

ANATOMICAL STUDIES IN THE GENUS *RUBUS*

PART IV. ANATOMICAL VARIATIONS IN THE LEAVES OF CULTIVATED VARIETIES OF *R. idaeus* L. AND *R. loganobaccus* L. H. BAILEY, AND OF CERTAIN SPECIES OF BRAMBLE

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Differential anatomical characters of the leaves of cultivated varieties of *R. idaeus* L. and *R. loganobaccus* L. H. Bailey, and of certain species of bramble, are described and illustrated. The comparative anatomical characters of raspberry, blackberry and loganberry leaves, including these varieties and species, is discussed.

THE anatomy of the leaves of wild plants of *R. idaeus* L., cultivated plants of *R. loganobaccus* L. H. Bailey and a wild bramble has been described in earlier communications (Fell and Rowson, 1956; 1957; 1960). There are many well-established cultivated varieties of *R. idaeus* and commercial samples of raspberry leaves are invariably derived from cultivated crops, the thornless variety of *R. loganobaccus* occurs in cultivation, moreover 386 species of indigenous brambles have now been recognised by Watson (1958). To determine if the leaves of certain of these varieties and species could be distinguished amongst themselves and from the materials already described, an investigation was carried out on leaves of eight well-established commercial varieties of *R. idaeus*, on the thornless variety of *R. loganobaccus*, and on seventeen species of bramble, representative of the seven Sections of the Sub-genera *RUBUS* (*MORIFERI* Focke), *GLAUCOBATUS* \times *RUBUS* and *IDAEOBATUS* \times *RUBUS* (Thoms, 1931).

MATERIALS

Authenticated leaf materials of *R. loganobaccus* L. H. Bailey, thornless variety and of *R. idaeus* L., commercial varieties *Lloyd George*, *Malling Enterprise*, *Malling Exploit*, *Malling Jewel*, *Malling Promise*, *Newburgh* and *Norfolk Giant* were supplied from the Gardens of the Royal Horticultural Society, Wisley, Surrey. Leaves of *R. idaeus*, variety *St. Walfried* were obtained from the East Malling Research Station, Kent. Leaves of the following British brambles were provided by a taxonomist specialising in this genus and selected to represent all seven Sections recognised by Watson (1958) in the three Sub-genera comprising this group (shown on following page.)

EXPERIMENTAL

The techniques employed for the examination of all materials were those previously described (Fell and Rowson, 1956; 1957; 1960).

Leaflets, rachis and stipules of the eight commercial varieties of *R. idaeus* were examined by means of surface preparations and by sections,

The subject matter of this communication forms part of a thesis by one of us (K.R.F.) accepted by the University of London for the Degree of Doctor of Philosophy.

Sub-genus	Section	Species
<i>IDAEOBATUS</i> <i>x RUBUS</i>	SUBRECTI	<i>R. fissus</i> , Lindl.
<i>GLAUCOBATUS</i> <i>x RUBUS</i>	TRIVIALES	<i>R. corylifolius</i> Sm., <i>R. bucknallii</i> White, <i>R. balfourianus</i> , Blox ex Bab.
<i>RUBUS</i> (MORIFERI Focke)	SYLVATICI	<i>R. schlechtendalii</i> Whe., <i>R. pyramidalis</i> Kalt, <i>R. hirtifolius</i> Muell. & Wirtg., <i>R. danicus</i> Focke., <i>R. pubescens</i> , var. <i>subinermis</i> Rogers, <i>R. cornubiensis</i> Rils.
	DISCOLORES	<i>R. lentiginosus</i> Lees
	SPRENGELIANI APPENDICULATI	<i>R. thurstonii</i> Rilst., <i>R. fuscus</i> , var. <i>macrostachys</i> , <i>R. nuticeps</i> , Barton & Ridd.
	GLANDULOSI	<i>R. koehleri</i> Weihe, <i>R. hystrix</i> Weihe, <i>R. cognatus</i> N.E. Br.

the rachis being cut above and below the point of insertion of the lateral leaflets. Particular attention was directed to the characters of the epidermal cells, cuticle, stomata, hydathodes and trichomes, and also the arrangement of vascular and fibrovascular tissue and meristemes. The majority of the structural characters of wild *R. idaeus*, already described

