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Histochemical Observation and HPLC Analysis of Phenolic Compounds at the Graft Union of Dwarf Peach Trees Grafted onto *Prunus tomentosa*

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Summary

Phenolic compounds extracted from the graft union of heterograft trees (*Prunus persica* / *P. tomentosa*) were analyzed by histochemistry and HPLC methods and compared with those on homograft trees (*P. persica*/*P. persica*). A DMACA (*p*-dimethylamino-cinnamaldehyde) staining method showed that at early stages after budding the phenols were localized mainly in the callus cells, in the new xylem and in the cortical tissues of the heterograft combination and persisted even 6 months after budding. These phenolic compounds frequently surrounded necrotic tissues which seemingly impeded the union between the scion and rootstock. From the HPLC analysis, four phenolic compounds were found as major components in both rootstocks, but two of them were not detected in the phloem of dwarfing *P. tomentosa*. The chemical nature of these peaks was unknown, except one was identified as catechin; however, based on their UV spectrum, behavior in TLC and retention time, our assignments are tentative. In general, the phenolic content in the phloem tissues was higher in homograft than in heterograft trees, particularly peaks # 1 and # 3, which we suppose are prunin and naringenin respectively. However, in the xylem tissues on heterograft trees the phenolic concentration was much smaller and the patterns from the stock to the scion were different from those observed on homograft trees. Peak # 1, prunin and Peak # 2 accumulated in the phloem above the heterograft union; whereas in the xylem, Peak # 1, prunin, and Peak # 3, naringenin, were present at low amounts. Catechin, a low molecular weight flavan occurred in similar amounts in the xylem of both combinations, accumulating at the graft union, whereas the phloem, of the homograft contained a larger amount of catechin.

Key Words: *Prunus tomentosa*, grafting, dwarfing rootstock, peaches.

Introduction

The graft incompatibility symptom observed on peaches grafted onto *Prunus tomentosa* Thunb. (Nanking cherry) often becomes apparent only after a few years when the trees suddenly die. The diminished growth of these trees could be attributed to graft incompatibility rather than to dwarfing *per se* as apple rootstocks. The causes of the graft incompatibility related to this rootstock for peaches are not known.

Researchers have attributed graft incompatibility of trees to the accumulation of phenolic compounds near the graft union zone (Errea et al., 1992a, b; Errea and Felipe, 1994; Cooman et al., 1996; Treutter and Feucht, 1988, 1991). We observed previously that the graft union of peaches on both *P. tomentosa* and *P. persica* rootstocks developed an abnormal pattern of vascular tissues even 4 years after grafting. However, necrotic cells were observed mainly in the union of peaches grafted on *P. tomentosa* at the early stages (1 to 6-month-old after budding) as well as in 2 and 4-year-old dwarfing trees (Salvatierra et al., 1998).

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Many phenolic compounds are natural constituents of plants; the release and eventual accumulation of phenols can occur as a response to tissue wounding, such as when grafting is performed; hence, the accumulation could be related to graft incompatibility. Prunin (naringenin-7-glucoside), a characteristic phenol of *Prunus* found in the phloem above the union in field-grown cherry trees was associated with the stock/scion incompatibility of the trees (Treutter and Feucht, 1988). Perhaps foreign phenols are released into the surrounding tissues when cut surfaces of the stock and scion are brought together. Then, the cells of both adjacent parts may be subjected to "foreign" substances and may not have the appropriate enzymes to break down the phenols or to convert them into a nontoxic form (Lockard and Schneider, 1981).

On the other hand, the phenolic compounds, depending on their chemical structure, have different roles in physiological processes. Some of them activate IAA oxidase, whereas others inhibit this enzyme (Lee et al.,1982). Thus, phenols that occur at the graft union may have favorable or noxious effects on the tissue development at the graft union.

This study aimed to determine the frequency and spatial location of phenolic compounds in the graft

union of trees budded onto *P. tomentosa*, compared to those budded onto *P. persica* and to identify and quantify the phenolic compounds in the graft union zone.

Materials and Methods

1. Histochemical localization of phenolic compounds at the graft union

Samples of graft union zone of cv. Kansuke Hakuto peach, grafted onto *P. tomentosa* and *P. persica* in August 1996, were collected at 4 and 15 days, as well as 1, 3, and 6 months after budding. The samples were fixed with 2.5 % glutaraldehyde in 0.1 M phosphate buffer at pH 7.5, under a moderate vacuum for three hours to remove intercellular air. The dehydration was performed by a sequence of ethanol solution, and the infiltration of samples was done with 20-40-60-80 % ethanolic glycol methacrylate (GMA) concentration series at 30 min. intervals. Then, the tissues were transferred to 100 % GMA solution for 48 hours and embedded into pre-polymerized GMA in gelatin capsules. The samples were put in an oven at 40 ℃ for one day, then at 60 ℃ until the fixative hardened. The embedded tissues were cut by a microtome at 25 μm thickness. The tissues were stained with 0.1 % DMACA (*p*-dimethylamino-cinnamaldehyde) dissolved in 0.5 M sulfuric acid in 1-butanol. The specimens were stained in a microwave oven for 15 sec until the solution began to boil; it was then washed with absolute ethanol three times and mounted on glass slides with Canada balsam (Gutmann and Feucht, 1991).

