Fruit Maturity and Calcium Affect Chilling Requirements and Ripening of 'd'Anjou' Pears

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Abstract. 'D'Anjou' pear (*Pyrus communis* L.) trees were sprayed with zero or 32.3 mM CaCl₂ during fruit development at 55, 86, 125, and 137 d from full bloom and harvested at 85% (immature), 100% (mature), and 110% (overmature) maturity stages. The fruit were stored in air at -1 °C for several periods to determine the effect of CaCl₂ treatments on chilling requirement to accomplish ripening during 11 d at 20 °C. Immature or mature unsprayed fruit required 55 d, while the overmature fruit required 40 d at -1 °C to gain the capacity to produce ethylene during ripening at 20 °C. Calcium sprays increased flesh firmness at harvest by 15 N, fruit Ca concentrations by an average of 0.01 mg·g⁻¹, fresh mass basis, and the chilling requirements by at least 15 d. Unsprayed immature fruit contained more Ca than the sprayed mature or overmature fruit, but their chilling requirement was similar. These results suggest that high Ca concentrations may increase the chilling requirement of 'd'Anjou' pears in a developmentally related manner.

While summer pears respond promptly to chilling by producing climacteric ethylene (Looney, 1972), winter pears require a considerable amount of chilling to gain the ability to produce climacteric ethylene and to ripen. The amount of chilling required depends on the storage temperature (Sfakiotakis and Dilley, 1974; Gerasopoulos and Richardson, 1995), the pear variety (Knee, 1973; Wang et al., 1985), the fruit Ca concentration (Richardson and Al-Ani, 1982), and the growing season. 'D'Anjou' pears normally require 50 to 60 d (Chen et al., 1981; Chen and Mellenthin, 1981) in cold storage at -1 °C to ripen upon rewarming. However, they may only require 30 d (Chen et al., 1982) or even no time (Wang et al., 1972) in cold storage at -1 °C to begin to ripen at 20 °C. Exogenous ethylene applied to immature and mature nonchilled 'd'Anjou' pears induces the respiratory and ethylene climacteric ripening and softening (Wang et al., 1972). Sensitivity of 'd'Anjou' pears to exogenous ethylene increases with time after anthesis. 'D'Anjou' pears harvested at four weekly intervals after the fruit attained full maturity, and then stored at -1 °C for 0 to 150 d, showed that the time required to satisfy the chilling requirements of the fruit decreased as harvest maturity was advanced (Chen and Mellenthin, 1982).

Increased Ca concentrations have been associated with reduced respiratory and ethyl-

ene production rates in apples (*Malus domestica* Borkh.) (Bangerth et al., 1972; Faust and Shear, 1972; Lieberman and Wang, 1982; Sams and Conway, 1984) and in pears (Vaz and Richardson, 1985). The effects of Ca on postharvest physiology of fruits are also related to increased flesh firmness and delayed softening of pears (Vaz and Richardson, 1985) and apples (Mason, 1976) during cold storage. 'D'Anjou' pear fruits high in Ca obtained by using preharvest sprays (Richardson and Lombard, 1979) required more time in cold storage to initiate ripening than did fruits low in Ca (Richardson and Al-Ani, 1982; Vaz and Richardson, 1985).

The purpose of this study was to determine the effect of maturity and fruit Ca concentrations on the 'd'Anjou' pear chilling requirement for ripening.

Materials and Methods

General procedures. Three groups of 10 mature 'd'Anjou' pear trees were selected at the Mid-Columbia Experiment Station, Hood

River, Ore., and sprayed with 32.3 mM CaCl₂ plus X-77 surfactant or surfactant only (control), four times (3 June; 3 July; 12 Aug., and 24 Aug.) during fruit development. Control fruit harvested 147 d from full bloom were considered as 100% mature (Wang et al., 1972). Fruits were harvested at three stages of maturity, 85%, 100%, and 110% (hereafter referred to as immature, mature, and overmature fruit, respectively). Fruits were placed directly into perforated polyethylene bag liners (folded over) in 20-kg cartons and stored in air at -1 °C. Fruits were not treated with fungicide after harvest to evaluate possible effects on storage rots separately; however, they were screened for cork spot incidence throughout the whole experiment. Only healthy fruit were used. Fruits from each harvest were stored for 25, 40, 55, 70, and 85 d and then placed in 20-L jars at 20 °C and ventilated with air at 1 L·min⁻¹; CO₂ accumulation did not exceed 0.5% and was monitored by infrared gas analysis.

