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Effect of DPA and 1-MCP on chemical compounds related to superficial scald of Granny Smith apples

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Abstract

Research was carried out to study the mode of action of diphenylamine (DPA) and 1-methylcyclopropene (1-MCP), on control of superficial scald of Granny Smith apples (*Malus domestica* Borkh.), and its relation with chemical compounds. Fruit was harvested from a commercial orchard in Chile, 182 and 189 days after full bloom and received the following treatments: DPA (2,000 ppm); 1-MCP (1.2 ppm) and control (no treatment). All fruit was stored for 4 or 6 months at 0°C. A completely randomized factorial design was used (2 harvest dates by 3 postharvest treatments). Monthly measurements were made on maturity indices, ethylene production rate (EPR), scald related compounds [α-farnesene (AF), conjugated trienes (CT), total anti-oxidants (AO)], and cell membrane stability. Following 4 and 6 months of storage, plus 7 days at 20°C, scald was evaluated. After 6 months, DPA-treated fruit, from both harvests, showed similar firmness, EPR and AO, compared to the control. However, AF and CT were lower, and cell membrane stability higher. Conversely, 1-MCP-treated fruit showed a noticeable EPR suppression and AF inhibition, along with higher firmness, lower CT and AO, compared to the control and DPA. Furthermore, cell membrane stability was superior to that of the control and similar to that of the DPA. Treated fruit (DPA and 1-MCP) showed an important reduction in scald compared to the control. The effect of 1-MCP on the investigated compounds and the reduction in scald, confirms that ethylene plays a major role on its development.

Additional key words: alpha-farnesene, anti-oxidants, cell membrane stability, conjugated trienes, ethylene.

Resumen

Efecto de la aplicación de DPA y 1-MCP sobre compuestos químicos relacionados con el desarrollo de escaldado superficial de manzanas Granny Smith

Se realizó una investigación para estudiar el modo de acción de difenilamina (DPA) y 1-metilciclopropeno (1-MCP) en el control de escaldado superficial de manzanas (*Malus domestica* Borkh.) Granny Smith y su relación con compuestos químicos. La fruta fue cosechada de un huerto comercial en Chile, 182 y 189 días después de plena flor y recibió los siguientes tratamientos: DPA (200 ppm), 1-MCP (1,2 ppm) y control (sin tratamiento). Toda la fruta fue almacenada de 4 a 6 meses a 0°C. Se utilizó un diseño factorial completamente al azar (2 fechas de cosecha × 3 tratamientos postcosecha). Mensualmente se realizaron mediciones de índices de madurez, tasa de producción de etileno (EPR), compuestos relacionados con el escaldado [α- farneseno (AF), trienos conjugados (CT), antioxidantes totales (AO)] y estabilidad de la membrana celular. El escaldado fue evaluado después de 4 y 6 meses de almacenaje, más 7 días a 20°C. Después de 6 meses, la fruta tratada con DPA, de ambas cosechas, mostró similar firmeza, EPR y AO en comparación al control. Sin embargo, AF y CT fueron menores y la estabilidad de la membrana celular mayor. Por otra parte, la fruta tratada con 1-MCP mostró una notable supresión de EPR e inhibición de AF, junto a una mayor firmeza, menor CT y AO, en comparación con el control y el tratamiento con DPA. Adicionalmente, la estabilidad de la membrana celular fue superior a la del control y similar a la de DPA. La fruta tratada (DPA y 1-MCP) mostró un desarrollo del escaldado reducido comparado con el control. El efecto de 1-MCP en los compuestos investigados y en la reducción del desorden confirma el papel preponderante del etileno en su desarrollo.

Palabras clave adicionales: alfa-farneseno, antioxidantes, estabilidad de la membrana celular, etileno, trienos conjugados.

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Abbreviations used: AF (α-farnesene), AO (anti-oxidants), CT (conjugated trienes), DPA (diphenylamine), EPR (ethyelne production rate), H1 (harvest date 1), H2 (harvest date 2), OD (optical density), RA (regular atmosphere), RH (relative humidity), TSS (total soluble solids), 1-MCP (1-methylcyclopropene).

Introduction

Superficial scald is one of the main physiological disorders affecting stored apples (*Malus domestica* Borkh.), (Ingle and D'Souza, 1989; Emongor *et al.*, 1994). Scald symptoms appear as irregular brown discolorations of the skin of the fruit, severely affecting the external appearance, and therefore, reducing its acceptability for the fresh fruit market.

Its origin is ascribed to the oxidation of α-farnesene, a natural compound present in the wax of the fruit, and its oxidation into highly reactive conjugated trienes (Chen *et al.*, 1990). These react with lipids and proteins altering cell membrane integrity (Abdallah *et al.*, 1997), allowing contact with oxidative enzymes and their respective substrates, causing the characteristic browning (Golding *et al.,* 1998) and even death of the hypodermal cells if the damage is severe (Ingle and D'Souza, 1989).

