Exothermic Characteristics of Dormant Buds of Persimmon (*Diospyros kaki* Thunb.) in Relation to Cold Hardiness

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Additional index words. *Prunus persica*, thermal analysis, thermoelectric module, 2,3,5-triphenyltetrazolium chloride

Abstract. Microcomputer-based thermal analysis (TA) was conducted on dormant mixed buds of Japanese persimmon ('Hiratanenashi'). The exotherms of buds were detected by thermoelectric modules. Flower buds of peach (*Prunus persica* Batsch cv. Shimizuhakuto) were also analyzed. When TA was used on a whole excised bud, including bud scales, the persimmon buds had only one exotherm at -14.3°C , while the peach buds had high and low exotherms at -8.4°C and -14.1°C , respectively. However, when the exotherm was measured for the primordium, with the bud scales and transitional leaves removed, each primordium showed only one exotherm at -20.7°C in persimmon and -11°C in peach. Determination of killing temperature by visual observation, electrolyte leakage method, and triphenyltetrazolium chloride test revealed that the primordium of the persimmon bud was killed at about -14°C as the excised whole bud or as the whole bud attached to the branch segment. Using the same method, the naked primordium was killed between -22°C and -25°C as the primordium was cooled. The peach primordium was killed at -14°C when examined as a whole bud and at -11°C as a naked primordium. Furthermore, the exotherm temperatures of persimmon buds and stem segments were measured at appropriate intervals during the two winter seasons 1993–95. Exotherm temperatures of persimmon buds were always higher than the low-temperature exotherm (LTE) temperatures of the stem segments and lower than the high-temperature exotherm (HTE) temperatures of the stem segments. LT_{50} of persimmon buds almost coincided with the exotherm temperatures of buds. A postulated role of bud scales in supercooling is discussed.

A low-temperature exotherm (LTE) has been associated with cold injury during controlled freezing of several woody plants. Since it was first shown that the LTE is involved in freezing injury of dormant *Rhododendron* flower bud primordia (George et al., 1974), thermal analysis has emerged as a simple and rapid technique for determining cold hardiness of flower buds (Biermann et al., 1979; Quamme, 1978). This analytical method has shown that flower buds of several important horticultural crops avoid freezing injury by deep supercooling (Ashworth et al., 1989; Biermann et al., 1979; Kader and Proebsting, 1992; Lipe et al., 1992; Quamme, 1974, 1978; Quamme et al., 1995; Wolf and Pool, 1987).

Although persimmon is native to temperate regions, buds and stems have been injured at -15 to -20°C (Leng et al., 1993; Zivotinskaja and Kui'kov, 1968). Some commercially important cultivars in Korea, such as 'Hiratanenashi' and 'Fuyu', are injured at -14°C to -18°C in winter, which limits their cultivation area (Hong and Hwang, 1980; Kim et al., 1988). Nevertheless, there is no report

using thermal analysis for evaluating cold hardiness of persimmon buds.

We investigated the supercooling ability of persimmon buds and its relationship to freezing resistance in comparison with those of peach flower buds, whose cold hardiness has been extensively evaluated with thermal analysis. Also, seasonal changes in supercooling of persimmon buds were measured during the two winter seasons.

Materials and Methods

Plant source. In early Dec. 1993, 1-year-old branches were harvested from mature persimmon ('Hiratanenashi') and peach ('Shimizuhakuto') trees growing at the experimental orchard of Kyoto Univ. To assess the seasonal changes in supercooling of persimmon buds and stems, 1-year-old branches of 'Hiratanenashi', 'Hanagoshō', and 'Tamopan' were also harvested at appropriate intervals during the two winter seasons from 1993 to 1995. Upon harvest, branches were used immediately or put into polyethylene bags and stored at 0°C until freezing tests. Duration of storage was not over 1 week; during that period, no changes in bud cold hardiness of persimmon and peach were observed.

