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1 **Understanding the key preharvest factors determining ‘Packham’s Triumph’ pear heterogeneity**  
2 **and impact in superficial scald development and control**

3

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26 **Abstract**

27 Although superficial scald (SS) is well characterized on apples, there is still few information regarding  
28 the influence that initial fruit heterogeneity may have on the development of this disorder on pears.  
29 Pears (*Pyrus communis* L.) cv 'Packham's Triumph' were picked during three consecutive seasons at  
30 three harvest maturities (H1, H2, H3) from different commercial orchards. Different SS control  
31 treatments (DPA vs. 1-MCP; season # 2) and storage scenarios (RA, CA and RA + stepwise cooling;  
32 season # 3) were evaluated. Superficial scald incidence, maturity indices and biochemical analysis  
33 associated with SS were carried out at harvest and periodically postharvest in all treatments. In  
34 general, bioclimatic indexes (GDA and HL10) were poorly correlated with SS incidence. Only in  
35 season #1, harvest maturity was positively correlated with SS after 140 and 180 d into storage ( $r_s$ =  
36 0.621\* and 0.620\*, respectively), the more mature fruit being more sensitive. The opposite was  
37 observed in season #3, and no pattern in season #2. There was a good and positive correlation  
38 between CTols dynamic ( $\delta$ CTols/ $\delta$ t) and SS development, with variation between seasons. DPA and  
39 1-MCP effectively reduced SS up to 180 d regardless of years and orchard location. In contrast, the  
40 beneficial effect of CA storage was orchard dependent and SWC strategy did not control SS and  
41 affected fruit quality. Collectively our results suggest that initial fruit heterogeneity at harvest is an  
42 important factor that modulate SS development in 'Packham triumph pears. Climatic and fruit  
43 maturity indexes are not reliable for a multi-year prediction of SS development. In contrast to CA  
44 storage that reduced the disorder in an orchard dependent manner, 1-MCP and DPA treatments  
45 effectively controlled SS independently of initial fruit heterogeneity.

46

47 **Keywords:** Physiological disorder; pome fruit; storage; fruit quality; fruit biochemistry, chilling injury

48

49

50 **1. Introduction**

51 Chilean pear (*Pyrus communis* L.) production is around 213,260 tons/year from which 68%, in  
52 average, is sold in fresh markets around the world (ODEPA-Ciren, 2016a, 2016b, 2015). To reach  
53 those markets most fruit must be cold stored for up to 7-8 months before shipment. However,  
54 during storage several physiological disorders causing peel and flesh discolorations may develop  
55 as a result of chilling injury thus affecting fruit quality and marketability. Superficial scald (SS) is  
56 considered the most important peel disorder in winter pears (Chen et al., 1990; Whitaker et al.,  
57 2009). Symptoms are characterized by brown necrotic patches affecting skin hypodermal cell layers,  
58 beginning in the neck of the fruit and later spread non-uniformly to the surface. It generally appears  
59 in shelf-life once the fruit has been removed from cold storage (Lurie & Watkins, 2012; Whitaker et  
60 al., 2009).

61 As in apples, SS in pears is associated with (*E,E*)- $\alpha$ -farnesene oxidation, a volatile sesquiterpene  
62 present in the cuticle wax, resulting in accumulation of conjugated trienols (CTols) which occurs with  
63 fruit maturation once in cold storage (Anet, 1972; Huelin & Coggiola, 1970; Whitaker, 2007).  
64 Nevertheless, CTols accumulation does not always correlate well with SS development (Calvo et al.,  
65 2015; Whitaker et al., 2000). Another end product of the  $\alpha$ -farnesene oxidation is 6-methyl-5-  
66 hepten-2-one (MHO); this ketone has been positively correlated with SS in pears (Hui et al., 2016).  
67 An important role has been given to the cell antioxidant system for SS suppression (Busatto et al.,  
68 2018; Rudell & Mattheis, 2009; Zhao et al., 2016), especially in pears (Larrigaudière et al., 2016).  
69 Synthetic antioxidants such as DPA and ethoxyquin applied right after harvest, effectively reduce SS  
70 development on both apples and pears (Lurie & Watkins, 2012).

