

Secondary thickening of stem in species of *Atragene* L. (= *Clematis* sect. *Atragene*, Ranunculaceae)

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Summary: The secondary thickening of the stem in woody vines of *Atragene* is maintained by the fascicular and interfascicular cambium and by the phellogen. The presence of the interfascicular cambium is confirmed, though it is hardly distinguishable from phelloderm and phellogen. This cambium mostly produces inner ray parenchyma. However, it yearly gives rise to the outer layer of parenchyma cells generating the basis for the next phellogen. The bark ring resultantly develops. A set of large tangential schizogene cavities is formed in every ray of the perennial stem. Ray dilatation results from the extension of periderm cells and not from the extension and proliferation of ray parenchyma cells. The ray cavities could be reservoirs for freezing tissue water to survive winter.

Keywords: Ranunculaceae, *Atragene*, secondary thickening, medullary ray, interfascicular cambium, phellogen, phelloderm, cork, bark

Atragene comprises about 10 species (YANG et al. 2009). It is a small northern temperate and subalpine segregate of mostly tropical *Clematis* s.l., the vast majority of which are woody lianas (REHDER 1949). Their stems were invariably reported to retain lifelong the fascicular stele (STERKX 1897; SMITH 1927; METCALF & CHALK 1950), but their secondary thickening was contradictory described. The interfascicular cambium was described to be inherent in some species and absent in others (METCALF & CHALK 1950).

All species of *Atragene* are deciduous woody lianas with dimorphic elongated and short shoots (Fig. 1) which usually live more than 10 years. They also lifelong retain the fascicular stele and have large cavities in their medullary rays (BARYKINA & CHUBATOVA 1983). Accordingly, *Atragene* species were thought to have no interfascicular cambium as some other *Clematis* species. However, the woody lianas retaining lifelong fascicular stele typically have both fascicular and interfascicular cambium (OBATON 1960). *Atragene* species are unlikely to maintain a secondary thickening without interfascicular cambium for a dozen of years. Therefore, the secondary thickening of their stem is worth being reinvestigated.

Materials and methods

The shoots of *A. speciosa* Weinm. (= *A. sibirica* L., nom. ambig. (SEROV & JARVIS 1988)) and *A. ochotensis* Pall. were sampled from naturally occurring plants in mixed forests near Lake Shchuchye, Buryatia and Sikhote Alin Reserve, Primorsky Region, in 1990. Shoots of the former species were subsequently sampled from plants grown from seeds in the Botanical Garden of Lomonosov Moscow State University.

Annual to triennial stems of both elongated and short shoots were fixed in 70% ethyl alcohol at the beginning and the middle of the growing season, transversely sectioned manually by razor blade and processed with phloroglucinol and hydrochloric acid and embedded into glycerol on