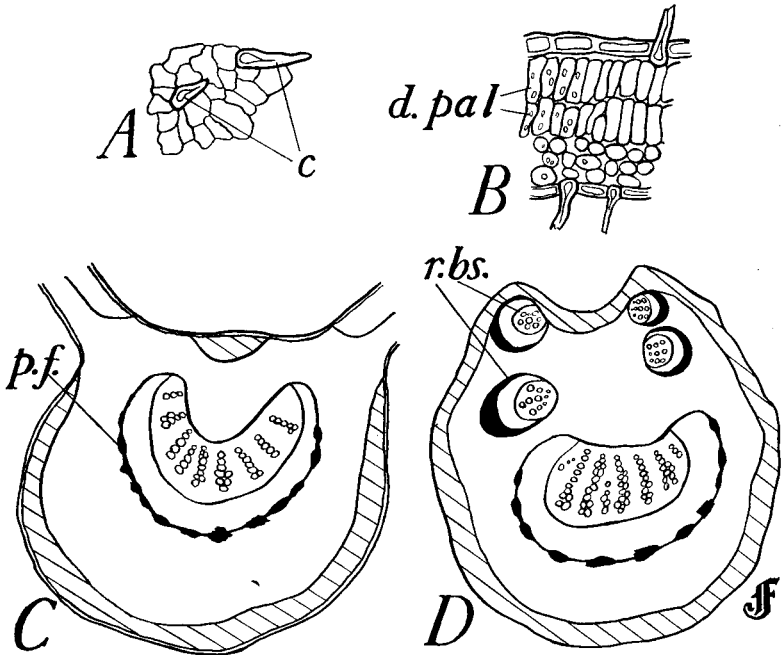


FIG. 1. Leaves of cultivated varieties of *R. idaeus* L. *A*, var. *Lloyd George*. Upper epidermis of internodal lamina, showing short, covering trichomes. *B*, var. *Malling Enterprise*. Transverse section of internodal lamina, showing well-formed double palisade. *C*, var. *Malling Jewel*. Transverse section of midrib of leaflet cut near base, showing pericyclic fibres. *D*, var. *Newburgh*. Transverse section of upper rachis, cut near the top, showing four fibro-vascular ridge bundles. *A* and *B* x 200; *C* and *D* x 25. *c*, covering trichomes; *d. pal*, double palisade; *p.f.*, pericyclic fibres; *r.bs.*, ridge bundles.

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and illustrated (Fell and Rowson, 1956), were found in the eight commercial varieties. Several differences from the wild material were also noted, these are given in Table I and are illustrated in Fig. 1.

Preliminary examination of the 17 bramble species showed a uniform anatomical pattern, similar to that already described and illustrated in this series of papers for a *Radula* sp. of bramble (Fell and Rowson, 1957). Some variations in armature have been reported by Thoms (1931) and Moeller-Griebel (1928a) and our bramble materials also showed such variations in trichome structures, thus detailed investigations were