Thermal analysis. Thermal analysis (TA) was performed by using thermoelectric modules (Komatsu Electronics, Tokyo) and a pro-

grammable freezer. The temperature control of the freezer and data acquisition of the measurement was controlled by a microcomputer. TA was performed on 5-mm-long internode segments, whole buds with or without subtending tissue, or naked bud primordia. For TA of the bud, mixed buds of the first and second position from the apex were used for persimmon, and lateral flower buds were used for peach. For TA of the persimmon naked bud primordia, the primordium was excised by removing the bud scales and several subtending leaf primordia. The peach primordium, with only the first and second bud scales subtending the floral primordium, was used for TA of naked primordium. The excised sample was mounted on one side of a thermoelectric module and placed in a glass jar that was used as a sample chamber. The chamber was then placed in a freezer and cooled at $6^{\circ}\text{C}\cdot\text{h}^{-1}$ after preconditioning at 0°C for 30 min. The temperature within the jar was monitored with a copper–constantan thermocouple at 4-s intervals. Silicone grease was used to facilitate contact between the thermoelectric module and the sample.

In preliminary tests, moist filter papers were applied to cut surfaces of excised persimmon buds, or branch segments with buds, to induce a high-temperature exotherm, but the papers did not change the exotherm temperatures of the buds. In all these experiments, therefore, the application of moist filter paper was omitted. To investigate the effect of bud scales on the LTE of persimmon bud, four or eight bud scales were removed from 'Hiratanenashi' buds and these buds were analyzed by the same method as above. The whole bud or the naked bud primordium was also subjected to TA for comparison. A minimum of 15 buds was measured for each sample.

Estimation of freezing injury. Experiments were conducted to evaluate the freezing resistance of persimmon and peach bud. Analysis of freezing resistance was performed on branch segments with two or three buds, excised buds, or naked primordia. Ten branch segments, 20 excised buds, or 20 primordia were exposed to selected temperatures. The branch segments with two or three buds or excised buds were wrapped in aluminum foil and placed in glass jars. The naked bud primordia were mounted on paraffin film and then placed in a small petri dish used as a sample chamber. Samples were placed in the freezer and cooled at $6^{\circ}\text{C}\cdot\text{h}^{-1}$. The temperature within the petri dish and glass jar was recorded with a copper–constantan thermocouple for sampling. A glass jar and a petri dish were removed from the freezer at 3°C intervals to evaluate freezing injury at -5°C in the case of visual observation, or at -7°C in the case of electrolyte leakage and TTC (2,3,5-triphenyltetrazolium chloride) tests for the excised buds or naked primordia. After sampling, glass jars and petri dishes containing the samples were placed in a refrigerator overnight at 5°C for slow thawing, and kept 1 day at 27°C for injury evaluation.

Half of the excised buds and branch segments with buds were dissected longitudinally

Received for publication 3 Sept. 1996. Accepted for publication 16 Jan. 1997. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

through the middle of the bud and evaluated for injury of the bud, bark, xylem, and pith tissues. Browning was used as visual evidence of injury where 1 = no browning and 2 = browning. Bud injury was evaluated by browning of the primordium. The undissected samples of branch segments with buds were placed in a vase with water for 3 weeks to evaluate budbreak or browning of primordia in buds showing no budbreak.

Injury of excised whole buds or naked bud primordia in response to freezing stress was evaluated visually, as explained above. In addition, the degree of injury was also evaluated by measuring solute leakage from the tissue and by vital staining with TTC. When whole buds were used for freezing, bud scales and subtending tissue of persimmon and peach were removed, and the primordia were transferred to test tubes containing 10 mL of deionized water and were vacuum infiltrated for 5 min. After incubation for 10 h, the electrical conductivity of the effusate was measured with an electrical conductivity (EC) meter. The test tubes were then autoclaved for 15 min at 120 °C. After leaving the samples for 10 h at room temperature, the EC was remeasured. The percentage of leakage and injury was calculated as described by Zhang and Willison (1987). With the TTC test, the primordia were placed in 0.5 mL of 0.8% (w/v) TTC solution in a phosphate buffer (pH 7.2) and incubated for 12 h at 27 °C in darkness. After incubation, the primordia were rinsed in distilled water and the reduced formazan was extracted with 1 mL of 95% ethanol. TTC reduction was determined by measuring absorbance at 530 nm (Towill and Mazur, 1975).

