Regulating Ripening of 'Bartlett' Pears Using Preharvest Plus Postharvest Aminoethoxyvinylglycine (AVG)

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Abstract: In many deciduous fruit growing regions of the world, because of such factors as lack of chilling hours during winter followed by protracted anthesis, variation in fruit maturity and ripening uniformity of 'Bartlett' (*Pyrus Communis*) pears is troublesome. Such variability makes timely harvest difficult, especially when labor is insufficient. Use of ReTain[®] (aminoethoxyvinylglycine, AVG, Valent Bioscience, Walnut Creek, CA) applied 4 weeks before harvest has mitigated this somewhat by delaying maturity development in treated blocks, thereby providing multiple harvest windows. To further regulate ripening of ReTain[®]-treated 'Bartlett' pears in cold storage, fruit were treated immediately after harvest by dipping fruit in aqueous solutions containing AVG at 0, 66, 132 or 264 mg·l⁻¹ and then kept in regular storage at -1 °C. Internal ethylene concentration of individual fruit was measured at harvest and monthly thereafter for 4 months. During the first 3 months in storage, ethylene production was well correlated with total (foliar plus dip) AVG applied. Treating 'Bartlett' pears after harvest with AVG appears more efficacious in reducing ripening variability during regular storage such that subsequent increase in ethylene is both consistent and predictable.

Key Words: Ethylene inhibitor, flesh firmness, Pyrus communis, ReTain[®], storage.

INTRODUCTION

Pears classified as "winter" varieties, such as 'Beurré Bosc' and 'Beurré d'Anjou', normally need a period of cold temperature after harvest to develop characteristics associated with ripeness such as change in peel color, flesh softening, aroma development and increased sweetness. In contrast, "summer" pears such as 'Bartlett' do not require cold temperature incubation to initiate similar ripening processes. Thus, harvest of 'Bartlett' pears must be carefully managed to avoid ripening on the tree or premature ripening in cold storage.

Important commercial indices for assessing maturity and ripeness of 'Bartlett' pears are firmness and peel ground color [1]. Because change in ground color can be influenced by ethylene produced within fruit tissue, preharvest management practices that influence fruit ethylene production may affect the rate of fruit ripening after harvest and during storage [2-5]. Harvested, mature 'Bartlett' pears are responsive to ethylene in the atmosphere, whether from fruit or other exogenous sources [6]. Indeed, postharvest ripening of several pear varieties can be hastened by applying exogenous ethylene [7-9]. It is not surprising that by reducing ethylene in storage, especially endogenous ethylene, change in ground color from green to yellow and loss of flesh firmness can be delayed, thereby extending storage life and affording more marketing options. AVG is often used in 'Bartlett' pear production as a preharvest foliar treatment because it temporarily inhibits fruit tissue ethylene biosynthesis, thereby delaying ethylene autocatalysis and, to some degree, fruit maturity [10]. Potential to delay maturity gives the grower greater flexibility in harvest scheduling and marketing, and often results in fruit with improved ripening uniformity and retention of quality [11, 12]. In larger 'Bartlett' orchards, given the possibility of insufficient labor, preharvest treatment with ReTain[®] is often vital to insure timely harvest of pears with optimum maturity for designated storage duration.

Efficacy of pre-harvest foliar application of ReTain® is often inconsistent because of three main factors. First, during the relatively long interval (about 4 weeks) between treatment and time of intended or anticipated harvest, there may be wide variations in climatic conditions (temperature, humidity, solar radiation, precipitation), cultural management (number and type of additional foliar applications, irrigation method and amount, nutrition) and plant dynamics (age, crop load, vigor, stress) between seasons and among orchards. Second, uniform fruit coverage by topical application may be difficult to realize due to restrictions of canopy size and density. Third, climate-induced protracted anthesis may result in a wide range of fruit maturity and size at harvest (Curry, personal observations). Although these factors can be anticipated and mitigated somewhat, uniform efficacy given such variance is difficult to achieve consistently. On the other hand, dipping or drenching fruit immediately after harvest would likely increase efficacy by improving coverage while, at the same time, minimizing other environmental variables. This study was undertaken to examine regulating ripening of 'Bartlett' pears by using both pre- and postharvest treatments of AVG.