Disorder susceptibility is associated with fruit maturity at harvest, *i.e.* the earlier the harvest, the more severe the damage (Ingle and D'Souza, 1989; Watkins *et al.*, 1993). Scald is effectively controlled with the synthetic anti-oxidant diphenylamine (DPA) (Huelin and Coggiola, 1970; Anet, 1974; Meir and Bramlage, 1988). Due to the possible restriction of its use, since it is considered to be a pollutant and a health hazard (Drzyzga, 2003), there is great interest in finding new control methods.

Presently, scald is thought to be a symptom of low temperature damage, as it induced by low temperature in storage (Lurie *et al.*, 1990; Watkins *et al.*, 1995; Lurie, 1998; Wang and Dilley, 1999). The latter supposes ethylene, a synthesized plant hormone, reacting to cold induced stress situations, plays some role (Looney, 1996). Larrigaudiere *et al.* (1997) showed that Granny Smith apples exposed to 20°C after a period under 1°C exhibited a sharp increase in ethylene production, whereas if kept always at 20°C, produced very little ethylene. On the other hand, the impact of reducing the ethylene level in the store atmosphere varies between seasons and authors; while Little *et al.* (1985) and Shorter *et al.* (1992) report slight scald development, Lau (1990) and Chellew and Little (1995) did not find differences. This could be attributed to the near impossibility of reducing ethylene production by the fruit to physiologically inactive levels (Johnson, 1994). However, using the ethylene inhibitor diazocyclopentadiene (DACP), Gong and Tian (1998) showed a significant reduction in scald incidence in Granny Smith.

Promising results in relation to the control of scald in Delicious and Granny Smith varieties were shown with another ethylene inhibitor, 1-methylcyclopropene (1-MCP) (Fan *et al.*, 1999b; Rupasinghe *et al.*, 2000). Furthermore, this compound caused a delay in fruit ripening by blocking ethylene synthesis, thereby extending both storage period and shelf-life (Fan *et al.*, 1999a; Watkins *et al.*, 2000; Zanella, 2003).

Although the effects of 1-MCP and DPA on control of superficial scald of apples have been extensively reported, there is less information on their effect on chemical related compounds, and especially on cell membrane stability. Articles that relate electrolyte leakage with 1-MCP application are mostly on fresh cut fruit (Jeong *et al.,* 2004; Mao *et al.*, 2007a,b). It is therefore important to study the changes occurring in the fruit during storage, especially over the first months, when the disorder is triggered.

The following research was carried out to compare the mode of action of DPA and 1-MCP on control of superficial scald of Granny Smith apples (a highly susceptible cultivar), based on their relation with ethylene production, chemical compounds and electrolyte leakage. This was achieved by applying DPA and 1-MCP treatments and determining their effect on maturity of the fruit, production of ethylene, α-farnesene, conjugated trienes, antioxidant and cell membrane stability. Additionally, the incidence and severity of the disorder were evaluated.

Material and methods

Plant material

Apple fruit (*Malus domestica* Borkh. cv. Granny Smith) were harvested from a commercial orchard in San Clemente, VII Region, Chile (35°33'S 27°24'W) from 10 yr-old trees, planted at 4.5 × 3.0 m, on MM 106 rootstock. Fruits were harvested 182 and 189 days after full bloom (1,440 and 1,550 accumulated degree days), denominated as H1 and H2, respectively and stored at Centro de Pomáceas, University of Talca, Chile, in regular atmosphere (RA) (0°C and 90 to 95% RH) prior to the treatments.

Treatments

Fruits of each harvest date were divided into three batches. One batch was kept in cold storage without

any treatment (control). A second lot, after having attained room temperature, was submerged for 20 s in a solution of 2,000 ppm diphenylamine (DPA) and after 12 h stored again in RA. The final lot was treated with 1.2 ppm 1-MCP for 12 h at room temperature in a hermetically sealed acrylic 0.3 m^3 chamber. 1-MCP was applied as a gas by dissolving 150 mg EthylBloc (0.43% 1-MCP) in a 0.9% (w/v) sodium hydroxide solution. Immediately after treatment the fruit was returned to RA storage.

Evaluations

Prior to treatments (considered to be the initial condition) and at monthly intervals during storage, fruit was evaluated for maturity indices, ethylene production rate, α-farnesene levels, conjugated trienes and total anti-oxidants. Additionally, at the same time, cell membrane integrity was evaluated. The incidence and severity of scald was determined after 4 and 6 months at 0°C, plus 7 days at 20°C.

