Healing process of the wounds of the branches of the Japanese persimmon that were caused by girdling, scoring, and strangulation

Kazutoshi Hamada[*,1], Tsuneo Ogata, Shinji Fujiwara, Kojiro Hasegawa

Faculty of Agriculture, Kochi University, Otsu 200 Monobe, Nankoku, Kochi 783-8502, Japan

[*]Corresponding author. E-mail hamadaka@cc.kochi-u.ac.jp

[1]Present address: Japan Science and Technology Agency, JST Innovation Satellite Kochi, Kochi, Japan

Abstract

Girdling is widely used for many crops, mainly in order to improve the fruit set, size, and quality and to increase the yield of flower buds. However, girdling may damage trees, and permanent injury can occur if callus bridges are not formed across the ring. We determined the regeneration of vascular bundles and the healing process of wounds caused by surgical treatments. It occurred as follows: necrosis of some cell layers on the edge of the cut, callus formation and dedifferentiation of the parenchyma cells, callus proliferation, contact between callus pads, callus bridge formation, and differentiation into the mature vascular bundle; however, the innermost callus cells remained undifferentiated. At 3 days after treatment (DAT), several phloem parenchyma cell layers showed symptoms of necrosis. At 10 DAT, callus formation started in the phloem tissue below the periderm. Initially, the callus cells were uniform; however, the callus tissue pad was distinguished into 2 layers based on color and firmness. After callus bridge formation, the inner callus differentiated into the mature xylem and the outer callus, into mature phloem. In strangulation, xylem elements were formed from the inner callus pad before callus bridge formation. The callus bridge formed within 6 weeks, 20–25 days, and 14 weeks after girdling, scoring, and strangulation, respectively.

Key words: Callus formation; Regeneration of phloem and xylem; Wounding

1. Introduction

Girdling is widely used for grapevines (Sidlowski et al., 1971; Yamane and Shibayama, 2006), citrus (Inoue et al., 1991; Rivas et al., 2006), apple (Arakawa et al., 1998), peach (Fernandez-Escobar et al., 1987; Onguso et al., 2004), and the Japanese persimmon (Naito et al., 1981; Yano et al., 1999), mainly in order to improve the fruit set, size, and quality and to increase the yield of flower buds. The mechanisms through which girdling operates are not yet fully understood (Goren et al., 2004). However, the effects of girdling are suggested to be involved in the accumulation of carbohydrates in fruits (Rivas et al., 2006) and that of indole-3-acetic acid above the girdle site (Dann et al., 1985).

Moreover, girdling may damage trees, and permanent injury can occur if callus bridges are not formed across the ring (Fernandez-Escobar et al., 1987). The use of girdling as a horticultural technique requires that the age, health, and vigor of the tree as well as the growth conditions be considered (Goren et al., 2004). The cultivator must take into account the girdling width, girdling time, location of the girdling, and cultivars employed while evaluating the anticipated benefits against the risks involved.

There are many similar techniques for girdling. Scoring—simple cut by a knife encircling the branch—has been as effective as ringing in improving the fruit size in loquat (Agustí et al., 2005). Strangulation with wire up to a width of 10 mm has demonstrated an increase in fruit size and soluble solid content and promotes fruit maturation in Japanese persimmon, similar

to girdling; better yield has been observed with strangulation up to a width of 5 mm (Hasegawa and Nakajima, 1992).

Numerous studies concerning the callusing of wounds caused by girdling have been conducted. Inoue et al. (1991) have reported that in the Satsuma mandarin, the girdle produced more callus and the wound healed more rapidly at 25°C than at 20°C, whereas no callus was formed at 15°C. In peaches and nectarines, the degree of callusing has been observed to depend on the width of the girdle and the cultivars used (Fernandez-Escobar et al., 1987). Sidlowski et al. (1971) have reported that callus bridges are formed across girdle wounds on grapevines within 16 days following girdling. Anatomical studies show that phloem connections are reestablished across such wounds within a few days following bridging by callus pads.