The DMACA method (Gutmann and Feucht, 1991; Teutter, 1989) selectively stains of flavan-3-ols and proanthocyanidins (oligomeric derivatives) blue, but because sulfuric acid is also present in the DMACA staining solution, the original blue of the DMACA/flavan-3-ols complex may have faded to a faint lilac tinge by the superimposed anthocyanidins (Gutmann, 1993; Gutmann and Feucht, 1994).

2. HPLC analysis

Plant material and extraction of phenolic compounds

Three 2-year-old peach trees grafted onto *P. tomentosa* (heterograft combination) and three on *P. persica* (homograft combination) growing in pots were sampled in July, 1996. The graft union zone was divided into three sections, 2 cm long i.e., below the union zone (stock sections), graft union (union itself) and above the union zone (scion sections). The sections were submerged in liquid nitrogen immediately and then freeze-dried. The epidermis (brown outermost layer) was discarded. The bark (phloem, phellem, phellogen, and cortex) and wood tissues (xylem) were scraped off the freeze-dried sections; 200 mg of this powder was extracted with 20 ml 80% acetone containing 0.4 % Triton X-100, for 10 days at 4 ℃ ; the solvent was changed twice. The filtrates were pooled and evaporated in vacuum at 40 ℃. The residue was dissolved in 2 ml methanol, containing 6-hydroxyflavone (0.5 mg/ ml) as internal standard. After these crude extracts were filtered through a 0.45 μm membrane filter (Sartorius), 10 μl of the filtrate were injected into HPLC (TOSOH, 8020 series, Japan).

Analytical Procedure

The phenolic compounds were separated by using a reversed-phase column Zorbax ODS (4.6 mm ID x 250), and detected by two methods. **1**. Chemical reaction detection (CRD) (Treutter, 1988, 1989; Treutter and Santos-Buelga, 1994); after separation through ODS column, the extracts were mixed with the staining reagent in a reactor consisting of a 40 m long capillary. The staining reaction was performed with 1 % DMACA reagent in 1.5 M methanolic sulfuric solution at 95 ℃. The compounds were detected at 640 nm. **2**. UV determination at 280 nm and 35 ℃ (Geibel et al., 1990; Martelock et al., 1994). A mobile phase flowed at 0.5 ml/min on both methods. Solvents: 1 % aqueous acetic acid (solvent A) and methanol: butanol 5:1, v/v (solvent B). The gradient (B in A) was programmed as follows : 0 min., 8%; 10 min., 10 % ; 20 min., 14 % ; 30 min., 17 % ; 40 min., 20 % ; 50 min., 27 % ; 60 min., 33 % ; 70 min., 49% ; 80 min., 62 % ; and 90-105 min., isocratic 80%.

A large peak, detected at 640 nm by method 1 (CRD), was identified as catechin by comparing the retention time with the known standard ((+)-catechin hydrate). The concentration of this peak was calculated as a proportion of the known concentration of catechin. The values were expressed by mg/g dry weight.