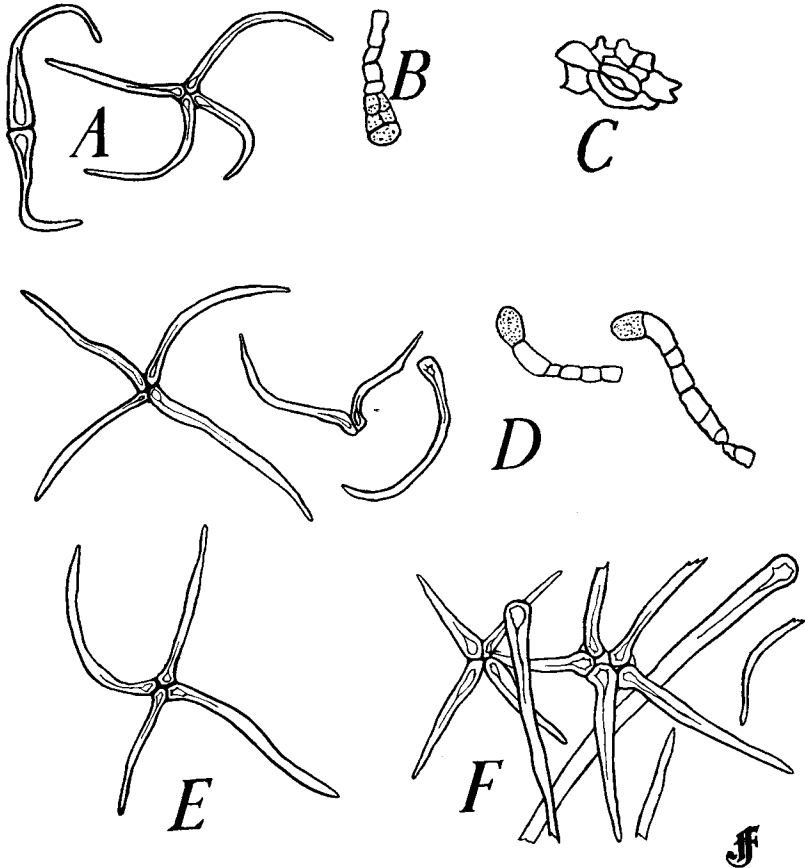


FIG. 2. Hydathode and trichomes from the leaves of certain bramble species. *A*, 2- and 4-celled covering trichomes from the lower epidermis of the lamina of leaflet of *R. corylifolius*. *B*, Glandular trichome from the lower epidermis of leaflet of *R. bucknallii*. *C*, Hydathode from upper epidermis of leaflet of *R. pyramidalis*, seen some distance in from the edge of the lamina. *D*, 1-, 2- and 4-celled, unligified, covering trichomes, and glandular trichomes from the lower epidermis of lamina and midrib, respectively, of leaflet of *R. hirtifolius*. *E*, 4-celled, stellate, covering trichome from the lower epidermis of leaflet of *R. danicus*. *F*, Fragment of the tomentum of lignified, covering trichomes from lower epidermis of leaflet of *R. thurstonii*. All x 200.

directed to the upper and lower epidermises of lamina and midrib of each species. The characteristic unicellular, lignified covering trichomes with well-marked crosslines and the different glandular trichomes of bramble (Fell and Rowson, 1957) were found in the 17 species examined. In addition, certain differential characters were noted and these are summarised in Table II and illustrated in Fig. 2.

TABLE I
ANATOMICAL CHARACTERS DISTINGUISHING CERTAIN CULTIVATED VARIETIES FROM WILD LEAVES OF *Rubus idaeus*

Variety	Covering trichomes of			Transverse section	
	Leaflet upper surface	Rachis	Stipules	Leaflets	Rachis
<i>Lloyd George</i>	30-50 μ long (Fig. 1, A)	Lignin \pm	Lignin very slight	Double palisade	
<i>Malling Enterprise</i>			lignin \pm	Very well-marked double palisade (Fig. 1, B)	
<i>Malling Exploit</i>		Lignin \pm	Lignin \pm	Well marked double palisade. Lignified pericyclic fibres also pitted vascular elements in some leaflets (Fig. 1, C)	
<i>Malling Jewel</i>	Lignin \pm		Well marked double palisade		
<i>Malling Promise</i>	Sparsely scattered		Well marked double palisade	At top of rachis two ridge bundles in each of two ridges commonly found	
<i>Newburgh</i>			Lignin \pm	Very well marked double palisade	
<i>Norfolk Giant</i>				Double palisade	
<i>St. Walfried</i>				Double palisade	

Note: lignin \pm means that the covering trichomes gave only a slight reaction for lignin.

The structure of the epidermises of the leaflet and rachis of the thornless variety of *R. loganobaccus* was investigated, the results are summarised in Table III.

DISCUSSION AND CONCLUSIONS

A. GENERAL

In the work described above, and in the earlier work, it has been shown that the morphological and anatomical characters of the leaves of *R. idaeus* L. (wild form and eight cultivated varieties), *R. fruticosus* L. (18 wild British species according to Watson's classification (1958)) and *R. loganobaccus* L. H. Bailey (two cultivated varieties) are similar in most general respects, but that there are differences in detailed structures, some of which are of diagnostic value.

B. MORPHOLOGY

The leaves of all varieties and species examined are imparipinnately compound, usually with five leaflets, but sometimes three or, occasionally,

