The season of tylosis development and changes in parenchyma cell structure in *Robinia pseudoacacia* L.

Minoru FUJITA, Keiko NAKAGAWA, Nanako MORI, and Hiroshi HARADA

ハリエンジュにおけるチロース発生の季節とそれに伴う柔細胞構造の変化

藤田　稔・中川啓子・森奈々子・原田　浩

Résumé

By periodical samplings, the autumn was found out to be the developing season of tyloses in *Robinia pseudoacacia* L. The tylosis buds were originated not from ray parenchyma cells but axial parenchyma cells which were contacting to vessel, so the contact cells were examined more precisely by electron microscopy. In September when tylosis buds did not occurred, the contact cells showed the dormant type. In October, the buds were ballooned actively from the contact cell to vessel lumen, and the interiors of the contact cells and buds were full of Golgi bodies, r-ER, mitochondoria and ribosomes. In December when vessels had been blocked by tyloses, the contact cells returned to the dormant type, and particles supposed to be by-products of tylosis development were accumulated in vacuole. These changes in the cytoplasmic structure are discussed in relation to tylosis formation.

要　　旨

ハリエンジュの樹幹から辺材部を定期的にサンプリングした。この光顕および走査電顕観察から，9月下旬から10月下旬にかけて当年輪の孔圏道管に，それに接した軸方向柔細胞からチロースが多く発生することが明らかとなった。つぎに，この軸方向柔細胞をはじめ，チロースおよび道管周辺の各種細胞の構造を透過電顕で調べた。チロース芽発生前の9月には細胞は休止型あるいは貯蔵型の構造を示し living fiber も starch grains と lipid droplets を保有していた。チロース芽が活発に発生している10月になると，道管に接した軸方向柔細胞とチロース芽にゴルジ体，r-ER，ミトコンドリア，リボゾーム等が充満しており，典型的な活動型の細胞構造となった。道管がチロースで閉そくされた12月には，道管に接した軸方向柔細胞ではゴルジ体，r-ER は減少しており，大径の脂質粒が現われた。また液胞中には代謝副産物と思われる顆粒が発生していた。

INTRODUCTION

Tyloses blocking vessel of many species in angiosperm have been a strong interest in the physiological point of view, especially in relation to heartwood formation.[1)2)] It is, however, very difficult to examine the actual developing phase because the development is proceeded in secret in the deep interior of tree trunk. So the relation to heartwood formation has been supposed only from the distribution of matured tyloses in the trunk. It is also well known that tyloses are caused by some injuries in sapwood of living tree, and further are formed in sapwood of logs which were cut down.[1)] Therefore the artificial tyloses are induced easily by a simple incubation method in which sapwood blocks collected from living tree are conditioned in a proper temperature and moisture.[3)] This method is very useful to observe precisely the developing stage. Meyer observed for the first time the cell structure of ray parenchyma cells which were originating tylosis buds in the incubated blocks collected from *Quercus alba,* and showed that cell organelles did not increased so much in the occurrence of buds.[4)] On the other hand, Fujita et al. reported the remarkable changes in ray and axial parenchyma cells sorrounding vessel of the incubated block at 26°C collected from *Quercus serrata* in winter.[5)] Golgi bodies, r-ER, mitochondoria and vesicles increased in vessel-contact ray cells and starch grains stored in non-contact parenchyma cells were consumed, and also some metabolites were accumulated in vacuole of these cells during development of tylosis buds. This developing stage were examined in the various conditions of incubation by Shibata et al.[6)]

However it remains still uncertain whether these changes reflect the cell metabolism in natural condition, so the natural developing phase must be examined and compaired with the changes in the incubated one described above. The natural tylosis development seems to be proceeded not throughout the year, but in the particular season, so the developing season must be caught first of all. But it was difficult to catch the season in *Quercus serrata* because of the wide sapwood and the irregular occurrence of tyloses in the sapwood. It is already known in the preliminary observation that *Robinia pseudoacacia* developed tyloses even in the current year annual ring,[7)] and the cell structure of parenchyma cells sorrounding vessel has been examined in detail by Czaninski.[8)] Therefore the season of natural tyloses development was examined in 1975 by periodical sampling, and then the changes in parenchyma cell during the developing season were observed precisely by electron microscopy in 1977.