Seasonal changes in supercooling of persimmon buds and stems. TA was conducted on the excised buds and stem segments collected during the two winter seasons from 1993 to 1995. Branches with two or three buds were collected at appropriate intervals during the first winter season, and freezing injury was estimated by cooling them at 6 °C·h⁻¹, as described above. Then, the branches with buds were held 3 weeks in a vase with water to evaluate budbreak or browning of primordia in buds showing no budbreak. LT₅₀ of buds also was calculated (Bittenbender and Howell, 1974).

Results

Thermal analysis (TA). When TA was conducted on whole excised buds, including bud scales, persimmon buds showed only one exotherm at -14.3 °C while peach buds had a HTE at -8.4 °C and a LTE at -14.1 °C (Fig. 1 A and E). Conversely, TA of the naked primordium produced only one exotherm at -20.7 °C in persimmon and -11 °C in peach (Fig. 1 B and F). The persimmon and peach internodes had a HTE and a LTE (Fig. 1 C and G). TA of the peach and persimmon bud with subtending tissue indicated that the presence of nodal tissue slightly complicated the exotherm profiles of the bud in either plants (Fig. 1 D and H).

The terminal buds of persimmon in which

Fig. 1. Typical exothermic response of a whole bud with or without nodal tissue, a naked primordium, and nodal tissue of persimmon 'Hiratanenashi' (A–D) and peach 'Shimizuhakuto' (E–H) collected in Dec. 1993. The naked primordia were prepared by removing the bud scales of mixed buds of the first and second position from the apex in the case of persimmon, and lateral flower buds in the case of peach.

a differing number of bud scales had been removed also had only one exotherm, irrespective of the number of bud scales. The exotherm temperature, however, occurred at a lower temperature as more bud scales were removed, and approached the temperature of the LTE of the naked primordia (Table 1).

Estimation of freezing injury. When freezing injury of the branch segments with buds was visually observed in persimmon, the bark tissues, including phloem and cambium and buds, were injured at a higher temperature than the xylem and pith. In peach, the bud, xylem, and pith were injured at the same temperature (Fig. 2). When budbreak was tested, sprouting was absent in treatments where injury was observed (data not shown).

The killing temperature of the buds was visually evaluated on a whole bud or a naked primordium (Fig. 3 A and D), by electrolyte leakage (Fig. 3 B and E), and TTC reduction (Fig. 3 C and F). In persimmon buds, death of the primordium began at about -14 °C as the excised whole bud was cooled. However, the primordium was killed between -22 °C and -25 °C as the naked primordium was cooled. The peach primordium was killed at about -14 °C when examined as a whole bud, and at about -11 °C when the primordium was excised.

Seasonal changes in supercooling of persimmon buds and stems. During the two winters, the excised whole buds of three persimmon cultivars produced only one exotherm (Fig. 4A) and the stem segments had a HTE and LTE (Fig. 4 B and C). The exotherm temperatures of buds and stem segments showed seasonal fluctuation, decreased as the winter progressed, and reached their mini-

Table 1. Exothermic response of persimmon ('Hiratanenashi') buds from which some bud scales were removed.

Tissue ^a	Exotherm (°C)
Whole bud	-14.3 ± 0.4 ^b
Four bud scales removed	-15.2 ± 0.5
Eight bud scales removed	-16.8 ± 0.6
Naked primordium	-21.2 ± 0.3

^aThe whole bud was an excised bud without subtending tissue; primordium indicates samples that had both the bud scales and the bud axis removed.

^bMean exotherm temperature ±SE measured on a minimum of 15 samples.

mum values between late December and early February, then increased toward early spring. The exotherm temperatures of buds were always higher than the LTE temperatures of stem segments, but lower than the HTE temperatures throughout the two winter seasons. LT₅₀ almost coincided with the exotherm temperatures of buds (Table 2).

Discussion

Cold injury of flower buds has been correlated with the freezing of supercooled water within a floral primordium of several plant species (George et al., 1974; Lipe et al., 1992), including *Prunus* species (Ashworth, 1982, 1984; Rajashekar and Burke, 1978). TA of buds typically reveals two distinct exotherms during cooling. The LTE corresponds to the freezing of supercooled water within the primordium and is associated with tissue injury. In our results, the excised peach buds showed a HTE and LTE, and the temperature of the LTE coincided with the temperature at which injury of the excised bud was visible. The

Fig. 2. Degree of freezing injury of each part of the branch segments with persimmon 'Hiratanenashi' (A) and peach 'Shimizuakuto' (B) estimated visually. Injury was estimated by the appearance of tissue browning, where 1 = no browning and 2 = browning.