To establish an appropriate method of treatment, determining the regeneration of vascular bundles and the healing process of wounds caused by these surgical treatments is important. However, detailed anatomical study on callus formation and differentiation of the vascular bundle is not yet fully understood.

In this paper, we explored the anatomical healing process of the wounds of the branches of the Japanese persimmon that were caused by general girdling, single/double scoring using a knife, and strangulation with wire.

2. Materials and Methods

2.1. Plant materials and treatments

Experiments were conducted on mature 'Hiratanenashi' (47-year-old, in 2004), 'Matsumotowase-Fuyu' (18-year-old, in 2007), or 'Fuyu' (18-year-old, in 2007) Japanese persimmon trees growing at the farm of the Faculty of Agriculture, Kochi University.

The treatments performed were as follows: (1) Girdling was performed with knife on May 31, 2007, and approximately 5 mm of the bark was removed from the 3-year-old branches of 'Fuyu' trees. The girdled branches were sampled 1, 2, 3, 6, 9, 12, and 15 weeks after treatment (WAT). (2) Single and double scoring was performed with a knife on July 3, 2007, without removing the bark of 2-year-old branches of 'Matsumotowase-Fuyu' trees. The treated branches were sampled 0, 3, 6, 10, 15, 20, 25, and 30 days after treatment (DAT) by single scoring and 0, 5, 10, 15, 20, 30, and 40 DAT by double scoring. (3) Strangulation was performed for the bark of the 3-year-old branches of 'Hiratanenashi' and 'Fuyu' using a vinyl chloride-coated steel wire of diameter 2 mm. We performed strangulation for 'Hiratanenashi' on April 28, 2004 and removed the wire on June 28, 2004. The treated branches were sampled 3 years after the treatment. We performed strangulation for 'Fuyu' on May 31, 2007 and removed the wire 8 WAT on July 24, 2007. The treated branches were sampled 0, 1, 2, 3, 8 (immediately after removing wire), 9, 10, 12, 14, and 16 WAT.

Six branches (two branches/tree) were sampled from three trees of each treatment at each

sampling time.

2.2. Tissue observation

The sampled branches were immediately fixed in formalin-acetic acid (FAA) preservative solution and vacuum-infiltrated. The fixed branches were cut lengthwise. The samples were dehydrated in a graded alcohol series, embedded in paraffin, and then cut into 20–24 μm longitudinal sections on a sliding microtome (Yamato Kohki Industry Co., Ltd., Japan). The sections were stained with safranin and fast green, and permanent mounts were prepared.

All the sections were observed under a light microscope (Olympus DX-50; Olympus, Tokyo, Japan) and stereomicroscope (Olympus SZX12; Tokyo); the digital images were then captured using a digital microscope camera (Olympus DP-12; Tokyo).

3. Results

3.1. Girdling

Several layers of phloem parenchyma cells became necrotic within 1 WAT. At 2 WAT, the periderm cracked slightly and callus formation began in the phloem tissue below the periderm. At 3 WAT, callus pads developed and spread approximately 3 mm downward from the upper cut and approximately 0.5 mm upward from the lower cut in the wound (Fig. 1). The callus

pad was distinguished from the 2 layers on the basis of color and firmness. The inner callus tissue was firmer and its color was light as that of the xylem tissue. The outer callus tissue was softer and its color was dark as that of the phloem tissue and periderm. At 6 WAT, a callus bridge was formed across the girdle wound; it mainly comprised the callus growing from the upper cut of the wound (Fig. 2). The girdled area was swollen due to the callus.