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MATERIALS AND METHODS

Small wood blocks containing current year annual ring were collected periodically

from April to December by increment borer from the stems of black locust (*Robinia pseudoacacia* L.) growing steadily at the campus of Kyoto University in 1975, and at Kamigamo experiment station of Kyoto University Forests in 1977, taking care not to confound the natural tyloses development with the traumatic one caused by the previous samplings.

Wood blocks collected in 1975 were observed by a scanning electron microscope (SEM: JSM-U3) after the glutaraldehyde fixation, critical point drying using CO_2, and Au-coating by ion spattering, and also they were observed by a light microscope after the stain of safranin-light green. Blocks collected in 1977 were fixed by the glutaraldehyde-osmium tetroxide combination and embedded in epoxy resin. Cross and tangential sections having 2 μm thickness and ca. 0.15 μm thickness were sliced off from the embedded materials respectively. The 2 μm sections were observed by a phase contrast microscope. The ultra-thin sections were examined by a transmission electron microscope (TEM: JEM-7).

RESULTS AND DISCUSSION

Vessel distribution and structure were beforehand examined in the material of April. Vessels in pore zone were very large in diameter, and distributed independently or occasionally accompanied by small vessels. Late wood vessels were small and clustered with one another. Terminal zone near the annual ring boundary was occupied almost by the very small vessels filing up radially. The small size vessels were ornamented by spiral thickenings without exception, and those near the annual ring boundary were hollow without tyloses at the last two year annual ring at least, and then were partitioned off horizontally by tylosis walls (Fig. 1). The inner surface of large size vessel at pore zone was sculptured by numerous pits whose shapes were various from slit-like to circular. These large vessels of the last year annual ring were already full of tyloses. So the large vessels at pore zone of the current annual ring, which must be closed by tyloses by April of the next year, were examined periodically by SEM. In 1975, tylosis buds were occurred toward the end of September and most large size vessels were blocked in October (Fig. 2). Thus the season of tylosis development was proved to be autumn. The development may be corresponding to the season when the leaves turn yellow. In 1977, some of the vessels was already blocked by tyloses in August. It was unusually so droughty in that summer that the leaves were slightly turned yellow. The rest of vessels were closed in October. It is of interest that the season of tylosis development was found to be autumn. Autumn is not so cold as the cell activity is interrupted, and photosynthetic products are stored adequately in tree stems, while cambial activity has been finished. So it may be convenient to heartwood formation. It may be concerned that the conduction is obstructed at the opening of the next year growth by the blockade of pore zone vessels even in the current annual ring. But the developed vessel groups near the annual ring boundary are proved to be well conducting in the current and the last year annual

ring by an experiment in which acid fuchsin was sucked up from the cut end.

Tylosis buds were ballooned at random from pits dispersed on the inner surface of the vessel, different from the case of *Quercus serrata* in which they had been derived only from the ray-vessel pittings, although they have an inclination to be derived from the large and circular pit apparture comparatively. These buds were proved by the observation of cross sections to be originated from axial parenchyma cells (Fig. 5). The fixed concept by the Cattaway's observation that tyloses are derived from ray cells must be corrected because of the apparent occurrence from axial parenchyma cells.

Cross sections of the embedded materials collected in September, October and December were examined. The vessel or the vessel groups were enclosed by a sheath composed of axial parenchyma cells. The vessel-contact cells are specialized differently from the ordinary non-contact axial parenchyma cells, as was pointed out by Czaninski[8]. That is, they have not so many starch grains as non-contact cells, and have the more dense cytoplasm. In the materials collected in September when tylosis buds were not occurred yet, the interior of non-contact cells were occupied by many and large starch grains (Fig. 3). Ray cells and living fibers were full of large starch grains and lipid droplets (Fig. 4). On the other hand, vessel-contact cells were often vacuolated and contained many small lipid droplets and a few small starch grains (Fig. 3). Golgi bodies, r-ER and mitochondoria were very poor. It is supposed from the cell structures that the vessel-contact cells are dormancy and non-contact cells and other cells are devoted to storage of starch and lipid.