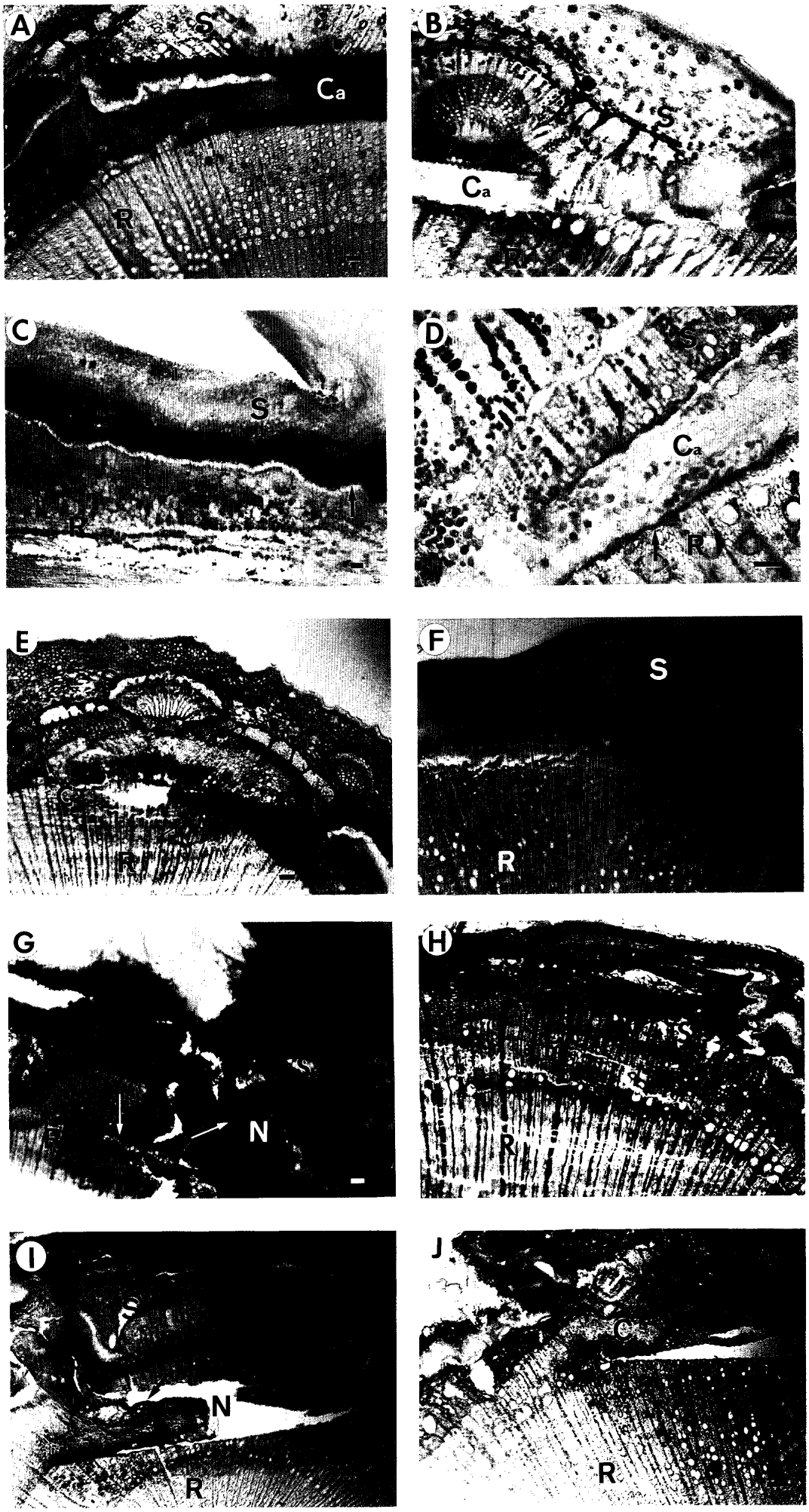
Peaks on the chromatogram detected at 280 nm by method 2 were integrated and measured with a photodiode-array-detector (JASCO, MD-910, Japan), then compared to known data from the literature (Treutter et al., 1985; Waterman and Mole, 1994). Fractions of Peak # 1 and # 2 were separated by two-dimensional chromatography on cellulose plates using the solvents (1) n-butanol-acetic acid-water (4:1:5, v/v upper layer) and (2) 15 % aqueous acetic acid. The fluorescence was detected under UV light after exposure to ammonium vapor. Chromogenic staining was performed with ferric chloride (2.5% in methanol) and diazotized sulphanilic acid (Waterman and Mole, 1994). The retention time was also considered in the tentative assignment of phenolic compounds.

Results

1. Histochemical localization of phenolic compounds at the graft union

Early stages after budding

At 4 days after budding, in the heterograft combination (*P. persica* / *P. tomentosa*), the callus cells which



developed between the scion and the stock were surrounded by stained cells (Fig. 1A), whereas on trees of homograft combination (*P. persica* / *P. persica*) the callus cells did not become stained (Fig. 1B). At the early stages the cambium is apparently active because new xylem was produced in the stock xylem, but near the stained cells, new xylem elements differentiated.

At 15 days after budding, the longitudinal sections of heterograft union revealed an isolated layer of stained cells between the scion bud and the stock (Fig. 1C). On the contrary, in comparable sections of homografts, some plugged cells could be seen in the callus (Fig. 1D).

One-month-old bud union

On the heterograft combination, stained cells surrounded the callus (Fig. 1E). At the union, one side seemed to be connected, whereas the other side containing pockets of stained cells did not. New xylem elements developed on the scion; but in the zone where there were stained cells, the connection was not clear (Fig. 1E, arrow). On homograft trees, the callus cells were reabsorbed differentiating to vascular tissues; only a thin layer of stained cells was observed in the intergraft zone (Fig. 1F).

Three-month-old bud union

The presence of red (proanthocyanidin) and blue (flavans) stained cells surrounding the callus (Fig. 1G, white arrows), reveal the distribution of phenolic compounds. The large amount of necrotic tissues (N) and suberin-like cells between the stock and the scion apparently prevent a continuous connection from forming between graft partners on heterograft trees (Fig. 1 G). Contrarily, in the homograft trees, the graft union was free of necrotic tissues, and only vessels with resin were observed in the stock. A new wound cambium differentiated vascular elements on both sides of partners (Fig. 1H).