Maturity indices

Ground colour was evaluated using a scale of 1 to 4 (green to yellow); flesh firmness (N) was measured with a pressure meter on a stand (FT 327, McCormick Fruit Tech, Yakima, Washington, USA) with an 11-mm tip, making two measurements on opposite sides of the equatorial area of the fruit; a temperature compensated refractometer (ATC-1E, Atago, Tokio, Japan) was used to determine total soluble solids (TSS, °Brix) and starch breakdown was assessed according to a scale proposed by Mitcham (1993) from 0 (no starch breakdown) to 6 (complete breakdown). For each treatment, four replicates of three fruits each, were used.

Ethylene production

Ethylene production rate (EPR, μL kg^{-1} h^{-1}) was quantified using three replicates of three fruits each. Individual fruits were placed in hermetically sealed acrilic mini-chambers (2,700 cm^3). After one hour a gas sample was taken of the chambers utilizing a 1-mL syringe and analysed through gas chromatography (Series II HP 5890, Hewlett-Packard, Palo Alto, California, USA), fitted with a flame ionization detector column, and a Porapak Q (Supelco, Bellefonte, Pennsylvania, USA) column, and helium as a carrier gas. Injector, column and detector temperatures were 80°C, 80°C and 155°C, respectively. For each treatment, three replicates of three fruits each, were used.

α-farnesene, conjugated trienes and total anti-oxidant analysis

Fifteen apple peel disks of 0.8 cm^2 cut from the equatorial region of three fruits per repetition (4 replicates per treatment) were submerged for 3 min in 25 mL n-hexane (Merck, Darmstadt, Germany). Aliquots of the extract were analyzed with a spectrophotometer (Milton Roy 1201, Milton Roy, NY, USA). The α-farnesene concentration (nmol cm^{-2}) was determined through its absorption at 232 nm, using an extinction coefficient of 29,000 (Anet, 1972). The content of conjugated trienes (nmol cm^{-2}) were calculated by subtracting absorptions at 258 nm (CT 258), 269 nm (CT 269) and 281 nm (CT 281) from absorbance at 290 nm, and using an extinction coefficient of 25,000 (Anet, 1972). Total anti-oxidants were measured by absorption readings at optical density (OD) peak at 200 nm, and expressed in OD · 1,000 · cm^{-2}, according to Meir and Bramlage (1988).

Membrane integrity

Disks of peel of equal dimension and quantity intended for α-farnesene and triene analyses were submerged in 40 mL distilled water and incubated for 6 h at room temperature. Electrical conductivity was measured initially, after 6 h and again after boiling for 5 min (0% stability), cooled down and brought back to its original volume. A conductivity meter was used (LF90, WTW, Weilheim, Germany) (Thomai *et al.*, 1998), and the results were expressed as percentage stability.

Scald evaluation

Scald incidence was determined visually, on four replicates of 15 fruits each and expressed as percentage of affected fruit. Severity, expressed as percentage of the fruit surface affected, was classified using a four point scale, where 0 = no injury; 1 = slight injury (1 to 25% of surface affected); 2 = moderate injury (26 to 50% of surface affected) and 3 = severe (> 50% of surface affected). A severity index was calculated as

follows: Index = [(% fruit grade 1) + (2 · % fruit grade 2) + (4 · fruit grade 3)]/4 (Lurie *et al.*, 1990).

Experimental design and statistical analysis

A completely randomized design with factorial 3×2 arrangement was used for comparison of the different treatments (Control, DPA and 1-MCP) and harvest periods (H1 and H2). To evaluate the variables, both parametric and non-parametric analyses were used. Parametric data was subjected to analysis of variance and followed, when corresponding, by means separation by Tukey test ($p \leq 0.05$) (Conover and Iman, 1981; Fisher and Van Belle, 1993). For variables expressed as percentages, angular transformation [Arcsine $(\%)^{0.5}$] was used, before analysis (Gómez and Gómez, 1984). Non-parametric variables (ground colour and starch breakdown) were analyzed using Kruskal-Wallis test ($p \leq 0.05$). Correlation analyses were performed to find associations between fruit compounds and scald development. Basic statistics, analysis of variance (ANOVA) and correlations were performed using SAS programs (SAS Institute Inc., Cary, NC, USA) and Sigma Plot (SPSS, Chicago, Ill, USA).

Results

The differences found for maturity and chemical compounds could mainly be ascribed to the application of DPA and 1-MCP. Harvest period and the interaction between anti-scald compounds and harvest period showed no significant differences. Therefore, except where indicated, only results related to the post-harvest treatments are reported.