In the October material, numerous tylosis buds were originated into the large vessels and also the accompanying ones from the vessel-contact cells through the pittings (Fig. 5). In this season, the vessel-contact cells changed the cytoplasmic structure remarkably. That is, Golgi bodies, r-ER, mitochondoria and ribosomes much increased and they were moved out to buds (Fig. 6). It is well known that they are relating to cell metabolisms and syntheses of cell wall materials. Therefore the increment of them seems to be concerned in tyloses formation. On the other hand, starch grains and lipid droplets preserved in the vessel-contact and non-contact cells were decreased. They may be consumed in the increments and the actions of cell organelles descrived above. Vacuole developed in the large buds and nucleus was observed occasionally in buds (Fig. 7). Tyloses will be expanded by the development of vacuole. And existence of nucleus in bud may suggest the possibility of proliferation of tyloses.

Most vessels were blocked by tyloses in the materials collected in December, Although the tylosis walls do not seem to possess the whole thickness (Fig. 8). The tyloses and the mother parenchyma cells were vacuolated and the cytoplasm were restricted in a narrow region along the wall. In the vessel-contact cells not deriving tylosis, Golgi bodies and r-ER decreased, while large lipid droplets were developed in the cytoplasm (Fig. 8). Electron-dense particles were often accumulated in the vacuoles of vessel-contact cells and also non-contact cells (Fig. 9). The particles may be polyphenolic by-product during tylosis development. Starch grains even in the non-contact cells decreased in number and became more small.

These changes in the vessel-contact and also in the non-contact cells are good consistent with the sequence in the incubated blocks collected from *Quercus serrata*. Vessel-contact cells without distinction of ray or axial parenchyma cell seem to be specialized from other parenchyma cells pointed out by Czaninski[8] and Fujita et al.[5] And these cells will play an important role in tyloses development, judging from the conspicuous changes in the cytoplasmic structure. It have been reported by Czaninski that these contact cells are constant in cell structure. But she would seem to fail in finding the tyloses developing season as descrived by herself.[9]

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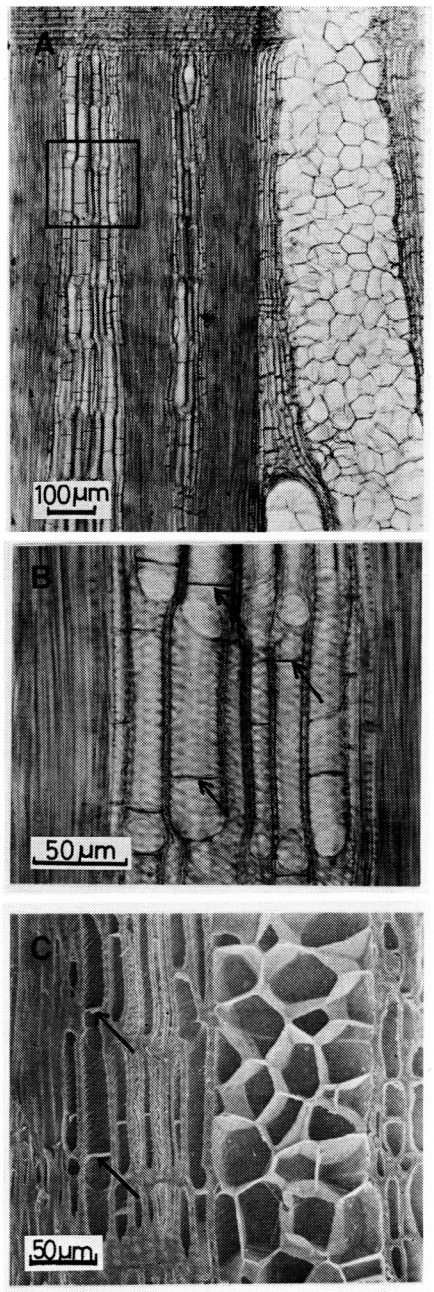

