

“RAY-INTRUSIVE” LATICIFERS IN SPECIES OF *CROTON* SECTION *CYCLOSTIGMA* (EUPHORBIACEAE)

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SUMMARY

A description of the occurrence and structure of “ray-intrusive” laticifers in the rays of species of *Croton* section *Cyclostigma* is provided. The systematic significance of laticifers within *Croton* section *Cyclostigma* is briefly discussed in relation to the section’s known production of red latex, commonly called “dragon’s blood”. A developmental hypothesis is offered and discussed in the context of the assumption that all laticifers in wood rays are non-articulated.

Key words: *Croton*, section *Cyclostigma*, Euphorbiaceae, laticifer, wood anatomy, articulated, non-articulated.

INTRODUCTION

Croton L. is one of the largest genera of plants, comprising over 1200 species that occur around the world in mostly tropical and subtropical zones. *Croton* section *Cyclostigma*, a group known as the dragon’s blood *Croton*, comprises 39–42 species growing in the Neotropics, confined mainly to the northern and central Andes, but widespread as well in southeastern Brazil and extending more sparingly throughout Amazonian South America and into Central America (Riina 2006). This circumscription (*Croton* sect. *Cyclostigma* s.s.) is distinct from the prior classification of the section (*Croton* sect. *Cyclostigma* s.l., Webster 1993) and is based on molecular phylogenetic information as well as morphological analyses. Riina (2006) characterized the section by a combination of morphological characters, one of which is the presence of red or reddish latex in the adult trunks. It is this latex that is used medicinally throughout much of the native range of the section (Meza 1999a, 1999b; Jones 2003) and is also of interest to pharmaceutical researchers (Borges & King 2000; Salatino *et al.* 2007).

Latex in plants is produced in specialized cells, laticifers, which are most commonly found within the primary plant body, especially in leaves, cortical tissues, and in areas near primary phloem (Evert 2006), presumably as a part of plant defense against herbivores and microbes (Rudall 1987). For those species that bear laticifers in the secondary plant body, they are most commonly found in the secondary phloem, and relatively infrequently in the wood (Esau 1965; Rudall 1987; Evert 2006). In a species such as *Hevea brasiliensis*, the commercial natural rubber tree, laticifers are common in the secondary phloem, and only rarely present in the wood itself (Rudall 1987; personal observation).

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Laticifers occur in two basic varieties, articulated and non-articulated, depending on their ontogeny and final structure (Rudall 1987, 1989; Evert 2006), with articulated laticifers developing as multicellular structures in which the component cells fuse and common walls between them are removed. Non-articulated laticifers are unicellular, often multinucleate, presumably tip-growing structures of indeterminate length and, in most tissues, frequently branching. With the exception of a single report for a specimen of *Croton conduplicatus* (Rudall 1989), non-articulated laticifers are thought to originate solely during embryogenesis, whereas articulated laticifers are commonly formed later in plant development as well. In wood, laticifers are usually found as a part of the radial system (Rudall 1987; IAWA 1989; Carlquist 2001) and are differentiable from normal ray cells by their typically larger diameter, lack of frequent end walls, and latex. They do not typically result in any apparent distortion of the adjacent cells seen in radial section, though in the tangential section there is often some lateral displacement of normal ray cells, much as is the case with radial resin canal complexes in fusiform rays of softwoods (Larson 1994; Wiedenhoef et al. 2002; Wiedenhoef & Miller 2003; IAWA 2004).

Rudall (1987) documented the presence of laticifers in the rays of *Croton draco* (syn. *C. panamensis*), a member of *Croton* section *Cyclostigma* s.s. The laticifer she showed appeared to have disrupted the adjacent ray cells with its growth. She referred to the laticifer as intrusive due to the apparent disruption of the organization of the end walls of adjacent ray cells, presumably due to its radial growth within the ray. A similar report was made for laticifers in the rays of another species in *Croton* sect. *Cyclostigma* s.s., *C. rusbyi* (Berry et al. 2005); in that work the laticifers were referred to as ray-intrusive, following the nomenclature of Rudall. Radial and axial laticifers in species of Moraceae, including a micrograph showing deflected end walls of adjacent ray cells in *Artocarpus communis*, were documented by Topper and Koek-Noorman (1981). Carlquist (2001) showed a similar laticifer in the wood of another euphorb, *Chamaesyce* (*Euphorbia*) *celastroides*, though it is not possible from the picture presented to confirm that it is of precisely the same type as those discussed here.

Ray-intrusive laticifers must originate from either the pith side or the bark side of the ray and then continue to grow in the radial direction. In none of the above cases did the authors address definitively the direction in which the laticifer was growing within the ray (centrifugally, from pith to bark, or centripetally, from bark to pith). Laticifers were reported in the rays of *Micrandra inundata*, but it was not determined whether the laticifers were articulated or non-articulated; in either case, the laticifers were not reported as ray-intrusive (Berry & Wiedenhoef 2004).