71 Ethylene is also a key player in SS development by triggering  $\alpha$ -farnesene synthesis (Gapper et al.,  
72 2006; Tsantili et al., 2007). Therefore, 1-methylcyclopropene (1-MCP), an ethylene perception  
73 inhibitor, prevents SS development by not only downregulating the expression of  *$\alpha$ -farnesene*

74 *synthase 1* gene (AFS1), but also enhancing other cold acclimation-related mechanisms (Busatto et  
75 al., 2018). In winter pears such as ‘Packham’s Triumph’ and ‘d’Anjou’, which require 30-70 d at low  
76 temperatures (-0.5 and -1 °C) after harvest to reach eating quality (Villalobos-Acuña and Mitcham,  
77 2008), 1-MCP applications may halt fruit ripening and softening (Candan and Calvo, 2011).

78 Among predisposing factors, genotype, preharvest climatic conditions and length of cold storage  
79 play a critical role (Ingle, 2001; Lurie & Watkins, 2012). Fruit maturity at harvest is also considered  
80 important. Conversely from apples, in which late harvested fruit exhibit less SS in long-term storage  
81 (Lurie & Watkins, 2012), European pears seem to have the opposite behavior. Despite this  
82 assumption, some contradictory results have been reported in different or even the same pear  
83 cultivars. In ‘d’Anjou’ and in ‘Packham’s Triumph’ pears Calvo et al (2015 and 2017, respectively)  
84 found higher SS in late harvested fruit, while Zoffoli et al. (1998) found higher SS in early harvested  
85 ‘Packham’s Triumph’. Growing conditions have also been shown to modulate cultivar susceptibility  
86 on apples (Whitaker et al., 2009).

87 Warming treatments during cold storage have effectively shown to reduced SS on apples with  
88 varying degrees upon cultivar and growing region (Watkins et al., 2000), but there is scarce  
89 information on pears. Other storage regimes such as controlled atmosphere (and ultra-low  
90 oxygen,ULO, in some instances) can reduce this disorder but not completely prevent it from  
91 occurring (Larrigaudière et al., 2019; Truter et al., 1994), especially when fruit is removed from these  
92 storage regimes and then kept at low temperature for 2-3 months more prior reaching final  
93 consumers (Torres & Hernández, 2015).

94 ‘Packham’s Triumph’ pears in Chile develop SS much later into the storage season compared to  
95 ‘Granny Smith’ apples, and randomly between seasons and orchards, which makes the cold chain  
96 hard to manage. Therefore, in this study we aimed to understand the effect of growing season and

97 site, harvest maturity, and their interaction with postharvest treatments on superficial scald  
98 development.

99

## 100 **2. Material and methods**

### 101 *2.1 Plant material, fruit sampling and storage conditions*

102 Pears (*Pyrus communis* L.) cv 'Packham's Triumph' were harvested from seven commercial orchards  
103 located in the main Chilean pear-growing region, characterized by a mediterranean climate. The  
104 study was conducted during three consecutive seasons: 2015/16 (#1), 2016/17 (#2), and 2017/18  
105 (#3). The first one included four orchards, named "Genova" (33°64'38"S, 70°75'99"W), "El Carmen"  
106 (34°60'51"S, 70°97'84"W), "Lo Carrizo" (34°45'3"S, 71°7'1"W), and "Pirhuin 3" (35°2'27"S,  
107 71°15'7"W). The second and the third season included three orchards, "Pirhuin 3", "Pirhuin 11" (35°  
108 2'46"S, 71°14'43"W), "Agrofruta" (35°35'12"S, 71°28'56"W), and "Talcaehue" (34°38'16"S,  
109 70°53'11"W). All orchards were managed conventionally with standard commercial practices.