Fig. 1. (A) Radial section of heartwood block. Large pore zone vessel is crowded with the polyhedron shape tyloses, but small size vessels near the annual ring boundary are partitioned off horizontally by tyloses. (B) Enlarged phtograph of photo-A. Arrows indicate the horizontal partitions (cf. photo-C). (C) Scanning electron micrograph of a similar region to photo-A.

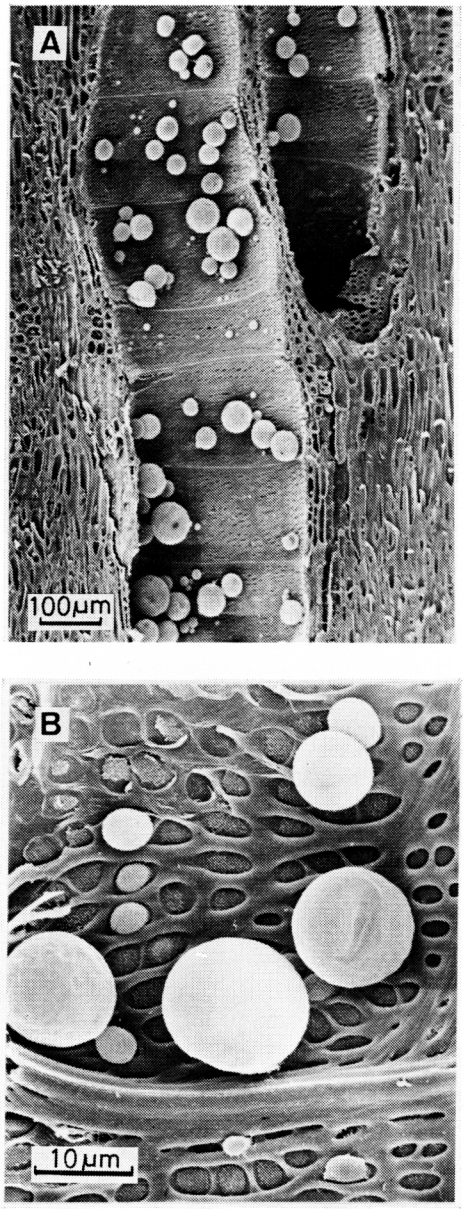

Fig. 2. Scanning electron micrographs of the inner surface of pore zone vessel collected at the beginning of October. (A) Tylosis buds are ballooned from pits all over the vessel inner surface, but (B) the occurrence is more abundant at the large and circular pits.

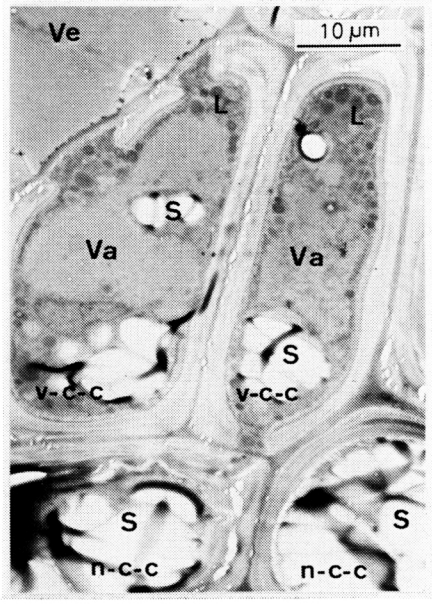

Fig. 3. Vessel-contact cells (v-c-c) and non-contact cells (n-c-c) in the September material. The formers are vacuolated and contain small starch grains (S) and lipid droplets (L). The latters are occupied by large starch grains. Va: vacuole, Ve: vessel.

