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ENDOGENOUS POLYAMINE CONCENTRATIONS DURING DEVELOPMENT, STORAGE AND RIPENING OF PEAR FRUITS*

ARAZDORDI TOUMADJE† and DARYL G. RICHARDSON

Department of Horticulture, Oregon State University, Corvallis, OR 97331, U.S.A.

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Key Word Index—*Pyrus communis*; Rosaceae; pear; low temperature requirements; ethylene.

Abstract—Bartlett and Comice pears both had about 270 nmol/g of putrescine (PUT) one month after full bloom (AFB) and decreased to a low level at harvest. However, at early stages of fruit development Comice pears contained more than twice as much spermidine (SPD) as did Bartlett pears. The spermine (SPM) was undetectable at early development in Bartlett pears but was 244 nmol/g in Comice pears. Polyamine concentrations were also determined during storage of D'Anjou pears at −1° for 74 days. SPD was the polyamine in highest concentration and SPM the lowest, early in storage; the latter decreased to undetectable levels after 60 days in storage. After 40 days at −1° polyamines decreased to about 3 nmol/g and this corresponded to the time when ethylene synthesis began. Anjou pears which had not fulfilled their chilling requirements were not able to ripen or synthesize ethylene when held 14 days at 20°. In these tissues, PUT and SPD decreased but SPM did not change in concentration significantly during 14 days at 20°. In 'Packham's Triumph' pears that had been chilled at −1° to produce ethylene and ripen normally on transfer to 20°, the SPM decreased to an undetectable level by day four of ripening. There were no significant changes in PUT concentration, but SPD decreased gradually during ripening. It appears that SPM may play some regulatory role in ethylene metabolism, but the role of PUT and SPD is less clear.

INTRODUCTION

Polyamines and ethylene have a common intermediate, *S*-adenosylmethionine (SAM) [1, 2]. SAM is a substrate for ACC synthetase in the synthesis of ethylene via 1-aminocyclopropane-1-carboxylic acid (ACC). SAM is also a substrate for SAM decarboxylase in a pathway which leads to the synthesis of polyamines, SPD being the first polyamine product via this pathway [3, 4]. Both pathways give rise to methylthioadenosine (MTA) and other intermediates in recycling back to methionine.

Normal ripening of pear fruits, i.e. to develop good dessert quality, is dependent on the biosynthesis of ethylene, a ripening hormone [5]. Maturing pears lack the ability to produce much ethylene until shortly before harvest, or in the case of winter pear cultivars, only after a period of cold storage has satisfied some chilling requirement [6–8]. The physiological and biochemical basis for this requirement is not known, although two systems of ethylene biosynthesis, one apparently active at low temperature (0–4°) and another at 15° in pears has been suggested [9].

Cold temperature induction of ethylene synthesis is also observed in immature Bartlett pears [1, 2] and cucumbers [11]. The chilling-induced ethylene pathway apparently follows the generally accepted pathway of methionine → SAM → ACC → ethylene [12]. ACC synthase activity, ACC content, and ethylene production was readily stimulated by chilling temperature in cucumbers [11, 12].

There was no increase in ribosomal RNA of mRNA of Passe–Crassane pear fruits that were held at 15° immediately after harvest [9]. However, after 12 weeks in low temperature storage, ripening and ethylene production were accompanied by increased ribosomal RNA and mRNA. Recently, fruit Ca^{2+} concentration has been found to exert some influence on the chilling requirement, low calcium concentration being associated with a shorter chilling requirement for producing ethylene [13].

Polyamines are apparently ubiquitous, but of fairly low concentration in mature tissues [15]. Enzymes of polyamine synthesis and polyamine concentration are high in immature avocado [16], mandarin [17] and decreased by maturation.

Exogenously applied polyamines retarded the senescence of leaves, stabilized protoplasts against lysis, inhibited the dark-induced rise in RNase and protease activity, and reduced the rate of chlorophyll loss in leaves and protoplasts [18, 20]. Sprayed polyamines on apple trees increased the number of fruit per tree and also weight per fruit [19].

