces are finally included, just before the junction of the rachis and stem (Fig. 6, J); the pericyclic fibres are absent again in this region.

The EPIDERMIS consists of cells having similar structure to those of the epidermis of the midrib. They are heavily cuticularised, elongated longitudinally, and measure about H 9 to 16 μ, Lev B 14 to 28 μ and Lev L 16 to 60 μ (Fig. 7, A, B and C); *stomata* of the anomocytic (ranunculaceous) type are present; they are elliptical in shape and measure about 30 μ in length and 20 μ in width (Fig. 7, C). *Covering trichomes*, generally similar in detailed structure to those on the lower surface of the midrib, occur frequently, but are occasionally bi- and even tri-cellular here; they vary enormously in size—from 100 to over 1000 μ in length, and 10 to 25 μ wide at their bases (Fig. 7, F). *Glandular trichomes* occur very frequently, particularly on the upper (adaxial) epidermis; they are multicellular and frequently multiseriate, with subspherical or irregularly-shaped multicellular heads; the smallest resemble the glandular trichomes of the midribs in appearance and size, but large glandular trichomes commonly up to 500 μ long with heads about 60 μ diameter are common on the rachis. The *prickles* are frequently larger than, but have similar structure to those on the lower surface of the midrib (Fig. 7, E).

The CORTEX, like that of the midrib, consists of two layers of tissue—an outer hypodermal layer of *collenchyma*, several cells wide, similar in character to the corresponding layer of the midrib; the cells measure about L 40 to 140 μ, R and T 14 to 40 μ, and contain chloroplasts, 3 to 6 μ in diameter (Fig. 6, B-J; Fig. 7, D). The inner cortex is *parenchymatous*, consisting of cells measuring about L 20 to 140 μ and R and T 20 to 120 μ; they frequently possess slightly pitted walls. As in the midrib, many cells of the innermost layer(s) of the cortical parenchyma are frequently lignified; in specimens where the innermost layers are non-lignified, minute starch grains are often seen, about 2 to 7 μ in diameter. Frequent idioblasts containing cluster crystals of *calcium oxalate*, about 18 to 50 μ in diameter occur in idioblasts throughout the cortical parenchyma, whilst many *reticulated cells*, similar to those of the midrib, are found in the inner region and around the small ridge bundles.