Ripening characteristics determinations. Twenty fruit at each harvest date and four fruit per treatment were taken from the 20-L jars every other day and used to measure the internal ethylene from a 1-mL internal atmosphere sample extracted from water-immersed pears. Ethylene was determined with a Carle Model 311 flame ionization gas chromatograph (Carle Instruments, Fullerton, Calif.) equipped with a 2-m, 80/100 mesh activated alumina column. While threshold ethylene may be in the range 0.05 to 0.5 µL·L⁻¹, for comparative purposes, an internal ethylene concentration of 1 µL·L⁻¹ was arbitrarily chosen as being above the concentration having threshold physiological activity (Gerasopoulos and Richardson, 1996). The same four fruits were used for flesh firmness measurements with a Hunter force gauge model LKG-1 12 kg tester equipped with an 8-mm tip (Western Industrial Supply, San Jose, Calif.), in a UC Davis apparatus using two pared surfaces on opposite sides at the equator.

Calcium determinations. At termination of each ripening experiment, all fruits of each 4-L jar were washed and calcium extracted by the method of Perring (1968), as modified by Richardson and Al-Ani (1982). Ten grams of a 1:1 fruit-water homogenate was transferred into a serum test tube and 10 mL of concentrated HCl (37.8% A.R.) was added and then the tube was capped. The suspension was boiled for 20 min in a water bath, cooled, then filtered through Whatman 41 low ash paper. Strontium chloride was added to a final con-

Table 1. Effects of stage of maturity and 32.3 mM preharvest CaCl₂ sprays on fruit mass, flesh firmness, fruit Ca concentration (fresh mass basis), and chilling requirements of 'd'Anjou' pears.

Maturity class	Ca spray	Fruit characteristics		Ca concn (mg/100 g)	Chilling requirement ² (d at 1 °C)
		Mass (g)	Firmness (N)		
Immature	—	117 c ²	89.9 b	8.37 b	55
	+	---	98.5 a	9.55 a	70
Mature	—	202 b	67.3 cd	5.59 ed	55
	+	---	71.7 c	6.59 c	70
Overmature	—	269 a	60.8 de	5.44 e	40
	+	---	66.7 cde	6.12 cd	55

²Obtained from Fig. 1.

³Mean separation in each column by Duncan's multiple range test when *P* ≤ 0.05.

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centration of 3% to all extracts to reduce atomic absorption mineral interference. The filtrate was diluted to 25 mL with distilled water, and calcium was determined in a Perkin Elmer Model 303 atomic absorption spectrophotometer (Perkin Elmer, San Jose, Calif.) calibrated against CaCl_2 standards in acid.

Statistical analysis. LSDs for comparisons between treatments at a particular time during ripening were calculated with the NCSS "Number Cruncher" (J. Hintze, Kaysville, Utah) statistical computer software. LSDs for comparing firmness and internal ethylene were obtained by the application of factorial analysis.

Results and Discussion

Fruit mass more than doubled with fruit maturation from immature to overmature fruit (Table 1). Flesh firmness of control and CaCl_2 -treated fruits at harvest decreased with fruit maturation by $\approx 32\%$ (Table 1). Fruit Ca concentration was 0.0873 and 0.0955 $\text{mg}\cdot\text{g}^{-1}$ on a fruit mass basis for the control and CaCl_2 -sprayed fruit, respectively, at the immature stage and decreased by 37.5% and 36% at the overmature stage, respectively (Table 1). Ca-treated fruit at the immature stage was firmer than control fruit. Control and Ca-sprayed fruit softened at similar rates (about $\geq 1\text{ N}\cdot\text{d}^{-1}$) during fruit maturation.

Firmness of pears of differing maturity stages at harvest or during ripening after -1°C storage was always higher at higher fruit Ca concentrations. We have found that fruit with high Ca levels soften more slowly than those with low Ca ones at similar rates of ethylene production (Gerasopoulos and Richardson, 1995). Several other reports support a strong association of Ca with firmness for apples (Vaz and Richardson, 1985) and pears (Mason and Welsh, 1970). There are several possible roles for Ca in the postharvest behavior of fruits. Ca interacts with the cell wall pectic substances, thus strengthening the cell walls (Bartley and Knee, 1982). Similarly, the cell membranes are affected by Ca, improving their integrity and reducing their permeability (Clarkson and Hanson, 1980).