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TABLE II

EPIDERMAL CHARACTERS DISTINGUISHING CERTAIN SPECIES OF BRAMBLE

Species	Upper epidermis	Lower epidermis	
		Stellate trichomes number of arms	Glandular trichomes
<i>Rubus fissus</i> ..		2	Multicellular head on multicellular uniseriate stalk, occasional collapsed cell in stalk (Fig. 2)
<i>R. corylifolius</i> ..		2-6	
<i>R. bucknallii</i> ..		2-5	
<i>R. balfourianus</i> ..		2	
<i>R. schlechtendalii</i> ..	Trichomes rare except on midrib and veins	2-8	
<i>R. pyramidalis</i>	Few trichomes on margins only Hydathodes prominent (Fig. 2)	Abundant unicellular trichomes varying much in length and in amount of lignification	Abundant with unicellular head on multicellular uniseriate stalk
<i>R. hirtifolius</i> ..	Trichomes rare except on midrib and veins	2-4 (on midrib lignin ±)	Unicellular head on multicellular uniseriate stalk, frequently with collapsed cell in stalk (Fig. 2) As <i>R. pyramidalis</i>
<i>R. danicus</i> ..		2-6 (Fig. 2)	
<i>R. pubescens</i> var. <i>subnervis</i> ..	Glandular trichomes rare	2-5	
<i>R. cornubiensis</i> ..	Trichomes rare	2-4	
<i>R. lentiginosus</i> ..	Few trichomes near the margin only	2	
<i>R. thurstonii</i>	Covering trichomes mainly confined to the veins	2-8 Covering trichomes forming a dense tomentum	Abundant on interneural surface and on veins
<i>R. fuscus</i> var. <i>macrostachys</i> ..		2-6 as <i>R. thurstonii</i>	Few
<i>R. ruticeps</i> ..		2-6	
<i>R. koehleri</i> ..		2-4	
<i>R. hystrix</i> ..		2-4	
<i>R. cognatus</i> ..		2-4	

TABLE III

ANATOMICAL CHARACTERS OF *R. loganobaccus* AND OF A THORNLESS VARIETY

	<i>R. loganobaccus</i>	<i>R. loganobaccus</i> (thornless)
1. <i>Sclereids</i>	Present, as described*	Absent
2. Upper epidermis of lamina	Cells beaded	Cells not beaded
Lower epidermis of lamina	Tomentum: hairs only give a positive reaction for lignin with phloroglucin and hydrochloric acid after preliminary treatment with ether	Very hairy tomentum; hairs give a positive reaction for lignin with phloroglucin and hydrochloric acid without difficulty and without preliminary treatment with ether
4. Lower epidermis of midrib	Cells beaded. Covering hairs give a positive reaction for lignin only after preliminary treatment with ether	Cells very slightly beaded, covering hairs plentiful, lignified and tending to be tomentose
5. Epidermis of rachis ..		Cells particularly well beaded; covering hairs lignified and very numerous

* Fell and Rowson, 1960.

seven. Individual *leaflets* have sharply pointed apices, the margin is coarsely serrate-dentate with mucronate teeth and the base cordate. The venation is pinnate; each vein terminates at the apex of one of the marginal teeth. The lamina is hairy in all species, but much more so on the lower surface; since the dense tomentum on the lower surface of the

lamina of leaflets of *R. idaeus* imparts a distinctly white colour to this surface, this latter is a valuable macroscopical diagnostic character, even when the leaves are in a coarsely chopped and dried condition, as is commonly the case with commercial material. This character is not seen in the leaves of *R. loganobaccus*; those of the bramble species vary in appearance (due to the greatly differing degrees of hairiness encountered in different species) but in no species does the underside of leaflets appear

TABLE IV
KEY FOR MACROSCOPICAL IDENTIFICATION OF SPECIES

1. Colour	Leaves, or fragments of lamina showing white lower surface	2
	Not as above	3
2. <i>R. idaeus</i> :	odour pleasantly aromatic; confirmed by histological examination (see Table V)	
3. Prickles	Present	4
	Absent	5
4. <i>R. loganobaccus</i>	or a species of bramble	6
5. <i>R. loganobaccus</i>	thornless variety: confirm by histological examination (see Table V)	
6. Leaves green;	leaflets (if leaves whole) large, and usually five in number <i>R. loganobaccus</i> —confirm by histological examination (see Table V)	—
	Leaves varying in colour from green to greyish-green, brownish-green, light brown or greyish-white; leaflets not large .. <i>Sp. of bramble</i> —confirm histologically (see Table V)	

white (see Table IV). Terminal leaflets are, in general, larger than the lateral ones and are symmetrical; lateral leaflets are slightly asymmetrical, and are sessile on the rachis in the cases of *R. idaeus* and *R. loganobaccus*, but this detail is very variable in the brambles, where (depending on the species) either or both lateral and basal leaflets may be stalked. Leaflets of *R. loganobaccus* were the largest of any species examined, but several

TABLE V
KEY FOR HISTOLOGICAL IDENTIFICATION OF SPECIES

1. All of the trichomes of the lower interneural epidermis unligified (even after treatment with ether or chloral hydrate solution) and forming a tomentum	2
Some, or all, ligified (after treatment with ether)	3
2. <i>R. idaeus</i> or variety.	
3. Trichomes of the lower interneural lamina ligified (after treatment with ether) and forming a loose tomentum. No stellate trichomes	4
Covering and glandular trichomes present, former frequently stellate; some covering trichomes, at least, ligified	7
4. Sclereids present	5
Sclereids absent	6
5. <i>R. loganobaccus</i> .	
6. <i>R. loganobaccus</i> , thornless variety.	
7. Species of bramble. Refer to Table II.	

leaflets of cultivated varieties of *R. idaeus* were almost as large, and mere size cannot be considered as a useful differential character between these two species.