Other taxa that commonly have laticifers in the rays (e.g. *Dyera costulata*, Apocynaceae) generally have much larger-diameter laticifers than those seen thus far in the rays of the *Croton*, and they generally do not show ray cell end wall deflection. It appears that most authors assume that laticifers in wood rays are non-articulated, but in our survey of the literature it has been impossible to find clear proof supporting this assumption (Rudall 1987, 1989, 1994; Carlquist 2001; Evert 2006). Some authors ignore (Topper & Koek-Noorman 1981; Sudo & Fujii 1987; Berry & Wiedenhoef 2004) and others sidestep the question of whether they are articulated or non-articulated (Metcalf

& Chalk 1983). A mature articulated laticifer with fully resorbed end walls will appear like a non-articulated laticifer in the absence of developmental data. Evert (2006) warns strongly against basing a determination of the type of laticifer on the apparent lack of cross walls in the mature structure for this reason. The lack of work clearly showing radial laticifers in wood to be either articulated or non-articulated indicates that this should be considered a question that still needs to be answered by developmental work on a taxon-by-taxon basis until another method to separate the two classes of laticifers can be found.

Traditional views on the origin of non-articulated laticifers in the embryo, generally in the vicinity of the cotyledonary node (Mahlberg 1961; Mahlberg & Sabharwal 1967, 1968; Evert 2006) would suggest a pith-origin (or primary phloem, adjacent to the pith) for the laticifers found in the rays of *Croton draco* (Rudall 1987) and the species reported here, and that once initiated the laticifers grow in a centrifugal direction. If this were the case, one would expect that only a relatively small number of laticifers could ever occur in rays, because at the initiation of secondary growth there are a fixed number of rays available to host the laticifers. It would be expected that as a stem increases in diameter, the number of laticifers appearing as a function of tangential distance around the circumference of the stem would drop precipitously, as an increasingly larger proportion of the rays seen at larger stem diameters would be rays added by the vascular cambium and those new rays do not have contiguity to the pith or primary phloem. Therefore, if laticifers originate only in the pith, one should predict a greater number of laticifers on the pith side of a specimen, rather than the bark side, and laticifers extending all the way to the vascular cambium would be increasingly rare as stem diameter increases.

A second possibility is the centripetal penetration of existing rays by intrusively or tip-growing laticifers, presumably entering from the secondary phloem. This is fairly unlikely, because ray cells, though parenchymatous in nature, are cells with strong, frequently lignified secondary cell walls embedded in the matrix of the axial system, and a centripetal penetration route would run counter to the centrifugal development of the normal ray cells. A last possibility is the *de novo* initiation of laticifers within the ray, presumably by the conversion of a ray initial, or a daughter cell derived from a ray initial, to a laticifer that would then grow centrifugally. Rudall (1989) reported a single laticiferous ray initial in *Croton conduplicatus* (section *Cascarilla*), though there was no indication that intrusive laticifers were present in the mature rays of that shrubby species. If the conversion of a ray initial to a laticifer is responsible for the laticifers reported here, it represents a minor paradox, as other than the report by Rudall, non-articulated laticifers are thought to enter secondary tissue, when present, by tip growth from laticifers formed in embryogenesis, whereas articulated laticifers arise later in plant development.

As a part of a larger work on the wood anatomy of *Croton*, all available samples of species in *Croton* section *Cyclostigma sensu lato* (Webster 1993; Riina 2006) were examined to determine the presence, abundance, and form of laticifers. Additionally, the woods of 127 other species representing 25 of Webster's 40 sections were examined to understand the utility of ray-intrusive laticifers as a character within the genus.

MATERIALS AND METHODS

All specimens studied were prepared for microscopic observation and measured following standard microtechnique protocols (as in Berry & Wiedenhoef 2004). Split radial faces of all specimens were studied with a stereomicroscope to confirm the orientation of the deflected ray cell end walls with respect to the radial pith-to-bark axis. Images were captured digitally. The relative abundance of laticifers in the rays at the pith compared to the bark was determined qualitatively by examining pith-to-bark split radial faces of specimens, and abundance was coded as either greater abundance on the pith side, bark side, or no difference between pith and bark sides of the specimens. Quantitative determinations of laticifer abundance were not attempted. Specimens for scanning electron microscopy were hand-sectioned with razor blades, placed on a glass slide under a weighted coverslip and heated at 80 °C for several minutes to flatten the section and dry the specimens. Specimens were then adhered to stubs, gold coated, and observed and photographed with a Zeiss EVO 40 SEM at 10kV accelerating voltage.