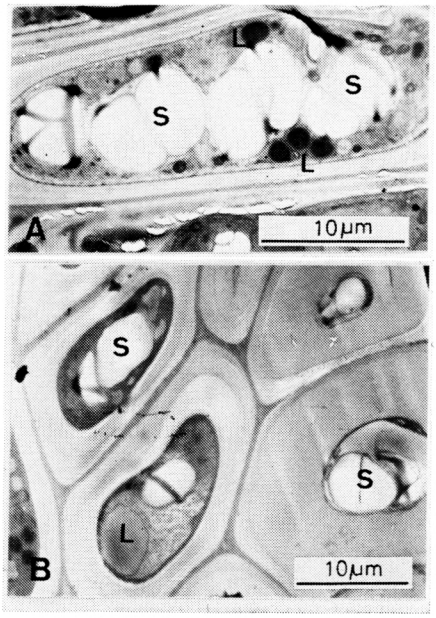

Fig. 4. Ray cell (photo-A) and living fibers (photo-B) in September material. Starch grains (S) and lipid droplets (L) are contained in them.

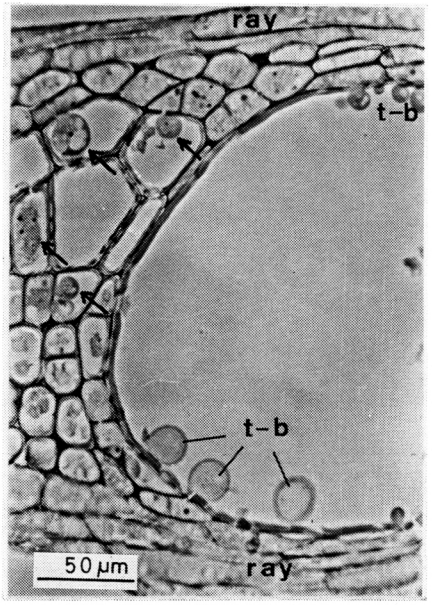

Fig. 5. Phase contrast micrograph of the cross section in October material. Tylosis buds (t-b) are ballooning into the lumen of large vessel and also small vessels (see arrows) from vessel-contact cells.

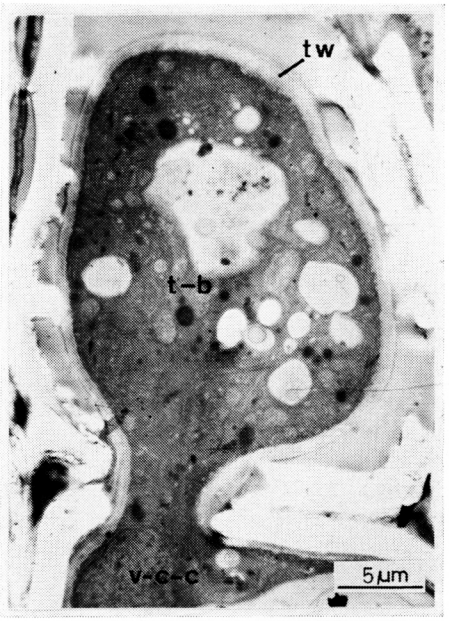

Fig. 6. Tylosis bud (t-b) occurred into the small size vessel in October material. The interior of bud is full of various cell organelles.
tw: tylosis wall.