110 Fruit was picked at three (Seasons #1 and #2) and two (Season #3) harvest maturity from each  
111 orchard, determined by maturity indices (flesh firmness, skin color, starch index) and days after full  
112 bloom (DAFB). They are referred as H1 (early-harvest, 120-132 DAFB, one week before commercial),  
113 H2 (commercial harvest, 127-138 DAFB) and H3 (late-harvest, 132-145 DAFB, one week after  
114 commercial harvest).

115 A total of 500 pieces of fruit per orchard and harvest maturity were stored in regular atmosphere  
116 (RA; -0.5 °C, >90 %RH) for up to 180 d, otherwise mentioned. Fruit maturity was determined at  
117 harvest and during cold storage after 1 and 14 d at 20 °C (shelf-life). Biochemical analyses on peel  
118 tissue were performed at harvest and after 30 and 60 d into cold storage.

119

### 120 *2.2 Postharvest treatments during seasons #2 and #3*

121 During season #2 fruit was picked from each orchard and harvest maturity, and treated with 1-MCP  
122 (SmartFresh®, AgroFresh, Spring House, USA; 0.3  $\mu\text{L L}^{-1}$ ) and DPA (DPA Shield, Pace International  
123 LLC, Wapato, USA; 1,200  $\mu\text{L L}^{-1}$ ) within 5 d of harvest. Treated (1-MCP and DPA) and non-treated  
124 (Control) fruit were stored in RA (-0.5°C, >90 %RH) for 180 d.

125 In season #3, three postharvest storage conditions were evaluated in fruit from three orchards and  
126 two harvest maturity. These storage conditions were regular atmosphere (RA at -0.5 °C and >90  
127 %RH), controlled atmosphere (CA; O<sub>2</sub>: 2.0 kPa, CO<sub>2</sub>: 1.0 kPa, -0.5 °C, >90 %RH) and stepwise cooling  
128 (SWC, 1 week at 5 °C, 1 week at 3 °C, 1 week at 2 °C and 1 week at -0.5 °C, >90 %RH). Five replicates  
129 of 100 fruit each per sampling date were used to evaluate fruit maturity and SS incidence.

130

### 131 2.3. Bioclimatic indexes

132 Growing degree-days accumulated (GDA; 10 °C base; Stanley, 2000) from full bloom to harvest, and  
133 Chill Units (hours < 10 °C, HL10) 45 d prior harvest (Merritt *et al.*, 1968; Thomai *et al.*, 1998) were  
134 calculated using air temperature obtained from weather stations located in each commercial site.

135

### 136 2.4 Maturity Indices

137 Maturity indices evaluated were: Peel color (Hue°), internal ethylene concentration (IEC), flesh  
138 firmness (N), starch index (SI, scale 1-6). Hue angle ( $H^\circ = \text{arccot} \frac{b^*}{a^*}$ ; McGuire, 1992) was  
139 measured using a colorimeter (Minolta CR200b, Tokyo).

140 Internal ethylene concentration (IEC) was performed according to Torres et al. (2013). Briefly, one  
141 ml of gas was obtained from the core of the fruit and then injected into a gas chromatograph system  
142 (Series II HP 589 Hewlett Packard, CA, USA). Ethylene concentrations were calculated using a  
143 standard curve (1, 10, and 100 ppm of ethylene). Flesh firmness was obtained by inserting an 8 mm  
144 plunger into opposites sides of each fruit using a Texture Analyzer (GS14, GÜSS Manufacturing,

145 Strand, South Africa), results were expressed in Newton (N). Twenty fruit were used to assess fruit  
146 maturity.

147

#### 148 *2.5. Superficial scald evaluation*

149 Superficial scald incidence was evaluated visually after 140 and 180 d of cold storage plus 14 d at 20  
150 °C (shelf-life). Scald incidence was expressed as percentage of fruit affected (#fruit with SS/total #  
151 fruit). One-hundred pieces of fruit per replicate were used for this evaluation.