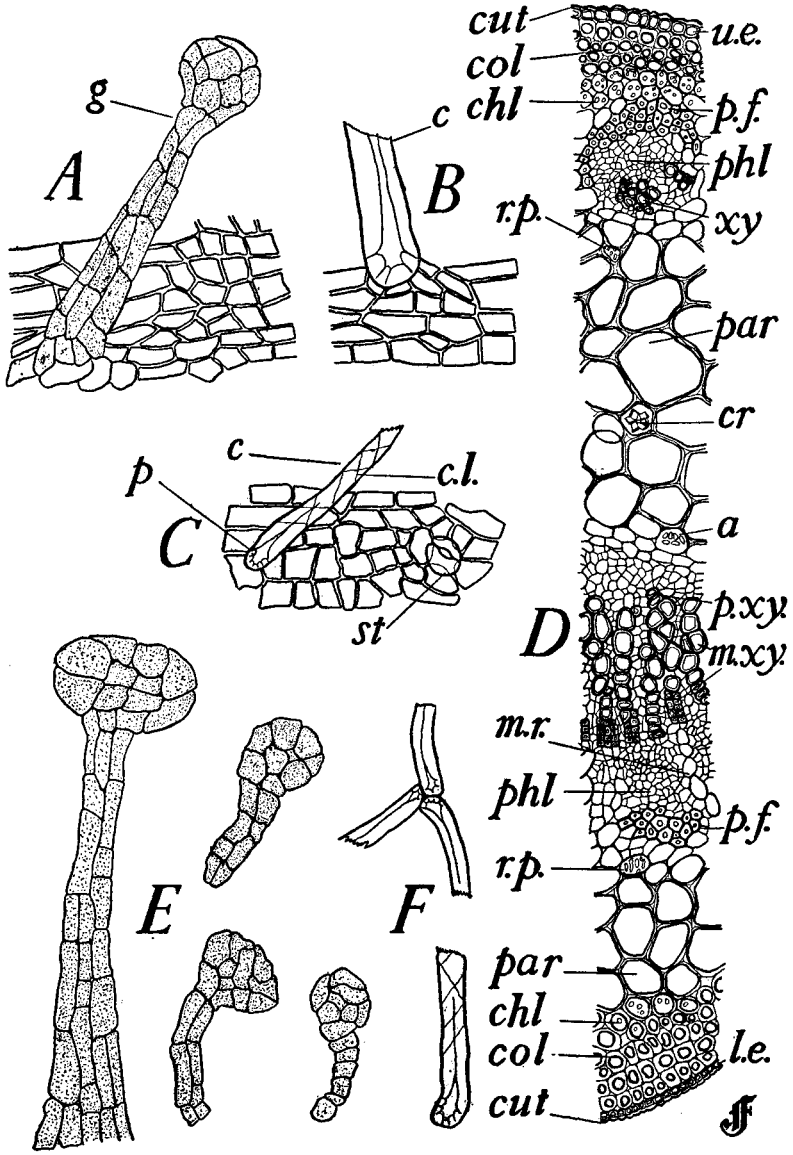

FIG. 7. Rachis of *Rubus fruticosus* L. *A*, upper epidermis with large glandular trichome. *B*, lower epidermis with base of a covering trichome. *C*, upper epidermis with stoma and base of a covering trichome. *D*, transverse section through central region of rachis at the point *D*, Fig. 6. *E*, glandular trichomes. *F*, bases of covering trichomes. *A*, *B*, *C*, *E* and *F*, x 200; *D*, x 130. *a*, starch; *c*, covering trichomes; *chl*, chloroplast; *c.l.*, crossed-line effect; *col*, collenchyma; *cr*, cluster crystal of calcium oxalate; *cut*, cuticle; *g*, glandular trichome; *l.e.*, lower epidermis; *m.r.*, medullary ray; *m.xy.*, metaxylem; *p*, pit; *par*, parenchyma; *p.f.*, pericyclic fibre; *phl*, phloem; *p.xy.*, protoxylem; *r.p.*, reticulate parenchyma; *st*, stoma; *u.e.*, upper epidermis; *xy*, xylem.