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MATERIALS AND METHODS

In 2003, two commercial 'Bartlett' pear orchards near Cashmere, WA, were selected from which to sample fruit. Both orchards had mature, well-established trees on seedling rootstock with moderate to heavy crops. Orchards were managed similarly; neither had been treated with bioregulators for 2 previous seasons. Trees comparable in size and bloom density were selected during anthesis. Approximately 4 weeks before anticipated harvest, all but 3 rows of trees in both orchards were treated (108 trees treated) with RetainTM at 66 mg· Γ^1 plus 0.05% Regulaid[®] (KALO, Inc., Overland Park, KS).

Behavior of Untreated Fruit Kept at 23°C

From the middle untreated row in each orchard, 14 fruit from 20 trees were picked on 16 Aug. Fruit were rinsed, randomized and placed on open trays in the dark at 23°C. Firmness, peel ground color, fruit weight and ethylene production were measured at harvest and every other day for 12 days from each of 20 fruit per orchard. Ethylene production was measured by enclosing a single pear in a clean 1.5-l glass jar for 50 min and withdrawing 1 ml headspace gas for analysis. Ethylene was measured using a GC-FID (Model 5880A; Hewlett Packard, Avondale, PA) equipped with a 1 m Poropak O column. Ground color was measured with a ColorFlex (Model 45/0; Hunter Labs, Reston VA) using the Hunter L*, a*, b* system, and hue angle (°h) calculated. Firmness was measured at two locations per fruit after removing the peel to a depth of ~2 mm, with a Texture Analyzer (TA-XT2; Texture Technologies, Scarsdale, NY) equipped with an 8 mm probe.

Postharvest Treatments with AVG

On 16 Aug, 40 fruit of similar size and color from 20 trees previously treated with ReTain® were harvested and taken to the laboratory. Fruit from each orchard were kept separate. All fruit were rinsed briefly with clean deionized water, sorted and randomized preparatory to treatment. Each treatment consisted of dipping 100 pears per orchard, in solutions of AVG at 0, 66, 132 or 264 mg·l⁻¹ (no additional surfactant) for 2 minutes and allowing them to air dry for 30 minutes. Fruit were put on dry fiber trays, placed within liners in closed cardboard boxes and stored in the dark at -1 °C until further evaluation. At harvest, and after 1, 2, 3 and 4 months, 20 fruit per treatment from each orchard were removed from storage and kept in the dark at 23 °C for 24 h, after which internal ethylene concentration (IEC) was measured from each fruit. An 18-gauge needle equipped with a rubber septum was inserted through the fruit calyx into the central cavity and withdrawing 0.5 ml of core gas for analysis using the method described previously. Nonlinear regression analysis of the data was performed using Systat (Systat Software Inc., Richmond, CA), and graphical analysis was performed using simple curve-fitting functions in TableCurve2D and TableCurve3D software (Systat Software Inc., Richmond, CA). Unless noted data from both orchards are combined.

RESULTS AND DISCUSSION

Normal Ripening Behavior of Untreated Fruit at 23°C

Fully mature 'Bartlett' pears harvested with an average firmness of 128 N and kept in the dark in ambient atmosphere at 23°C exhibited an initial increase in ethylene production at the rate of 1.5 μ l·kg⁻¹·h⁻¹ for the next 4 days (Fig. **1A**, R_{0A}). Data are suggestive of a respiratory minimum during this preclimacteric or resting phase. Thereafter, the rate of ethylene production increased about 12-fold to 18.8 μ l·kg⁻¹·h⁻¹ (R_{1A}) for two days, after which it returned to a modest 1.1 μ l·kg⁻¹·h⁻¹ for 6 days R_{2A}). Because the pears selected were of similar size in a year when the bloom period was relatively synchronous (~6 days from first bloom to 90% blossoms open) the uniformity of ripening as indicated by the close grouping of ethylene values on a given day over the 12–day examination is quite good (Fig. **1A**).