At 3 WAT, microscopic examination showed that the callus pad comprised 2 layers: the inner xylem elements and the outer elements (Fig. 1). The outer callus contained more tanniferous cells than the inner callus, which was similar to intact tissues. The boundary between the 2 different calluses was lined by depressed cells with definite nuclei. The exposed xylem of the girdle area did not form a callus, but a few layers became necrotic. After healing of the wound, the inner callus differentiated into mature xylem and the outer callus, into mature phloem (Fig. 3). Finally, the outermost callus tissues differentiated into the phellem, phellogen, phelloderm, and cortex. However, the newly formed callus and exposed xylem did not fuse and persisted as a scar. The callus tissue adjacent to the scar remained as an undifferentiated cell mass.

3.2. Single and double scoring

The bark tissue did not change immediately after scoring by a knife and was without injury (Fig. 4A). At 3 DAT, several layers of phloem parenchyma cells showed symptoms of necrosis (Fig. 4B). Moreover, the vascular cambium became inwardly necrotic. Therefore, the

results of scoring were almost similar to those of 2-mm wide girdling. At 10 DAT, the periderm began to swell due to callus growth, resulting in the division of dedifferentiated phloem parenchyma cells (Fig. 4C and D). The callus bridge formed partially within 20 DAT and completely within 25 DAT. At 30 DAT, the callus differentiated into the vascular bundle (Fig. 5).

The patterns of the healing process following single and double scoring were almost similar. Callus formation was always more extensive in the upper cut than in the lower cut of the wound; however, the formation of a callus bridge was not always more rapid in the upper cut as compared to in the lower cut of the wound.

3.3. Strangulation with wire

Immediately after strangulation, the bark was partially cut by the wire. At 1 WAT, the bark was depressed by the wire and became necrotic accompanied by growth of the branch (Fig. 6A). The bark above the wire was warped. At 4 WAT, callus formed under the warped bark (Fig. 6B). Thereafter, the callus continued to enlarge and spread almost over the wire between 10 and 12 WAT, which was until when the wire remained. After removing the wire at 8 WAT, the callus enlarged further, bridging the gap caused by the wire (Figs. 6D and 8). A callus bridge was formed 6 weeks after the removal of the wire. The callus hump was more prominent following strangulation than girdling.

At the time of removal of the wire, the callus tissue pad was distinguished into 2 layers,

and the inner callus had already differentiated into mature xylem tissue (Figs. 6C and 7). At 6 weeks after removing the wire, callus differentiated into mature vascular bundles, before the complete healing of the wound. At 10 weeks after removing the wire, callus tissues differentiated into the phellem, phellogen, phelloderm, and cortex (Figs. 9 and 10).

At 3 years after strangulation, although the scar persisted at the inner side of the strangulated branch, growth was almost similar to that of the intact tissue.

4. Discussion

The healing response of the wound caused by these surgical treatments was observed in the functioning and developing tissue of the phloem or vascular cambium and not in the phellem, phellogen, phelloderm, cortex, and non-functioning and degenerating tissue of the phloem and xylem. The process demonstrated the following sequence of events: necrosis of some cell layers on the edge of the cut, callus formation accompanied by dedifferentiation of the phloem parenchyma cells, proliferation of the callus cells, contact between the upper and lower callus pads, formation of a callus bridge, and differentiation into the mature vascular bundle. However, in strangulation, the inner callus had already differentiated into xylem elements before a callus bridge was formed. It is interesting that this healing process was almost similar among the surgical treatments. The healing process may be common to plant trees.

The formation of necrotic layers at the girdle interface was the first wound response. The necrotic layers, which are formed as a general response to any injury, act as a temporary simple barrier against foreign enemies and prevent the outflow of water (Ogata, 2005). In this study, symptoms of necrosis first appeared at 3 DAT, and a few layers of the phloem parenchyma cells, cambium, and exposed xylem became necrotic.

The parenchyma cells of only the functioning phloem dedifferentiated; thereafter, callus was formed. Proliferation of callus cells was observed within 14 and 10 DAT with girdling and scoring, respectively. Sidlowski et al. (1971) reported that the cells were occasionally observed to proliferate slightly from the exposed xylem. However, no response was observed from the exposed xylem without necrosis.