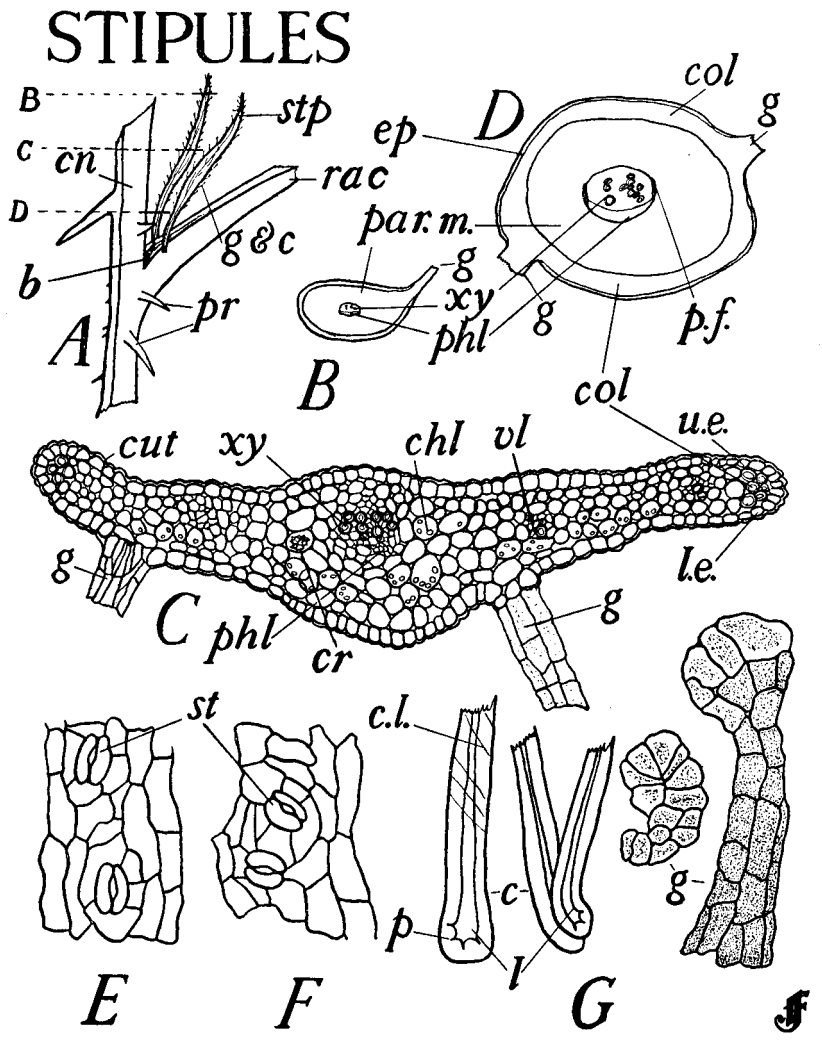

FIG. 8. Stipules of *Rubus fruticosus* L. *A*, paired stipules at base of rachis. *B*, *C* and *D*, transverse sections cut at the positions indicated in Fig. *A*. *E*, lower epidermis. *F*, upper epidermis. *G*, covering and glandular trichomes. *A*, x 2½; *B* and *D*, x 75; *C*, x 130; *E-G*, x 200. *b*, bud; *c*, covering trichomes (or fragment of); *chl*, chloroplast; *c.l.*, crossed-line effect; *cn*, cane; *col*, collenchyma; *cr*, cluster crystal of calcium oxalate; *cut*, cuticle; *ep*, epidermis; *g*, glandular trichome; *l.e.*, lower epidermis; *par.m.*, parenchymatous mesophyll; *p.f.*, pericyclic fibre; *phl*, phloem; *pr*, prickle; *rac*, rachis; *st*, stoma; *stp*, stipule; *u.e.*, upper epidermis; *vl*, veinlet in transverse section; *xy*, xylem.

PERICYCLIC FIBRES occur below the arc of the meristele throughout the rachis except at the junction with the lateral petioles and at the base of the rachis; they measure up to several mm. in length and 7 to 18 μ in diameter; they exhibit thick, smooth walls and pointed apices (Fig. 7, D).

The vascular tissue of the central MERISTELE is arranged in a crescent and in general the structure of the vascular elements approximates closely to those of the midrib, except that those of the rachis are all somewhat larger. The PHLOEM consists of groups of *sieve-tubes* about 80 to 100 μ long and 4 to 8 μ wide, frequently accompanied by small rectangular parenchymatous cells arranged in longitudinal files, many of which contain microclusters of *calcium oxalate* about 9 μ in diameter. Medullary rays traverse the phloem; they are usually one or two cells wide and their cells often contain minute starch grains about 3 μ in diameter. XYLEM is well-defined, the elements being arranged in radial rows. Its general structure is very similar to the xylem of the midrib, except that there is a much greater proportion of pitted and reticulate vessels. There is but little xylem parenchyma; the medullary rays are clearly seen alternating with the rows of tracheids and vessels; again, many of the cells often contain minute starch grains (Fig. 6, B-J; Fig. 7, D).

The fibrovascular bundles on the adaxial side each exhibit similar structure to that shown by the main meristele.

The reaction of sections of the fresh rachis with ferric chloride solution is similar to that observed in the midrib.

(*c*) STIPULES

The paired stipules are adnate to either side of the base of the rachis. They are about 10 to 20 mm. long and 1 to 2 mm. wide, subulate or linear, and hairy (Fig. 1, A; Fig. 6, A and Fig. 8, A).

EPIDERMAL CELLS of both upper and lower surfaces are small and elongated, sometimes with very slightly beaded anticlinal walls, measuring about H 10 to 24 μ, Lev B 8 to 30 μ and Lev L 20 to 70 μ. *Stomata* are present on both surfaces; they are usually elliptical in outline and measure about 30 μ in length and 20 μ in width (Fig. 8, E and F). Numerous *covering trichomes* occur, especially around the edges of the stipules; they measure about 600 to 800 μ in length and 12 to 22 μ wide at the bases and possess the general characters of those of the upper epidermis of the leaflets. Many large *glandular trichomes* are present, particularly around the edges; these are similar in character to those of the rachis (Fig. 8, G).

