

Growth, Structure and Lignin Localization in Rose Prickle

Gyoichi ASANO, Ryohko KUBO and Shizufumi TANIMOTO

(Plantech Research Laboratory)

Received November 28, 2007

Summary

Initiation of prickles of *Rosa hybrida* cv. "Laura" began almost simultaneously with leaf primordia. No regularity of prickle positioning was found on the stem, but a few roses have some regularities. No vascular tissue was found in the prickle. Interestingly, a layer which seems to be the same as well-known abscission layer of deciduous leaves was observed not only in the prickle tissue of *R. hybrida* cv. "Laura" but also in that of *R. × hybrida* cv. "Shortcake". Young and old prickles were easily broken off with fingers from the layer. Lignin localization was observed in the layer. Accumulation of lignin begins from top portion and spreads down to entire portion of prickle. This suggests that hardening of a prickle is closely related with lignin accumulation. These evidences suggest that rose prickles should be leaf spines which are homologous to leaves.

Key words: abscission layer, leaf spine, lignin, prickle, rose.

Introduction

It is already known that a prickle is a sharply pointed spine lacking vascular tissue and grows from tissue under the outer layer of plant.

Prickles are more commonly used than thorns as far as roses are concerned. Scientifically the word "emergence" was used in general for spines of plants, such as prickles and thorns but now it is classified into two categories, stem spines (ex. Japanese quince spines) and leaf spines (ex. Cactus needles) which are homologous to small branches and leaves respectively (Hara, 1993). There are few reports on rose prickles, so we studied to make sure that rose prickles belong to leaf spines or stem spines.

In horticultural and commercial viewpoints, hardening of prickle is one problem. The hardening may be due to accumulate lignin. Therefore, we examined lignin accumulation in prickle.

Materials and Methods

Plant materials

Rosa hybrida cv. "Laura" was used as a main material. Miniature rose, *Rosa × hybrida* cv. "Shortcake" and "Mini cocktail", *Zanthoxylum piperitum* and *Citrus junos* were also used as materials for comparative studies.

Measurement of relative growth of prickle

Three prickles were arbitrarily chosen from three newly developing shoots of “Laura” for relative growth measurement. All three tops of prickles were painted red and bottoms were painted black on the 1 st day, in order to make clear which portion of prickles grow dominant. It was difficult to measure prickle length directly with a slide caliper because of its small size and dramatic change of shape during growth period, while diameter and internode length were easy to measure directly with a slide caliper. So prickle length was measured indirectly from magnified prickle pictures taken with a digital camera, which were shown on the display of a personal computer.

Observation of prickle distribution and measurement of prickle length on a shoot surface

After taking all leaves off, from a fully developed shoot, cortex was cut manually with a razor blade from the top straight down to the bottom of the shoot then stripped off and flattened. Cortex was easy to strip off. Prickle distribution on the flattened stem surface was recorded and prickle length was measured directly with a slide caliper.

Observation of prickle tissue structure

After being fixed with mix solution of acetic acid and ethanol (3:1) , the explant was soaked in 100% ethanol to replace water in it, then 100% ethanol sequentially replaced with n-butanol and 100% n-butanol was replaced with wax. Finally explant embedded in wax was sliced to be 15 µm thick with a rotary microtome. Sliced tissues were stained with safranin and fast green, then observed with an optical microscope.

Lignin localization in prickle tissue

Prickles were cut longitudinally with a razor blade and stained with phloroglucinol for 30 - 50 minutes (Ruzin, 1999). Lignified cell walls were stained red.

Results

Observation in early developmental stage of prickles of “Laura”

As shown in Fig. 1 A, the shape of large size prickles in initial stage looked like a micro dome which emerged almost simultaneously with leaf primordia. Two types of prickles were observed, large size and small size ones. These small size prickles may be named to acicles. All large size prickles in early stage stood upward, parallel with the stem (Fig. 1 B) and developmentally began to bend downward, finally became sharp pointed hooks. On the other hand, interestingly acicles (small size prickles) in early stage had a spheric, rather flat point (Fig. 1 C) and finally became very sharp pointed. Not only a stem but also a leaf had prickles on a petiole and leaf veins of abaxial leaf side and they were found to belong to the small size group.

Relative growth of large size prickles of “Laura”

As shown in Fig. 2, patterns of three relative growth curves of prickles were essentially similar, but maximum values were quite different among them. Their rapid growth finished almost within two weeks, and of course it depended upon circumstance conditions, such as temperature, moisture, daylight span, etc., and after that slow growth approaching to saturation values was seen. Relative growth curves of prickles corresponded to diameter and internode length (Fig. 2).