Polyamines have shown inhibitory effects on ethylene synthesis in a variety of plant tissues [16, 21–25]. Also, some of these exogenously applied compounds decreased ethylene evolution from pear discs as well as from intact fruit [26]. Since polyamines inhibit ethylene biosynthesis, it might be expected that their endogenous concentration would decrease by the time pear fruits begin to synthesize ethylene. This paper describes changes in endogenous PUT, SPD, and SPM during pear fruit development, maturation, storage, and ripening.

†Present address and to whom correspondence should be addressed: Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, U.S.A.

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RESULTS AND DISCUSSION

Changes in endogenous concentration of PUT, SPD, and SPM during fruit growth and development of Comice and Bartlett pears are shown in Fig. 1. Cadaverine or agmatine were not detectable during any stage of pear development and storage. Number of days from full bloom to harvest were 145 for Bartlett and 157 for Comice.

The first measurement of Bartlett and Comice was from samples taken 34 and 25 days after full bloom (23 and 16% of total development time), respectively. The initial concentration of PUT and SPD were high in both Bartlett and Comice pears and decreased during fruit development (Fig. 1). This parallels decline of PUT and SPD during development of avocado fruit [16]. Spermine was undetectable initially in Bartlett pears and accumulated slowly during development and attained a level of 10 nmol/g fresh weight 20 days before harvest. However, Comice pears contained 244 nmol/g fresh weight SPM early in fruit development and declined to a very low level by harvest (Fig. 1). It is reported [16] that in avocado fruits, unlike Comice pears, SPM did not change in concentration during fruit development. This might be related to the continuous cell division of avocado fruits during the entire developmental period. Unlike avocados, after *ca* 7–9 weeks of cell division pears shift to cell enlargement.

Comice pears contained 45 nmol SPM/g fresh weight 41 days after full bloom (26% development time) compared with Bartlett pears with SPM that was undetectable at 34 days after full bloom (23% development time). At these stages of development, Bartlett pears, although at an earlier stage of development, contained less SPM than Comice pears.

These two cultivars are different from the standpoint of initiation of ethylene synthesis after harvest. Bartlett pears, a summer type, begin to synthesize ethylene immediately after harvest in contrast to Comice pears (a winter type) that usually requires 40–45 days of low temperature storage. As far as the changes in concentrations of PUT, SPD, and SPM are concerned, the only pronounced difference between these two cultivars was the high concentration of SPM at early stages of development in Comice pears compared to an undetectable level in Bartlett pears.

D'Anjou pears typically require 50–60 days of low temperature storage before they initiate any ethylene synthesis [26]. Some evidence suggests that fruit calcium concentrations may be related to differing chilling requirements [13]. Earlier studies showed parallel effects of Ca and polyamines (especially spermine) on apple disc ethylene biosynthesis [23].

Endogenous polyamines and ethylene synthesis of stored D'Anjou pears for 1983 are shown in Fig. 2. These fruits began to produce ethylene in storage two weeks earlier than average. The endogenous concentration of PUT in flesh tissues of D'Anjou pears after seven days $-1°$ storage was 10 nmol/g fresh weight and eventually