Laticifer height was measured from the radial section to ensure that a true laticifer was measured in each case; this method may under-report laticifer height in cases where the section was not medial through the full height of the laticifer. Given the small size of the laticifers and a section thickness averaging 20 µm, however, this bias toward under-representing the height of the laticifers is probably negligible. Ray cell end wall deflection was measured in degrees by projecting an image of the specimen onto an electromagnetic digitizing tablet and then using the angle measuring function to determine the angle of the vertical end wall with respect to the horizontal wall of the ray cell-laticifer boundary; an angle of ~90 degrees would be a normal measurement, and any measurement less than this reflects an abnormal degree of cell wall deflection. In some end walls, the laticifer-proximal portion of the wall was at a greater angle than the distal portion of the wall; in such cases, angles were determined on the basis of the angle formed by the proximal portion of the wall measured at the inflection point where the angle changed leading to the distal portion of the wall (Fig. 1). Measured angles were averaged, and the averages were evaluated against an assumed angle of 90 degrees using a one-tailed t-test (Sokal & Rohlf 1995; Rohlf & Sokal 1995).

The number of rays present at the pith and at the boundary with the vascular cambium was calculated by multiplying the rays per linear millimeter as measured from tangential sections with the calculated circumference of the stem at each position.

RESULTS

Laticifer distribution

Table 1 lists all the specimens examined in which laticifers were observed, and Appendix 1 provides a brief description of the laticifers in each specimen. Specimens of *Croton* section *Cyclostigma s.s.* that lacked laticifers are listed in Appendix 2. Laticifers were found only in the rays of species belonging to *Croton* sect. *Cyclostigma s.s.* None of the 127 other species from other sections bore laticifers in the rays. All laticifers observed in rays were unbranched, and the ray cells adjacent to them generally showed

Table 1. List of all species and specimens of *Croton* in which laticifers were observed, ray cell end wall deflection measured in degrees, calculated number of rays at pith and bark, percent of rays at the bark face that could have originated at the pith, and laticifer height.

Taxon	Collector	Abundance, end wall angle	# of rays (pith)	# of rays (bark)	% rays with pith origin	Laticifer height (µm)
<i>C. echinocarpus</i>	Riina 1318	p, 47***	228	947	24	8–12
<i>C. echinocarpus</i>	Irwin 2084	b, 54***	207	1059	20	8–12
<i>C. gossypifolius</i>	Williams, Ll. 9539	n, 30***	691	2206	31	8
<i>C. huberi</i>	Riina 1275	n, 58*	238	761	31	8
<i>C. macrobothrys</i>	Reitz & Klein 3910	n, 30**	113	1330	8	15–20
<i>C. macrobothrys</i>	MADw 14543	n, 53*	57	1068	5	15–25
<i>C. macrobothrys</i>	Berry et al. s.n.	b, 25***	141	1512	9	18–25
<i>C. mutisianus</i>	R. Riina 1416	b, 31***	173	3325	5	8–10
<i>C. mutisianus</i>	Cuatrecasas 15247	n, 31***	141	10146	1	8–10
<i>C. mutisianus</i>	MADw 29934	n, 10***	251	1608	16	8–10
<i>C. rusbyi</i>	Riina 1481	p, 18***	165	1577	10	8
<i>C. urucurana</i>	Riina 1351	p, 45***	332	1134	29	8–12
<i>C. vulneriarus</i>	Cordeiro 2793	n, 62**	159	373	43	5

* significant at 0.05 level, ** significant at 0.01 level, *** significant at 0.001 level.
p indicates a pith-side abundance of laticifers, b a bark-side abundance, and n an even abundance.

some deflection from normal. Based on stereomicroscopic examination and confirmation with transmitted light microscopy, the direction of ray cell end wall deflection suggested a centripetal penetration of the ray by the laticifer; that is, the ray cell end walls adjacent to the laticifers appeared “pushed” toward the center of the stem (Fig. 1).

Within the stems of some specimens, there appeared to be a greater abundance of laticifers on the bark side of the stem in small stems, and no apparent difference in abundance between either the pith or bark side in specimens of larger diameter. In others, there was a greater abundance on the pith side of the stem. Qualitative observations to this effect are reported in Table 1.

In all specimens examined, pitting between the laticifers and the adjacent ray cells was rare or lacking (Fig. 2), observed from both radial and tangential sections, except for sporadic blind pits, *i.e.* pits on the parenchyma side of the double wall, with no corresponding pit on the laticifer side.

A description of the ray cells associated with the laticifers in each of the specimens in Table 1 follows as Appendix 1. Note that in all cases the apparent direction of penetration into the ray was centripetal based on the angle of the ray cell end walls, but many specimens observed on the transverse section with a stereomicroscope show laticifers in contact with the cambial zone, and Figure 3 clearly shows that two laticifers cross the cambial zone and are continuous into the secondary phloem.