Fig. 3 consists of consecutive growth pictures of the same prickle for 6 days. Top portion of

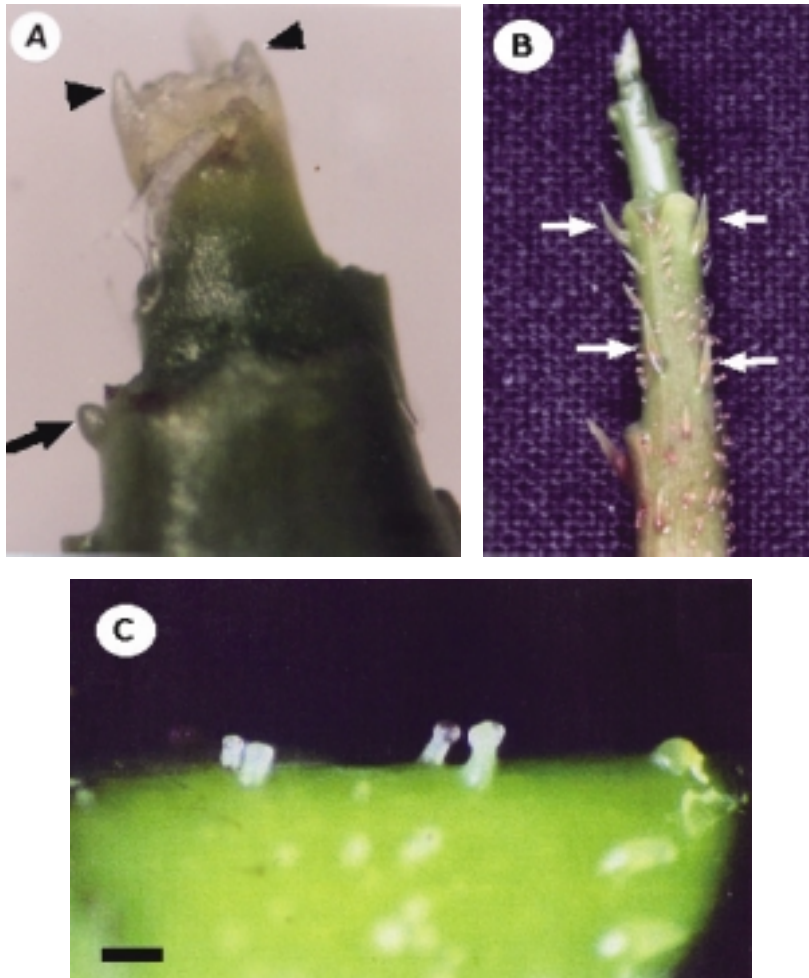


Fig. 1 Prickles in early stage of *R. hybrida* cv. "Laura".

A: A newly developing 3 . 5 mm long stripped lateral bud, initiation of large size prickle (arrow), and leaf primordia (arrowheads) were indicated.

B: 2 cm long stripped shoot, large size prickles (arrows) and many small size prickles (acicles) were shown.

C: Details of acicles in early developmental stage. Bar: 1 mm.

prickles painted red on the first day, changed little, while bottom portion painted black grew dominant, elongated, and diffused very much. Rose prickles grew up from bottom portion not from top.

Prickle distribution and size on a fully matured shoot of "Laura"

As shown in Fig. 4, it seems almost impossible to find out any regularity in their positioning of both small (acicles) and large size prickles on a stem surface, but some roses preserve regularities, for example "Mini cocktail" has one or two straight upward prickles just beneath the basal portion of petiole, almost same as *Zanthoxylum piperitum*.