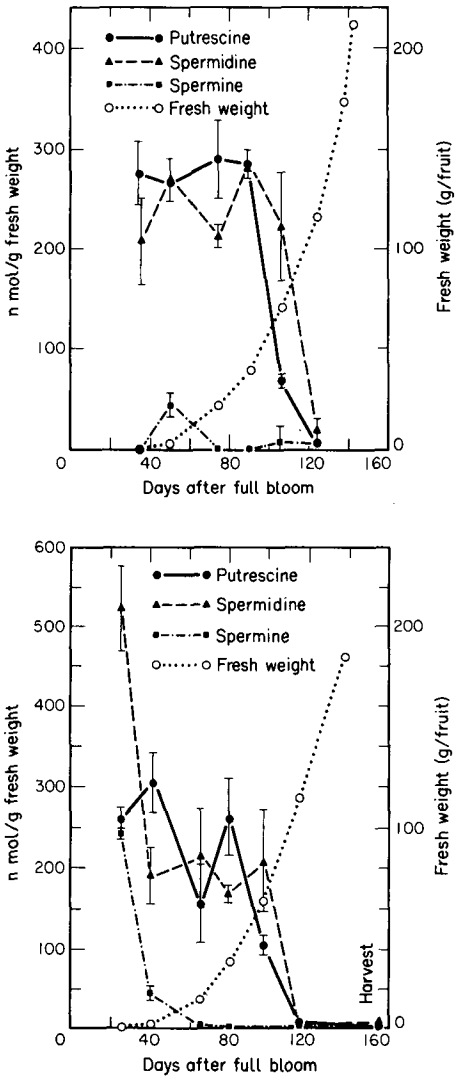

Fig. 1. Changes in polyamines during fruit growth of Bartlett pears (a) and Comice pears (b). Vertical bars are ± 1 s.e. of three replicates.

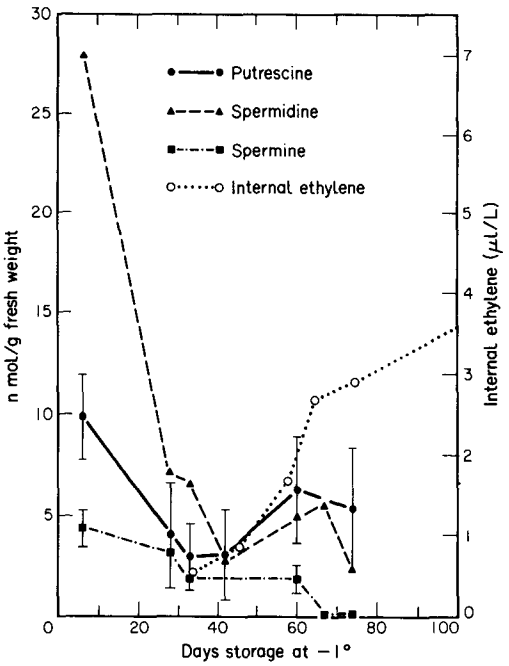

Fig. 2. Changes in polyamines and internal ethylene during storage of Anjou pears at $-1°$. Vertical bars are ± 1 s.e. of three replicates.

declined to 3 nmol by day 42. However, it gradually increased back to the initial level by 74 days in storage.

Initial concentration of SPD was 28 nmol/g fresh weight and steadily decreased to 2 nmol by day 74. D'Anjou pears contained smaller amounts of SPM initially in comparison to PUT and SPD. After 7 days storage, SPM concentration was 4 nmol/g fresh weight and decreased to 2 nmol between 28 and 60 days of storage, whereupon it became undetectable after day 67. This coincided with the time that D'Anjou pears produced maximum ethylene. In general, SPD and SPM decreased continuously during storage (Fig. 2). Also, PUT and SPD decreased even in D'Anjou pears that do not produce any ethylene at 20° because of the unfulfilled low temperature requirement (Fig. 3). D'Anjou pears that had been in storage at −1° only for one week were tested for ripening at 20° for 14 days. No ethylene was detected during this period. However, PUT decreased 71% by day 5 and SPD decreased 81% by day 9 during this period. SPM, on the other hand, did not change significantly during this period. Therefore, in these fruits whose ethylene generating system is not yet active at 20°, endogenous concentration of SPM remains almost the same, unlike PUT and SPD which decrease. However, if winter pear fruits (e.g. Packham's) have been in storage long enough (e.g. 100 days) to meet the low temperature requirement for ethylene synthesis, SPM became undetectable during ripening at 20° (Fig. 4A).

Packham's Triumph pears after 100 days at −1° storage, had already satisfied their chilling requirement and produced large amounts of ethylene at 20° and softened very fast (Fig. 4B). During six days ripening of these fruits, there were no significant changes in endogenous concentrations of PUT and SPD. However, SPM decreased to undetectable levels on day 4 of ripening. D'Anjou and Packham's pears are both winter pears, that normally require a cold storage period before they produce any ethylene. D'Anjou pears were chosen to monitor the relation of ethylene production and polyamine concentration during storage at −1° and ripening at 20°. However, these fruit after about 74 days of storage at −1° do not contain any detectable SPM to monitor its change during ripening at 20°. For this reason, Packman's pear that still contained measureable SPM after 100 days at −1° storage were used to follow the changing trends of polyamines concentrations and ethylene production during ripening at 20°. The assumption was made that all winter type pears share common ethylene regulatory systems.

