Relationship between production of ethylene and \( \alpha \)-farnesene in apples, and how it is influenced by the timing of diphenylamine treatment

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Abstract

The relationship between ethylene and peel \( \alpha \)-farnesene concentrations was examined by applying diphenylamine (DPA) and the ethylene analogue, propylene at varying times after harvest to superficial scald (scald) susceptible ‘Granny Smith’ apples (\( Malus domestica \) Borkh.) stored at 10°C. Delaying DPA application after harvest had no large effect on ethylene or on peel \( \alpha \)-farnesene production. Propylene advanced fruit ripening and promoted an increase in peel \( \alpha \)-farnesene concentration before endogenous internal ethylene production, suggesting that ethylene has an important regulatory role in \( \alpha \)-farnesene production, but their biosynthetic pathways are controlled independently. The effect of delayed DPA application (4 and 7 days after harvest) on the relationship between ethylene and peel \( \alpha \)-farnesene was further examined at both a scald-inducing temperature (0°C) and a non-scald-inducing temperature (10°C) with ‘Granny Smith’ and the scald resistant ‘Crofton’ cultivar. Similarly a delayed DPA application had only minor effects on internal ethylene and peel \( \alpha \)-farnesene concentrations. The relationship between internal ethylene and peel \( \alpha \)-farnesene concentration was dependent on storage temperature, and the type of relationship was independent of cultivar. However, the magnitude of the relationship between cultivars was significantly different (‘Granny Smith’ produced significantly more \( \alpha \)-farnesene than ‘Crofton’) and may be related to scald development. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Superficial scald (scald) is a physiological storage disorder of apples and pears and its development has been associated with the naturally occurring volatile sesquiterpene \( \alpha \)-farnesene (Huelin and Coggiola, 1970). It has been shown...
that cuticular \( \alpha \)-farnesene concentration increases with the ethylene climacteric during ripening (Meigh and Filmer, 1969; Du and Bramlage, 1994; Whitaker, 2000), although the precise nature of this relationship is unclear. Several authors have recently shown that inhibitors of ethylene action delay the rise in the production of \( \alpha \)-farnesene, reduce the accumulation of its oxidation products and prevent scald in ‘Granny Smith’ and ‘Red Chief Delicious’ apples (Gong and Tian, 1998; Fan et al., 1999).

Postharvest application of diphenylamine (DPA) is an important commercial scald control treatment and there is considerable evidence that DPA must be applied as soon as possible after harvest to successfully suppress scald development (Little, 1985). However, our knowledge about the changes in peel physiology that occur during this critical time is poor. DPA not only prevents the oxidation of \( \alpha \)-farnesene but also has numerous physiological affects. It has been consistently observed that DPA suppresses the oxidation of \( \alpha \)-farnesene and prevents scald (Huelin and Coggiola, 1968, 1970), although the reported effects of DPA treatment on the production of \( \alpha \)-farnesene are variable (Huelin and Coggiola, 1968; Lurie et al., 1989; Du and Bramlage, 1994; Whitaker, 2000). It can be postulated that DPA application must occur before the ethylene climacteric to effectively control scald, where DPA suppresses the oxidation, and/or the production of \( \alpha \)-farnesene.

To test this hypothesis, DPA was applied at regular intervals before and during the ethylene climacteric and the concentrations of internal ethylene and peel \( \alpha \)-farnesene were measured during storage. To accelerate physiological changes in the fruit, the first experiment was conducted at 10°C. Although scald does not develop at this temperature (Watkins et al., 1995), it is easier to compare ethylene and \( \alpha \)-farnesene production because the rates should be approximately twice those at 0°C.

A problem with the majority of scald (and apple) experimental studies has been the lack of a consistent and reliable physiological marker of fruit age. Calendar date, starch content and firmness are general guides for commercial maturity but are not good indicators of physiological age. Propylene, an active analogue of ethylene which is not produced by the fruit, provides a convenient means of advancing the autocatalytic production of ethylene without interfering with the measurement of endogenous ethylene production (McMurchie et al., 1972). Thus propylene can be used to hasten and synchronise the climacteric and thereby reduce variability within the treated fruit population.

To further examine the relationship between internal ethylene and peel \( \alpha \)-farnesene production and the timing of DPA application, a second experiment was conducted in air at both a scald-inducing temperature (0°C) and a non-scald-inducing temperature (10°C) on a scald-resistant cultivar, ‘Crofton’, and a scald-susceptible cultivar, ‘Granny Smith’.

