Temperature and Exposure Time during Ethylene Conditioning Affect Ripening of Bartlett Pears

I. Tayfun Agar,† William V. Biasi,‡ and Elizabeth J. Mitcham*,‡

Department of Horticulture, University of Çukurova, 01330 Adana, Turkey, and Department of Pomology, University of California, Davis, California 95616

Freshly harvested early- and mid-season Bartlett pears (Pyrus communis) were treated with ethylene (air plus 10 Pa C2H4) or air at 5, 10, and 20 °C for 24 and 48 h (experiment 1) and at 5 and 10 °C for 48, 72, and 96 h and at 20 °C for 24 h (experiment 2). Following C2H4 or air treatment at different temperatures and durations, pears were transferred to 20 °C in air for ripening. Bartlett pears were evaluated for firmness, color, respiration, C2H4 production, and activities of 1-aminocyclopropane-1-carboxylic acid synthase (ACC-S) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACC-O). Ethylene action was temperature dependent. The duration of C2H4 conditioning needed to fully induce ripening was longer at lower temperatures: 72 h at 5 °C, 48 h at 10 °C, and 24 h at 20 °C. Cold storage in air for as little as 3–4 days at 5 or 10 °C appeared to hasten subsequent ripening, but to a lesser extent than pears kept for 2 weeks at −1 °C in air. Despite a significant increase in ACC-S activity in pears treated with C2H4 at 5 °C, there was not a simultaneous increase in ACC-O activity, resulting in low C2H4 production that was insufficient to generate the threshold endogenous levels of C2H4 required for ripening. Contrary to previous findings with pears, these data indicate that ACC-O could be a rate-limiting step in C2H4 biosynthesis.

INTRODUCTION

Freshly harvested, mature-green, early- and mid-season Bartlett pears ripen nonuniformly and typically fail to achieve good color, texture, and flavor when ripened at 20 °C immediately after harvest (Hansen, 1939; Puig et al., 1996). Initiation of ripening in pear, a climacteric fruit, is induced by a threshold concentration of internal ethylene (C2H4) (Chen and Mellenthin, 1981). Mature-green, early- and mid-season pears do not have the capability to exhibit autocatalytic C2H4 production (Pech et al., 1994). Mitchell (1990) showed that 2 weeks of cold storage at −1 °C induced C2H4 production and uniform ripening in Bartlett pear fruit. Sfakiotakis and Dilley (1974) stated that storage of Bosc pears at 5 or 10 °C for 7 days was equally effective in initiating C2H4 production.

Alternatively, conditioning the fruit with a C2H4 treatment at harvest may substitute for cold storage in early-season Bartlett pears to initiate endogenous C2H4 production and ensure more uniform ripening upon transfer of the fruit to 20 °C (Mitchell and Mayer, 1976; Mitchell and Thompson, 1996; Agar et al., 1999). Autocatalytic C2H4 production requires upregulation by C2H4 of the enzymes involved in the rate-limiting reactions in C2H4 biosynthesis, namely 1-aminocyclopropane-1-carboxylic acid synthase (ACC-S) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACC-O) (Lelievre et al., 1997a). Low temperatures or exogenous C2H4 treatment act to stimulate ACC-S and ACC-O activity, resulting in endogenous C2H4 production and uniform ripening when fruit are placed at 20 °C (Liu et al., 1985; Lelievre et al., 1997b; Chen et al., 1997; Agar et al., 1999).

A 24–48 h treatment of freshly harvested Bartlett pears with 10 Pa (100 ppm) C2H4 at 20 °C prior to fruit cooling and shipment to market has been shown to substitute for cold storage and stimulate C2H4 production and ripening upon rewarmed of the fruit at retail markets (Mitchell and Thompson, 1996). Despite this research, fruit harvested during the first 2 weeks of the season are often not cold-stored or treated with C2H4 because delays in shipping are unacceptable due to the high market demands for Bartlett pears at that time.