Prior to the application of the treatments, H1 fruit showed a higher EPR than H2 (Table 1). This was not expected since EPR should be lower in less mature fruit; the result could be explained by the high variability that is usually found among ethylene measurements, suggesting that for this index, a higher number of replicates should be used. However, the rest of the maturity indices did not exhibit a more advanced maturity level. Regarding chemical concentrations, α-farnesene levels were higher in H1, whereas conjugated trienes and total anti-oxidants were similar on both harvest dates (Table 1).

Maturity indices

After the second month of storage, significant differences in flesh firmness were observed. Firmness loss was lower in 1-MCP treated fruit (Fig. 1). This difference increased after the 4th month; at that stage, firmness was about 13 N higher than the control and the DPA treatment. Consequently, after 180 days of storage 1-MCP treated fruit had a firmness of 64.5 N, whereas DPA and control fruit had less than 52.0 N. Concerning the rest of the indices (data not shown), the TSS of 1-MCP showed a higher concentration after two and

Table 1. Condition of Granny Smith apples, prior to treatments

Variable evaluated	H1[a]	H2[a]	Significance[b]
Maturity			
Ground colour[c]	2.5	2.7	ns
Firmness (N)	79.8	75.6	ns
Total soluble solids (°Brix)	13.3	13.3	ns
Starch breakdown index[d]	4.2	4.4	ns
EPR (mL kg^{-1} h^{-1})[e]	9.9	1.5	**
Compounds related to scald[f]			
AF(nmol cm^{-2})	67.6	25.0	**
CT 258 (nmol cm^{-2})	4.0	4.5	ns
CT 269 (nmol cm^{-2})	2.6	2.9	ns
CT 281 (nmol cm^{-2})	0.4	0.4	ns
AO (OD · 1,000 cm^{-2})	143.4	141.7	ns

[a] H1, H2: harvested 182 and 189 days after full bloom, respectively. [b] ns: not significant; **: highly significant ($p \leq 0.01$). [c] Scale 1-5 (green to yellow). [d] Scale 0 (no breakdown) – 6 (complete breakdown). [e] EPR: ethyelene production rate; [f] AF: α-farnesene; CT: conjugated trienes; AO: anti-oxydants.

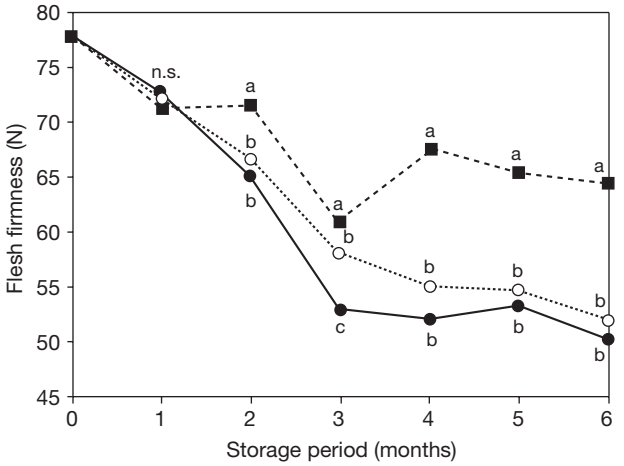

Figure 1. Effect of Control (●), DPA (○) and 1-MCP (■) applications on flesh firmness (N) of Granny Smith apples, stored during 180 days at 0°C. At each date, means followed by the same letter do not differ according to the Tukey test ($p \leq 0.05$).

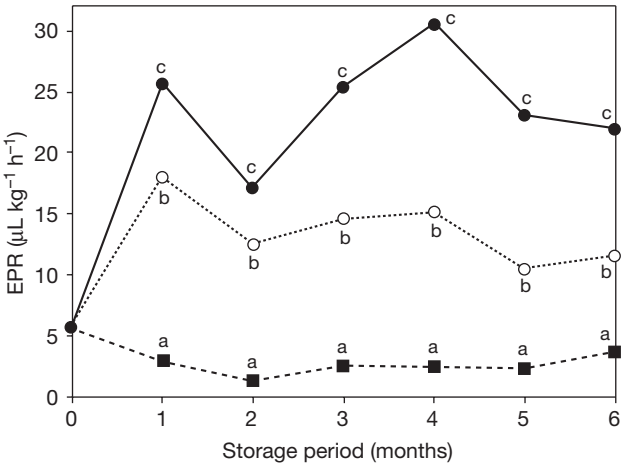

Figure 2. Effect of Control (●), DPA (○) and 1-MCP (■) applications on ethylene production rate (EPR, µL kg^{-1} h^{-1}) of Granny Smith apples, stored during 180 days at 0°C. At each date, means followed by the same letter do not differ significantly according to the Tukey test ($p \leq 0.05$).

three months of storage. Regardless of the post-harvest treatment, ground colour changed to yellow (mean value of 3.6 on the colour scale, after 6 months). Starch breakdown was high (4.3), and after three months all treatments had reached total hydrolysis.