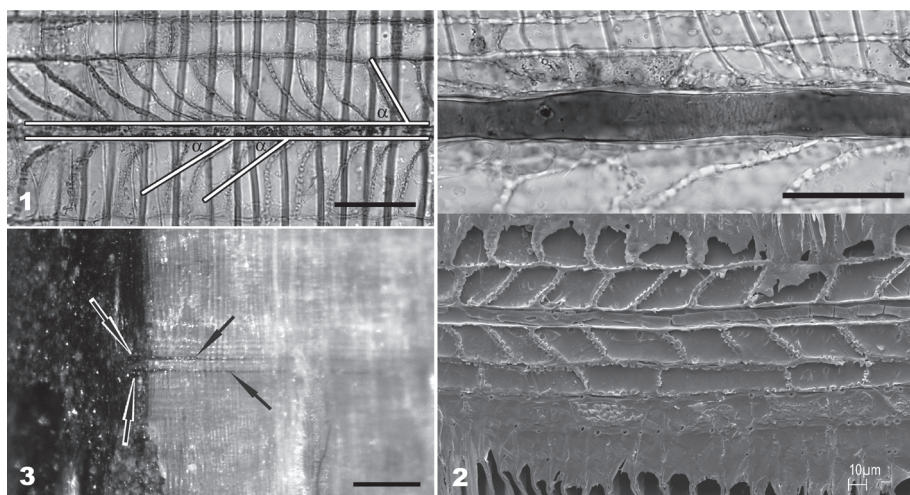


Figure 1. Light micrograph of a laticifer in *Croton urucurana* (Riina 1351) overlaid with examples of how ray end wall deflection angles (a) were measured, with the pith to the right of the image and the bark to the left (not pictured). Note that on the two lower ray cell end walls with overlays, the angle was determined by the angle of the wall prior to the inflection point in the curvature. The upper angle is a straight-line measurement. – Scale bar = 50 μm . — Figure 2. Top: Light micrograph of a laticifer in a ray of *C. macrobothrys* (Reitz & Klein 3910). Note that the horizontal walls of the laticifer show no pit pairs with the common walls to the normal ray cells. – Scale bar = 50 μm . — Bottom: Scanning electron micrograph of a laticifer in a ray of *C. echinocarpus* (Irwin 2089). As above, the horizontal walls of the laticifer are unpitted, whereas the vertical walls of the ray cells are highly pitted one to another. The ray cell end wall deflection is also clearly shown. — Figure 3. Photomicrograph of two laticifers (black arrows) in a ray of *C. echinocarpus* (Irwin 2089). They cross the vascular cambium and clearly continue (black arrows with white borders) into the secondary phloem. – Scale bar = 200 μm .

Ray cell end wall deflection and ray numbers

Ray cell end wall angles were significantly different from 90 degrees, with p values varying from $p < 0.05$ to $p < 0.001$, depending on the specimen (Table 1). Disjunctive ray end walls (IAWA 1989) were absent in ray cells with deflected end walls, despite their common presence in ray cells without deflected end walls (Fig. 4).

Table 1 presents an estimate of the number of rays available at the initiation of secondary growth at the pith, compared to the estimated number of rays occurring at the boundary between the xylem and the vascular cambium, as well as the height of the laticifers and the angle of deflection of the ray cells adjacent to laticifers. Even in the smallest stems, less than half the rays estimated to be present at the boundary with the vascular cambium could have been initiated at the boundary with the pith, assuming all rays continue and never die out, an assumption that is likely to overestimate the number of rays continuous to the pith. In the two specimens with the most laticifers, *C. echinocarpus* (Riina 1318 and Irwin 2084), a maximum of roughly one quarter and one fifth, respectively, of the rays present at the boundary with the vascular cambium could have originated at the pith.

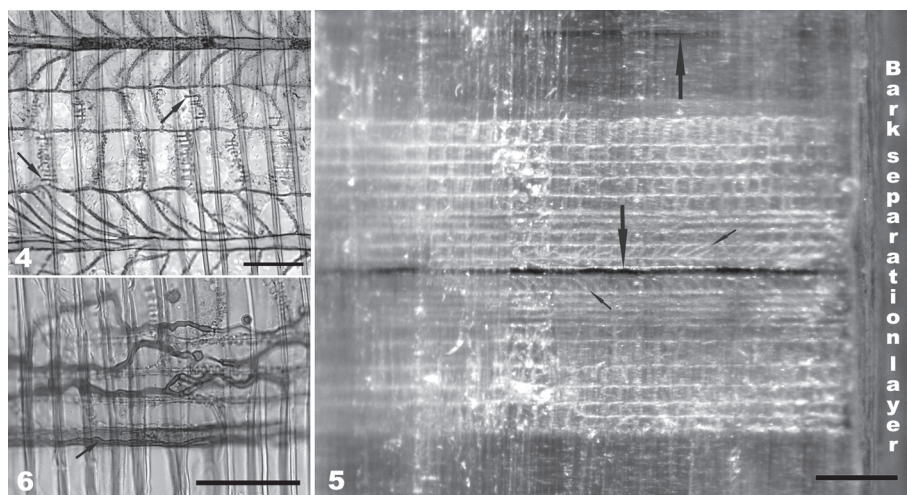


Figure 4. Light micrograph of part of a ray in *Croton urucurana* (Riina 1351) with two laticifers (top and bottom of frame). Note that the upright ray cells in contact with the laticifers have deflected end walls with small simple pit-pairs. In the upright cells not in contact with the laticifers, disjunctive ray cell end walls (arrows) are clearly present. – Scale bar = 50 μm . — Figure 5. Photomicrograph of *C. urucurana* (Riina 1351), showing a laticifer (large arrow, center), ray cell end wall deflection (small arrows), and the bark separation layer on the outer tangential face of the block (labeled). Note also the laticifer (large arrow, top) visible out of the plane of focus. – Scale bar = 200 μm . — Figure 6. Light micrograph of thick, pigmented (blue-stain type) hyphae in the normal ray cells and in a laticifer (arrow) in *C. echinocarpus* (Irwin 2089). – Scale bar = 50 μm .