The rachis does not exhibit any significant variation in external structure; in specimens examined, its dimensions always lay between limits of 4 to 15 cm. in length, and 1 to 5 mm. in width, the latter increasing from top to base. It is terete except for a single groove running down the middle of the upper surface.

The elongated-conical, laterally compressed prickles found on the midrib and rachis are common to all species and varieties examined with

the single exception of the thornless variety of *R. loganobaccus* (see Table IV). The precise shape, size and frequency of the prickles vary with the species or variety, but such variations have not been used to distinguish between the 28 species or varieties possessing prickles and described in this series of papers.

It is not considered that the *stipules* have any differential diagnostic significance in the species and varieties so far examined.

The *sensory characters* are of greatest value in distinguishing commercial samples of leaves of *R. idaeus*, fragments of which show a green upper surface and a white lower surface. The colour of bramble leaves varies greatly with the species and age of the specimen—from green to greyish-green, brownish-green, pale brown, and, in the case of the lower surface, occasionally greyish-white (but not white); leaves of *R. loganobaccus* are green. The taste, for all species and varieties, is astringent, due to the high tannin content, the odour is distinctive in the case of *R. idaeus*, dried samples of which are quite pleasantly aromatic; the texture of all leaves in the dried condition is fragile. These differential morphological and sensory characters are summarised in Table IV as a key for the identification of the species here considered.

C. ANATOMY

A number of anatomical characters are common to the 29 species and varieties examined, these are the general distribution of tissues, similarity in sizes of corresponding cells (except trichomes, see below), stomatal type and distribution, hydathodes, also the cell inclusions of calcium oxalate and starch granules. Characters which may be employed for the distinction of the species and varieties from each other are principally those of the epidermis (trichomes, epidermal cells and prickles) but also include certain characters of the mesophyll, cortex and stele. They will be discussed under the following headings, and they are employed in Tables II and V.

(a) *Epidermal tissues*

(1) *The trichomes*. The present work and other records (Thoms, 1931; Moeller-Griebel, 1928b) show that, in general, the *types* of trichome found on the leaves of the brambles serve adequately to distinguish them from the leaves of *R. idaeus* and *R. loganobaccus* but, as shown previously (Fell and Rowson, 1957) and in Table II, there is a considerable variation in character (e.g., whether single or stellate), location, frequency and distribution of both covering and glandular trichomes between leaves of different species of the brambles. It is possible that still further variation in the epidermal characters exists in leaves of other indigenous species of bramble recognised by Watson (1958). The considerable infra-specific variation in detailed anatomical characters, especially of the trichomes, which we have found within *R. fruticosus* L. does lend support to the work of Watson in splitting up this Linnaean species.

Covering trichomes only were found on the *upper epidermis* in the *interneural* region of leaflets of all leaflets examined; they were abundant

except in *R. idaeus* var. *Malling Promise*, in *R. schlechtendalii* and in *R. lentiginosus* where they were rare and in *R. cornubiensis* where they were confined to the margin of the leaflet. The trichomes are unicellular, lignified (except in *R. idaeus* var. *Malling Jewel* which only gave a slight reaction for lignin), thick walled, the cross-line effect being well marked in the brambles and in *R. loganobaccus*, less well-marked in cultivated varieties of *R. idaeus* and absent in the wild form of this species. The trichome apex is often solid, the lumen extending only about half-way up the arm of the hair; the base is thickened and pitted, adjacent epidermal cells being arranged in a radiate manner around the base, this being well-marked in *R. idaeus* and *R. loganobaccus*. The lengths of the hairs varied between species and varieties, in *R. idaeus* they were 200 to 500 μ (except var. *Lloyd George* which were 50–60 μ), 300 to 1,000 μ in the brambles and 180 to 1,400 μ in *R. loganobaccus*.