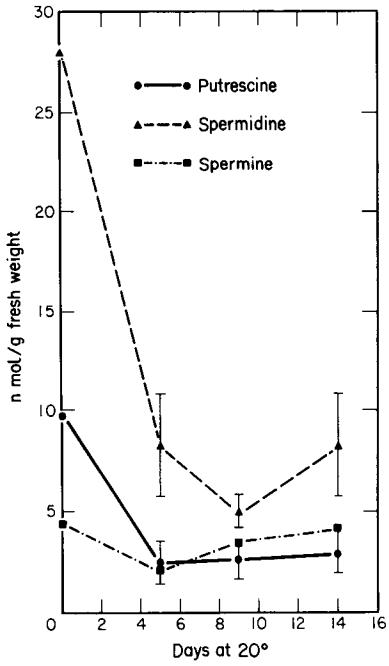

Fig. 3. Changes in polyamines of Anjou pears at 20° after seven days at −1° storage. Vertical bars are ± 1 s.e. of three replicates.

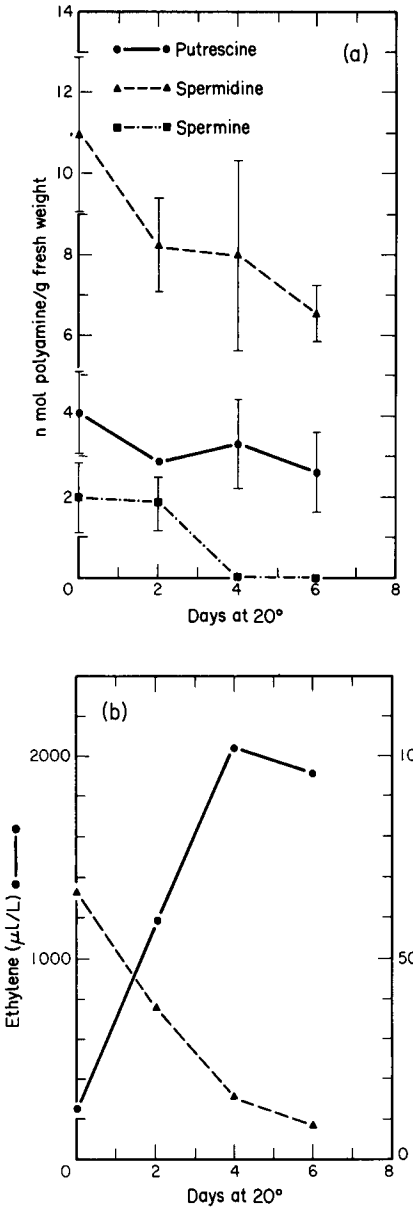

Fig. 4. Changes in polyamines (a) and internal ethylene and firmness (b) during ripening of Packham's Triumph pear at 20°. Fruits have been stored at −1° for 100 days. Vertical bars are ± 1 s.e. of 3 replicates.

Naturally occurring polyamines have been shown to play an important role in the regulation of cell division and growth in higher plants [14]. The cell division period for pear fruit is *ca* 7–9 weeks after anthesis or 45% of total development time. In immature pear fruits during the cell

division period, the concentrations of polyamines were high and decreased by maturation. The high concentration of polyamines during the cell division period of pear fruits is similar to other reports on avocado and mandarin orange that these compounds are involved in growth and cell division [16, 19]. Polyamines, especially SPM, have been shown to inhibit ethylene production in higher plants [16].