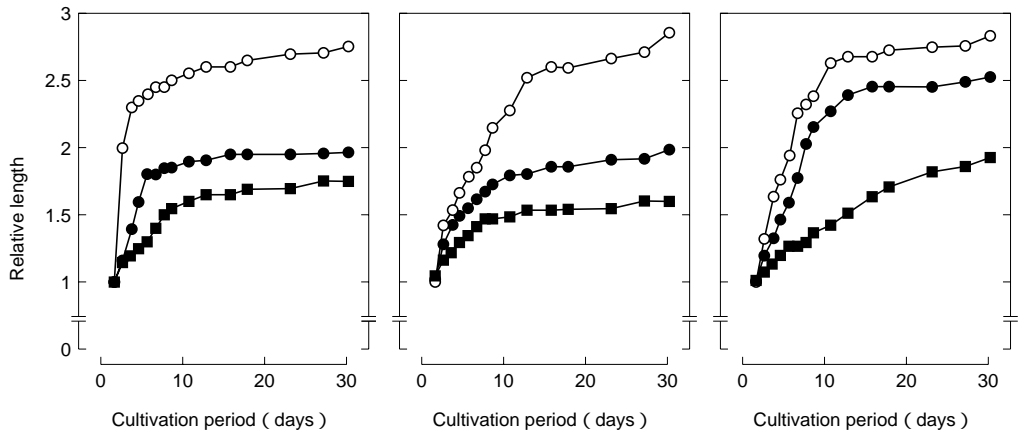


Fig. 2 Growth curve of three independent shoots, for a month, of *R. hybrida* cv. "Laura". Three prickles were arbitrarily chosen from three newly developing shoots for relative growth measurement. The internode length (), large size prickle length (), and stem diameter () were measured.

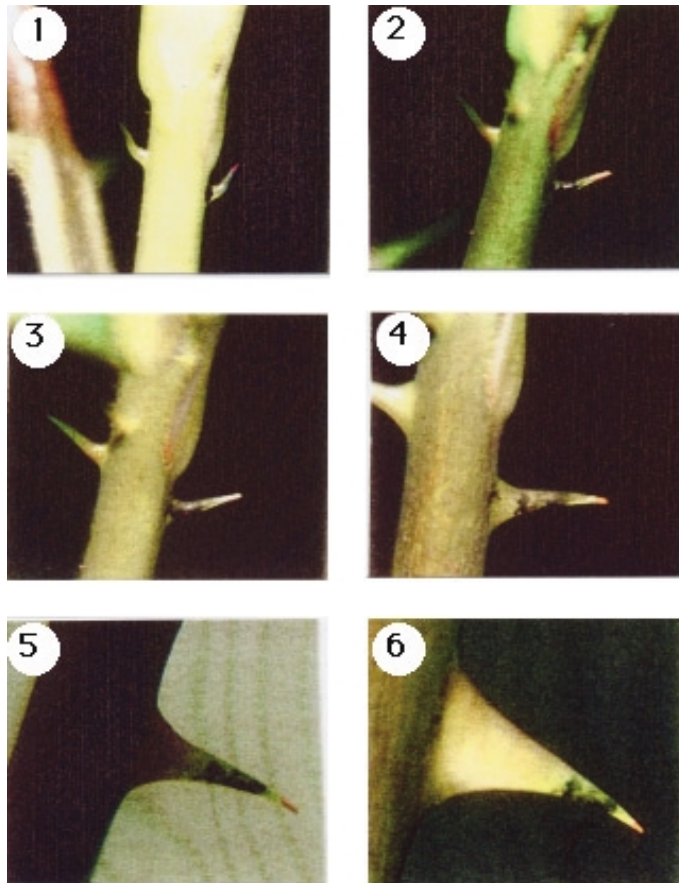


Fig. 3 Consecutive 6-day-pictures (1 to 6 indicates the 1 st to 6 th day) of a large size prickle of *R. hybrida* cv. "Laura" in rapid growth stage. The prickle top was painted red and the bottom was painted black on the 1 st day.

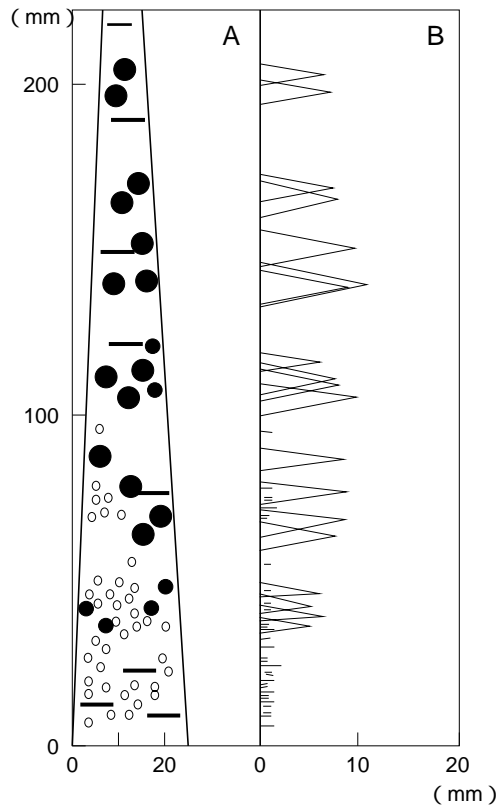


Fig. 4 Schematic prickle distribution on a stripped and unrolled cortex of stem (A) and prickle length (B) of *R. hybrida* cv. "Laura".