2. Methods

2.1. Delayed DPA application to propylene-treated ‘Granny Smith’ apples at 10°C

Preclimacteric ‘Granny Smith’ apples (Malus domestica Borkh.) were harvested from a local orchard on March 23, 1994 at Bilpin, NSW. Apples were stored at 10°C and continuously treated with propylene at 100 \( \mu \)L L\(^{-1}\) (equivalent to about 1 \( \mu \)L L\(^{-1}\) ethylene) (Burg and Burg, 1967). Fruit were dipped in a solution of DPA (14.7 mM ‘Shield-Brite Corporation’, Washington, USA) either at harvest, or 0, 1, 2, 3, 5, 7, 10 or 14 days after the commencement of the endogenous ethylene climacteric. The commencement of the ethylene climacteric was judged as 1 \( \mu \)L L\(^{-1}\) internal ethylene concentration and began 8 days after propylene treatment. In addition, samples of fruit were not treated with DPA and stored in either air or propylene (100 \( \mu \)L L\(^{-1}\)) ripening environment. Eight single apple replicates per treatment were used for the destructive measurement of internal ethylene concentration and the peel from four of these fruit was excised, frozen in liquid nitrogen and stored at −30°C for later \( \alpha \)-farnesene analysis.
2.2. DPA application to ‘Granny Smith’ and ‘Crofton’ apples at 0°C and 10°C

‘Granny Smith’ and ‘Crofton’ apples were harvested on April 15, 1994 from a commercial orchard at Orange, NSW. Apples were stored at either 0°C or 10°C in boxes lined with polyethylene film. DPA (14.7 mM ‘Shield-Brite Corporation’, Washington, USA) was applied at room temperature at harvest, or at 4 or 7 days after harvest. A control treatment (no DPA) was included at both storage temperatures for both cultivars. Fruit from each treatment were removed from cold storage at 2 week intervals. Eight single apple replicates per treatment were used for the destructive measurement of internal ethylene concentration and the peel from four of these fruit was excised, frozen in liquid nitrogen and stored at −30°C for later α-farnesene analysis.

Regression of best fit (ranked $r^2$) was determined with Table Curve 2-D (Ver. 4, Jandel Scientific, San Rafael, CA).

2.3. Measurement of internal ethylene and peel α-farnesene concentrations

The internal concentration of ethylene was measured on a gas sample withdrawn via a syringe and hypodermic needle inserted in the calyx end of the fruit. Ethylene was determined with a GowMac Model 580 GC fitted with an activated alumina column (2 m x 2 mm ID, stainless steel) and FID detector with nitrogen carrier gas at 28 mL min$^{-1}$, hydrogen 20 mL min$^{-1}$, air 300 mL min$^{-1}$, and oven temperature at 100°C. One mL gas samples were used and the lower detection limit for ethylene was 0.01 μL L$^{-1}$.

α-Farnesene was extracted from frozen ground apple peel (1 g) using hexane (5 mL, HPLC grade) as a solvent by sonicating (Soniclean 120T) at room temperature for 10 min. Peel from each of the four apples were treated as replicates. Dodecane (20 μg C12 mL$^{-1}$ hexane) was added as an internal standard. The extract was injected directly into a Hewlett Packard (HP) 5890 GC with an injector temperature of 250°C. The samples were separated on a fused silica SE-30 capillary column and detected with a flame ionisation detector held at 280°C. The column was maintained at 60°C for 1 min then programmed at 10°C min$^{-1}$ to 200°C and held for 5 min. Data were acquired with HP 3365 series II ChemStation software. Quantification was obtained by calculating the response factor of an external standard of pure synthetic α-farnesene (kindly supplied by D. Rowan).

3. Results and discussion

Considering the universal importance of ethylene in ripening climacteric fruit and the apparent role of α-farnesene in scald development, it is surprising how little work on scald has explored the detailed relationship between ethylene and α-farnesene. Fig. 1 illustrates the normal development of ethylene and peel α-farnesene during ripening in ‘Granny Smith’ apples in air at 10°C, and shows that the increase in peel α-farnesene was coincident with the increase in internal ethylene concentration. This is in agreement with other studies (Meigh and Filmer, 1969; Watkins et al., 1993; Whitaker, 2000) and suggests that α-farnesene production is dependent on endogenous ethylene production.