DISCUSSION

Laticifer distribution and abundance

Not every species of *Croton* section *Cyclostigma* s.s. bore laticifers (Appendix 2), but all *Croton* species that did bear them were members of this section. None of the species belonging to *Croton* section *Cyclostigma* s.l. (Webster 1993) that were subsequently excluded from the section by Riina (2006) were found to bear laticifers. Presence of laticifers in the rays of *Croton* is thus far found to be restricted to section *Cyclostigma* s.s. While the systematic utility of such a polymorphic character may not be significant, laticifers in the rays represent another character specific to section *Cyclostigma* s.s., thus increasing the number of features that can be used to identify this group within the genus.

The different patterns in the abundance of laticifers at the bark side and pith side of the specimens suggest different origins for the laticifers; in specimens with an abundance on the pith side and a dearth on the bark side, the tacitly assumed pith origin for the laticifers is a sound explanation. For the other species, in which there is a general tendency toward more abundant laticifers at the bark side, a pith origin of laticifers is much less likely. As mentioned above, even in the smallest stem, the relative proportion of rays that could be of pith origin is less than half, and in some of the larger stems that proportion drops to less than 10 % (Table 1). For pith-originating laticifers to be the only

source of laticifers in the rays, every ray would need to bear a laticifer, and even then there would be an increasingly lower abundance as one looked toward the bark. In no specimen did every ray at the pith boundary bear laticifers, and so we dismiss the pith as the sole source of laticifers in the rays for those species with a greater abundance of laticifers on the bark side of the specimen.

Another line of evidence that would appear to suggest a non-pith origin for the laticifers is the fact that in all specimens the apparent direction of penetration of the laticifer into the rays is centripetal (Fig. 5). Previous reports in the literature (Rudall 1987; Berry *et al.* 2005) of these ray-intrusive type laticifers did not report a pith or bark directionality, and the apparently intrusive nature of the laticifer seemed self-evident (Rudall 1989; Berry *et al.* 2005). With the understanding that, at least in the species studied here, the laticifers appear to enter from the bark side of the xylem, it is clear that an intrusive form of growth cannot explain the observations. Rays develop by the cell division, elongation, and maturation of ray initials from the vascular cambium (Larson 1994), yielding a structure that grows centrifugally in a manner somewhat analogous to a root tip. Since the direction of cell elongation and maturation is centrifugal, the centripetal penetration of a laticifer would push almost immediately into mature tissues, and though there are unmistakable disruptions of ray structure, there is not sufficient damage to support the hypothesis of intrusive, centripetal penetration of a mature ray.

Ray cell pitting

Ray cells in *Croton* section *Cyclostigma s.s.* are typically highly pitted, one to another, but the common wall between ray cells and laticifers do not have obvious pits, much as in *Pimelodendron* (Sudo & Fujii 1987). This could be a reflection of the contents of the laticifer; secretory structures are commonly symplastically isolated from the nearby tissue (Esau 1965; Evert 2006); however, lack of pits to adjacent normal ray cells cannot prove symplastic isolation.

Putative function of laticifers

The function of laticifers is unknown. Esau (1965) included laticifers in the excretory system of plants, a concept that we do not accept as such. Studies in leaf-feeding caterpillars (Dussourd & Denno 1994; Dussourd 1999) have shown that laticifers in leaves can play a role in reducing herbivory by insects, except in insects with modified behavior that sever the laticifers, and thus avoid additional contact with laticifer contents. No such studies exist for wood, but Rudall (1987) postulated an anti-microbial function, and the latex from species of *Croton* in this section is used on lesions and wounds by local peoples to protect against infection (Meza 1999a, b; Riina 2006; Salatino *et al.* 2008). In some specimens of *Croton* studied here, the presence of blue-stain type hyphae in the vicinity and in the interior of a laticifer (Fig. 6) was noted. This suggests that at least for one or more species of blue-stain fungus, the putatively antimicrobial role of laticifers in plant defense is not robust. Of all the specimens examined, the one with the most laticifers, *C. echinocarpus* (Irwin 2089), also had the most extensive colonization by blue-stain type fungi.