152

#### 153 *2.6. Biochemical assays ( $\alpha$ -farnesene and CTols)*

154 Measurements of  $\alpha$ -farnesene and CTols, compounds associated to scald development, were  
155 carried out according to Isidoro & Almeida (2006) and Calvo et al. (2015). Evaluations were done at  
156 harvest and during postharvest. For this, 10 peel discs of 1 cm<sup>2</sup> (and fleshless) were taken along the  
157 equatorial zone of five pears per replicate, with a total of 20 fruit obtained from four replicates. Ten  
158 discs were immersed in 5 ml of hexane (HPLC-grade; Merck, Germany) and extracted for 10 min at  
159 25 °C under stirring (50 rpm) on an orbital shaker (model ES-20/60). Following extraction, solvent  
160 was filtered on Whatman cellulose paper and the final volume adjusted to 5 ml with hexane.  
161 Absorbance of 1 ml of extraction was recorded at 200, 232, 258, 269, 281 and 290 nm using a  
162 spectrophotometer (Biosan, Pharo 3000, Riga, Latvia) according to Giné Bordonaba et al. (2013).  
163 Concentration of  $\alpha$ -farnesene was determined at 232 nm using molar extinction coefficient (27,700)  
164 according to methodology proposed by Huelin & Coggiola (1968) and expressed as  $\mu\text{mol m}^{-2}$  peel  
165 tissue. Whereas concentration of CTols, was calculated as the difference between each OD and 290  
166 nm, and expressed as CT258, CT269, and CT281 using molar extinction coefficient of 25,000.  
167 Evolution of CTols accumulation dynamics was calculated as reported by Giné Bordonaba et al.



168 (2013). Total antioxidant activity (AO) was determined at 200 nm according to Meir & Bramlage  
169 (1988) and expressed as  $AO_{200} \times 1000$ .

170

## 171 *2.7. Statistical analysis*

172 Correlation analysis between different variables and SS incidence was done using Spearman's rank  
173 correlation coefficients ( $r_s$ ). Maturity indices were subjected to analyses of variance (ANOVA) to  
174 determine differences between treatments. Mean separation was carried out using Tukey's  
175 multiple range test. All statistical analyses were performed using OriginPro version 2019 (OriginLab  
176 Corporation, Northampton, MA, USA).

177

## 178 **3. Results**

### 179 *3.1. Preharvest factors affecting SS development*

180 Only in season #1 harvest maturity (H1, H2, and H3) was well correlated with SS incidence after 140  
181 and 180 d into storage ( $r_s = 0.621^*$  and  $0.620^*$ , respectively; **Table 1**). Nevertheless, this correlation  
182 was not observed in season #2 nor #3 or with all of them together (**Table 1**). HL10 was inversely  
183 correlated with SS, but only in season #1 with incidence after 180 d ( $r_s = -0.631$ , **Table 1**). Fruit from  
184 "Pirhuin 3" had the highest averaged SS incidence among all sites and seasons (Table 1). In this  
185 site, the average GDA increased with each season (843, 940, and 1,062 in seasons #1, #2, and #3,  
186 respectively) as it did the average SS incidence (51.7 %, 64.5 %, 93.9 % in seasons #1, #2, and #3,  
187 respectively).

188 The interaction Orchard x Harvest maturity was significant for flesh firmness in seasons #1 and #3  
189 at harvest, and after 140 and 180 d into storage in all three seasons (**Table 2**). As expected, flesh  
190 firmness decreased as harvest date increased in all orchards, except 'Lo Carrizo' in season #1. This  
191 trend was maintained after 140 and 180 d into storage (**Table 2**). In general, SI increased as harvest

192 maturity increased but not always consistently and significantly different within orchards in any of  
193 the seasons (**Table 2**). The effect of harvest date over skin color (Hue°) change from green to yellow  
194 was not consistent in any of the orchards, harvest date or seasons (**Table 2**).

195 In all cases SS incidence increased as time in storage did. In season #1, fruit harvested the latest (H3)  
196 had higher SS incidence, although not always statistically different (**Table 2**). The opposite was  
197 observed in season #3, and no pattern at all in season #2 (**Table 2**).