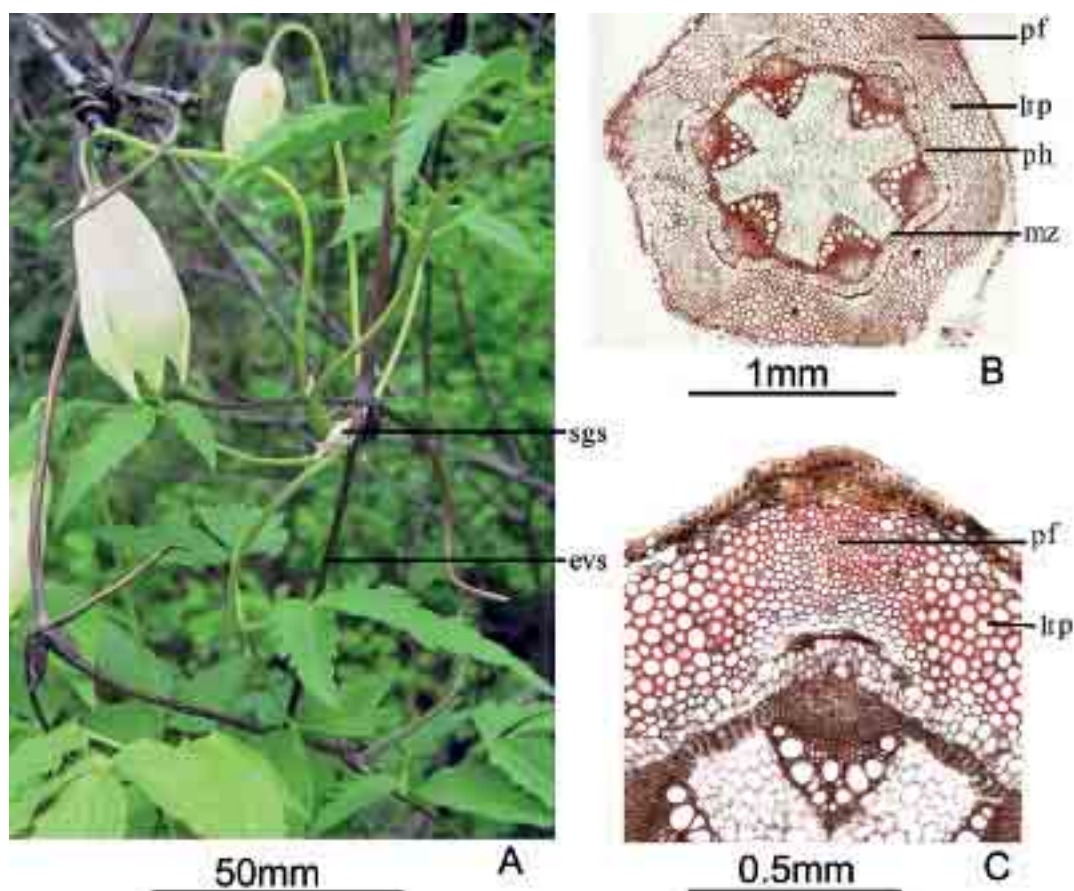


Figure 1. *Atragene speciosa*. A – dimorphic shoots; B – cross section of the annual stem of elongated shoot; C – mechanical ring. evs – elongated vegetative shoot; sgs – short generative shoot; pf – protophloem fibers; lrp – lignified ray parenchyma; ph – cork; mz – meristematic zone.

the slides according to standard technique (BARYKINA et al. 2004). The sections were examined under Axioplan-2 Imaging light microscope, digital photographs were taken with built-in AxioCam MRc camera and edited with Adobe Photoshop to remove external debris and to enhance contrasts but not otherwise manipulated.

Results

Annual stems of elongated and short shoots (Fig. 1A) have invariably 6 collateral vascular bundles separated by wide and high medullary rays of roundish parenchyma cells (Fig. 1B) the inner ones being slightly lignified. A peripheral ring of mechanical tissue consists of amalgamated protophloem fibers and lignified outer ray parenchyma in between the protophloem strands (Fig. 1C).

The secondary thickenings of elongated and short shoots differ only quantitatively, the former exceeding much more. The fascicular cambium is distinguishable in stems collected in early growing season though it develops a 5–7-layered zone of similar meristematic cells by the middle of growing season when real cambial initials become indiscernible. This cambium produces only vertical constituents of the secondary xylem and secondary phloem, but no secondary ray cells (Figs 1B; 4A, B).