, > : large size prickle ; , - : small size prickle (acicle) ; ○ : phyllotaxis.

Structure of prickle tissue of "Laura" and "Shortcake"

Fig. 5 shows longitudinally (Fig. 5 A) and transversely (Fig. 5 B) sliced tissue structure of matured prickles of "Laura". It was clear that no vascular tissue was found. More interestingly a layer was observed which seemed to be the same as abscission layer of deciduous leaves (Esau, 1977), but prickles in early stage did not have their layer yet (Fig. 5 C). However mechanically soft and flexible young prickles already had the layer. Both matured and young prickles were easily broken off with fingers from the layer. This layer was not specific to "Laura" but it was observed also in prickle tissue of "Shortcake" (Fig. 5 D). As a new shoot began to develop fully in spring, soft and flexible young prickles became hard, consequently matured in late spring, and eventually died in late autumn. The layer in longitudinally sliced old prickle tissue of "Laura" was strongly stained with safranin. Almost all leaves died and fell down from stem in autumn, but dead prickles did not.

Lignin localization in prickle tissue of "Laura"

Fig. 6 shows lignin localization in longitudinal cross sections of prickles, fully stained with

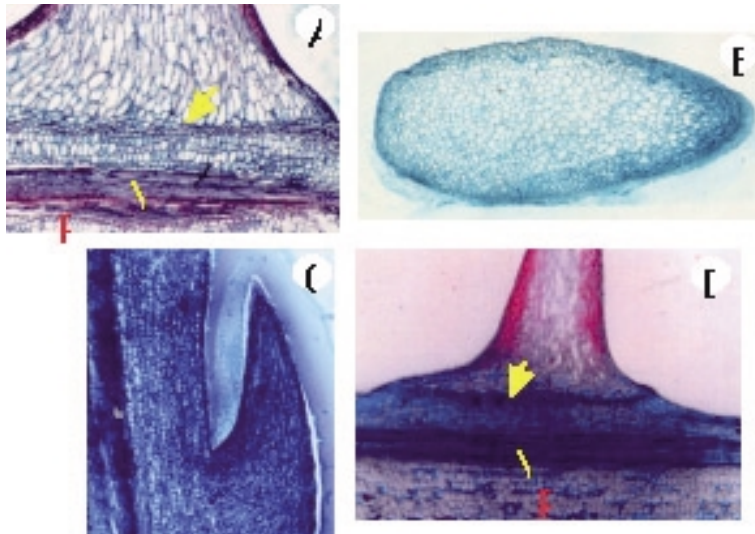


Fig. 5 Sections of prickles in *R. hybrida* cv. "Laura" and *R. x hybrida* cv. "Shortcake".

Longitudinally sliced (A) and transversally sliced (B) large size prickle tissues in mature stage of *R. hybrida* cv. "Laura". Longitudinally sliced prickle tissue in early stage of *R. hybrida* cv. "Laura" (C). Longitudinally sliced large size prickle tissue in matured stage of *R. x hybrida* cv. "Shortcake" (D). Separation layer (arrow), vascular tissue (V), and pith tissue (P) were indicated.

phloroglucinol for 50 min., in three stages: a soft and flexible young prickle (Fig. 6 A, left), a matured and hard one (Fig. 6 A, middle), and an old one (Fig. 6 A, right). These three pictures suggest that lignin accumulation begins from the top of a prickle and spreads down to the bottom.

Fig. 6 B shows lignin localization in longitudinal cross section of an old prickle stained with phloroglucinol for 30 min. Lignin was rich in the layer.

Discussion

Initiation of large size prickles began almost simultaneously with leaf primordia (Fig. 1 A). Distribution of prickle position on stem surface had no regularity such as phyllotaxis, as far as "Laura" was concerned (Fig. 4), but a few roses such as "Mini cocktail" and *R. hirtula* "Nakai", had one or two prickles just beneath basal portion of petiole, almost same as spines of *Zanthoxylum piperitum*. It seems that many rose breeders have crossed numerous roses to improve their qualities for more than 100 years and every time regulatory of prickle arrangement might be mixed together and consequently lost.