Thereafter, in the cases of girdling and scoring, the callus spread downward from the upper cut and upward from the lower cut of the wound. In the case of strangulation, the callus spread to the outer side, covering the wire. After removing the wire, newly formed callus filled the gap caused by the wire. Callus formation from the upper cut was always more rapid than from the lower cut of the wound, which was in agreement with the report of Sidlowski et al. (1971). Onguso et al. (2004) reported that the starch and sugar contents in the bark were higher in the upper part than in the lower part of the ring, which promoted callus formation from the upper cut of the wound.

The callus tissue pad was distinguished from the 2 layers on the basis of color and firmness until 20 DAT. Under the microscope, it was observed that the outer callus tissue

comprised numerous tanniferous cells similar to those in the intact phloem tissue. Holden (1930) found abundant tanniferous cells in the callus tissue. On the other hand, the inner callus tissue had hardly any tanniferous cells, similar to as in the intact xylem tissue. Therefore, the callus had already differentiated into the inner xylem elements and outer phloem elements. The cambium was the boundary between the 2 different calluses.

The upper and lower callus pads met and merged immediately. We suppose that the outer callus differentiates into phloem immediately after the merging of the callus pads, which would be able to resume translocation across the wound. Short sieve tubes, which were slightly longer than other callus cells, were observed in callus tissue adjacent to a newly formed vascular cambium. Sidlowski et al. (1971) observed sieve tube cells with sieve plates at the end walls or lateral sieve areas in all the callus tissues investigated. However, we did not observe cells with sieve plates. After callus enlargement ceased, the thickness of the phloem was almost similar to that of the intact phloem.

Naito et al. (1981) reported that no callus bridge formed in the trunk girdle of the Japanese persimmon until 6 month unless the girdle was wrapped with a vinyl tape. The vinyl tape was used to prevent drying and enhance callus formation (Inoue et al., 1991). When the girdle was covered with vinyl tape, healing of the wound caused by 10-mm wide girdling was established within approximately 1 month (Naito et al., 1981; Yano et al., 1999). In this experiment, when the lateral branches were treated, a callus bridge was formed even if the wound was not covered with vinyl tape, suggesting that the younger wood healed earlier with

girdling. Fernandez-Escobar et al. (1987) have also observed that girdling young wood is preferred to ensure rapid, complete callusing. We observed callus bridge formation within 6 weeks, 20–25 days, and 14 weeks after girdling (width, 5 mm), scoring, and strangulation treatments, respectively.

A completely healed area can be observed with a typical bulge (Sidlowski et al., 1971). The bulge caused by strangulation was the largest. However, the bulge relatively reduced in size every year and was almost flattened 3 years after strangulation. Although the scar persisted on the inner side of the strangulated branches, the branches had not snapped.

On the other hand, Hasegawa and Nakajima (1992) demonstrated that strapping (i.e. strangulation) and girdling in 10 mm width were greater in fruit quality than girdling in 3 mm width. Moreover, Taylor (2004) reported that one or two cable ties (similar to strangulation) resulted in peach fruit size and yield similar to or better than that of standard knife girdling. However, as mentioned above, girdling may damage trees, and permanent injury can occur if callus bridges are not formed across the ring (Fernandez-Escobar et al., 1987). Our study would contribute to understand the process of healing and can help the growers to realize a better treatment of girdling, scoring or strangulation. However, further detailed studies are required to establish the relationship between fruit quality and healing.

References

Agustí, M., Gariglio, N., Juan, M., Almela, V., Mesejo, C., Martínez-Fuentes, A., 2005. Effect of branch scoring on fruit development in loquat. J. Hort. Sci. Biotechnol. 80, 370–374.

