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Relationships of Ethanol Production by Seeds of Different Types of Japanese Persimmons and Their Tannin Content

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Abstract. **Ethanol production in the seeds and its accumulation in the flesh were compared among 47 Japanese persimmon cultivars (*Diospyros kaki* L.) in relation to their degree of astringency. Those which produce relatively high amounts of ethanol and accumulate it in the flesh, i.e. pollination-variant/ nonastringent (PVNA) cultivars, lose astringency on the tree, while those producing less ethanol, i.e. pollination-variant/ astringent (PVA) ones, remain astringent. Pollination-constant/ nonastringent (PCNA) and pollination-constant/ astringent (PCA) cultivars generally produced little ethanol in the seeds and accumulated small amounts or none in the flesh. Thus, 2 different mechanisms exist that are involved in the loss of astringency. One is associated with PVNA, PVA, and PCA types and is dependent upon the production and accumulation of ethanol and presumably acetaldehyde. The second is associated with PCNA types which apparently do not produce these volatile substances.**

Japanese persimmon cultivars are classified into 4 different types depending upon the ways or degrees their fruits lose astringency on the tree and upon flesh color: 1) pollination-constant and nonastringent (PCNA), 2) pollination-constant and astringent (PCA), 3) pollination-variant and nonastringent (PVNA) and 4) pollination-variant and astringent (PVA) (2, 5). Fruits of types PCNA and PVNA naturally lose astringency in the process of growth and ripening and become edible at maturity while other 2 types, PCA and PVA, have to be treated after harvest with warm water, alcohol, and carbon dioxide before they become sweet and edible.

There have been many reports concerning the possible mechanism of the removal of astringency following various artificial treatments (1, 2, 3, 4, 5, 6). Such treatments can stimulate the accumulation of volatiles such as ethanol and acetaldehyde in the flesh tissue and these substances, especially acetaldehyde, induce soluble tannins to coagulate and form insoluble complexes, thereby resulting in the loss of astringency. The removal of astringency from naturally nonastringent-type fruits has been assumed to follow the same mechanism. Our previous paper (7) dealt with the natural disappearance of astringency on the tree in nonastringent-type fruits in which ethanol and/or acetaldehyde produced in the seeds was thought to be responsible for the loss of astringency with PVNA fruits, but not with PCNA fruits. To support this postulation, differential production of ethanol in the seeds and flesh among additional cultivars belonging to the 4 different types was examined.

Weekly determinations of ethanol and soluble tannins were conducted with 7 cultivars starting on July 1 and ending on September 30 for soluble tannins and October 21 for ethanol. On September 4, nearly mature fruits from 40 other cultivars were sampled and analyzed for ethanol and soluble tannins. Fruits were picked from mature trees growing in the orchard of Kyoto University in Kyoto. For ethanol determination, 2-or 4-g slices of seeds and 5 g of flesh tissue between 2 adjacent, seeded loculi were taken from 5 to 7 fruits, cut into small pieces quickly, and immersed in 10 ml of acetone in separate test tubes with screw caps. The contents were subjected to ultrasonic waves for 1 min and stored in a −20°C freezer until analysis. Two µl of acetone extract were injected into Shimadzu gas chromatograph GC-4CM equipped with flame ionization detector. Column was 1 m × 3 mm i.d. glass packed with 50/80 mesh Porapak Q. Column and detector were kept at 125° and 160°, respectively. The carrier gas was N_2 at a flow rate of 50 ml min^{-1}. Amount of ethanol was expressed as µl g^{-1} fresh weight of seeds or flesh. Flesh tissue between 2 adjacent seeds was removed from 10 fruits of uniform size and maturity. On mixing, 2 g were weighed for soluble tannins determination. Soluble tannins responsible for the astringent taste were determined by Folin-Denis method (8) and expressed as the percentage of tannic acid on fresh weight basis.

Seven cultivars used for weekly determinations of ethanol and soluble tannins were 'Fuyu' (PCNA), 'Hana-gosho' (PCNA), 'Sangokuichi' (PVNA), 'Ohniwa' (PVNA), 'Aburatsubo' (PVNA), 'Aizu-mishirazu' (PVA), and 'Yotsuya-saijo' (PCA). Increase in the ethanol content in the seeds preceded that in the flesh by several weeks (Fig. 1).