The relationship between firmness and peel color (hue angle, °h) on the same pears is shown in Fig. (1B). Because the preclimacteric phase is not apparent with respect to ground color, it is assumed that R_{0B} is either absent or trivial. Initial changes in ground color are, thus, associated with large decreases in flesh firmness. The phase of rapid ripening characterized by the slope R_{1B} , of 13.3 N.°h⁻¹ is at least 10-fold greater than the final rate, R_{2B} , of 1.3 N.°h⁻¹ (Fig. 1B). Similarity of the slopes of ethylene production vs. time (R_{1A}) and firmness vs. hue (R_{1B}) is illustrated by the symmetry in Fig. (2).

This model is represented by the equation of the form, $\ln(z) = a + be^{(x/wx)} + ce^{(y/wy)}$ where z = firmness (N), x = ethylene production [ETH (μ l·kg⁻¹·h⁻¹)], y = hue (°) and a, b, and c are the constants 1.78, 2.03 and 2.99 x 10⁻⁶, respectively (adj. R² = 0.95). The terms wx and wy are related to the limits over which x and y data have relevance in this instance; that is, the range of ethylene production and hue angle for which flesh firmness predictions for this formula make biological sense; namely, a single value of -17.1 for wx= Xrange/ln(ZatXmax/ZatXmin) and 7.89 for wy=Yrange/ln (ZatYmax/ZatYmin). Thus, the simplified formula within the physiological framework of this experiment, becomes ln(firmness) = 1.77 + 2.03e^{(ETH/-17.1)} + 2.99 x 10⁻⁶e^(hue/7.89). This proposed model suggests both IEC and hue are weighted approximately the same as indices of firmness loss.

Ripening of Untreated Pears in Regular Storage -1 °C

IEC of untreated 'Bartlett' pears 24 h after harvest and 24 h after storage at -1 °C at monthly intervals indicates a relatively constant rate of ripening to peak ethylene production between 2 and 3 months, followed by a relatively slow decrease (Fig. 3).

This curve is similar to IEC of ripening fruit at harvest with the exception of an expanded time scale and a decrease in IEC after peak production; both likely the result of protracted substrate depletion due to low temperature. Change in peel ground color also progressed with increasing IEC, similar to that of pears ripened at harvest (i.e., hue angle decreased from 118° to 93°, data not shown).

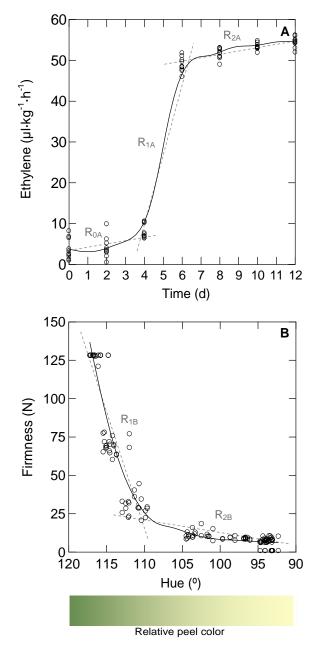


Fig. (1). Ethylene production vs. time (**A**), and firmness vs. hue (peel color) (**B**), for untreated 'Bartlett' pears kept in the dark at 23°C immediately following harvest. R_0 , R_1 and R_2 (dashed lines) indicate slopes of initial phase, ripening phase and post-climacteric phases, respectively.