The MESOPHYLL has very simple structure, is undifferentiated and consists of rounded or somewhat elongated cells, measuring about H 10 to 30 μ, Lev B 10 to 30 μ and Lev L 10 to 30 μ, and containing chloroplasts about 4 to 8 μ in diameter. Occasional idioblasts occur, containing small cluster crystals of calcium oxalate about 10 to 35 μ in diameter. Towards the base of the stipule, the hypodermal tissue near the margin is collenchymatous (Fig. 8, B, C and D).

The VENATION is very simple, consisting of a central midrib and minute, pinnate, slightly anastomosing secondary veinlets. The midrib consists of a few xylem elements about 10 μ in diameter; there is but little phloem; the pericycle is evident and consists of a very few fibres at the base of the stipule, but of lignified parenchyma only throughout almost its whole length (Fig. 8, C).

POWDER

A No. 60 powder is green in colour; it has a very slight odour and a not unpleasant, very slightly astringent taste. When some of the powder is mixed with ferric chloride solution, a greenish-black colour is observed.

To examine the powder for structural features, it was mounted in 50 per cent v/v glycerol solution, solution of chloral hydrate, or phloroglucin and hydrochloric acid. The diagnostic characters (Fig. 9) are:—

Numerous large, straight or slightly curved, lignified fragments of *covering trichomes* from both surfaces of the lamina, up to about 40 μ wide, apical fragments being acutely pointed and solid, whilst basal fragments are hollow and pitted with simple pits; most fragments show double-spiral markings very prominently; *fragments of the lamina*, showing a

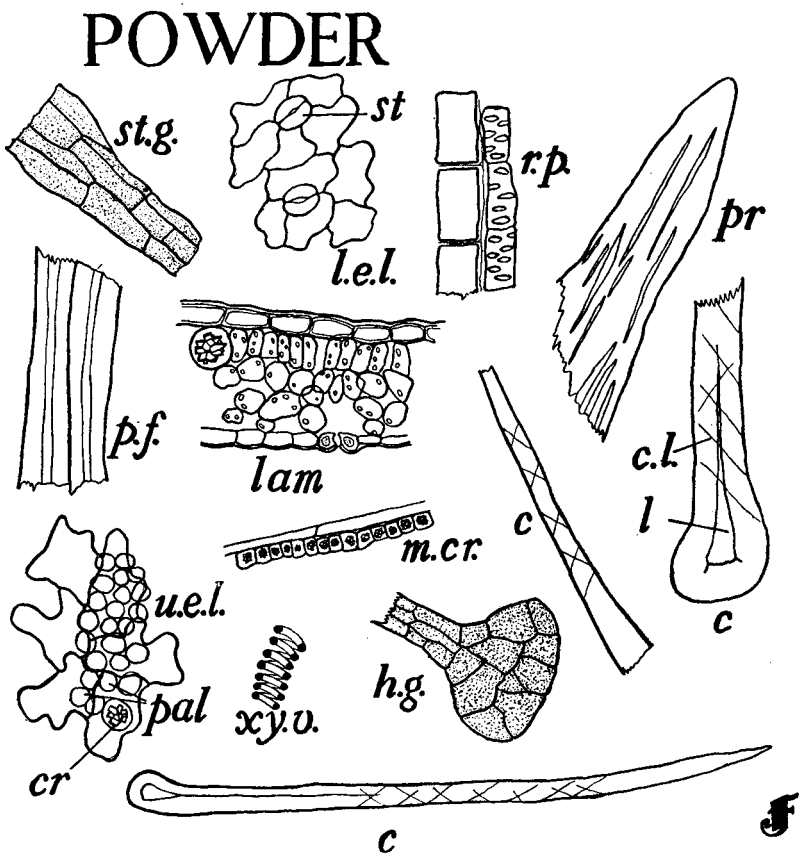

FIG. 9. Powder of *Rubus fruticosus* L. All x 200. *c*, covering trichome; *c.l.*, crossed-line effect; *cr*, cluster crystal of calcium oxalate; *h.g.*, head of large, glandular trichome; *l*, lumen; *lam*, fragment of lamina in transverse section, showing upper and lower epidermises, stoma, idioblast with cluster calcium oxalate crystal, palisade and spongy mesophyll; *l.e.l.*, lower epidermis of lamina; *m.cr.*, file of microcluster crystals of calcium oxalate, adjacent to phloem tissue; *pal*, palisade; *p.f.*, fragments of pericyclic fibres; *pr*, apex of prickle; *r.p.*, reticulate parenchyma; *st*, stoma; *st.g.*, stalk of glandular trichome; *u.e.l.*, upper epidermis of lamina; *xy.v.*, fragment of xylem vessel.