Six-month-old bud union

On heterograft trees, the xylem of the scion and the stock developed vessels with resin-like substance. A layer of necrotic cells surrounded the callus and stained cells seemingly separated the old and new xylem of scion. Although the development of the new xylem reflects cambial activity, no clear vascular connection

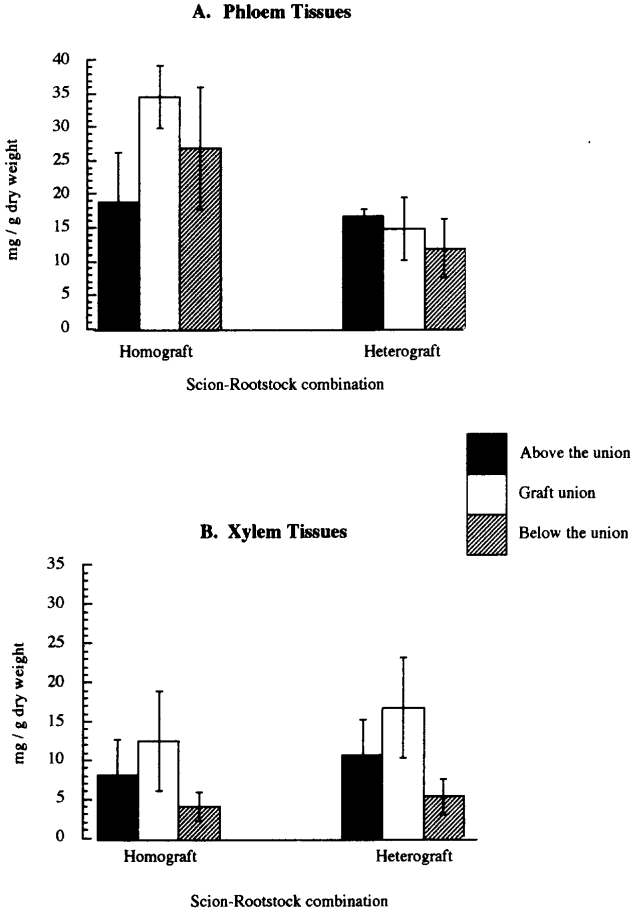


Fig. 2. Mean values of catechin (mg/g dry weight) of phloem and xylem tissues from graft union of peach trees onto *P. persica* and *P. tomentosa*. Vertical bars= SE.
Homograft combination: *P. persica* / *P. persica*
Heterograft combination: *P. persica* / *P. tomentosa*

Fig. 1 Photographs of graft union of peach bud-grafted onto *P. tomentosa* and onto *P. persica* rootstocks.

(A) 4 days after budding, strongly stained cells are present at the graft union in heterograft trees.

(B) 4 days after budding, lesser-stained cells are in callus between the scion and rootstock on homograft trees.

(C) Longitudinal sections of graft union 15 days after budding, stained cells are present between the *P. tomentosa* rootstock and scion.

(D) The callus is surrounded by a slight suberin-like cell layer on homograft trees.

(E) The graft union between the rootstock and scion 1 month after budding on heterograft trees. Stained cells surround the callus.

(F) A slight stained cells layer was observed between the rootstock and scion 1 month after budding on homograft trees.

(G) Transverse section of graft union 3 months after budding on heterograft trees. Necrotic cells are surrounded by stained cells (white arrows).

(H) Transverse section of graft union 3 months after budding on homograft trees the cambium is connected together and stained cells indicating phenolic compounds are only present in the epidermis.

(I) After 6 months on heterograft trees a strongly stained cell layer is present in the new scion xylem and in the intergraft zone separating scion and rootstock parts.

(J) After 6 months homograft trees display no necrotic cell layer and the cambium is continuous between the scion and stock.