To ensure good ripened quality and also timely delivery of early-season fruit to distant markets, pears could be conditioned with C2H4 during transit. To be commercially feasible, C2H4-conditioned pears must remain yellow-green and firm to resist bruising during shipping and distribution, ripen rapidly upon transfer to room temperature at retail markets, and become juicy and flavorful when ripe. The recommended procedures for conditioning with C2H4 (24–48 h at 20 °C) may not be optimum during transit, as the fruit will continue to ripen at 20 °C, possibly resulting in physical damage or over-ripening.

If fruit could be shipped and C2H4-treated at lower temperatures to suppress ripening after C2H4 conditioning, a commercially feasible system could be developed. However, the response of Bartlett pears to C2H4 conditioning at temperatures <20 °C has not been investigated. The objective of this study was to determine suitable temperature and C2H4 exposure time combinations for conditioning of fruit during transit to distant markets, ensuring that the pears remain firm enough.

Keywords: Pyrus communis; pear; firmness; color; respiration; ACC synthase; ACC oxidase
to be distributed undamaged and will ripen uniformly to good eating quality upon transfer to ripening temperatures.

MATERIALS AND METHODS

Mature-green Bartlett pears (Pyrus communis L.) were harvested on July 21 and August 14, 1998, from 15 trees (5 trees/replicate) in commercial orchards in Sacramento and Mendocino Counties, California, respectively, at the initiation of commercial harvest in each location. Fruit were transported the same day to the University of California, Davis, in an air-conditioned vehicle. Pears were sorted to eliminate damaged fruit and to obtain fruit of uniform size and color.

**Experiment 1.** Pears from the early California growing district in Sacramento County were randomly divided into groups for exposure to C2H4 at 5, 10, and 20 °C for 24 and 48 h. In addition, for each group ripened with C2H4, a control group of pears was exposed to air without C2H4 at the same temperatures and times (Table 1). Bartlett pears were evaluated for firmness, color, respiration, C2H4 production, and ACC-S and ACC-O activities immediately after harvest and after 3, 5, and 10 days (5 °C-conditioned), 3, 5, and 7 days (20 °C-conditioned) at 20 °C following treatment with C2H4 or air.

**Experiment 2.** Pears from Mendocino County were randomly divided into groups for exposure to C2H4 at 5 and 10 °C for 48, 72, and 96 h or at 20 °C for 24 h. In addition, for each group ripened with C2H4, a control group of pears was exposed to air without C2H4 at the same temperatures and times (Table 1). Pears from this group were evaluated for firmness and color immediately after harvest and after 3, 5, and 7 days at 20 °C following C2H4 or air treatment.

Six randomly selected pears from each group were placed into 3.7 L glass jars as one replicate and ventilated with a flow of humidified air or air plus 1 Pa (100 μL L−1) C2H4 at 500 mL min−1 to ensure that CO2 concentration was maintained below 0.3 kPa. Following C2H4 or air treatment at different temperatures and for different durations, pears were moved to 20 °C for ripening under a flow of humidified air at 500 mL min−1. Three replicates were used per treatment, and all data are reported as the means and standard error (SE).

Flesh firmness was determined with a University of California firmness tester (Western Industrial Supply Co., San Francisco, CA) fitted with an 8 mm probe. Skin was removed with a pearler from six pears and assayed immediately upon removal from air plus C2H4 or air treatments. ACC-O activity was also assayed in triplicate for each treatment of Sacramento County fruit according to the method described by Gorny and Kader (1997). Pear skin tissue (5 g) was collected with a pearler from six pears and assayed immediately upon removal from air plus C2H4 or air treatments.

Data were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure (SAS Institute, Cary, NC), followed by Duncan’s multiple-range test (DMRT) with a significance level of P < 0.05.