transverse sectional view, about 90 μ thick, the palisade containing rounded idioblasts, each containing a crystal of *calcium oxalate* about 12 to 50 μ in diameter; particles showing in surface view the wavy-walled cells of the *upper interneural epidermis* of the lamina and, usually, immediately beneath it, the palisade with its idioblasts; fragments showing the *lower interneural epidermis* consisting of wavy-walled cells with numerous anomocytic (ranunculaceous) stomata which are sometimes surrounded by radiating epidermal cells; fragments of the *veins* showing small lignified vascular elements showing annular, spiral, reticulate and pitted thickenings, and occasional files containing microclusters of *calcium oxalate*; *reticulated parenchymatous* cells in vertical files; fragments of prickles consisting of fibre-like *lignified sclereids* with oblique or transverse linear pits; numerous, usually broken, *glandular trichomes*, with multicellular, multiseriate stalks and yellowish-brown multicellular, glandular heads.

Discussion

We have found that the histological structure of the leaf of *R. fruticosus* L. is basically similar to that of *R. idaeus* L. Certain diagnostic characters, such as the incidence of hydathodes in the marginal teeth, of anomocytic (ranunculaceous) stomata on the lower epidermis of the lamina and of a smooth cuticle on the epidermises of the lamina, are common to leaves of both species. There are, however, several well-contrasted features; these are found in (*a*) the epidermal characters, and (*b*) the tissues of the midrib and rachis.

(*a*) *Epidermal characters*

The anticlinal walls of the cells of the upper epidermis of the leaflets of *R. fruticosus* are beaded, whilst the covering trichomes show the double-spiral markings very prominently, when mounted in chloral hydrate solution; neither of these features are normally observed in *R. idaeus*. The tomentum of hairs of the lower epidermis of the leaflets of the latter is absent from *R. fruticosus*, but the occasional two-celled covering trichomes, and the *bent* glandular trichomes found commonly on the lower surface of the midribs of the blackberry leaflets afford further differential characters; the epidermises of the rachis and stipules are also strongly characterised by the great numbers of *large*, multiseriate glandular trichomes.

(*b*) *Midrib and Rachis*

The inner cortex of the midrib and rachis of *R. fruticosus* contains many reticulated, slightly lignified, parenchymatous cells; these are absent from *R. idaeus*, but the detection of rachis in blackberry leaf powder cannot be based on the presence of pericyclic fibres, because in this case they are also present in the leaf midrib and veins; the presence of the large, multiseriate glandular trichomes mentioned above affords better evidence of the presence of rachis. The latter usually shows, moreover, an almost complete ring of vascular bundles in transverse sections.

It must be emphasised that the foregoing anatomical description applies only to the leaves taken from one particular clone of plants belonging to the section *Radulae* of the fruticose brambles. From other descriptions[11,12,22], and from our own observations on leaflets from other plants, it is apparent that considerable variations in the detailed structure are probable throughout the 300 species which have now been named. The diagnostic characters listed must not, therefore, on any account be taken as specific for all forms of *R. fruticosus* L.

Summary

Blackberry leaves collected from wild plants were used for this investigation, and, although their anatomy is described under the specific name of *R. fruticosus* L., they have been identified as belonging to the section *Radulae* Focke, described by Watson[19]. They are characterised by the following features:—

1. The *epidermises* of the leaflets. The *upper epidermis* consists of cells with wavy anticlinal walls and bears scattered, unicellular, lignified, *covering trichomes* with acute, solid apices and thickened, pitted bases. The *lower epidermis* consists of cells with slightly beaded, wavy anticlinal walls and also bears *covering trichomes* similar to those on the upper epidermis. *Glandular trichomes* are present on the lower epidermises of the midrib and main veins; they consist of a uniseriate, multicellular stalk of about 6 to 10 cells, and a two- to four-celled glandular bead, and frequently are bent over; occasional biseriate glandular trichomes also occur.