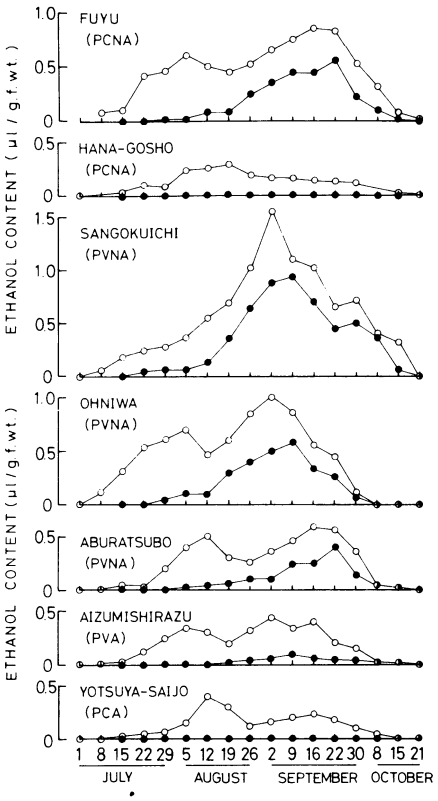

Fig. 1. Seasonal changes in ethanol contents in the seeds (○) and flesh (●) of 7 Japanese persimmon cultivars.

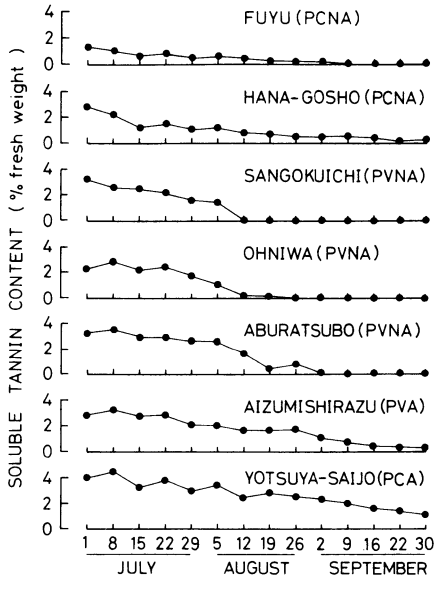

Fig. 2. Seasonal changes in soluble tannin contents in the flesh of 7 Japanese persimmon cultivars.

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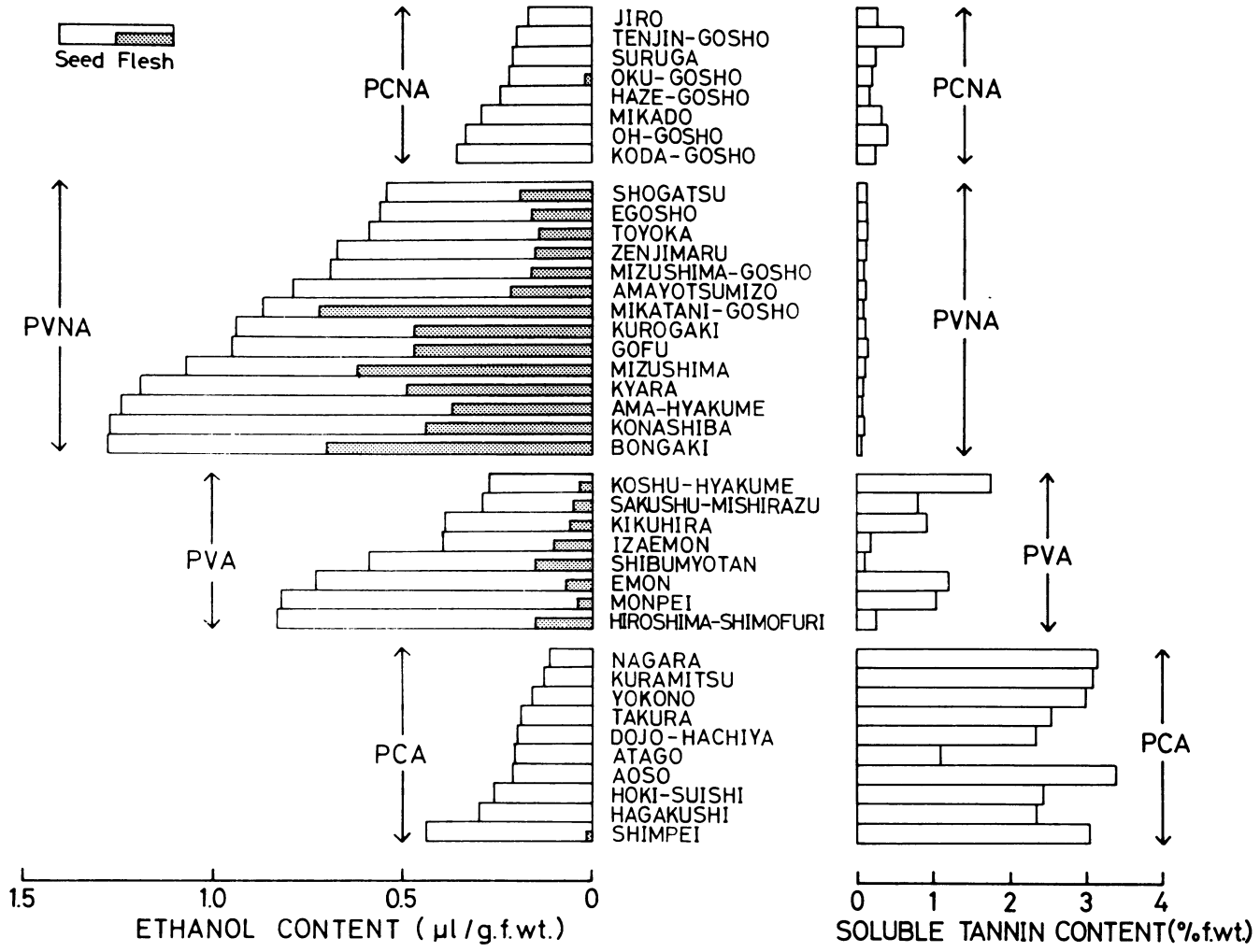