RESULTS

**Ethylene Conditioning of Pears at 5 °C.** Changes in fruit firmness, color, and C2H4 production were negligible in Sacramento-grown pears conditioned with C2H4 at 5 °C, irrespective of C2H4 exposure time (Figure 1A,D,G). Pears treated with C2H4 for 24 h remained firm (Figure 1A) and green (Figure 1D) and exhibited very low ACC-S (Figure 2A) and ACC-O (Figure 2D) activities resulting in <100 pmol kg−1 s−1 C2H4 production (Figure 1G) during 5 days of subsequent ripening at 20 °C. Ethylene production increased slightly thereafter, resulting in minor firmness and skin color changes after 10 days of ripening at 20 °C. Similarly, pears treated with C2H4 for 48 h showed no ripening-associated changes for 5 days, but after 10 days at 20 °C they were 15 N softer and slightly less green than pear treated with C2H4 for 24 h (Figure 1A,D). Pears exposed to air at 5 °C for 48 h had 2.5-fold higher ACC-S activity (Figure 2A), 1.5-fold higher ACC-O activity (Figure 2D), and 3-fold higher C2H4 production rates (Figure 1G) as compared to pears exposed to C2H4 for 24 h at 5 °C. ACC-S and ACC-O activities were similar for pears exposed to 5 °C for 48 h, regardless of whether they were treated with C2H4 (Figure 2A,D). Whereas ACC-S activity increased during pear conditioning at 5 °C, ACC-O activity and C2H4 production remained relatively low even after 10 days at 20 °C. All pears conditioned at 5 °C had firmness levels >60 N after 10 days of ripening at 20 °C, irrespective of treatment (Figure 1A).

For Mendocino-grown pears, a 48 h C2H4 conditioning at 5 °C had only a slight effect on fruit softening (Figure 3A) or color change from green to yellow (Figure 3D) after 7 days of subsequent ripening at 20 °C as compared to fruit exposed to air for 48 h at 5 °C, which showed no signs of ripening. However, C2H4 conditioning for 72 or 96 h significantly increased the rate of softening and color change during ripening at 20 °C compared to 72 h air and 48 h C2H4 treatments. Pears treated with C2H4 for 72 and 96 h softened to 36 N (8 lb-force), the firmness recommended for retail display, in 7 and 5 days, respectively. Ripening of the 48 and 72 h C2H4 conditioned pears was comparable with the 72 and 96 h air treated fruit, respectively.

**Ethylene Conditioning of Pears at 10 °C.** Sacramento-grown pears that were treated with C2H4 for 24 h at 10 °C exhibited no ripening-related changes for 5 days but softened from 93 to 63 N (Figure 1B), changed

---

**Table 1. Hours of Exposure to Air or Air plus Ethylene (C2H4) at 5, 10, and 20 °C prior to Ripening at 20 °C**

<table>
<thead>
<tr>
<th>temp (°C)</th>
<th>Sacramento</th>
<th>Mendocino</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C2H4</td>
<td>air</td>
</tr>
<tr>
<td>5</td>
<td>24, 48</td>
<td>24, 48</td>
</tr>
<tr>
<td>10</td>
<td>24, 48</td>
<td>24, 48</td>
</tr>
<tr>
<td>20</td>
<td>24, 48</td>
<td>24</td>
</tr>
</tbody>
</table>
color from 114 to 109 h° (Figure 1E), and increased in C2H4 production rate to 300 pmol of C2H4 kg−1 s−1 (Figure 1H) between 5 and 8 days of ripening at 20 °C.

The 24 h C2H4 treatment at 10 °C and the 48 h C2H4 treatment at 5 °C softened the pears to similar firmnesses after 8 and 10 days of ripening at 20 °C, respectively (Figure 1A,B). Pears treated with C2H4 for 48 h at 10 °C exhibited nearly 1.5-fold higher ACC-S activity (Figure 2B), 3-fold higher ACC-O (Figure 2E) activity, and 3-fold higher C2H4 production (Figure 1H) as compared to fruit treated with C2H4 for 24 h at 10 °C, resulting in 3.5-fold softer pears after 7 days of ripening at 20 °C (Figure 1B). Pears treated with C2H4 for 48 h at 10 °C reached 36 N (8 lb) in 7 days. The pears treated with C2H4 for 24 h at 10 °C reached 36 N (8 lb) in 7 days.

Ethylene Conditioning of Pears at 20 °C.