Arakawa, O., Kanetsuka, A., Kanno, K., Shiozaki, Y., 1998. Effects of five methods of bark inversion and girdling on the tree growth and fruit quality of 'Megumi' apple. J. Japan. Soc. Hort. Sci. 67, 721–727 (In Japanese with English abstract).

Dann, I. R., Jerie, P. H., Chalmers, D. J., 1985. Short-term changes in cambial growth and endogenous IAA concentrations in relation to phloem girdling of peach, *Prunus persica* (L.) Batch. Austral. J. Plant Physiol. 12, 392–402.

Fernandez-Escobar, R., Martin, R., Lopez-Rivares, P., Paz Suarez, M., 1987. Girdling as a means of increasing fruit size and earliness in peach and nectarine cultivars. J. Hort. Sci. 62, 463–468.

Goren, R., Huberman, M., Goldschmidt, E. E., 2004. Girdling: Physiological and horticultural aspects. Hort. Rev. 30, 1–36.

Hasegawa, K., Nakajima, Y., 1992. Effects of girdling and strapping of lateral branches on fruit growth in persimmon cv. Nishimurawase. Bull. Fac. Agric. Kochi Univ. 41, 39–45 (In Japanese with English abstract).

Holden, H. S., 1930. Observations on some wound reactions in the aerial stem of *Psilotum triquetrum*. Ann. Bot. 44, 285–296.

Inoue, H., Ikoma, Y., Kataoka, I., 1991. Effects of ringing and temperature on flower bud differentiation of satsuma mandarin. J. Japan. Soc. Hort. Sci. 60, 275–284 (In Japanese

with English abstract).

Naito, R., Ueta, H., Yamamura, H., 1981. Promotion of early fruiting in ‘Saijo’ Japanese persimmon I. Effects of girdling, bark inversion and SADH spray on vegetative growth, flowering and yield. Bull. Fac. Agr. Shimane. Univ. 15, 12–21 (In Japanese with English abstract).

Ogata, T. 2006. Physiological aspects of grafting. Regulation of Plant Growth & Development 40, 131–138 (In Japanese).

Onguso, J. M., Mizutani,F., Hossain, A. B. M. S., 2004. Effects of partial ringing and heating of trunk on shoot growth and fruit quality of peach trees. Bot. Bull. Acad. Sin. 45, 301–306.

Rivas, F., Erner, Y., Alós, E., Luan, M., Almela, V., Agustí, M., 2006. Girdling increases carbohydrate availability and fruit-set in citrus cultivars irrespective of parthenocarpic ability. J. Hort. Sci. Biotechnol. 81, 289–295.

Sidlowski, J. J., Phillips, W. S., Kuykendall, J. R., 1971. Phloem regeneration across girdles of grape vines. J. Amer. Soc. Hort. Sci. 96, 97–102.

Yamane, T., Shibayama, K., 2006. Effects of Trunk Girdling and Crop Load Levels on Fruit Quality and Root Elongation in ‘Aki Queen’ Grapevines. J. Japan. Soc. Hort. Sci. 75, 439–444.

Yano, T., Moriguchi, K., Shinkai, A., 1999. Investigations on the control of physiological fruit dropping in the ‘Tonewase’ Japanese persimmon (1) Effects of plant growth regulators

and girdling on fruit set and fruit quality. Bull. Ehime pref. 13, 11–18 (In Japanese with English abstract).

Figure captions

Fig. 1. Longitudinal section of the girdle of a 3-year-old lateral branch in ‘Fuyu’ 3 weeks after girdling. The left and right sides are the upper and lower cuts of the wound, respectively. The callus pads (cp) comprise 2 layers, and the outer layer contained more tanniferous cells than the inner layer. The inner callus comprised xylem elements (xy) and the outer, phloem elements (ph); the boundary between the 2 layers is the cambium (cm). Newly differentiated phellogen (pg) also produced on the surface of callus pads. Necrotic tissue persists between the periderm and callus pads. Bar: 500 μm.