2. The epidermises of the rachis and stipules bear *large*, characteristic, multicellular, multiseriate, glandular trichomes.

3. The *lamina* of the leaflet is thin and dorsiventral, with a single row of palisade in which are rounded idioblasts, each containing a cluster crystal of *calcium oxalate*.

4. The *midrib* of the leaflet contains a meristele consisting of annularly and spirally thickened *protoxylem tracheids*, spiral and sometimes reticulate and pitted *vessels*, and fibre-like, pitted *tracheids* of the metaxylem, a phloem of simple sieve-tubes with transverse or oblique sieve-plates, and rows of parenchymatous cells in longitudinal files, each cell containing a micro-cluster of *calcium oxalate*.

5. *Prickles* of midrib and rachis are composed of lignified, fibre-like sclereids.

6. Lignified pericyclic fibres are abundant and common to both leaflets and rachis; the latter may be recognised when in powder, however, by the presence of slightly larger and more extensively thickened *vascular elements* and by the numerous, *large*, multicellular, multiseriate, glandular trichomes.

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REFERENCES

1. *The Greek Herbal of Dioscorides*; Englished by John Goodyear, 1655; edited and first printed, 1933, by Robert T. Gunther, Oxford, 1934, p. 432.
2. *Ibid.*, p. 431.
3. *The Herbal of Apuleius Barbarus* (M.S. Bodley 130); Described by R. T. Gunther (a facsimile), Oxford University Press, 1925, p. 26.
4. Gerard, *The Herbal or, Of the History of Plants*, Norton and Whittakers, London, 1633, p. 1271.
5. Cruso, *A Treasury of Easy Medicines*, London, 1771, p. 250.
6. Grieve, *A Modern Herbal*, Jonathan Cape, London, 1931, Vol. I, p. 108.
7. Sowerby's *English Botany*, edited by J. T. B. Syme, 3rd Ed., Vol. III, Hardwicke, London, 1864.
8. Bentham and Hooker, *Handbook of the British Flora*, 7th Ed., Reeve, Ashford (Kent), 1930, p. 139.
9. Clapham, Tutin and Warburg, *Flora of the British Isles*, Cambridge University Press, 1952, p. 463.
10. Clapham, Tutin and Warburg, *ibid.*, 1952, p. 466 *et seq.*
11. Thoms, *Handbuch der praktischen und wissenschaftlichen Pharmazie*, Vol. V, Urban & Schwarzenberg, Berlin und Wien, 1929, p. 1024.
12. Moeller-Griebel, *Mikroskopie der Nahrungs- und Genussmittel aus dem Pflanzenreiche*, 3rd Ed., Springer, Berlin, 1928, p. 366.
13. Wasicky, *Leitfaden für die Pharmakognostischen Untersuchungen in Unterricht und in der Praxis*, Vol. II, Franz Durticke, Leipzig und Wien, 1936, p. 57.
14. Jaretsky, *Lehrbuch der Pharmacognosie*, 2nd Ed., Vieweg, Braunschweig, 1949, p. 191.
15. Gilg, Brandt und Schurhoff, *Lehrbuch der Pharmacognosie*, 4th Ed., Springer, Berlin, 1927, p. 188.
16. Tucakov, *Pharmacognosy*, Scientific Books, Belgrade, 1948, p. 402.
17. Horhammer, *Teeanalyse*, 1st Ed., Institut für Pharmazeutische Arzneimittellehre der Universität, Munchen, 1955, p. 10.
18. Berger, *Handbuch der Drogenkunde*, Band II, Maudrich, Wien, p. 288.
19. Gassner, *Microskopische Untersuchung pflanzlicher Nahrungs- und Genussmittel*, 3rd Ed., Fischer, Stuttgart, 1955, p. 237.
20. Clapham, *Check-List of British Vascular Plants* (Reprinted from the *Journal of Ecology*, **33**, No. 2 (1946), 308–47), Cambridge University Press, 1946.
21. Fell and Rowson, *J. Pharm. Pharmacol.*, 1956, **8**, 334.
22. Fell and Rowson, *J.R. micr. Soc.*, 1955, **75**, 111.
23. Engard, *Organogenesis in Rubus*, University of Hawaii Research Publication, No. 21, Honolulu, 1944, p. 220.