Sacramento-grown pears treated with C2H4 for 48 h at 20 °C exhibited the fastest softening for this group of pears, reaching 36 and 13 N firmness after =4.5 and 7 days, respectively, whereas fruit exposed to air for 48 h softened only to 65 N after 7 days (Figure 3B). Moreover, the 48 h C2H4 conditioning at 10 °C resulted in faster softening than the 72 h C2H4 treatment at 5 °C and was similar to the 96 h C2H4 treatment at 5 °C (Figure 3A,B). Ethylene conditioning for 72 or 96 h at 10 °C resulted in similar ripening behavior with the fastest firmness loss and skin color change among the six treatments at 10 °C (Figure 3B,E). Pears from both the 72 and 96 h C2H4 treatments softened to 36 and 13 N in 3 and 5 days, respectively. Ripening of pears was also enhanced by the 72 and 96 h air treatments at 10 °C, as compared to the 48 h air treatment. Firmness of pears from 72 and 96 h air treatments were 31 and 24 N, respectively, after 7 days of ripening at 20 °C. After 7 days at 20 °C, fruit C2H4 conditioned for 48, 72, or 96 h reached similar firmness (13 N) and color (94 h°) values (Figure 3B,E).

For Mendocino-grown pears, the 48 h C2H4 conditioning at 10 °C resulted in significant ripening, reaching 36 and 13 N firmness after =4.5 and 7 days, respectively, whereas fruit exposed to air for 48 h softened only to 65 N after 7 days (Figure 3B). Moreover, the 48 h C2H4 conditioning at 10 °C resulted in faster softening than the 72 h C2H4 treatment at 5 °C and was similar to the 96 h C2H4 treatment at 5 °C (Figure 3A,B). Ethylene conditioning for 72 or 96 h at 10 °C resulted in similar ripening behavior with the fastest firmness loss and skin color change among the six treatments at 10 °C (Figure 3B,E). Pears from both the 72 and 96 h C2H4 treatments softened to 36 and 13 N in 3 and 5 days, respectively. Ripening of pears was also enhanced by the 72 and 96 h air treatments at 10 °C, as compared to the 48 h air treatment. Firmness of pears from 72 and 96 h air treatments were 31 and 24 N, respectively, after 7 days of ripening at 20 °C. After 7 days at 20 °C, fruit C2H4 conditioned for 48, 72, or 96 h reached similar firmness (13 N) and color (94 h°) values (Figure 3B,E).
to 36 N 2 days more quickly than fruit treated with C2H4 for 24 h at 20 °C. Untreated pears exhibited little evidence of ripening, with firmness and color values similar to the initial values after 7 days at 20 °C. Pear fruit treated with C2H4 for 48 h exhibited 3-fold higher activities of ACC-S (Figure 2C) and ACC-O (Figure 2F) and 2-fold higher C2H4 production rate (Figure 1I) at the peak levels compared to the 24 h–C2H4-treated pears. Pears conditioned with C2H4 for 24 h at 20 °C softened at a similar rate (Figure 1C) and exhibited
similar skin color (Figure 1F), activities of ACC-S (Figure 2C) and ACC-O (Figure 2F), and C2H4 production rate (Figure 1I) as compared to fruit conditioned with C2H4 for 48 h at 10 °C (Figures 1B,E,H and 2B,E). At the end of the ripening period pears held for 24 h at 20 °C in air exhibited 3- and 12-fold lower ACC-S activity and 2- and 23-fold lower ACC-O activity as compared to fruit held for 24 h in air at 10 and 5 °C, respectively. Despite the higher enzyme activity at lower temperatures, none of the fruit held for 24 h in air ripened satisfactorily during 7–10 days of subsequent ripening at 20 °C.

Mendocino-grown pears exposed to a 24 h C2H4 treatment at 20 °C exhibited ripening similar to that of pears conditioned with C2H4 for 96 h at 5 °C, both reaching 20 N after 7 days of ripening at 20 °C (Figure 3A,C). However, ripening following a 24 h C2H4 treatment at 20 °C was slightly slower for the first 5 days than fruit treated with C2H4 for 48 h at 10 °C (Figure 3B), although both ripened to similar firmness at 20 °C. Although the fruit treated with C2H4 for 24 h at 20 °C were similar in firmness after 7 days of ripening to fruit treated with C2H4 for 48 h at 10 °C, the color change from green to yellow was not as complete in the fruit treated for 24 h at 20 °C, and fruit were greener after 7 days of ripening (h° = 101). Untreated pears exhibited no evidence of ripening as indicated by firmness and color levels close to initial values after 7 days at 20 °C (Figure 3C,F).