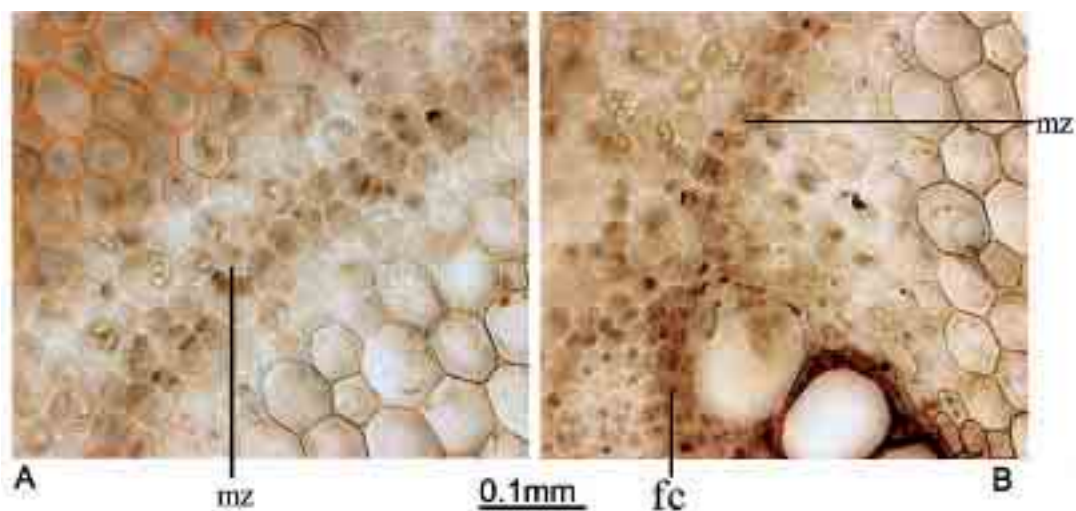
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Figure 2. Annual stem of *Atragene ochotensis*. A – the development of interfascicular meristem; B – the connection between the interfascicular meristem and the fascicular cambium. mz – meristematic zone; fc – fascicular cambium.

One- to three-layered zone of tangentially flattened meristematic cells can be seen in the medullary rays of the stem at the beginning of the growing season. This zone is formed by nearly periclinal divisions of the un lignified ray cells (Fig. 2A). It is continuous with the distinctive fascicular cambium (Fig. 2B). The meristematic zone grows up to 5–6-layered at the middle of the growing season. Its cells are in radial rows throughout (Fig. 3). Distinctive cork of 2–3 layers of uniform, mostly radially elongated dead cells with suberized walls develops by the middle of the growing season. It runs through the rays outside the multilayered meristematic zone and crosses the bundles just inside their protophloem masses. The cork is underlaid by a 2-layered zone of much smaller tangentially flattened living cells in the bundles (Fig. 3). The cork cells are in the same radial rows as their counterparts of the inner cell zone. Schizogenuous cavities are formed in rays under the meristematic zone already in the annual stems (Fig. 3).



Figure 3. Meristematic zone in medullary ray of annual stem of *Atragene speciosa*. lrp – lignified ray parenchyma; ph – cork; phg – phellogen; phd – phelloderm; ic – interfascicular cambium; sc – schizogenuous cavities; mr – medullary ray.