R; Rootstock; S: Scion; Ca: Callus; N: Necrosis; C: cambium. Bars= 0.1 mm

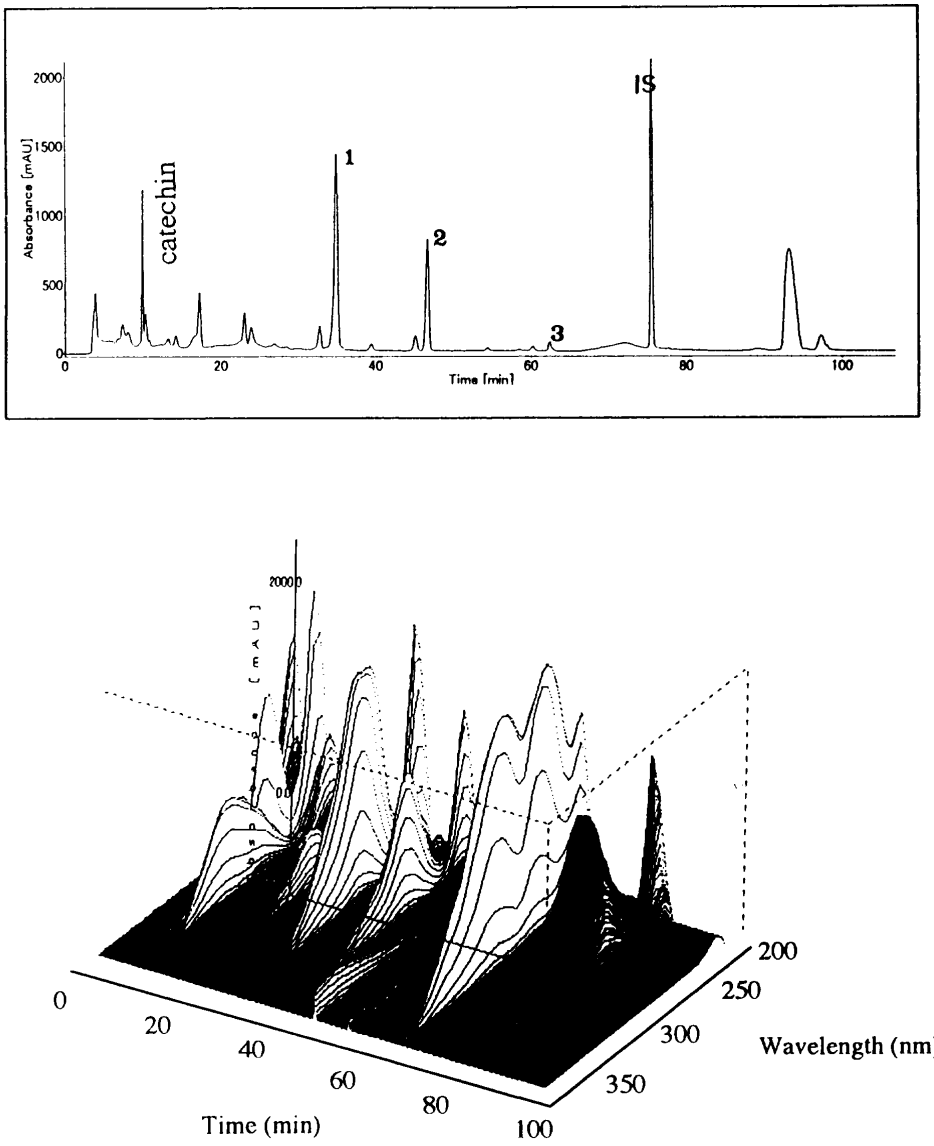


Fig. 3. Chromatographic tracing of phenolic compounds detected at UV photo-diode-array HPLC system in the phloem tissues of graft union of homograft trees (upper). Three dimensional assay of phenols (lower).
IS: Internal standard.

between the scion and rootstock was observed (Fig. 1I).

On homograft trees, numerous blue stained cells existed in the bark tissues which reveal the natural abundance of diverse phenolic compounds in these rootstocks. The vessels of the stock xylem at the cut surfaces seemed to be plugged with resin substances. No callus cells was observed at this stage, indicating that they completely re-differentiated into connective tissues (Fig. 1J).

2. HPLC analysis

Method 1 : HPLC-CRD

The catechin was identified and quantified by the HPLC-CRD method because of the high specificity of DMACA with flavan-3-ols and proanthocyanidins (Treutter, 1988).

Catechin in the phloem tissues

A smaller amount of catechin and a different accumulation pattern were found in the heterograft combination than in homograft trees. Catechin, in the phloem tissues of the graft union (Fig. 2A) tended to accumulate equally above and below the graft union in homograft trees, whereas in the heterograft combination catechin accumulated to a higher degree above than below the graft union.

Catechin in the xylem tissues

The amount of catechin and its accumulation pattern in the xylem (Fig. 2B) of graft union zone were similar in both stock/scion combinations. In the heterograft combination, the amount found at the graft union tended to be higher than that found at the same section on the homograft combination but the catechin levels in the rootstock section were lower than those found in other sections of graft union in both rootstocks.

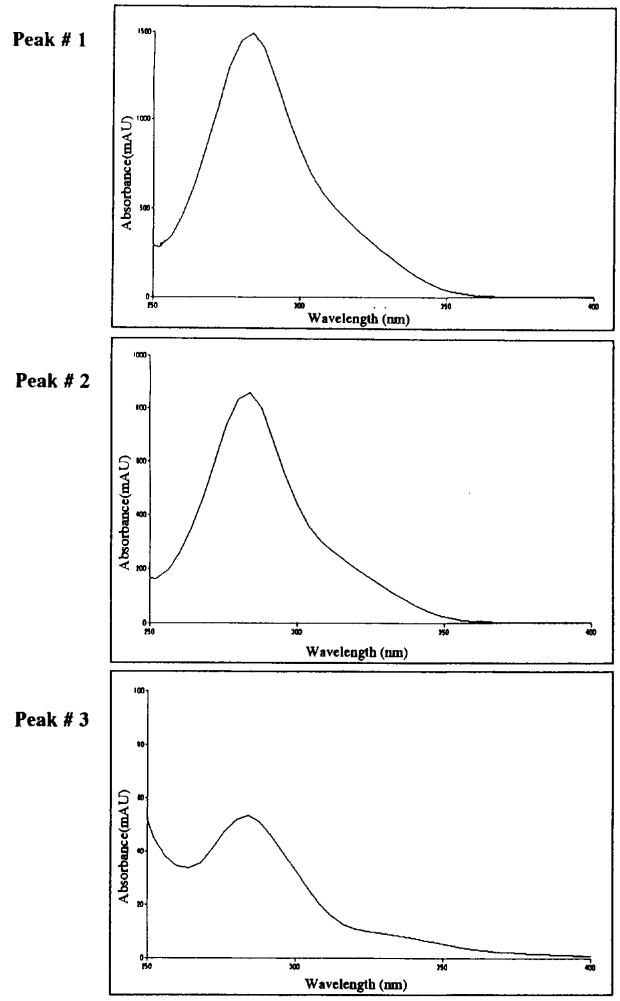


Fig. 4. UV spectrum, attained from UV photo-diode-array HPLC system, of phenolic compounds from the graft union tissues.