Fig. 1B illustrates the influence of application of the ethylene analogue, propylene on ripening of ‘Granny Smith’ apples stored at 10°C. Propylene significantly advanced ripening, as indicated by internal ethylene concentration, and also increased the onset of α-farnesene production, and their respective rates of production were not significantly different from those in the air controls. However, the rise in peel α-farnesene preceded ethylene production in propylene-treated fruit (Fig. 1B). This earlier production of α-farnesene in the presence of propylene shows that α-farnesene production is triggered by the ethylene analogue, and suggests that endogenous ethylene has a fundamental role in α-farnesene synthesis, but that the biosynthetic pathways for their production are independent. This was similarly demonstrated by Ju and Curry (2000) who showed that the application of lovastatin (a specific inhibitor of hydroxymethylglutaryl coen-
Fig. 1. Internal ethylene and peel α-farnesene concentrations in 'Granny Smith' apples stored at 10°C. Control apples were not treated with DPA and stored in air (A). Apples were either not treated with DPA (B) or treated with DPA (14.7 mM) at harvest (C) and stored in propylene ripening environment (100 μL 1⁻¹). Bars show the standard error of the means (SEM; n (ethylene) = 8; n (α-farnesene) = 4); when absent the SEM bars fall within the dimensions of the symbol.
zyme-A reductase) to ‘Golden Supreme’ apples inhibited \( \alpha \)-farnesene production, without affecting ethylene synthesis.

Cultivar is the most important determinant of ethylene and peel \( \alpha \)-farnesene concentrations and is probably related to scald development. Scald-susceptible ‘Granny Smith’ generally had lower internal ethylene concentrations and higher peel \( \alpha \)-farnesene concentrations (Fig. 3), whilst scald-resistant ‘Crofton’ had higher internal ethylene concentrations and significantly lower concentrations of peel \( \alpha \)-farnesene (Fig. 4). As expected, storage temperature had a significant effect on ethylene production and \( \alpha \)-farnesene accumulation. The higher storage temperature (10°C) stimulated higher internal ethylene concentrations in both ‘Granny Smith’ and ‘Crofton’ apples, which resulted in high peel \( \alpha \)-farnesene concentrations (up to 500 mg g\(^{-1}\)) in ‘Granny Smith’ apples, but relatively low concentrations in ‘Crofton’ peel. Indeed, the maximum concentration of \( \alpha \)-farnesene reached in the peel of ‘Crofton’ apples was 77 mg g\(^{-1}\), irrespective of storage temperature and internal ethylene concentration.

Interestingly, the relationship between the concentration of internal ethylene and peel \( \alpha \)-farnesene in ‘Granny Smith’ and ‘Crofton’ was similar at each storage temperature, although the absolute concentrations were significantly different. Regression analysis of combined data for removal times and delayed DPA application at 0°C showed that irrespective of cultivar, there was a positive linear relationship between the concentrations of peel \( \alpha \)-farnesene and internal ethylene (‘Granny Smith’ \( y = 8 + 7.9x, r^2 = 0.73 \); ‘Crofton’ \( y = 0.5x, r^2 = 0.59 \)), whilst an asymptotic-type relationship best described the relationship at 10°C (‘Granny Smith’ \( y = -19 + 11 \ (lnx)^2, r^2 = 0.79 \); ‘Crofton’ \( y = -2 + 0.95 \ (lnx)^2, r^2 = 0.61 \)). This suggests that at 10°C there is a significant change in metabolism and biosynthesis and raises some interesting physiological observations and questions. For example, in ‘Granny Smith’ apples, increasing the storage temperature from 0 to 10°C resulted in a 20-fold increase in ethylene production but only a doubling of the concentration of peel \( \alpha \)-farnesene, whilst in ‘Crofton’ apples, the ethylene production increased 9-fold and the concentrations of peel \( \alpha \)-farnesene remained the same. It is well known that ethylene production is autocatalytic which generally increases logarithmically (Burg and Burg, 1962), whilst peel \( \alpha \)-farnesene production appears to follow a \( Q_{10} \) relationship. However, it is important to note that this method of \( \alpha \)-farnesene extraction and quantification measures peel \( \alpha \)-farnesene accumulation, and storage of fruit at 10°C would result in higher losses of peel \( \alpha \)-farnesene, than storage at 0°C, due to the greater vapour pressure at the higher storage temperature.