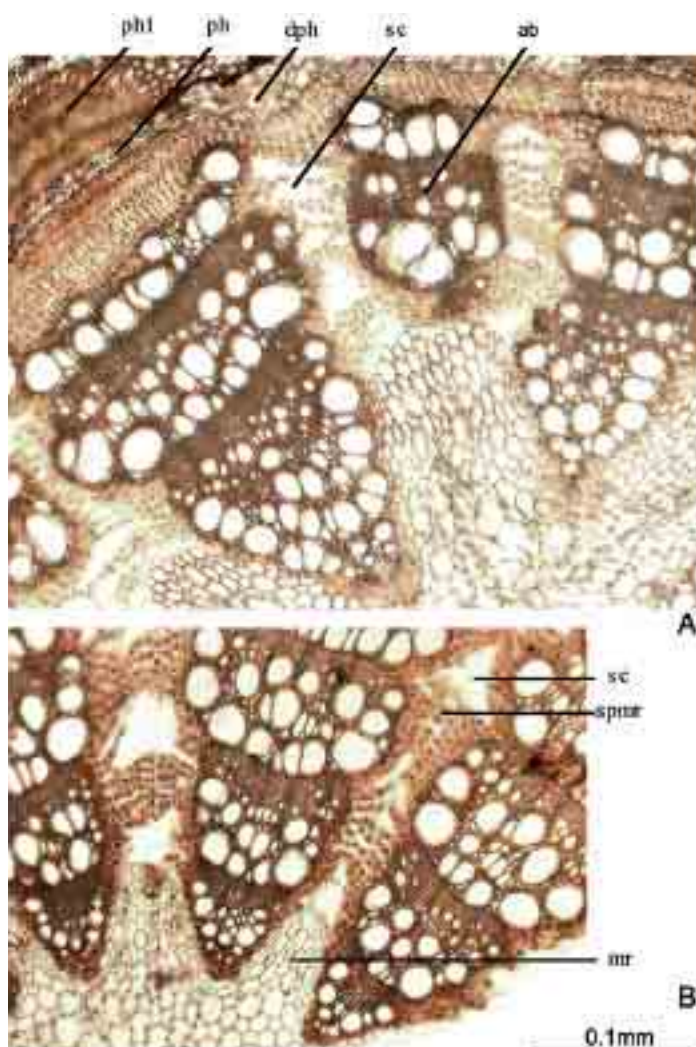


Figure 4. Steles of perennial stems of *Atragene ochotensis*. A – short shoot; B – elongated shoot. phl – phloem; ph – cork; dph – dilated cork cells; sc – schizogeneuous cavities; ab – the additional bundle; spmr – secondary parenchyma of medullary ray; mr – medullary ray.

Stems mostly have 6 vascular bundles, but those in some short shoots occasionally have additional small bundles in the medullary rays (Fig. 4A). The interfascicular zone looks bifurcate. Secondary rays are neither in the primary nor in the additional bundles.

The fascicular cambium yearly produces up to 16 layers of secondary xylem cells inwards (Fig. 4B) and 12–15 cell layers of secondary phloem outwards. Six to eight unlignified parenchyma cells of the medullary rays are in radial rows. Besides, a set of tangentially flattened schizogeneuous cavities is also discernible in every ray of perennial stems (Figs 4A, B; 5A), no one adjoining the outer zone (Figs 3; 5B).

New periderm arises just inside the previous one every year. Each periderm consists of 2–3 layers of suberized cork cells and 2 inner layers of smaller lignified cells, all being in the same radial rows (Fig. 5A). Thus, the bark ring of successive cork layers is intercalated by 2-layered lignified tissues in rays and lignified phloem in the bundles (Fig. 4A).

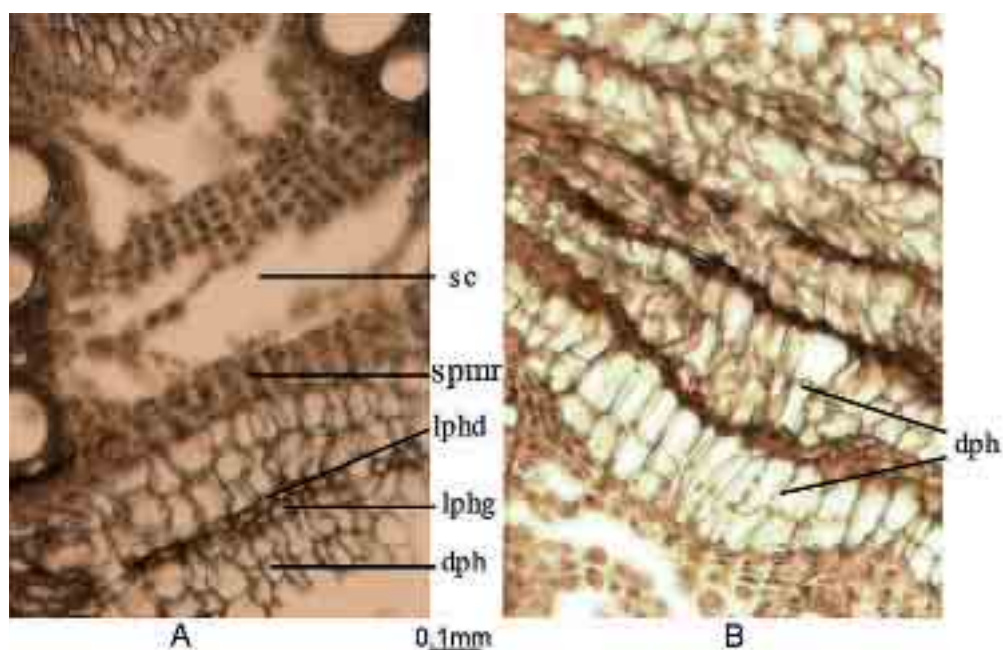
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Figure 5. Medullary rays of the elongated shoots of *Atragene speciosa*. A – biennial stem; B – perennial stem. sc – schizogene cavities; spmr – secondary parenchyma of medullary ray; lphd – the lignified phelloderm cells, lphg – the lignified phellogen cells, dph – dilated cork cells.