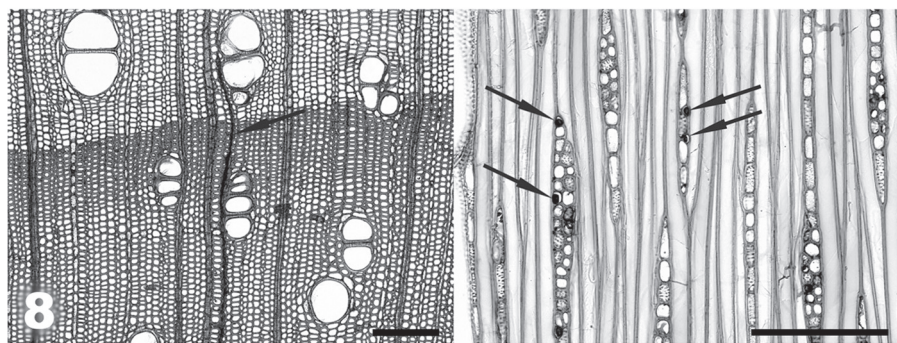
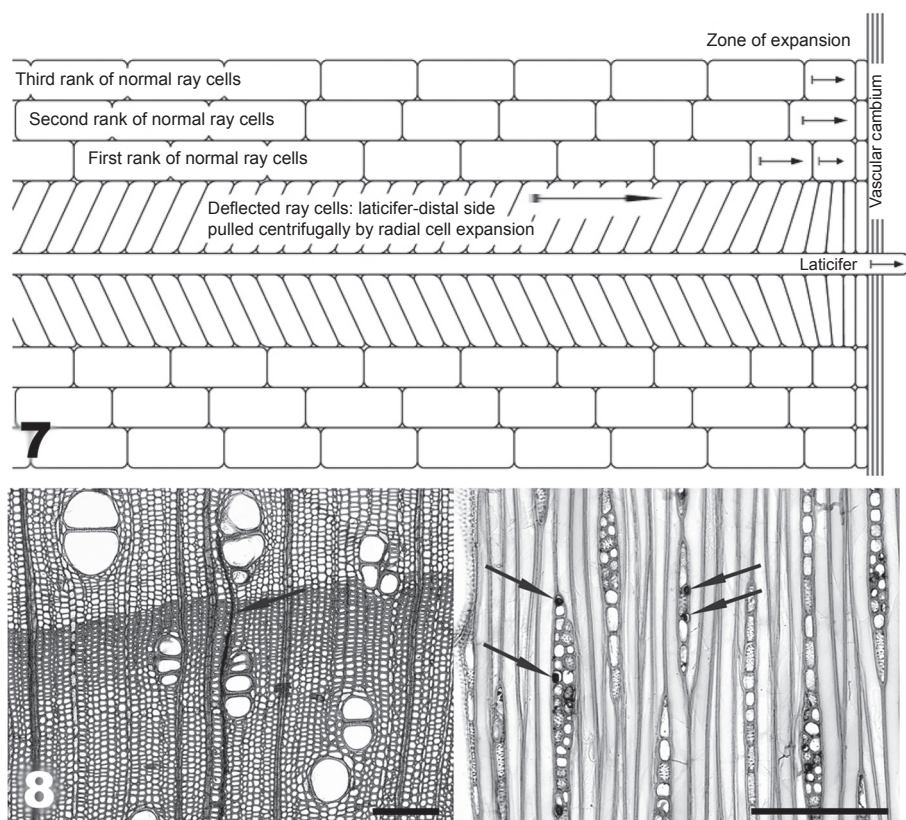


Figure 7. Cartoon of hypothetical laticifer development within a ray. The laticifer elongates (arrow) by tip or intercalary growth in the vicinity of the vascular cambium. The adjacent ray cells have their end walls deflected by the coordinated centrifugal cell expansion (arrows) in the first, second, and third ranks of normal ray cells, resulting in deflected ray cell end walls, and yielding the appearance of a ray penetrated centripetally by a laticifer. Note that the geometry of the cells adjacent to the laticifer in the vicinity of the vascular cambium and in the zone of expansion are purely speculative, serving as an illustration of a possible mode of cell division leading to the observed phenomenon. Tip growth is shown, or intercalary growth could maintain connectivity to the laticifer system of the secondary phloem. — Figure 8. Light micrographs of *Croton echinocarpus* (Riina 1318). — Left: transverse section showing a laticifer (arrow) crossing a growth ring boundary. — Right: tangential sections showing laticifers (arrows) associated with the rays. — Scale bars = 100 μm .