Fig. 3. Ethanol and soluble tannin contents in the fruits of 40 Japanese persimmon cultivars picked on September 4.

In 'Hana-gosho' and 'Yotsuya-saijo', in which the level in the seeds was low, no accumulation was accompanied in the flesh. This suggests that ethanol produced in the seeds diffuses out to the flesh tissue. Some cultivars exhibited double-peaked curves for ethanol level in the seeds with maximum peaks in August and September. With all cultivars, ethanol was not detected at harvest both in the seeds and flesh. Though 'Fuyu' and 'Hana-gosho' are both PCNA-types, whose astringency is lost regardless of the presence of seeds, 'Fuyu' accumulated much more ethanol in the seeds and flesh than 'Hana-gosho'. But such high accumulation of ethanol occurred only after substantial reduction in soluble tannins occurred (Fig. 2). Three cultivars of PVNA-type showed higher content of ethanol both in the seeds and flesh, while 'Aizumishirazu' (PVA) and 'Yotsuya-saijo' (PCA) accumulated less ethanol. An inverse relationship between the ethanol content in the flesh and tannin content was found with PVNA cultivars. It was not as good with PVA cultivar (Fig. 1 and 2). PVNA fruits rapidly lost their astringency when the ethanol content in the flesh approached 0.1 $\mu l\ g^{-1}$ fresh weight.

Among the 40 additional cultivars, the PVNA-types generally showed the highest contents of ethanol in the seeds and flesh and the least amount of soluble tannins (Fig. 3). PVA cultivars contained intermediate levels of ethanol and moderate amounts of tannins. PCNA and PCA cultivars showed lowest alcohol levels in the seeds with almost no accumulation in the flesh. Thus no relation was observed between the ethanol level in the flesh and astringency in PCNA cultivars. The high ethanol level in 'Fuyu' fruit (Fig. 1) is considered an exception compared to low levels of other PCNA cultivars (Fig. 3). This high level of ethanol is probably responsible for relatively abundant brown tannin spots in the flesh of 'Fuyu' fruit.

Our findings support the previous postulation (7) that 2 different mechanisms are involved in the loss of astringency in Japanese persimmon fruits; one with a close association with ethanol and presumably acetaldehyde production in the seeds, and the other having no association with these volatile chemicals. Yonemori et al. (9, 10) demonstrated that tannin compositions of PCNA fruits are considerably different from those of the other 3 types and suggested that this may account for the difference in the ease of removing astringency.

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The Effect of Artificial Shading on Cold Hardiness of Peach and Sour Cherry

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Abstract. **Shade treatments giving 36% full sun or less reduced both hardiness and shoot cross-sectional area of one-year-old sour cherry (*Prunus cerasus* L. cv. Montmorency) and peach [*Prunus persica* (L.) Batsch cv. Redhaven]. Shade significantly reduced soluble carbohydrate in 'Redhaven'.**

Light level can be a limiting factor in cold resistance (7, 11, 14) and numerous morphogenic and reproductive responses of trees are affected by solar radiation. Shading reduces flower bud formation, fruit size and quality, total dry weight, and photosynthetic rate (4, 5, 8, 9, 10). Since practices that alter the exposure of plants to light or that emphasize the importance of healthy leaves may affect hardiness through their influence on carbohydrate production and accumulation (14), it is logical to predict that shading may have a detrimental effect on cold hardiness. The objective of this study was to determine the effect of shade on shoot growth, water status, carbohydrates, and cold hardiness of peach and sour cherry.