During storage of D'Anjou pears, SPM concentration decreased to an undetectable level by the time the fruits produce high ethylene. However, its concentration did not show any significant change during ripening at 20° of non-ethylene producing D'Anjou pears due to unfulfilled low temperature requirement. Spermidine and PUT decreased in both situations. Similarly, SPM declined significantly during the ripening of ethylene producing Packham's Triumph pears, but PUT and SPD did not. In another of our studies, SPM (43% inhibition) was the most effective polyamine as an ethylene inhibitor in pear discs [26] compared to PUT and SPD. Spermine was also shown to be the most potent ethylene inhibitor among polyamines tested on avocado fruits [16]. These observations suggest that there may be a relationship between the SPM concentration and ethylene production in pear fruits.

EXPERIMENTAL

Extraction. Flesh tissues of Bartlett, Comice, and D'Anjou cultivars of pear fruits were used for determination of polyamines during development, storage, and ripening. All analyses were performed in triplicate (one fruit per replicate at each sampling date).

The method of ref. [27] was used to identify and quantify different polyamines during fruit development. Fruit tissues (1 g fr. wt) were homogenized in 10 ml of 5% cold $HClO_4$ with a Polytron. The macerated extracts in polypropylene centrifuge tubes were left at 0° for 1 hr, then centrifuged 20 min at 23 500 *g*. The supernatant phase containing the 'free' polyamines was used immediately for benzoylchloride derivatization or stored at −20° in a plastic vial.

Due to the low concons of polyamines as fruits reached maturity and during storage and ripening of fruit, the method of ref. [28] was used to extract and concentrate polyamines. Flesh tissues (20 g) were homogenized in 160 ml of 5% cold $HClO_4$ in a Waring blender, left at 0° and centrifuged as described above. The supernatant was added to 3 g of Dowex 50W-X8 (20–50 mesh) ion exchange resin (H^+ form) in a 500 ml plastic bottle. Fruit extract and resin were shaken for 1 hr. Unbound extract was aspirated from the resin, followed by a brief H_2O wash which was also removed by aspiration. Bound polyamines were released from the resin by addition of 10 ml 11 M HCl and 2 hr shaking. The HCl-eluted polyamines were collected by aspiration through Whatman no. 1 filter paper and the soln (the amine fraction) was concd to dryness at 60°. After cooling, the amine fraction was dissolved in 1 ml 0.01 M HCl. This fraction was filtered through a 0.45 μ Metricel membrane and Metrigard filter (Gelman).

Benzoylation and HPLC analysis. The method of ref. [29] was used to benzoylate the extract and polyamine standards in preparation for HPLC separation and quantification. One ml of 2 M NaOH was added to 1 ml of extract or amine fraction followed by 10 μl of benzoylchloride (Eastman). After vortexing for 10 sec and standing for 20 min at room temp., 2 ml of saturated NaCl was added. Benzoylated polyamine derivatives were extracted in 2 ml Et_2O (anhydrous grade, Baker) that had been cleaned through activated Alumina F-1, 80/100 mesh column (Supelco, Inc.). After 5 min centrifugation at 1500 *g*, 1 ml of the Et_2O phase was transferred to a 1.5 ml HPLC standard vial and evapd to dryness under a stream of air, then dissolved in 100 μl MeOH (HPLC grade). A 50 μl aliquot of this soln was injected to HPLC immediately or stored at −20° for not more than 2 days before use.

HPLC analysis was done with a solvent programmable Beckman-Altex Model 421 Controller. The solvent system consisted of 60% MeOH: 40% H_2O, run isocratically at 1 ml/min. Solvents were filtered through 0.45 μ pore size membrane filters (Altech) and degassed before use. The benzoylated extracts were eluted at 30° through a 4.6 × 250 mm, 10 μ reverse-phase (C_{18})-column (Altex-octadecylsilane) and detected at 254 nm. Under these conditions, retention times for PUT, cadaverine (CAD), SPD, and SPM were: 8.18 ± 0.13, 9.65 ± 0.09, 14.2 ± 0.3, and 24.6 ± 1.26 min, respectively. Peak areas were integrated by Columbia Scientific CSI-208 digital integrator and molar concentrations calculated from standard curve responses of the known polyamines (Sigma).

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