Longitudinally sliced prickle tissue showed clearly that a prickle grew from under tissue of outer layer, lacking vascular tissue (Fig. 5 A , 5 B). A prickle in the early stage had no separation layer. However, interestingly, a soft and flexible young prickle already had the separation layer which is same as the abscission layer of a deciduous leaf (Esau, 1977). Old as well as young prickles of roses were easily broken off with fingers from the separation layer. Dead prickles did

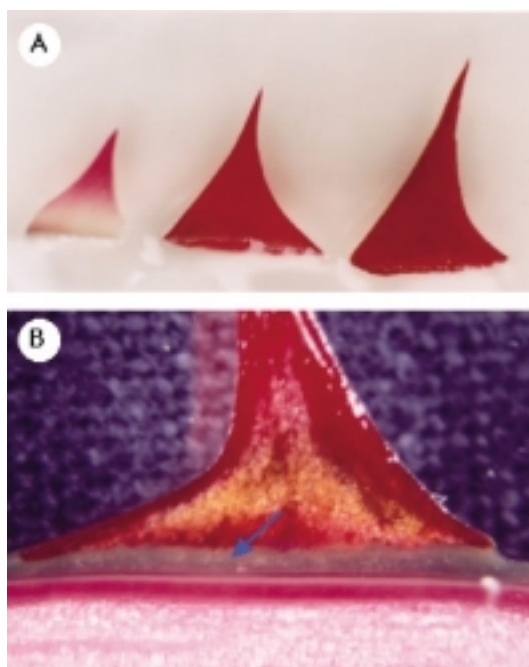


Fig. 6 Lignin localization in large size prickles of *R. hybrida* cv. "Laura".

A: Longitudinal cross section of young (left), matured (middle) and old (right) prickle tissues, stained fully with phloroglucinol for 50 min.

B: Longitudinal cross section of old prickle tissue, stained with phloroglucinol for 30 min.

Separation layer (arrow) was indicated.

not fall off in autumn as leaves did, but this might be advantageous for protecting them from their enemies. It is well known that deciduous trees form abscission layer in joint portion between stem and petiole from when dead leaves fall off in autumn, but exceptionally dead leaves of *Lindera glauca* "Blume" do not fall down until next spring. As to characteristics, rose prickles were very similar to spines of *Zanthoxylum piperitum*, but on the contrary quite different from *Citrus junos* spines which had vascular tissue and no separation layer, were difficult to take off with fingers, and lived long.

The separation layer of old prickles of roses were strongly stained with safranin which tends to stain old tissue red. Accumulation of lignin begins from top portion and spreads down to entire portion of prickle. This suggest that hardening of prickle is closely related with lignin accumulation. Moreover lignin localization in the separation layer of old prickle of "Laura" was observed (Fig. 6 B).

These evidences strongly suggested that separation layer found in prickle tissue of "Laura" and "Shortcake" should be identical with abscission layer of deciduous leaves, consequently rose prickles should not be stem spines but leaf spines.

Present report should be greatly helpful to eliminate sharp pointed prickles from rose plants or make them harmless, that is, soft and flexible or round pointed, by using biotechnological

techniques in the near future.

Acknowledgment

We thank Prof. N. Nitoh (Kinki University) and Prof. Y. Ozeki (Tokyo University of Agriculture and Technology) for great helpful advise and valuable suggestions on this manuscript. We also thank Mr. S. Saisho for preparing specimens.

References

- Esau, K.(1977). *Anatomy of Seed Plant*. John Willey & Sons, Inc., New York.
Hara, N.(1993). *Plant Morphology*. Shokabo, Tokyo. (Japanese)
Ruzin, S. E.(1999). *Plant Micro Technique and Microscopy*. Oxford University Press, London.

バラのトゲの成長，構造，リグニンの局在

浅野 暁一・久保 了子・谷本 静史
(植物工学研究室)

平成19年11月28日 受理

摘 要

バラ (*Rosa hybrida* cv. “Laura”) のトゲは葉原基とほぼ同時に成長を開始した。茎上のトゲの位置には特に規則性は認められなかったが、同時に調べた他の品種では規則性を持つものもあった。維管束組織はトゲに見られなかった。興味深いことに、落葉時の葉の離層と同様の層が“Laura”ばかりでなく“Shortcake”にも見られた。トゲはこの層から容易に指ではずされるのであろう。リグニンの蓄積がこの層に認められた。リグニンの蓄積はトゲの先端部から始まり全域に及ぶようであった。このことはトゲの硬化にリグニンの蓄積が深く関与していることを示唆する。以上の結果は、バラのトゲが葉を起源としていることを示している。