The amounts of catechin in the three sections of phloem tissues were generally higher than those found in the xylem tissues, particularly in the homograft trees.

Method 2 : HPLC - UV determination

From chromatographics tracing of UV determination at 280 nm, three quantitative and qualitative relevant peaks were prominent. Based on retention time (Fig. 3), UV spectrum (Fig. 4), and TLC behavior, the tentative assignments for Peaks # 1 and # 3 are prunin and naringenin, respectively. Peak # 2 showed the same spectrum as Peak # 1, but the retention time was dissimilar to those shown by standards tested. As Peaks # 1 and # 2 have the same spectrum, both were expressed as units of rutin. Peak # 1 is yellow under UV light and with methanolic ferric chloride, and Rf values: 0.61 and 0.92, whereas Peak # 2 is red with diazotized acid and Rf values : 0.51 and 0.46.

Epicatechin was identified as one of the other peaks observed in the Fig. 3. However, this particular compound and the other unidentified peaks were not systematically detected in all the samples; thus, these peaks were not considered in this analysis.

The phloem tissues

The relative concentration of each peak was expressed on the basis of a chemical standard of each component after conversion, except Peak # 1 which was calculated as that of rutin.

The selected peaks in the phloem extracts were not found in those from below the graft union (rootstock section) on heterograft trees (Fig. 5A), except a trace amount in Peak # 1. The amount of the compound in Peak # 1 was higher on homograft than on the heterograft.

The compound in Peak # 1, tentatively identified as prunin, tended to accumulate at the graft union of homograft trees, whereas in heterograft trees it accumulated above the graft union, increasing from the graft union to the section above it.

The compound in Peak # 2, showed an increase above the union on heterograft trees; the amount was slightly larger than the corresponding part of the homograft. This phenolic compound had a rising gradient from the graft union to the scion on the heterograft combination but it was undetected in the stock. The inverse trend was observed on the homograft combination.

The amount of naringenin in Peak # 3 was much lower than those of prunin and peak # 2 in both graft combinations; the amounts in the three parts were similar, except that they were undetected in the *P. tomentosa* stock.

The xylem tissues

The amounts of phenolic compounds in the xylem tissues were much lower than those found in phloem tissues in both combinations except for Peak # 3 on homograft trees (Fig. 5B). Peaks # 2 and # 3 had the smallest amounts of phenolic compounds at below the union of the heterograft combination; in which Peak # 1 was undetected.

More prunin was accumulated in homograft than it was in heterograft combination; it tended to concentrate more at the graft union than above the union, contrary to that found in phloem tissues. In homograft trees, this phenol tended to accumulate at and below the graft union.

Less phenolic compounds were found in Peak # 2 than were found in the phloem; this compound did not accumulate at the graft union in both rootstocks. Much more naringenin in the xylem tissues was accumulated in the homograft than in the heterograft; it tended to accumulate at the graft union.

Discussion

The specific staining method by DMACA for flavan-3-ols and proanthocyanidins (Gutmann and Feucht, 1991; Gutmann, 1993) clearly indicated that soon after budding, the graft union of heterograft trees frequently had more stained cells than those in homograft trees; these stained cells mainly surrounded the callus (Errea et