198 In general, fruit from “Talcarehue” in season #2 exhibited, on average, the lowest incidence of SS  
199 after 180+14 d (33 %), while that from “Pirhuin 3” the highest (64 %). The same trend was observed  
200 in season #3, but with much higher average incidences (70 % and 94 %, respectively). Conversely, in  
201 season #3 early-harvested fruit (H1) showed higher SS incidence than late-harvested one (H3, **Table**  
202 **2**).

203 When all seasons were combined together, fruit firmness, skin color (Hue°) and SI at harvest were  
204 poorly correlated with SS after 140 or 180 d in storage (**Table 2**). Nonetheless, when seasons were  
205 separated, flesh firmness showed good correlations with SS after 140 ( $r_s = -0.678^*$ ) and 180 d ( $r_s = -$   
206  $0.770^*$ ) but only for season #1 (**Table 2**). In the case of skin color, it was poorly and negatively  
207 correlated with SS after 180 d when all seasons were included ( $r_s = -0.432^*$ ), but well correlated whe  
208 only season #2 was analyzed (**Table 2**). Overall, SI was not correlated with SS incidence postharvest  
209 (**Table 2**).

210

### 211 *3.2. $\alpha$ -farnesene, CTols and antioxidant activity in fruit peel*

212 The accumulation of compounds associated to SS was studied over three seasons. The evolution of  
213  $\alpha$ -farnesene and AO capacity during cold storage are shown in **Fig. 1**. In general, there was a peak  
214 on  $\alpha$ -farnesene production around 60-75 d into storage in all three seasons, with the highest values  
215 for season #3 (**Fig. 1A, B, C**). Antioxidant activity progression during storage varied between seasons

216 and growing sites with no clear overall pattern, except in season #3 were they followed the same  
217 pattern as  $\alpha$ -farnesene accumulation (**Fig 1D, E, F**).

218 Conjugated trienols were explored using correlation analysis between the rate of production and SS  
219 incidence after 180 d (**Fig. 2**). Spearman correlation ( $r_s$ ) were significant when all seasons were  
220 combined together (**Fig. 2A**) or for seasons #2, and #3, when separated (**Fig. 2C, D**). CTols rates were  
221 not correlated with SS 140 d ( $P=0.103$ ). Overall, CTols accumulation dynamic were not statistically  
222 different between harvest maturity ( $P=0.084$ ).

223

### 224 3.3. *Effect of 1-MCP and DPA treatments on superficial scald development*

225 Both 1-MCP and DPA were effective controlling SS development after 140 and 180 d in storage  
226 (**Table 3**). Alpha-farnesene rates were reduced, in average, by 45 % and 89 % in DPA and 1-MCP-  
227 treated fruit, respectively, compared to untreated Control (**Fig. 3**). Consequently, CTol rates were  
228 also reduced by 91 % and 95 % in DPA and 1-MCP-treated fruit, respectively, compared to the  
229 Control treatment (**Table 3**). In general, CTols accumulation rate ( $\delta\text{CTols}/\delta t$ ) showed a good and  
230 positive ( $r_s > 0.6$ ) correlation with SS (**Table 3**). Antioxidant activity varied between treatments and  
231 orchards with no clear pattern throughout storage (**Fig. 3**).

232 Internal ethylene concentration in 1-MCP-treated fruit was significantly lower than those of DPA-  
233 treated and Control from all sites, although not always significantly different (**Table 4**). In general,  
234 DPA or 1-MCP did not significantly change fruit firmness or fruit green color (Hue°) after 140 d or  
235 180 d in RA regardless of harvest date in any of the sites (**Table 4**).

236

### 237 3.4. *Effect of CA storage and SWC treatment on superficial scald development*

238 Controlled atmosphere storage significantly ( $P \leq 0.05$ ) reduced SS incidence after 140 d and 180 d  
239 in all sites, except in "Pirhuin 3" (**Table 5**). The overall reduction in  $\alpha$ -farnesene production was, on

240 average, 41 % for CA-stored fruit compared to the untreated Control, except for that of “Pirhuin 3”  
241 site (**Fig. 4**). In general, fruit in SWC treatment produced as much  $\alpha$ -farnesene, and earlier than that  
242 stored in air (RA) (**Fig. 4**). The same pattern as previously described for  $\alpha$ -farnesene was observed  
243 in peel AO (**Fig. 4**).