Ripening Behavior of Pears Treated with Pre- and Postharvest AVG

IEC of fruit treated 4 weeks before harvest with 66 mg·l⁻¹ ReTain[®] plus postharvest by dipping for 2 minutes with AVG at 0, 66, 132 and 264 mg·l⁻¹ and stored up to 4 months at -1 °C is shown in Fig. (4). Compared with untreated controls, fruit receiving a postharvest dip of AVG at 0 mg·l⁻¹ (i.e., only treated with preharvest foliar RetainTM) showed an initial delay in IEC increase of about 3-4 weeks (Fig. 4A).

After 4 months at -1 °C, IEC was still increasing, which suggests fruit had not yet reached peak ethylene production.

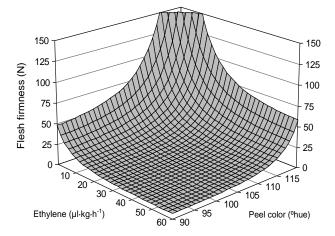


Fig. (2). Fruit flesh firmness plotted as a function of ethylene production and peel color following harvest for untreated 'Bartlett' pears kept in the dark at 23°C. Graph is represented by the equation, $\ln(\text{firmness}) = 1.77 + 2.03e^{(\text{ETH/-17.7})} + 2.99 \times 10^{-6}e^{(\text{hue/7.89})}$ (see text for derivation and explanation).

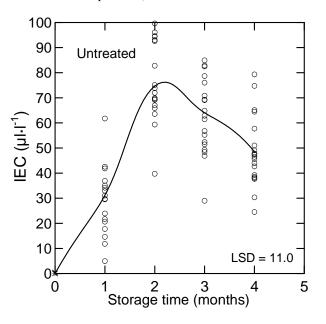


Fig. (3). Internal ethylene concentration (IEC) of untreated 'Bartlett' pears kept at 23°C for 24 h after harvest or after removal from storage at -1 °C.

Fruit receiving a postharvest dip of AVG of 66 or 132 mg·l⁻¹ showed an initial delay in IEC of about 5-7 weeks compared with untreated fruit (Fig. **4B,C**). Peak IEC after 4 months storage at -1 °C was about 40 and 38 µl·l⁻¹ for fruit receiving 66 and 132 mg·l⁻¹, respectively. IEC of fruit receiving the highest rate of AVG, 264 mg·l⁻¹, showed a delay in initial IEC similar to fruit receiving AVG at 66 and 132 mg·l⁻¹, however, peak IEC after 4 months at -1 °C was 31 µl·l⁻¹, about 40% of peak IEC of untreated fruit (Fig. **4D**). Data represented in Figs. (**3** and **4**) were modeled multi-dimensionally (Fig. **5**) and, although the model mimics the individual curves in these figures, the low R² value (< 0.69) indicated variability was excessive for a derived function to be used predictively (function not shown).

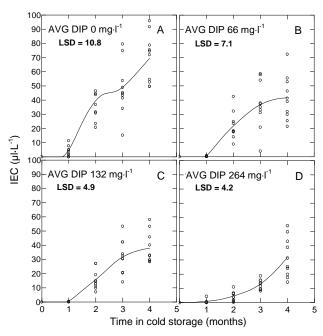


Fig. (4). Internal ethylene concentration (IEC) of 'Bartlett' pears treated 4 weeks before harvest with ReTain[®] and immediately after harvest by dipping in solutions of AVG. IEC was determined after 24 h at 23°C following removal from storage at -1 °C.

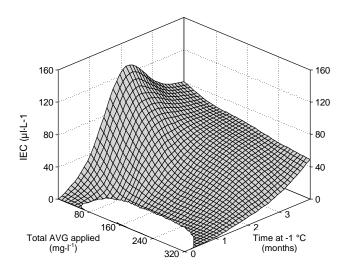


Fig. (5). Internal ethylene production (IEC) plotted as a function of time in storage at -1 $^{\circ}$ C and total AVG (pre- and postharvest) applied (data from Figs. 3 and 4).

Predictive value may be improved somewhat using robust nonlinear regressions of IEC vs. total AVG applied (pre- and postharvest) derived for each of the 4 storage periods as shown below (Fig. 6).