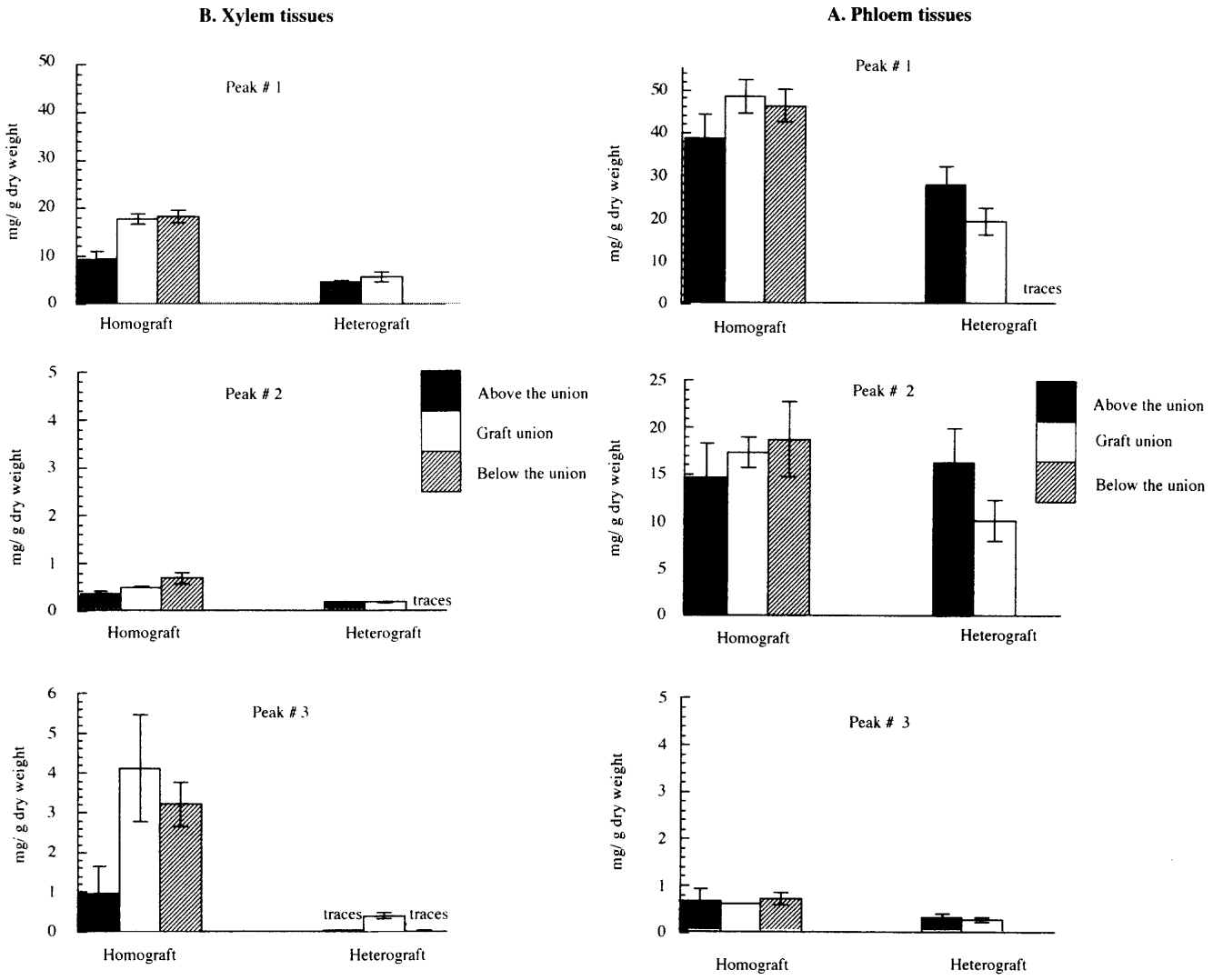


Fig. 5. Mean values of phenolic compounds (mg/g dry weight) found at phloem (A) and xylem tissues (B) from graft union of heterograft and homograft trees.
Vertical bars= SE.
1. Peak # 1, prunin
2. Peak # 2, calculated as rutin
3. Peak # 3, naringenin
Homograft combination : *P. persica* / *P. persica*
Heterograft combination: *P. persica* / *P. tomentosa*

al., 1994). The stained cells were also found in the new xylem and in the cortex between the scion and the rootstock even on 6-month-old budded trees. One month after budding, the necrotic tissues, surrounded by stained cells adjacent to the callus in the heterograft combination, seemingly impeded callus differentiation. Therefore, it is possible that catechin and other phenolic compounds which accumulated at the graft union could be the first biochemical response to incompatibility (Cooman et al., 1996). Subsequently, the callus cells differentiate slower about the graft union of heterograft than those in the homograft trees (Errea et al., 1994). Six months after budding, the callus cells as well as a necrotic layer bordering the callus were still visible on heterograft combination. This necrotic tissue extendible through the cortex and the phloem apparently impeded the formation of a good vascular connection between the stock and scion. However, the cambium is probably still active because new xylem was differentiated in both scion and rootstock. The callus, surrounded by 1) stained cells, indicate the presence of phenolic compounds, and 2) necrotic cells, may be unresponsive to growth promoting compounds, thus impeding vascular differentiation and, subsequently, limiting the supply of water and nutrients to the scion.

Our histochemical observations reveal a significant difference in the distribution of phenolic compounds between homograft and heterograft combinations. On heterograft trees, the phenols were found mainly in the undifferentiated callus, adjacent necrotic cells, in the bark tissues and in the new xylem of the scion (Fig. 1), whereas in homograft combination, a small amount

occurs in the callus. In *Prunus armeniaca* used as a rootstock, an accumulation of phenolic compounds occurs during the early stages of graft establishment which was related to the problems in differentiation of the callus (Errea et al., 1994).

Catechin, is a flavanol recognized as a protective agent of IAA oxidation (Lee et al., 1982) or as a radical scavenger. In addition, an exogenous application of catechin improved the growth of callus cells by increasing the cellular division (Feucht and Treutter, 1995). Catechin, together with IAA, may play a regulatory role in the growth of shoots. In fact the more vigorous trees of *P. avium* contained comparatively more catechin than did smaller trees of *P. fruticosa* (Feucht and Nachit, 1977).

In this study, catechin contents were higher in the vigorous homograft combination than in the smaller heterograft. The lower levels of catechin found in phloem tissues of 2-year-old heterograft trees might imply that the cells of *P. tomentosa* lack a protective action of catechin because of a difference in enzymatic activity between the partners.