**DISCUSSION**

Bartlett pears, like other climacteric fruit, are characterized by autocatalytic C2H4 production during ripening. In this study, Bartlett pears exhibited different rates of C2H4 production and ripening after exposure to C2H4 at three temperatures. With decreasing temperature during the C2H4 conditioning period there was an increasing lag time at 20 °C before ripening changes were detected, indicating that the pears needed more time to respond to the C2H4 treatment for the initiation of ripening. For Sacramento-grown pears treated for 48 h, when C2H4 conditioning was at 5, 10, and 20 °C, the lag periods before C2H4 production rate increased were 7, 4, and 1 day, respectively. Ripening-dependent changes at 20 °C occurred more slowly when C2H4 conditioning occurred at lower temperatures and were enhanced by increasing conditioning temperature and exposure time.

Sacramento-grown pears were not responsive to 24 or 48 h of C2H4 conditioning at 5 °C. Resistance to ripening of fruit treated with C2H4 for 48 h at 5 °C was likely a result of very low ACC-O activity. Treatment with C2H4 for 48 h at 5 °C was similar to the 24 h C2H4 treatment at 10 °C, and the 48 h C2H4 treatment at 10 °C was similar to the 24 h C2H4 treatment at 20 °C stimulating fruit ripening. From the two experimental groups of fruit used in this study, distinct differences in ripening characteristics were evident. In general, Mendocino-grown pears were more responsive to C2H4 conditioning than Sacramento-grown pears. As with Sacramento-grown fruit, a 48 h C2H4 conditioning at 5 °C had negligible effects on ripening of Mendocino-grown fruit. Ethylene conditioning for 96 h at 5 °C resulted in a satisfactory rate of ripening. Similar to Sacramento-grown fruit, a 48 h C2H4 conditioning at 10 °C resulted in fruit ripening within 7 days at 20 °C. This conditioning treatment resulted in fruit that ripened similarly to fruit treated with C2H4 for 24 h at 20 °C or for 96 h at 5 °C and more quickly than fruit treated with C2H4 for 72 h at 5 °C. The similar rates of ripening that were achieved by C2H4 conditioning for 72 and 96 h at 10 °C might be an indication that ripening was fully induced with the 72 h treatment and, therefore, longer exposure periods had no further effect. The differences in response to C2H4 treatment of the pears from the two growing locations is likely related to a more advanced physiological maturity of the fruit from Mendocino County, resulting in pears that have a higher capacity to ripen. Advanced maturity of the Mendocino-grown fruit was indicated by the firmness measured at harvest (86 N, 7 N softer than Sacramento-grown fruit). Moreover, cooler preharvest temperatures in Mendocino County may induce higher ACC-S and ACC-O enzyme activities, resulting in higher endogenous C2H4 production, which triggers ripening (Streif, 1976).

ACC-O activity of Bartlett pears was very low at harvest. Our results suggest that the activity of ACC-O was not significantly induced by a 24–48 h C2H4 treatment at 5 °C. Even though ACC-S activity did increase significantly in pears C2H4 conditioned 48 h at 5 °C, the lack of a significant increase in ACC-O activity resulted in low C2H4 production, which was insufficient to stimulate ripening. These results suggest that there may be a differential response of the C2H4 synthesizing enzymes to low temperatures. According to J. Brecht and A. A. Kader (personal communication, 1998), this differential response may result in an accumulation of ACC. Our results on ACC-O activity at low temperatures demonstrate that ACC-O could also be a rate-limiting step in C2H4 biosynthesis in Bartlett pears, in contrast to the results of Blankshe and Richardson (1985), who concluded that ACC-S was the rate-limiting step in Anjou pears.