Fig. 3. Freezing injury of the primordium of persimmon 'Hiratanenashi' and peach 'Shimizuakuto' estimated visually (A and D), by measurement of electrolyte leakage (B and E), and the triphenyltetrazolium chloride (TTC) reduction test (C and F). Whole buds or naked primordia were cooled to selected temperatures. Arrows indicate the exotherm temperatures of excised buds (closed) without nodal tissue or naked primordia (open). Each data point was the mean of four measurements. Vertical bars indicate standard error. O.D. was measured in wavelengths of 530 nm.

flower primordium of peach flower buds is isolated from ice crystals or other ice nucleators, permitting water to supercool in the primordium (Quamme, 1974, 1978). This characteristic seems to be due to the lack of xylem continuity in the dormant flower primordium (Ashworth, 1984).

The persimmon bud, however, showed only one exotherm when the excised whole bud was cooled. This exotherm temperature coincided with the killing temperature of the primordium

of the excised whole buds and the whole buds attached to the branch segments. Flower buds of some species belonging to subgenus *Padus* of the genus *Prunus* freeze without supercooling (Rajashekar and Burke, 1978). This subgenus is characterized by flower buds borne laterally on spurs with a large racemose inflorescence. In such plants, xylem vessels extend the length of the raceme and into the pedicels of individual florets, so that supercooling is prevented by the propagation of ice from the

bud scales to the inflorescence through its xylem elements (Ashworth, 1984). It was clear that peach had the barrier such as a provascular strand and a cell zone at a flower base, preventing ice propagation from the flower bud axis to the flower (Quamme et al., 1995). In our preliminary observation, however, persimmon buds had no barrier at the base of buds such as observed in peach buds. Further, we saw no xylem vessels extending to the primordium.

The exotherm temperature of the persimmon bud became lower when several bud scales were removed. Furthermore, the naked primordium with the bud scales and transitional leaves removed showed a relatively low exotherm temperature. The primordium of persimmon bud itself could supercool until -20.7°C , and was killed between -22°C and -25°C . Quamme et al. (1995) showed that the presence of the first and second bud scales in the peach flower bud was important to fully express supercooling. In our experiment, the killing temperature was relatively high when the naked primordium of the peach flower bud was cooled, indicating a positive effect of the bud scales on supercooling of the peach flower primordium. Conversely, it seemed likely that the scales of persimmon buds act as an inoculator in the freezing of the primordium, probably due to its relatively high freezing temperatures.

According to our results, the bud and the bark were two of the most sensitive tissues to cold injury in persimmon. The thermal analysis of nodal tissue showed two distinct peaks in persimmon, and the LTE was much lower than the killing temperature of the bud, indicating the uselessness of nodal tissues for evaluation of cold hardiness in persimmon (Hong and Hwang, 1980). In thermal analysis on three persimmon cultivars throughout the two winter seasons, LT_{50} of buds also coincided with the exotherm temperatures of buds rather than the LTE temperatures of stem segments. Therefore, whole buds can be used to evaluate cold hardiness in persimmon by TA.

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Fig. 4. Seasonal changes in the exotherm temperatures of persimmon buds (A) and stems (B and C) during the two winters (1993–95) estimated with thermal analysis (TA). TA was conducted on a minimum of 10 apical buds or stem segments at a freezing rate of 6 °C·h⁻¹. Vertical bars indicate standard errors.

Table 2. Changes in cold hardiness (LT₅₀) of persimmon buds harvested during the first winter season (1993–94).

Sampling time	LT ₅₀ (°C)		
	Cultivar		
	Hiratanenashi	Hanagosho	Tamopan
1993			
Mid-November	-10.9	-12.3	-13.5
Mid-December	-14.2	-14.4	-15.1
1994			
Mid-January	-13.4	-13.8	-15.1
Mid-February	-13.5	-12.5	-13.7

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