The grafting process implies decompartmentation of cellular components by wounding. The main phenolic compounds implicated, in enzymatic browning are catechin (flavans) or hydroxycinnamic acid derivatives (Florence and Gauillard, 1997) which come in contact with oxidative enzymes (peroxidases or polyphenol oxidase) and result in the formation of *o*-quinones which subsequently polymerize and lead to brown pigments (Errea, 1998) or condensed tannins (Hillis and Isoi, 1965). The newly formed quinones may lead to protein precipitation that result in cellular necrosis (Errea and Felipe, 1994). The browning of tissues, a common symptom in the graft union of heterograft trees, could be similar to the enzymatic browning of fruits and vegetables. In addition, the oxidation of lignin precursors (hydroxycinnamic acid derivatives) would affect the process of lignification of the tissues (Errea, 1998).

When we analyzed the phloem and xylem tissues separately, we found that the graft partners have quantitatively and qualitatively different phenolic compounds and the pattern varies depending on tissues. The lack of some phenolic compounds below the union of the heterograft trees, characterized this rootstock. In the bark of *P. persica* and other *Prunus* species phenolic compounds, such as naringenin, prunin, and dihydrokaempferol have been isolated (Bohm, 1975; Treutter et al., 1985).

In phloem tissues of heterograft trees, the increase in prunin and the compound in Peak # 2 above the union could indicate that the rootstock influences the normal gradient of phenolic compounds. These results are similar to the finding of Errea et al. (1992a, b) and Treutter and Feucht (1991) in *Prunus*. Feucht et al. (1988) noticed that prunin caused less callus growth and inhibited xylogenesis and peroxidase activity.

In xylem tissues, prunin and naringenin which accumulated at the graft union of heterograft trees could affect wound healing. Although less naringenin than prunin was found, the relatively small amount of naringenin may be enough to induce dysfunction at the interface between two cells (Errea and Felipe, 1994). Naringenin was found to function as an inhibitor of the growth promoting activity of gibberellin, while stimulating IAA oxidase (Feucht et al., 1988).

These results indicate that phenolic compounds have a possible role in causing graft incompatibility. The cells of *P. tomentosa*, which lack some phenolic compounds, could be damaged by the foreign phenols released by peach cells at the graft union . This may contribute to the formation of an isolation layer that finally would promote an accumulation of these toxic substances above the union and graft union. According to Wang and Kollmann (1996), this isolation layer is composed of necrotic material derived from wall remnants, contents of cut cells, and pectinaceous as well as phenolic compounds secreted by callus cells.

The intense production of new phenolic compounds during the early steps in the graft establishment has been reported (Kahl, 1978; Treutter and Feucht, 1991); their accumulation could be an unspecified response because it occurs in stressed tissues after wounding (Errea and Felipe, 1994; Treutter and Feucht, 1991). In the homograft combination, the phenolic compounds released as a wounding response practically disappeared from the union zone, where the necrotic layer was broken by callus proliferation. In the heterograft combination, the establishment of unions may be influenced by the difference in cellular metabolism of the graft component, leading to necrosis and slower cell differentiation. We suspect that the failure or lack of adaptation between stock and scion results in the accumulation of phenols. When these phenols are converted to quinones, they oxidize other cells constituents (Errea, 1998). According to Treutter and Feucht (1991), such oxidation would lead to a necrotic line at the union; or depending on the nature of the phenolic substances, they would stimulate IAA oxidase (Lee et al., 1982), thereby affecting IAA contents at the graft union (Santamour, 1996).

In the future, the additional phenols occurring at the graft union of *P. persica* on *P. tomentosa* need to be identified and the enzymatic activity which detoxify them need further study.

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Prunus tomentosa 矮性台モモ樹の接木部に蓄積するフェノール物質の組織化学的観察およびHPLCによる分析

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摘　要

ユスラウメ (*Prunus tomentosa*) 台モモ樹 (矮性台樹) の接木部のフェノール物質含量とその組織内分布について高速液体クロマトグラフ (HPLC) および組織化学的手法で調査し，共台樹と比較した．

DMACA (*p*－dimethylamino cinnamaldehyde) 染色の結果，矮性台樹の接木部のカルス，新生木部および皮層組織に接木後6ヶ月の間フェノール物質が局在して蓄積することが示された．このフェノール物質は穂木台木間の接着を遅らせるものと思われた．

HPLC分析により，穂木接着に関係すると思われる4つのフェノール物質が検出された．1つのピークを除きカテキン，プルニン，ナリンゲニンと推定された．このうち2成分は矮性台樹の台木部師部では検出されなかった．共台樹では師部フェノール含量が矮性台樹に比べて多く，とくにプルニン (ピーク #1成分) およびナリンゲニン (#3)で著しいことが認められた．

また矮性台樹では木部フェノール含量も少なく，とくに台木部で微量もしくは検出されない成分があった．次いで接木部，穂木部の順にフェノール含量が多く，共台樹のパターンと異なっていた．すなわち，プルニン (#1)と未同定の #2成分が接木部より上部の師部に蓄積するが，木部においてはプルニン (#1)成分およびナリンゲニン (#3)成分が共台樹に比べ少ないことが判明した．

低分子フラボノールであるカテキンは，とくに矮性台樹の接木部木部組織で多かった．師部のカテキン含量は矮性台樹よりも共台樹の接木部でその含量が多かった．