Resistance to ripening of pears not conditioned with C2H4 at 10 or 20 °C is likely a result of low ACC-S or ACC-O activity and therefore low rates of C2H4 biosynthesis (Yang and Hoffman, 1984). Our results agree with those of Lelièvre et al. (1997b), who reported that ACC-O gene expression and activity can be induced by either chilling or short-term exogenous C2H4 treatment in Passe-Crassane pears. Lelièvre et al. (1997b) found that ACC-S gene expression and ethylene biosynthesis may not be regulated only by exogenous C2H4 in nonreceptive fruits but that a chilling treatment may also be required prior to the C2H4 treatment. In contrast, our findings with Bartlett pear suggest that treatment with C2H4 at temperatures ≥10 °C together with longer treatment durations at lower temperatures stimulates ACC-S activity more rapidly upon immediate transfer of the fruit to 20 °C than does holding the fruit at 5 °C in air prior to transfer to 20 °C.

It appears that holding pears for 48–96 h in air at 5 or 10 °C induced some ripening activity in Bartlett pears. Holding pears at the intermediate temperature of 10 °C resulted in lower ACC-S and ACC-O activities in pears held at 5 °C but in higher enzyme activity than in pears held at 20 °C. Storage of Mendocino-grown pears in air at 5 or 10 °C for 72 or 96 h also promoted ripening upon transfer of the fruit to 20 °C. These results are similar to those of SFakiotakis and Dilley (1974) with Bosc pears, who found that storage at 5 °C for 1.5 or 3 days was sufficient to establish the full potential for C2H4 production, although 3 days was more effective than 1.5 days. These authors also found that when Bosc pears were stored at 5 °C for up to 6 days,
the longer the fruit were held at 5 °C, the shorter was the lag period at 23 °C before C2H4 production was detected. Our results showing that only 2–4 days of storage at 5 or 10 °C stimulated pear ripening are in contrast to those of Mitchell (1990), who found that at least 2 weeks of storage at −1 °C was necessary for adequate Bartlett pear ripening.

CONCLUSIONS

Ethylene action was shown to be temperature dependent. The duration of C2H4 conditioning required to fully induce autocatalytic C2H4 production and fruit ripening of Bartlett pear was longer at lower temperatures: 72 h at 5 °C, 48 h at 10 °C, and 24 h at 20 °C. Cold storage for at least 3–4 days at 5 or 10 °C also appears to induce ripening, whereas 2 weeks is needed at −1 °C. The effect of air storage for up to 7 days at 5 or 10 °C on Bartlett pear ripening merits further research.

Exposing freshly harvested and nonchilled Bartlett pears to exogenous C2H4 for 48 h at 10 °C may offer a new approach to condition pear fruit for ripening, effectively substituting for treatment with C2H4 for 24 h at 20 °C and allowing C2H4 conditioning during transit to market. Because the rate of Bartlett pear ripening at 10 °C is much slower than at 20 °C, this should be a feasible temperature for C2H4 conditioning during transit as little fruit ripening would occur while the fruit were held at 10 °C prior to marketing. We were able to induce autocatalytic C2H4 biosynthesis in freshly harvested Bartlett pears with a 48 h exogenous C2H4 treatment at 10 °C, providing a satisfactory rate of ripening for commercial practice. Treatment with C2H4 at 5 °C would require 96 h, which is longer than most domestic commercial shipments.

ACKNOWLEDGMENT

We gratefully acknowledge invaluable help and advice of Murray Clayton on statistical analysis. We thank David Bouck, George Sakaldasis, and Michelle Lauer for their excellent technical assistance. We also thank Doug Hemly of Greene and Hemly, Inc., David Elliot of David J. Elliot & Sons, and Tom Thomas of Alex R. Thomas & Co. for their cooperation and donation of fruit used in this study.

LITERATURE CITED


Mitchell, F. G.; Mayer, G. Temperature management of deciduous fruits for processing. In Canners League of California Report; Department of Pomology, University of California: Davis, CA, 1976; p 19.


Received for review May 5, 1999. Accepted November 10, 1999. Research supported in part by the California Pear Advisory Board.