Developmental hypothesis for laticifer ontogeny within the rays

Although we present no developmental work as proof, it seems apparent that the deflection of the end walls of the ray cells could not be due to disruption by a centripetally penetrating laticifer, but rather must be the result of a process that occurs in concert with the centrifugal development of the ray, as only prior to maturation could the observed phenomenon take place. The simplest hypothesis is that, rather than the laticifer pushing the ray cell end walls toward the pith at the proximal side of the

cells, the distal side of the normal ray cells are being pulled centrifugally by the radial expansion of the next rank of adjacent normal ray cells, thus resulting in deflected ray cell end walls (Fig. 7). The place where the vertical end wall joins the horizontal wall adjacent to the laticifer does not elongate radially at the same rate nor to the same total extent as the opposite side of the cell that is adjacent to normal ray cells, therefore there is differential elongation leading to the angling of the ray cell end wall. This explanation is predicated on the assumption of minimal radial laticifer expansion in the zone of normal ray cell expansion interior to the vascular cambium; lack of laticifer elongation in the zone of cell expansion ensures that the ray cells adjacent to the laticifer do not expand centrifugally at the same rate on the laticifer proximal side of the cell as the distal portion that moves centrifugally with the expanding normal ray cells. Such a pattern could be established by the coordinated tip growth of the laticifer into the cambial zone as xylem is added and the stem diameter is increased, or by radial extension (intercalary growth) within the body of the laticifer in the cambial zone for those laticifers that continue into the phloem (*e.g.* Fig. 2 & 7). Such modes of extension would explain the observed cell deflections, and the latter mode would allow for maintenance of continuity with the laticiferous system in the secondary phloem (Fig. 3), if such connections are vital. The lack of pitting between laticifers and the adjacent ray parenchyma cells also suggests a possible lack of intercellular connectivity that could result in uncoordinated cell expansion. Regardless of the cell-to-cell signaling issues, a simple tip-growing (and thus non-articulated, by definition) laticifer could develop in a semi-coordinated way with ray initials or their derivatives in the cambial zone, and the differential rates of radial expansion across the two sides of the laticifer-adjacent ray cells would explain the observed ray cell end wall deflection without recourse to post-maturity penetration of a ray by the laticifer. This hypothesis needs to be confirmed or rejected by detailed ontogenetic work in which the development of rays with and without laticifers are compared.

The above ontogenetic hypothesis suggests three interesting corollary points. The first is that one might expect to see greater cell wall deflection in species with a high radial growth rate. Though we did not test this explicitly, we found no significant relationship ($p \gg 0.01$) between the average angle of deflection of the ray cell end walls and specimen wood specific gravity (data not shown), which in some species correlates with radial growth rate (Wiemann & Williamson 1988, 1989). If this hypothesis is correct, wood specific gravity may be too blunt a proxy for radial growth rate at the scale of laticifers in rays; it is also possible that the developing ray and laticifer are coordinated to a great enough degree not to reflect these changes. The related hypothesis is that the degree of radial expansion of the second and third ranks of ray cells (Fig. 7) might also influence the degree of deflection in a similar way.

The second corollary is the unexpected absence of disjunctive ray cell end walls in the cells adjacent to the laticifers, but their common presence in the end walls of rays without laticifers, or in ray cells not in contact with laticifers (Fig. 4). Disjunctive ray cell end walls are implicated in the maintenance of cellular connections between disturbed or partially disjoined cells (IAWA 1989), and the deflected cell end walls seen here should be just such an instance, but the end walls have normal simple pits. In rays

without laticifers in these species, disjunctive ray end walls are often present (Fig. 4). It seems that the conceptual definition and imputed function of disjunctive end walls does not correspond to their distribution within the wood of these species of *Croton*.

The third corollary is that the relative increase in proportion of laticifers closer to the bark in some species implies the initiation of new laticifers by some method, and conversion of ray initials or their derivatives to laticifers is one possibility (as in Rudall 1989). It is also possible, however, that a tip-growing laticifer could enter a ray at the vascular cambium and re-orient itself to grow centrifugally in coordination with the developing ray, and such a method could account for the initiation of laticifers in the rays, though there is no microscopic evidence in this study to support this hypothesis. The spontaneous generation of laticifers in *Croton* section *Cyclostigma*, presumably by the conversion of a ray initial or one of its derivatives to a laticifer, is also unsupported by direct microscopic evidence. All such hypotheses will necessarily remain speculative until definitive ontogenetic work is done to support or refute the developmental process outlined above and clarify the nature of laticifers in these and other species that bear them.

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ADDENDUM

Subsequent to the acceptance of this manuscript for publication, small portions of laticifers, too infrequent to permit characterization of their dimensions and abundance, were observed in the rays of the following species and specimens: *Croton alchorneicarpus* Brazil São Paulo Campos de Jordão (Riina 1531); *Croton charaguensis* Bolivia (Riina 1513); *Croton draco* Panama (Stern, Chambers, Dwyer, Ebinger 1637); *Croton floccosus* Ecuador (R. Riina & L. López 1404); *Croton glaziovii* Brazil (Riina 1521); *Croton hibiscifolius* Colombia (Cuatrecasas, J. 14525); *Croton jimenezii* Costa Rica (RBHw 19930); *Croton lechleri* Peru (Riina 1449); *Croton piluliferus* Bolivia (Riina 1508); *Croton piluliferus* Argentina (Zuloaga 8554); *Croton rimbachii* Peru (Riina 1437); *Croton speciosus* Venezuela (Riina 1278); *Croton speciosus* Venezuela (Williams, Ll. 9923)

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Appendix 1

Brief descriptions of the laticifers. All specimens are located in MADw-SJRw.