Ethylene production rate

EPR of the control and DPA treatment increased progressively during the first month, decreasing slightly after the fourth month (Fig. 2). On the other hand, the 1-MCP treatment had a low EPR (for both harvest dates), with values not exceeding 5 µL kg^{-1} h^{-1} until the end of the 6-month storage period.

Evaluation of α-farnesene, conjugated trienes and total anti-oxidants

With lengthening of the storage period, α-farnesene concentration increased in the control treatment. The highest level (109.2 nmol cm^{-2}) was exhibited on the 3rd month of storage, decreasing towards the final evaluation (Fig. 3a). Conversely, application of 1-MCP

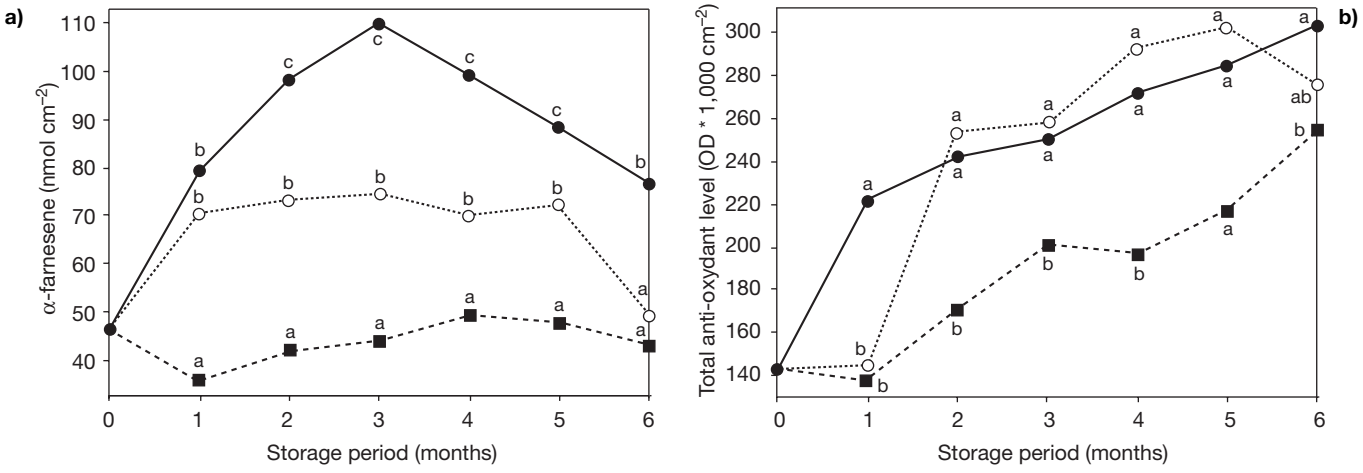

Figure 3. Effect of Control (●), DPA (○) and 1-MCP (■) applications on the α-farnesene (A) – and total anti-oxidant level (b) of Granny Smith apples, stored during 180 days at 0°C. At each date, means followed by the same letter do not differ significantly according to the Tukey test ($p \leq 0.05$).

prevented the accumulation of α-farnesene, compared to the control, maintaining relatively constant levels (±44 nmol cm^{-2}) during the whole period. DPA-treated fruit results were intermediate, reaching a maximum of 70 nmol cm^{-2} in the first month, staying almost constant until the fifth month and dropping thereafter. CT 258 and CT 269 (Figs. 4a and 4b) increased as storage period progressed, independent of the treatment applied. Nevertheless, 1-MCP treated fruit showed the lowest concentration, *i.e.* compared to the control; levels were on average 47% lower for CT 258 and 54% for CT 269. Compared to the control, DPA resulted in reduced CT 258 synthesis for the first, fifth and sixth month. As for CT 269, this effect could only be shown at the end of the 3rd storage month. DPA and 1-MCP treatments suppressed CT 281 accumulation found in control fruit, although 1-MCP was more effective than DPA (Fig. 4c). DPA and 1-MCP treatments only showed an increase during the second month but remained relatively constant and below 2.5 nmol cm^{-2}. Contrary to the CT 258 and CT 269 levels both treatments (DPA and 1-MCP) showed similar concentration of CT 281. Independent of the treatment applied, total anti-oxidants showed an upward trend during storage (Fig. 3b). During the major part of the trial, fruit of the control and DPA treatments showed higher levels (without differences between the two) than the 1-MCP treatment. Anti-oxidant activity of the latter was 25.7% lower than the control.