244 The CTols accumulation dynamics were only reduced in CA-stored fruit by 66 % from that observed  
245 in the Control (**Table 5**), with the least reduction for “Pirhuin 3”’s fruit (**Table 5**). Overall,  $\delta$ CTols/ $\delta$ t  
246 was well and positively correlated with SS ( $r_s > 0.6$ , **Table 5**).

247 There was no consistent effect of CA or SWC on fruit firmness (**Table 6**). CA-stored fruit from  
248 “Agrofruta” and “Talcarehue” sites was less yellow than those from the other 2 sites, and had less  
249 IEC, mostly when they came from the early harvest (H1) (**Table 6**). In contrast, fruit from SWC  
250 treatment had generally higher IEC than other treatments (**Table 6**).

251

## 252 **4. Discussion**

### 253 *4.1. Preharvest factors affecting SS development*

254 Climate during the growing season has an important role in fruit susceptibility to physiological  
255 disorder’s development postharvest. Although the biochemistry behind this observation is still  
256 under investigation in many of them, fruit acclimation to chilling in cold storage appears to be of  
257 great importance (Lurie and Watkins, 2012). Superficial scald is a manifestation of chilling injury  
258 during cold storage in pome fruit, modulated by multiple pre-and postharvest factors including  
259 environmental conditions during the growing season (Emongor et al., 1994; Smock, 1953). Scald  
260 susceptibility has been shown to vary between seasons, growing conditions, and harvest maturity  
261 (Wilkinson and Fidler, 1973). On apples, low preharvest temperatures (<10°C) 2-3 weeks before  
262 harvest decrease fruit susceptibility to the disorder (Barden and Bramlage, 1994; Blanpied et al.,  
263 1991; Merritt et al., 1961), whilst hot and dry climate in this period, as well as warm nights, increases

264 fruit susceptibility (Fidler, 1957; Little and Taylor, 1981). Low temperature preharvest would induce  
265 fruit acclimation to later chilling temperatures in storage by increasing antioxidants and total lipids,  
266 especially unsaturated fatty acids, to cope with this stress (Diamantidis et al., 2002; Thomai et al.,  
267 1998).

268 In our work, none of the growing sites had more than 37 h of HL10 accumulated within 45 d prior  
269 harvest, which is around a third of that required for scald-free fruit (Barden and Bramlage, 1994;  
270 Thomai et al., 1998). This would indicate that our experimental sites had an overall warmer climate,  
271 conducive to SS development regardless of the seasonal climatic variation. Nevertheless, this was  
272 only true when fruit was stored for more than 140 d (**Table 1**). Overall, HL10 was only fairly  
273 correlated with SS incidence, except in season #1 where this index was well and negatively  
274 correlated with SS after 180 d in storage ( $r_s = -0.631$ ) (**Table 1**). This suggest that this bioclimatic  
275 index would not be suitable to predict SS sensitivity on Packham's Triumph pears grown in warm  
276 growing areas in Chile, as it had been found for other winter pears cultivars such as 'd'Anjou' pears  
277 grown in cooler climates. For example, HL10 has been well correlated with scald susceptibility on  
278 'd'Anjou' pears grown in OR, USA, where HL10 is around 175 h, in average, prior fruit harvest (Ma  
279 et al., 2001).

280 Although we found no correlation between GDA and SS when all seasons and orchard sites were  
281 combined ( $r_s = 0.386$ ; **Table 1**), we did see a good linear correlation ( $R^2 = 0.974$ ) of this bioclimatic  
282 index with SS incidence after 180 d in "Pirhuin 3" site, which was the one sampled in all three  
283 seasons.