There may also be direct and indirect effects of Ca on the ethylene biosynthesis pathway.

The internal ethylene concentration increased above $1\text{ }\mu\text{L}\cdot\text{L}^{-1}$ for control immature (Fig. 1A) or mature (Fig. 1B) fruit after 55 d of -1°C storage. After 70 or more d of -1°C storage and 11 d ripening at 20°C , internal ethylene rose to $35\text{ }\mu\text{L}\cdot\text{L}^{-1}$ or more for immature (Fig. 1A) or mature (Fig. 1B) fruit. Thus,

we considered the chilling requirement of immature or mature fruit to be 55 d (Table 1). Pears in all cases softened in response to the produced internal ethylene. However, after 40 d at -1°C , control overmature fruits began the climacteric internal ethylene production upon transfer to 20°C (Fig. 1C). Internal ethylene of all Ca-sprayed fruit rose above $1\text{ }\mu\text{L}\cdot\text{L}^{-1}$ 15 d later than for the control fruit (Fig. 1). Loss

Fig. 1. Internal ethylene concentration (IEC) and flesh firmness of immature (A), mature (B) and overmature (C) of 'd'Anjou' pear fruit during ripening for 11 d at 20°C after storage at -1°C for various durations. Data represent control fruit (open symbols) or fruit preharvest sprayed with 32.2 mM CaCl_2 (closed symbols).

in firmness was also delayed by about 15 d (Fig. 1).

Low storage temperature retards fruit softening as ethylene synthesis capability continues to develop (chilling requirement satisfaction). Upon rewarming, once the chilling requirement is satisfied, ethylene production and softening develop concurrently (Blankenship and Richardson, 1985; Gerasopoulos and Richardson, 1995). Storage at chilling temperatures has been reported to stimulate ACC synthase and ACC oxidase activity (Blankenship and Richardson, 1985; Larrigaudiere and Vendrel, 1993) in addition to changes in the mRNA population (Wilson et al., 1990), including ACC oxidase and ACC synthase mRNA transcripts (Lelievre et al., 1996). However, external application of calcium decreases the activity of the ACC oxidase in apples (Guan et al., 1991) and increases the chilling requirements of 100% mature winter pears by at least 15 d (Gerasopoulos and Richardson, 1995; Richardson and Al-Ani, 1982; Vaz and Richardson, 1985).

Preharvest CaCl_2 sprays increased fruit Ca content by about 13% and chilling requirements by 15 d (Table 1). The Ca content of the control immature fruit was higher than that of mature and overmature fruit (Table 1) but this increase was not accompanied by analogous increase in chilling requirement (Fig. 1 A–C). Apparently, the externally applied Ca positively influenced the chilling requirement of immature, mature, or overmature fruit. Perhaps the concentrations of levels of endogenous Ca are in balance with other (unknown) factors affecting the chilling requirements during fruit development. The increase of fruit Ca concentration via externally applied Ca may drive the balance towards increased chilling requirement and resistance to ripening.

Although we expected that the immature fruit would have a higher chilling requirement than mature fruit, this turned out not to be the case. The control or Ca-sprayed mature fruit had a similar chilling requirement to that of the immature fruit (55 or 70 d, respectively). Perhaps the factors determining the amount of chilling requirement act during fruit growth and remain unchanged until the fruit has reached maturity (67 N firmness). However, both control or Ca-sprayed overmature fruit showed decreased chilling requirements compared to mature or immature fruit, indicating a decrease of the chilling requirement when the fruit becomes overmature. Chen and Mellenthin (1981) have also shown that 100% or 104% mature 'd'Anjou' pears, corresponding to mature pears for our experiment, required

60 d at -1°C , while 109% or 114% mature pears, corresponding to overmature pears for our experiment, required only 30 d to satisfy chilling. This decrease of chilling requirements with advancement of pear maturation from the mature to overmature stage correlates with an increase in the sensitivity of the fruit to ethylene-induced ripening (Wang et al., 1972). While an interaction with other factors such as endogenous Ca is possible, the sensitivity of the fruit to ripening via chilling requirement satisfaction may either increase with the advancement of fruit maturation or, alternatively, the chilling requirements may have been partially satisfied by cold weather during late harvest, or both.

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