Membrane integrity

From the third month onwards fruit of the control showed a decrease in membrane integrity (from 40 to

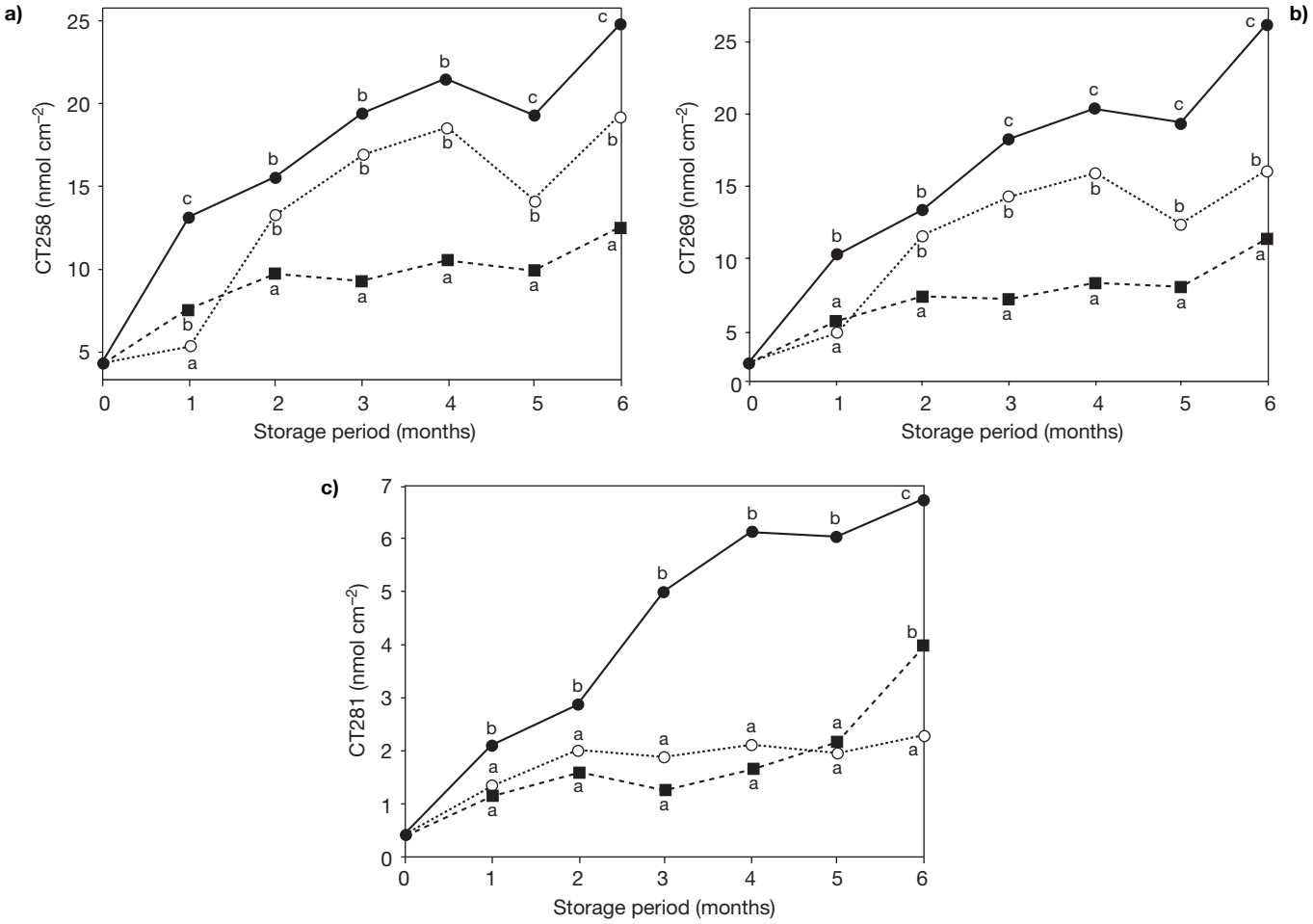

Figure 4. Effect of Control (●), DPA (○) and 1-MCP (■) applications on the concentration of conjugated trienes: CT258 (A), CT269 (B) and CT281 (C) of Granny Smith apples, stored during 180 days at 0°C. At each date, means followed by the same letter do not differ significantly according to the Tukey test ($p \leq 0.05$).