DPA has been used commercially for many years to control scald in apples, but its physiological and biochemical effects are not well understood. For example the application of DPA can either lower (Huelin and Coggiola, 1968; Du and Bramlage, 1994), have no effect (Meigh and Filmer, 1969) or increase \( \alpha \)-farnesene production/accumulation (Lurie et al., 1989) and these responses seem dependent on concentration, cultivar, maturity and time in storage (Huelin and Coggiola, 1968). In this experiment, the effects of DPA application at harvest, 3, 7 and 14 days after the initiation of the ethylene climacteric on the internal ethylene and peel \( \alpha \)-farnesene concentrations in ‘Granny Smith’ apples at 10°C in a propylene atmosphere are shown in Fig. 2. The data for delayed DPA application on day 1, 2, 5 and 10 are not shown, but trends in the changes in internal ethylene and peel \( \alpha \)-farnesene concentrations were similar to those presented in Fig. 2. These data show that \( \alpha \)-farnesene concentration in the peel began to increase rapidly before there was a significant increase in internal ethylene concentration. \( \alpha \)-Farnesene concentration reached a maximum 35–45 days after harvest in most treatments, whereas ethylene continued to rise until at least day 50 (when the experiment was terminated). Delaying application of DPA up to 14 days after harvest did not affect the amount of \( \alpha \)-farnesene produced. Its effects on ethylene production were less clear. There was an initial transient inhibition of ethylene production after DPA application (about 5–6 days), which was more evident with the 10 and 14 day delay in DPA application (Fig. 2D). However, in the first experiment when DPA was applied after harvest and
immediately before the apples were placed in the propylene atmosphere, the patterns of internal ethylene and peel α-farnesene concentrations were similar, but the peel concentrations of α-farnesene of these apples were lower than in apples not dipped in DPA (Fig. 1B) or those dipped during the climacteric (Fig. 2). Generally in the second experiment (Fig. 3 and Fig. 4), DPA application on fruit stored at 10°C suppressed peel α-farnesene production during storage, but did not affect ethylene production. However at 0°C, the effects of DPA were not significant on either internal ethylene or peel α-farnesene concentrations. Compared to treating the fruit with DPA at harvest, a delay of 4 or 7 days in applying DPA after harvest did not significantly alter the internal ethylene and peel α-farnesene concentrations (Figs. 3 and 4).

Thus, our results show that the effects of the timing of the postharvest application of DPA on peel α-farnesene and ethylene production depend on the storage temperature, cultivar and ripening environment. It is concluded that the suppression of α-farnesene production by DPA contributes little to its action as a scald inhibitor.

Experiments to further examine the role of ethylene and DPA in a wide range of both scald susceptible and tolerant apple cultivars are needed to increase our understanding of peel physiology during the early stages of scald induction. These studies would also include the use of inhibitors of

![Graphs showing internal ethylene and peel α-farnesene concentrations](image-url)
J.B. Golding et al. / Postharvest Biology and Technology 21 (2001) 225–233 231

Fig. 3. Internal ethylene and peel \( \alpha \)-farnesene concentrations in ‘Granny Smith’ apples stored in air at 0°C (A, C, E, G) and 10°C (B, D, F, H) for 12 weeks. Apples were treated with DPA (14.7 mM) at harvest (C, D), 4 days (E, F) and 7 days (G, H) after harvest. Control apples (A, B) were not treated with DPA. Bars show the standard error of the means (SEM; \( n \) (ethylene) = 8; \( n \) (\( \alpha \)-farnesene) = 4); when absent the SEM bars fall within the dimensions of the symbol.

ethylene action, such as 1-methylcyclopropene and specific inhibitors of \( \alpha \)-farnesene biosynthesis, such as lovastatin.

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Fig. 4. Internal ethylene and peel α-farnesene concentrations in ‘Crofton’ apples stored in air at 0°C (A, C, E, G) and 10°C (B, D, F, H) for 12 weeks. Apples were treated with DPA (14.7 mM) at harvest (C, D), 4 days (E, F) and 7 days (G, H) after harvest. Control apples (A, B) were not treated with DPA. Bars show the standard error of the means (SEM; n (ethylene) = 8; n (α-farnesene) = 4); when absent the SEM bars fall within the dimensions of the symbol.
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