Fig. 2. Longitudinal section of the girdle of a 3-year-old lateral branch in ‘Fuyu’ 6 weeks after girdling. The left and right sides are the upper and lower cuts of the wound, respectively. The callus pads (cp) spread and merge. A few layers of exposed xylem become necrotic (n). The inner callus comprised xylem elements (xy) and the outer, phloem elements (ph); the boundary between the 2 layers is the cambium (cm). Bar: 500 μm.

Fig. 3. Longitudinal section of the girdle of a 3-year-old lateral branch in ‘Fuyu’ 9 weeks after girdling. The left and right sides are the upper and lower cuts of the wound, respectively. The outer callus differentiated into mature phloem (ph’) and the inner callus, into mature xylem (xy’). The elongation cell was located immediately lateral to the neocambium (cm), which

would form the phloem tube element. Aging cells stained with safranin was dotted within the phloem (arrows). The innermost callus cells remained undifferentiated (c). A few layers of exposed xylem become necrotic (n). Bar: 500 μm.

Fig. 4. Longitudinal section of a 2-year-old lateral branch in 'Matsumotowase-Fuyu' 0, 3, and 10 days after single scoring. At 0 days after scoring (A), the wound formed was approximately 0.08 mm in width. At 3 days after scoring (B), some layers of parenchyma cells showed signs of necrosis. At 10 days after scoring (C and D), phloem parenchyma cells (ph) dedifferentiated and began forming callus (c). Phloem parenchyma cell and vascular cambium became necrotic but periderm remained unchanged. Bars: 200 μm. xy: xylem.

Fig. 5. Longitudinal section of a 2-year-old lateral branch in 'Matsumotowase-Fuyu' 30 days after single scoring. The right and left sides are the upper and lower cuts of the wound, respectively. The wound healed completely. Callus differentiated into mature xylem (xy'), mature phloem (ph') and cambium (cm), but the innermost callus cells remained undifferentiated (c). The injured xylem turned brownish and became necrotic (n). Bar: 500 μm.

Fig. 6. Stereomicroscopic appearances of a 3-year-old lateral branch in 'Fuyu' 1, 4, 8, and 14 weeks after strangulation. The right and left sides are the upper and lower cuts of the wound,

respectively. Arrows show depression occurring due to the wire. At 1 week after strangulation (A), the wire depressed the bark to become necrotic, and the bark above strangulation was warped (arrowhead). At 4 weeks after strangulation (B), callus was formed below the warped bark (arrowhead). Photo obtained immediately after removing the wire 8 weeks after strangulation (C). Callus spread outward along with the wire. The color of the outer and inner callus was brown and pale brown, respectively. The inner callus had already lignified (arrowhead). At 14 weeks after strangulation (D), newly formed callus (arrowhead) spread further bridging the gap caused by the wire. Bars: 2 mm.

Fig. 7. Longitudinal section of a 3-year-old lateral branch in 'Fuyu' 8 and 14 weeks after strangulation. (A) Section of branch shown in Fig. 6C at the time of wire removal (8 weeks). The inner callus began to differentiate into mature xylem (xy') with ray tissue. The callus pad (cp) comprised phloem elements (ph) and cambium (cm). (B) Section of branch shown in Fig. 6D at 6 weeks after wire removal (14 weeks). Newly formed callus (c) spread forward to bridge the gap. Bar: 500 μm.

Fig. 8. Longitudinal section of a 3-year-old lateral branch in 'Fuyu' 18 weeks after strangulation (10 weeks after the removal of wire). The right and left sides are the upper and lower cuts of the wound, respectively. Callus completely differentiated into mature xylem (xy'), mature phloem (ph'), and periderm (pr), but the innermost callus remained

1 undifferentiated (c). The injured xylem turned brownish and became necrotic (n). Bar: 500

2 μm.

3 Fig. 9. Transverse section of the dashed line shown in Fig. 8. Bar: 500 μm.