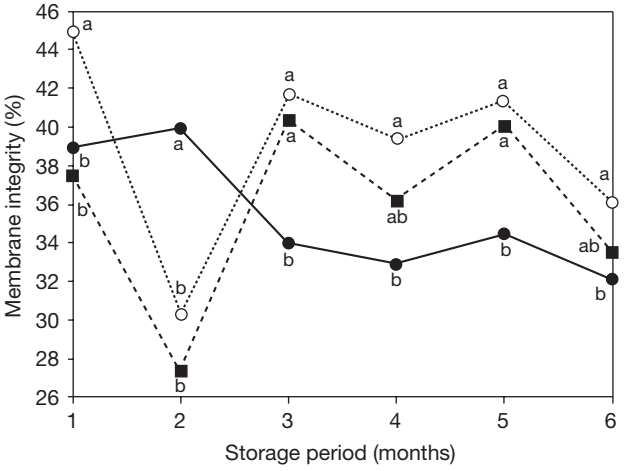

Figure 5. Effect of Control (●), DPA (○) and 1-MCP (■) applications on the membrane integrity (%) of Granny Smith apples, stored during 180 days at 0°C. At each date, means followed by the same letter do not differ significantly according to Tukey test ($p \leq 0.05$).

34%), remaining the same for the rest of the trial period (Fig. 5). Overall, DPA and 1-MCP treatments showed higher membrane stability percentages than the control, except during the second month, where the lowest values for these treatments were found, as well as the highest for the control.

Scald measurements

The most prominent results in scald control were obtained with DPA and 1-MCP applications, simultaneously reducing both the incidence and severity of the disorder after 4 and 6 months of storage (Table 2). Incidence in treated fruit (DPA and 1-MCP), of both harvest dates, did not show, on average, higher values than 6.7 and 10.4% after 4 and 6 months, respectively. At both evaluations scald in the control reached levels up to 55% after 6 months and was higher in fruit from H1 (Table 2).

Discussion

The pattern of α-farnesene accumulation and the concentrations detected were similar to those reported

Table 2. Effect of post-harvest applications and harvest date, on the incidence (%) and severity (index) of superficial scald[a] in Granny Smith apples. Evaluation after 4 and 6 months of storage at 0°C plus 7 days at room temperature

	4 months + 7 days		6 months + 7 days	
	Incidence (%)	**Severity[b]**	**Incidence (%)**	**Severity[b]**
Treatment[c]				
Control	35.8b	9.0b	44.8b	13.2b
DPA	5.8a	1.5a	10.0a	2.5a
1-MCP	7.5a	1.9a	10.8a	2.3a
Significance[d]	**	**	**	**
Harvest date[e]				
H1	20.0b	5.0b	24.4	6.9
H2	12.8a	3.2a	19.3	5.0
Significance[d]	*	*	ns	ns
Interaction				
H1-CONTROL	45.0	11.3	55.0c	16.3
H1-DPA	6.7	1.7	8.3a	2.1
H1-1MCP	8.3	2.1	10.0a	2.5
H2-CONTROL	26.7	6.7	34.6b	10.1
H2-DPA	5.0	1.3	11.7a	2.9
H2-1MCP	6.7	1.7	11.7a	2.1
Significance[d]	ns	ns	*	ns

[a] Data transformed to Arcsine $(\%)^{0.5}$, prior to analysis. [b] Severity index = (1*% fruit grade 1+2*% fruit grade 2 + 4*% fruit grade 3)/4. [c] Treatment, compound applied post-harvest: DPA: diphenylamine; 1-MCP: 1-methylcyclopropene. [d] Significance: ns, not significant; *, significant ($p \leq 0.05$); **, highly significant ($p \leq 0.01$). Treatment and interaction means, separated by Tukey test ($p \leq 0.05$). [e] H1, H2: harvested 182 and 189 days after full bloom, respectively.

by Anet and Coggiola (1974) and Lurie *et al.* (1989). At the same time, the patterns on conjugated trienes also reflected those previously published by Meir and Bramlage (1988) and Barden and Bramlage (1994).

Compared to the control, DPA and 1-MCP applications proved to be equally effective controlling the incidence and severity of superficial scald. However, the two products control the disorder in different ways. Compared to the control, fruit treated with DPA reduced the accumulation of α-farnesene (Huelin and Coggiola, 1968; Du and Bramlage, 1994) and conjugated trienes (Huelin and Coggiola, 1970; Anet, 1974), especially CT 269 and CT 281, and increased the total anti-oxidant concentration. DPA's effect on scald development is associated with the inhibition of α-farnesene oxidation and to some extent to a reduced synthesis of the compound (Du and Bramlage, 1993, 1994), as well as the fruit's higher resistance to cell damage incited by the conjugated trienes (Du and Bramlage, 1995). Conversely, 1-MCP prevents the accumulation of α-farnesene and reduces conjugated trienes (Rupasinghe *et al.*, 2000; Watkins *et al.*, 2000) and total anti-oxidants, simultaneously noticeably inhibiting ethylene synthesis and firmness loss (Watkins *et al.*, 2000).