Both covering and glandular trichomes were found on the *upper epidermis of the midrib* in all materials except *R. pyramidalis*. The covering trichomes are similar to those of the interneural lamina; the glandular trichomes consist of a subspherical, multicellular glandular head about 60 μ in diameter, on a uni-or bi-seriate stalk, 3 to 7 cells long.

The trichomes of the lower epidermises of the laminae and midribs give the clearest demarcation between leaves of *R. idaeus* and its cultivated varieties, the brambles and *R. loganobaccus*. Thus *R. idaeus* is characterised by the presence of abundant *unlignified* covering trichomes forming a tomentum. They are unicellular, about 150 to 500 μ long, usually with the lumen obliterated at the upper end only. *The brambles* show great variation within the 18 species examined; the covering trichomes differ much in size and are either unicellular or 2 to 8 stellate, the former being the more common on interneural laminae whilst the latter are usually found over the veins. The glandular trichomes also vary in these species. Table II indicates the range of variation and by comparing it with the tabulation of materials examined it is seen that no relationship exists between trichome structure and the taxonomic divisions of species, Sections and Sub-genera. As might be expected from its hybrid nature, the trichomes of the lower lamina of *R. loganobaccus* are somewhat intermediate in character between those of *R. idaeus* and the brambles (Fell and Rowson, 1960). They are mainly of the covering type, about 300 to 700 μ long, and form a tomentum which is less dense than that found in *R. idaeus*. They give a positive reaction for lignin with phloroglucinol and hydrochloric acid, only after preliminary treatment with ether or chloral hydrate solution, whereas the trichomes of the lower surface of the leaflet of *R. idaeus* will not stain, even after this treatment, and the corresponding trichomes of the brambles give a positive reaction without any such preliminary treatment (see Table V).

The trichomes on the *rachis* are of the covering type in *R. idaeus* and *R. loganobaccus*, where they resemble those on the midrib. Both covering and glandular types were seen in the *Radula* sp. of bramble examined earlier (Fell and Rowson, 1957), where it was shown that the glandular trichomes were of larger size and different character from those present

on the midrib of the leaflet, thus providing a useful method for detecting rachis in powdered bramble leaflet.

The trichomes of the *stipules* are mainly of the covering type in *R. idaeus* and *R. loganobaccus* but large numbers of both types occurred in the *Radula* sp. of bramble where the glandular ones were large, resembling those of the rachis. Covering trichomes are lignified and show the "cross-line" effect even in the case of the wild specimen of *R. idaeus*.

(2) *Epidermal cells*. In all species examined, the cells of both upper and lower epidermises of the lamina exhibit wavy anticlinal walls, the waviness being more pronounced on the cells of the lower epidermis. Slight beading was observed on cells of the *Radula* sp. of bramble; beading is well-marked on cells of both upper and lower lamina of *R. loganobaccus*, where it forms a distinctive character.

(3) *Prickles*. These are very common throughout the genus. In the species examined, they consist of elongated or fibre-like sclereids, lignified, and with pitted walls. The presence of these sclereids, or their fragments, is therefore to be expected in powders of all species examined, with the single exception of *R. loganobaccus*, thornless variety (see Table V).

(b) *Mesophyll*

In all cases, the mesophyll is well differentiated; in *R. loganobaccus* and all cultivated varieties of *R. idaeus*, there is a well-marked double palisade; in the wild plant of the latter, there is a tendency towards this condition, but the double palisade appears to be a feature associated with cultivation. Calcium oxalate is very common.

(c) *Cortical tissues*

The cortical tissues of both rachis and midrib follow a similar pattern in all species examined. The *endodermis* is rather ill-defined, and its cells contain minute starch grains; in the *Radula* sp. of bramble it is further characterised by the presence of lignified reticulate parenchyma which we have not observed in specimens of *R. idaeus* or *R. loganobaccus*.

(d) *Stelar tissues*

The *xylem* and *phloem* elements are similar in all species examined. *Pericyclic fibres* have also been found in every specimen of rachis examined. In the midrib, pericyclic fibres were found in some, but not all leaflets of *R. idaeus* var. *Malling Jewel*, they were absent from the other seven cultivated varieties and the wild form of this species; they were also present in some, but not all leaflets of *R. loganobaccus* (Fell and Rowson, 1960) and were always present in the *Radula* sp. of bramble (Fell and Rowson, 1957). Since the amount of fibre present in a leaf can vary with age, the diagnostic significance of presence or absence of pericyclic fibres in the leaf midrib is not stressed.

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