Discussion

The stems of investigated *Atragene* species remain quite thin because of the shortened period of cambial activity, which is associated with a short growing season, low activity of fascicular and interfascicular cambium and phellogen. However, they peculiarly change as follows.

The secondary thickening does not change the fascicular structure of the stem in the two investigated *Atragene* species what is typical of rather many lianas (ESAU 1953). This thickening is certainly maintained by 2 different meristems in the vascular bundles because the two are obviously separated by the phloem. The inner one (cambial zone) bidirectionally produces secondary xylem and secondary phloem whereas the outer counterpart produces cork unidirectionally outwards. Besides, the 1-layered phelloderm adjoins it inside. Thus, the outer meristem is worth being considered as phellogen.

There is a rather thick meristematic zone in medullary rays which looks like a single structure in the middle of the growing season. As it directly adjoins the (innermost) cork there, it is undoubtedly the phellogen in this zone which gives rise to the cork. If the phellogen were the only proper meristem in this zone, the ray parenchyma would be considered as multilayered phelloderm. The multilayered phelloderm is described in some dicotyledonous plants (ESAU 1953), but such a phelloderm is unlikely to be inherent in *Atragene* species investigated.

The cork layers of the bark are nearly invariably intercalated by 2-celled layers. This constant structure would be hardly understandable if the phellogen produced multilayered phelloderm. These cell layers are certainly remnants of the outer phellogen and the inner single-layered phelloderm, respectively. If so, the phellogen would directly adjoin the cork. Such a periderm

would be identical with its counterparts in the bundles. We believe the phellogen is the outermost cell layer of the apparently meristematic cells. It is underlaid with a single-layered phelloderm.

The inner part of this zone joins the evident fascicular cambium (Fig. 3). Such a junction seems to be quite natural for cambia (ESAU 1953), but not for phelloderm. Occasionally there are additional vascular bundles in the rays of perennial stems of short shoots. As far as we know, the phelloderm has never been reported to produce (additional) vascular bundles, whereas the interfascicular cambium is known to form such bundles in rather many plants (ESAU 1953). Therefore, the real interfascicular cambium must be in the apparently meristematic zone inside the phellogen and phelloderm in investigated species. Thus identified interfascicular cambium is nearly unidirectional. It gives rise mostly to inner secondary ray parenchyma. However, both phellogen and phelloderm become lignified, when the bark ring develops (Fig. 4A). That is the reason why the interfascicular cambium must yearly produce outwards at least one layer of ray parenchyma cells to generate the basis for regular annual phellogen. Otherwise, there were no tissues in the rays which could generate the new phellogen and the bark ring would not develop.

The outer ray cells have thick lignified cell walls, whereas their innermost counterparts have thinner lignified ones. The ray cells in between remain unlignified. These cells are in distinctive radial rows. Consequently, these cells are of different origin. The inner and outer ray parenchymas are certainly primary tissues and the middle one is worth being interpreted as secondary tissue produced by the interfascicular cambium. However, the secondary ray parenchyma is not a dense tissue. Schizogene cavities periodically develop within the rays (Fig. 4A, B). The cavities arisen subsequently enlarge to compensate radial increasing of the stem during secondary thickening. Such rays crossed by cavities seem to be unable to conduct solutions in radial direction, but they could facilitate *Atragene* species to survive under extreme environments of highlands and tundra. The ray cavities could be spaces, where cells could evacuate excessive water to prevent water freezing in cells and thus their damaging (BERESINA & AFANASEVA 2009).

The increasing of stem circumference is compensated by the dilatation of the periderm cells and not by the parenchyma of medullary rays. The periderm dilatation results from the stretching of the phellem cells in radial and tangential direction (Fig. 5A, B). Thus, the investigated species sharply contrast with most of woody plants in the mode of increasing their medullary rays.

The bark ring layers persist for many years, though its outermost layers fissure and gradually peel off.

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