Membrane stability showed deterioration in the control treatment, compared to DPA and 1-MCP, from the 3rd month on, which coincided with the maximum α-farnesene accumulation in the control fruit. Thomai *et al.* (1998) showed that electrolyte leakage significantly increased during storage of Granny Smith apples that were more susceptible to scald development. Unexpectedly, fruit from both DPA- and 1-MCP-treated fruit showed a decrease on month 2, but thereafter stability levels were higher than the control. These results would lead us to suggest that changes leading to membrane damage could be triggering at this moment, but response on the control fruit was delayed because of a higher physiological activity (visibly higher EPR). At the same time DPA- and 1-MCP-treated fruit were inhibited in their physiological response, thus, showing an initial damage (as evidenced by lower membrane integrity on month 2), that would be reversible after this time. Further research is needed to understand these changes.

The indications of better maintenance of membrane stability following 1-MCP treatment are likely due to inhibition of ethylene-induced α-farnesene formation and subsequent prevention of the formation of conjugated trienes. Considering that changes in the cell membrane, expressed as electrolyte release, is a characteristic of tissues susceptible to low temperature (Lyons, 1973; Wang, 1982), the loss of cell stability observed in the control fruit supports the hypothesis that scald, as suggested by Watkins *et al.* (1995), is a low temperature damage. According to these results 1-MCP effectiveness to control scald is due to its capacity to inhibit ethylene production and consequently the production of α-farnesene and its damaging conjugated trienes, a finding confirmed by Ju and Curry (2000a) who showed that ethylene regulates α-farnesene accumulation. Scald development during storage has been associated with the production of α-farnesene (Huelin and Coggiola, 1968; Meir and Bramlage, 1988; Ju and Curry, 2000b), which in the presence of oxygen readily oxidizes to free radicals and conjugated trienes (Chen *et al.*, 1990; Rowan *et al.*, 1995). Of the three triene types studied during this research, the most closely related with the induction of scald was CT 281 (*r* values ranging from 0.78** to 0.98**). These findings are similar to those reported by Anet and Coggiola (1974), Meir and Bramlage (1988) and Du and Bramlage (1993). As a matter of fact, whether the fruit was treated with DPA or 1-MCP, the effect of CT 281 concentrations on the incidence and severity of scald proved to be similar. This suggests that its accumulation during storage is related to cell damage that leads to the browning of the fruit. Therefore, α-farnesene contributes to the development of scald by acting as a substrate for the formation of CT 281. Our recent studies have indicated that measurement of TC281 during the second or third month of storage could serve as an indicator of scald risk of the fruit, proving the efficacy of the anti-scald initial treatment, and therefore leading to better decisions on the fruit destiny (unpublished data).

Another effect initiated by the application of 1-MCP is the reduction of the total antioxidant level suggesting that ethylene is associated with its synthesis. The lower anti-oxidant capacity in the 1-MCP treatment, would explain why, although α-farnesene was low in this fruit, it reached a similar CT 281 level as DPA (Anet, 1974; Barden and Bramlage, 1994). It has been suggested that ethylene plays a fundamental role in the development of scald (Du and Bramlage, 1994), not only by acting through the α-farnesene metabolism but, also, by regulating the natural anti-oxidant concentration in the fruit. The relation between the total anti-oxidant levels and the production of ethylene suggests the requirement for further research.

Fan *et al.* (1999b) results, working with 1-MCP, indicate a total absence of scald in Granny Smith apples after 6 months of storage. In this study, although com-

pared to the control, the incidence of the disorder was significantly reduced; it did not result in complete control. This outcome is attributed to a reduced effectiveness as the delay between harvest and application increases. Mattheis *et al.* (2002) report that 100% control is possible with applications at harvest. According to the authors, however, a two-week delay results in 10% damage and four weeks delay resulted in 100% scald incidence.

As reported by Blankenship and Unrath (1998) and Fan *et al.* (1999a), the application of 1-MCP, due to a reduction of the ethylene synthesis, has a positive effect on maintaining firmness. Contrary to what was expected, ground colour development was similar for all treatments. This could have been because DPA and 1-MCP were applied only after a period of storage and not immediately after harvest. Concurring with the results found by Fan *et al.* (1999a), TSS was not affected by 1-MCP applications. Based on the results reported here one may conclude that ethylene affects, either directly or indirectly, all compounds associated with scald development (α-farnesene, conjugated trienes, and endogenous anti-oxidants). Therefore, the use of 1-MCP is a feasible alternative for the control of scald, reducing the disorder with efficiency comparable to the application of DPA, with the advantage of very low residues on the fruit and the environment. At the same time, it significantly extends the fruit's post-harvest life by delaying maturity.

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