284 Superficial scald on apples has also shown to decrease with harvest maturity (Dilley, 1969; Huelin &  
285 Murray, 1966). This relationship would be, in part, attributed to antioxidants/ $\alpha$ -farnesene and CTols  
286 ratios (Huelin & Murray, 1966). In pears, the influence of harvest maturity on SS susceptibility is  
287 much less clear and consistent. Later-harvested winter pears ('Beurre d'Anjou', 'Packham's

288 Triumph') have shown to be more susceptible to SS than earlier and less mature ones (Calvo et al.,  
289 2017, 2015; Wang & Arzany, 2019). Nevertheless, Boonykiat et al. (1987) and Zoffoli et al. (1998)  
290 found the opposite in 'd'Anjou', Isidoro & Almeida (2006) in 'Rocha', and Zoffoli et al. (1998) also in  
291 Bartlett and 'Packham's Triumph'. In our study, harvest maturity (H1, H2, and H3) was positively  
292 correlated with SS incidence after 140 and 180 d into storage ( $r_s = 0.621^*$  and  $0.620^*$ , respectively;  
293 **Table 1**) only in season #1. This was not observed in seasons #2 or #3 or all of them together (**Table**  
294 **1**). This suggests that the effect of harvest maturity over SS development would depend on other  
295 growing conditions modulating fruit biochemistry (including maturation), which may explain  
296 contrasting results from different studies using smaller number of sites and seasons. Furthermore,  
297 in our study, flesh firmness used as a maturity index was not correlated with SS incidence when all  
298 seasons and locations were combined together, but it was significantly and negatively correlated in  
299 season #1, where early-harvested fruit (H1) showed higher incidences of SS (**Table 2**). Wang &  
300 Arzany (2019) also reported a good and negative correlation between flesh firmness at harvest and  
301 SS development on 'd'Anjou' pears.

302

#### 303 *4.2. Superficial scald incidence and the relationship with $\alpha$ -farnesene, CTols and antioxidant activity*

304 The current mechanism involved in SS development initiates with the accumulation of (*E,E*)- $\alpha$ -  
305 farnesene, a key sesquiterpene compound part of the lipidic and waxy layer of pome fruit skin, that  
306 oxidizes into CTols, responsible for SS symptoms (Ingle and D'Souza, 1989). Nevertheless, in both,  
307 apples and pears (*E,E*)- $\alpha$ -farnesene and CTols accumulation does not always correlate well with SS  
308 development (Calvo et al., 2015; Gapper et al., 2006; Lindo-García et al., 2020; Whitaker et al.,  
309 2000). In addition to these group of compounds, antioxidants have been found to play also an  
310 important role counteracting oxidative stress and SS development apples (Busatto et al., 2018;  
311 Rudell and Mattheis, 2009; Zhao et al., 2016), as well as on pears (Larrigaudière et al., 2016).

312 The dynamics of accumulation of all these compounds in the fruit peel vary between cultivars,  
313 environmental conditions, harvest maturity, storage regimes and postharvest treatments,  
314 modulating its susceptibility to SS (Emongor et al., 1994; Lurie and Watkins, 2012; van der Merwe  
315 et al., 2003).

316 Conjugated trienols (CTols) include hydro-and endoperoxides with peaks in the UV spectra at 258,  
317 269, and 281 nm. In pears, CT258, CT269, and CT281 have shown to increase during cold storage  
318 specially during the first 2-3 months before SS symptoms appear (Calvo et al., 2015; Larrigaudière  
319 et al., 2016; Zoffoli et al., 1998). In agreement with these authors, we found that CTols increased  
320 during cold storage, and their accumulation dynamic ( $\delta\text{CTols}/\delta t$ ) was positively correlated with SS  
321 incidence when seasons, orchards and harvest maturity were combined together ( $r_s = 0.739$ ; **Fig.**  
322 **2A**). When seasons were separated, season #1 in contrast to seasons #2 and #3, showed a poor  
323 correlation (**Fig. 2B**). The CTols accumulation rates we found were significantly lower (>10-times)  
324 than those reported in apples (Giné Bordonaba et al., 2013), and close to zero when 1-MCP or DPA  
325 was applied at harvest (**Table 3**).