- Croton draco* (Williams 9539): A single laticifer was observed, associated with square ray cells. On the tangential section, laticifers not distinct from ray cells with dark contents. Laticifer height 8 μm .
- Croton echinocarpus* (Riina 1318): Laticifers are numerous, often more than one in a single ray, occurring both in the body and at the margins of the rays, and thus associated with upright, square, and procumbent cells. On the tangential section, laticifers appear as round dark-staining cells, appearing as integrated parts of the ray (Fig. 8). Marginal laticifers 12–15 μm , body laticifers 8–12 μm tall.
- Croton echinocarpus* (Irwin 2084): Laticifers are numerous, associated with upright, square, and procumbent cells, in the body and at the margins of the rays. Clear and distinct on the tangential section, differentiated by dark contents and rounded, slightly enlarged outline. Laticifers range from 8–12 μm tall.
- Croton huberi* (Riina 1275): Laticifers are sparse, associated with upright cells. Laticifers were not evident on tangential section, indicating that they do not cause obvious disruption, and are rare. Laticifers 8 μm tall.
- Croton macrobothrys* (Reitz & Klein 3910): Large laticifers are present in the body of rays, typically associated with square cells. On the tangential section, the laticifers appear as large round to oval ray cells with dark contents. Biseriate rays allow more space for the larger cells with less apparent distortion in that plane of section. Laticifers 15–20 μm tall.
- Croton macrobothrys* (MADw 14543): Large laticifers are present on the outer margins of the rays. On the tangential section, laticifers appear as large, round cells, clearly differentiated from the normal ray cells by their shape and dark contents. Laticifers 15–25 μm tall.
- Croton macrobothrys* (Berry *et al.* s.n.): Large laticifers are present on the outer margins and within the rays. On the tangential section, laticifers appear almost as procumbent cells with dark-staining contents. Cellular disruption is not marked on the tangential section. Laticifers 18–25 μm tall.
- Croton* aff. *mutisianus* (Riina 1416): Laticifers are associated with square and procumbent cells in the body of the ray. On the tangential section, laticifers appear as small dark ray cells, without evident disruption of the ray. Laticifers 8–10 μm tall.

Croton mutisianus (Cuatrecasas 15427): A single laticifer was observed, associated with square cells in the body of the ray. On the tangential section, laticifers appear as small ray cells, not greatly disrupting the organization of the ray. Laticifers 8–10 μm tall.

Croton mutisianus (MADw 29934): Laticifers are associated with upright and square cells on the outer margins and in the body of the ray. On the tangential section, laticifers appear as small round cells, distinguished from the normal ray cells by the lack of axial flattening and the presence of dark contents. Laticifers 8–10 μm tall.

Croton rusbyi (Riina 1481): Laticifers are present in the rays, typically in association with upright cells at the margins of rays, or with procumbent cells in the body of the ray. On the tangential section, the laticifers show relatively little ray disruption. Laticifers 8 μm tall.

Croton urucurana (Riina 1351): Laticifers are generally associated with upright cells, in the body of the ray. On the tangential section, laticifers are not evident. Laticifers 8–12 μm tall.

Croton vulneriarus (Cordeiro 2793): Laticifers are present in rays, typically in association with upright cells. On the tangential section, the laticifers appear as small round dark-staining cells. In some cases the laticifer occupies the entire width of the (uniseriate) ray, and there is no apparent dislocation of the common walls. Laticifers 5 mm tall.

Appendix 2

Seventeen specimens of *Croton* sect. *Cyclostigma* s.s. that lacked laticifers:

Croton aff. *coriaceus* Ecuador (R. Riina & N. Saltos 1430); *Croton draco* Costa Rica (Stork 4129); *Croton draco* Panama (Stern, Chambers, Dwyer, Ebinger 1637); *Croton draco* Colombia (SJRw 20960); *Croton draco* Costa Rica (Dayton & Barbour 3157); *Croton draconoides* Brazil (Capucho 382); *Croton draconoides* Brazil (MADw 22060); *Croton gossypifolius* Venezuela (Williams, Ll. 12931); *Croton hibiscifolius* Ecuador (R. Riina & L. López 1413); *Croton lechleri* Ecuador (R. Riina & L. López 1393); *Croton lechleri* Peru (Riina 1494); *Croton perspeciosus* Peru (Riina 1435); *Croton perspeciosus* (Williams, Ll. 7378); *Croton rimbachii* Ecuador (R. Riina & L. López 1402); *Croton rimbachii* Peru (Riina 1438); *Croton speciosus* Venezuela (Riina 1278); *Croton speciosus* Venezuela (Williams, Ll. 9923); *Croton urucurana* Argentina (Anon. 48); *Croton urucurana* Argentina (Belgrano, M.J. 288); *Croton urucurana* Bolivia (SJRw 50160).