Robust regression analysis affords an alternative to the least squares regression model when data do not meet the assumption that variance of the error term is constant for all values of x and, therefore, there is uniformity to the confidence intervals along the predicted line. When data contain outliers, least squares estimation can be biased because the predictions are pulled towards those outliers thereby artificially inflating the variance in the estimates [13]. Robust estimation allows the variance to be dependent on x, which is more accurate and often more realistic. Such is the case with data in this experiment.

It is apparent, especially for the first 3 months of storage at -1 °C (Fig. **6A-C**), that variability in IEC is greatest for fruit treated with 66 mg·l⁻¹ AVG and that this variability decreases as dosage increases. After 30 days at -1 °C most of the fruit treated with AVG showed little IEC. Although the robust R² (0.69) is higher than that derived from least squares regression (R²=0.41), it is nevertheless low because of both the variability in IEC of untreated fruit and the preponderance of IEC values at or approaching 0 μ l·l⁻¹ from fruit receiving more than 66 mg·l⁻¹ ReTain[®].

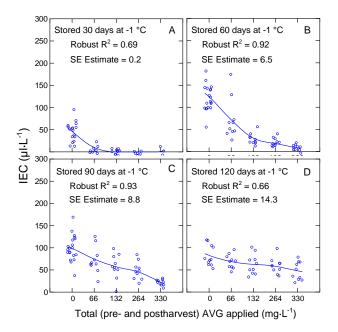


Fig. (6). Robust non-linear regressions of internal ethylene production (IEC) measured after 1-4 months at -1 $^{\circ}$ C vs. Total AVG applied (preharvest ReTain[®] plus postharvest dip).

Clearly, there is a strong correlation between rate of AVG in the postharvest dip and the reduction in IEC in months 1-3 (Fig. 6). After 4 months in storage, variation in IEC measurements was more uniform and mean differences were less significant. Peel color of fruit 24 h after removal from storage was directly related to IEC for months 1-3, whereas in month 4 the correlation was low (data not shown).

IEC of untreated fruit stored at -1 °C and measured monthly for 4 months was 11.0 (Fig. 3). When ReTain[®] was applied 4 weeks before harvest at 66 mg·l⁻¹, onset of ethylene autocatalysis and time to peak IEC production were delayed by 1 and 2 months respectively, whereas LSD was not significantly affected (Fig. **3A**). Increasing the rate of AVG in the postharvest dip to 66, 132 and 264 mg·l⁻¹ reduced the LSD to 7.1, 4.9 and 4.2, respectively (Fig. **3B-D**).

CONCLUSIONS

Mature 'Bartlett' pears having similar anthesis, growing conditions, exposure and size, such as those used in this study, usually ripen in a uniform manner when held in the

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dark at room temperature, as exhibited by the relatively tight clusters of data in Fig. (1). Placing such fruit in storage at -1 °C, on the other hand, expands the time of ripening about 10-fold, (that is, from 12 to 120 days) and increases the variance within the data. Moreover, though variability in IEC for untreated 'Bartlett' pears increased in cold storage over that of fruit ripened at room temperatures, it decreased when AVG was used as a postharvest dip at any rate.

Although the model in Fig. (5) depicting IEC as a function of time in storage at -1 °C and total AVG (pre- and postharvest) applied had a lower R² than was anticipated to use it predictively with high statistical confidence, increasing the sample number and eliminating untreated fruit from the data would likely improve its utility considerably.

Both regulation and uniformity of fruit ripening in cold storage are important marketing tools. Regulating ripening would allow extended marketing during which fruit could be shipped before shelf-life diminished, whereas uniform ripening would help reduce early fruit senescence and pathogenesis. ReTain[®] applied preharvest may be more or less effective because of the inconsistency of myriad factors during the interval between application and harvest. In contrast, AVG applied by dipping or drenching immediately after harvest and before cold storage insures better coverage and, therefore, greater efficacy.

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