One-year-old 'Redhaven' peach on Halford rootstock and 'Montmorency' sour cherry on Mahaleb rootstock were pruned to one 20–25 cm shoot, and planted in 18-liter pots in 1 sand:1 sphagnum peatmoss:1 soil (by volume) on May 5, 1979. Fertilizer, pesticides, and water were applied as required. Trees were grown under full sun until June 20, 1979, when 10 trees of each species were placed under shade equivalent to 36%, 21%, or 9% of full sun. The youngest expanding leaf below the terminal was tagged to distinguish pre- from postshade growth. Controls were grown without shading. Solar radiation was reduced with pipe frame structures (3.7 × 2.4 × 1.8 m) covered with black polypropylene shade fabric (A.H. Hummert Co., St. Louis, Mo.) which transmitted an average of 36%, 21% or 9% PAR (photosynthetically active radiation measured with a LI-COR LI-1905 quantum sensor connected to a Model LI-500 integrator) when compared to full sum. Ventilation prevented temperature differences of greater than ± 3°C and relative humidity differences greater than ± 5%. Spectral radiometer (ISCO Model SR portable spectroradiometer, Lincoln, Neb.) determinations confirmed that all wavelengths in the 380–750 nm range were reduced equally.

Shoot length above the tag and shoot cross-sectional area at the point tagged (to distinguish pre- from postshade growth) were recorded on September 11 and 12, 1979.

Hardiness was determined on November 29, 1979 using procedures similar to those described previously (13). Briefly, postshade terminal shoot sections (15 cm) were collected to provide 6–8 sections per treatment per tree per species. Stem pieces were taped to aluminum foil strips, labeled, and rolled into a bundle. A 26-guage copper–constantan thermocouple was attached with tape to one stem piece in the center of the bundle, and the latter was placed in a vacuum flask. The thermocouple was connected to a potentiometer with a pen recorder that monitored temperature in the foil roll. The flasks were placed in a Revco "Ultralow" freezer and temperature declined at a rate of about 2–3°C hr^{-1}. This rate allowed for uniform temperature decline of all twig pieces in a bundle. As the stems cooled, flasks were removed at 2.5° intervals within a predetermined temperature stress range so that warmest temperatures would cause damage and the coldest would kill all tissues. Tissue browning was used as the basis for viability evaluation (12). The T_{50} value was calculated using the Spearman-Kärber method (1), and viability values were separated by the modified Friedman test (3).

The anthrone test was used to measure soluble carbohydrate. Shoot tissue was freeze-dried at −40°C, then ground in a #20 mesh Wiley mill. The tissue was further ground (100 mg/liter) with 80% EtOH in mortar and pestle and the suspension centrifuged at 1500 rpm for 5 min. The supernatant was diluted with water (0.5 mg tissue/ml H_2O), a 1-ml extract was placed in a 10-ml test tube, and 5 ml anthrone reagent (2 g anthrone/liter 95% H_2SO_4) was added to each extract. Test tubes were placed in a water bath (70°) for 15 min., then cooled at 20° for 20 min. Absorbance was determined at 620 nm for the 1-ml extract using water as a blank. A standard curve was obtained using α-D-glucose at 20, 40, 60, 80, and 100 μg/ml. Data are expressed as mg soluble carbohydrate per gram dry weight of the tissue.

Shoot section fresh weight, dry weight, and percent water content (fresh-weight basis) were determined by weighing the samples shortly after excision and again after 60 hr at 80°C.

Wood and bud hardiness of both sour cherry and peach were reduced significantly by shading (Tables 1 and 2). For cherry, T_{50} values rose in both shoots and buds from −22.5°C for 100% full sun (FS) to −15.5° for 9% FS; values for all shade treatments were significantly different from those of the controls. Shoot cross-sectional area, but not shoot length, was reduced significantly by shading. In peach, T_{50} rose from −22.5° to −13.0° and from −17.5° to −13.0° for wood and vegetative buds, respectively. However, the effect was not significant at light levels above 21% FS. Hardiness was consistently greater in wood than in buds except at 9% FS. Shoot cross-sectional area and soluble carbohydrate decreased with shading (significant only at 9% FS), whereas shading tended to increase shoot length.

Significant negative correlation coefficients existed for solar radiation vs. T_{50} values for wood and bud hardiness and percent full sun, and shoot cross-sectional area for both peach and cherry (Table 3). Soluble carbohydrate and water content were also correlated negatively with T_{50} values for peach

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