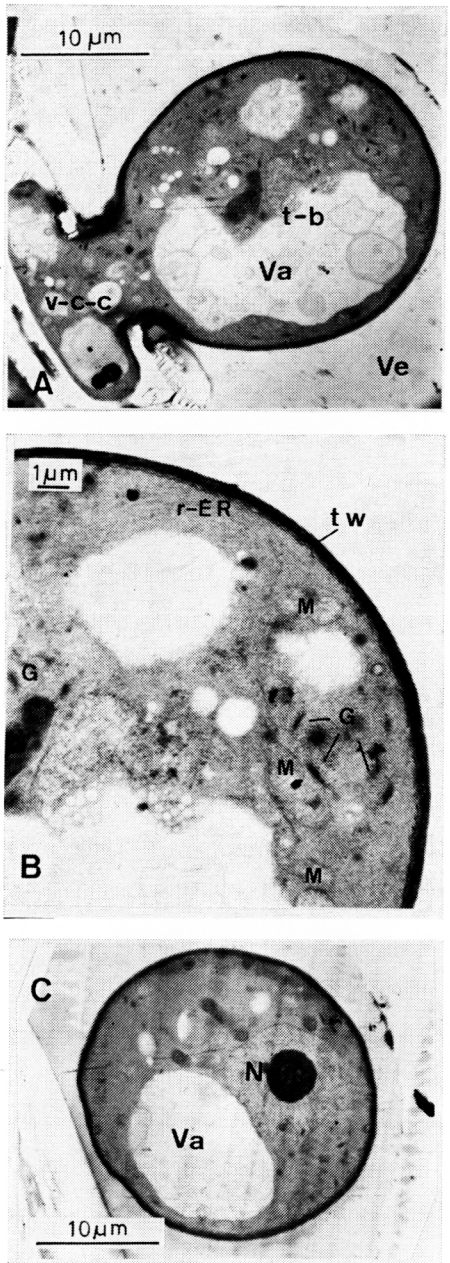

Fig. 7. (A)Tylosis bud (t-b) ballooned into large vessel(Ve) from vessel-contact cell (v-c-c) in October material. Vacuole(Va) are developing in the bud. (B) Enlarged portion of Fig. A. Golgi bodies (G), r-ER and mitochondoria (M) are abundant in the cytoplasm.(C) Same tylosis bud sliced at the other portion to that of Fig. A. Nucleus(N) is moved into bud.

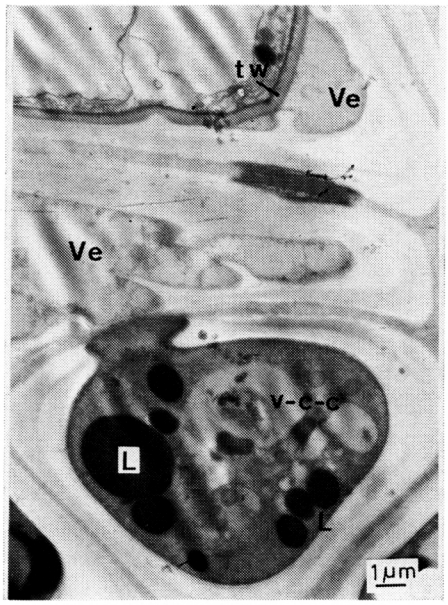

Fig. 8. Vessel-contact cell (v-c-c) and a portion of tylosis wall (tw) expanding in the small vessel (Ve) in December material. Large lipid droplets (L) are contained in the v-c-c. He: herical thickening of small vessel.

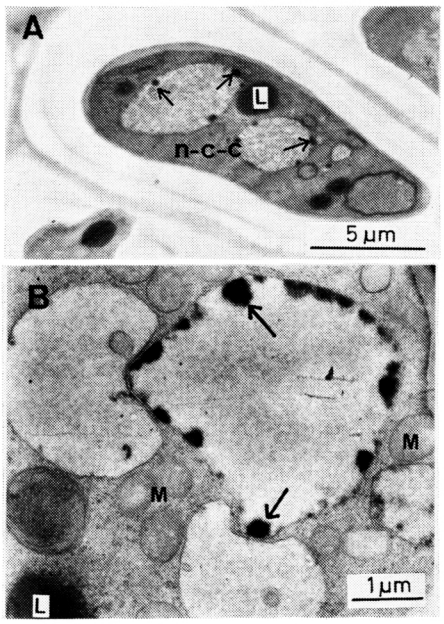

Fig. 9. Non-contact cell (n-c-c)(photo-A) and vessel-contact cell (v-c-c) (photo-B) in December material. Electron-dense particles are accumulating in the vacuole along the tonoplast (see arrows).