326 The accumulation rates of  $\alpha$ -farnesene found in fruit from all seasons and treatments followed the  
327 same pattern previously reported on pears (Isidoro and Almeida, 2006) and apples (Whitaker, 2007).  
328 They steadily increased until 60-75 d and then declined (**Fig. 1 and 3**). The higher  $\alpha$ -farnesene levels  
329 observed in season #3 (**Fig. 1**) coincided with the highest SS average incidence (63 and 78% after  
330 140 and 180 d, respectively) among seasons, but with comparable CTols rate as season #2 (**Fig. 2**).

331 An important role has been given to the cell antioxidant system for SS suppression on apples and  
332 pears. Nonetheless, Calvo et al. (2015) found that later-harvested (more mature) fruit peel had  
333 higher antioxidant potential at harvest, but more or as much SS than less mature fruit. These authors  
334 also found that a decrease on antioxidant capacity prior SS appearance (120 d into storage) would  
335 play a more important role than the overall antioxidant capacity. In our study we found no

336 relationship between AO and SS development on fruit from different growing sites, harvest maturity  
337 or seasons (data not shown), but different methodologies were used in these studies. In fact, AO  
338 remained overall relatively stable throughout storage in seasons #1 and #2 following a normal  
339 distribution, and increased until 100 d into storage, on season #3 (**Fig. 1**).

340

#### 341 *4.3. Effect of 1-MCP and DPA treatments on superficial scald development*

342 The use of synthetic antioxidants, such as DPA and ethoxyquin has been the primary method to  
343 control SS on pears in different pear-producing countries. In agreement with previous reports (Drake  
344 et al., 2006; Isidoro and Almeida, 2006), DPA-treated fruit had shown a significant reduction in SS  
345 incidence. In this work, we found 99% reduction (in average) in SS development after 6 m of storage  
346 (**Table 3**). CTols accumulation rate was 30 % of that obtained in Control fruit (**Table 3**), evidencing  
347 the antioxidant action of DPA (Huelin & Coggiola, 1968). Similar results have been reported by  
348 Isidoro & Almeida (2006) in 'Rocha' pears, and 'Granny Smith' apples (Lurie et al., 1989; Moggia et  
349 al., 2010). In general, the overall correlation found in this work between  $\delta\text{CTols}/\delta t$  and SS incidence  
350 after 140 and 180 d in storage was moderate and positive ( $r_s= 0.659^*$  and  $r_s= 0.758^*$ , respectively)  
351 (**Table 3**).

352 Ethylene is a key player in SS development by triggering  $\alpha$ -farnesene synthesis (Gapper et al., 2006;  
353 Tsantili et al., 2007). Therefore, 1-MCP, an ethylene perception blocker, prevents SS development  
354 by not only downregulating the expression of  *$\alpha$ -farnesene synthase 1* gene (AFS1), but also  
355 enhancing other cold acclimation-related mechanisms (Busatto et al., 2018). In our study 1-MCP  
356 applications effectively reduced SS incidence to nearly 0 % in most cases (**Table 3**), along with  $\alpha$ -  
357 farnesene levels (**Fig. 3**) and subsequent CTols accumulation rate (**Table 3**). The latest were zero for  
358 1-MCP treated fruit from H1 and H2 in all sites except "Pirhuin 11" (**Table 3**). These results are in  
359 agreement with those reported for 'Gem' (Dong et al., 2018), 'Rocha' (Isidoro & Almeida, 2006),



360 'd'Anjou' (Gapper et al., 2006; Lu et al., 2011; Zhi and Dong, 2018), and 'Packham's Triumph' (Calvo,  
361 2003) pears.

362 Although the literature indicates that 1-MCP-treated pears have shown higher antioxidant capacity  
363 and content than untreated ones (Zhi & Dong, 2018), in our study we did not find any consistent  
364 differences in peel AO between treatments (**Fig. 3**),

365 In agreement with previous results, 1-MCP treated fruit produced significantly lower amounts of  
366 ethylene in most cases, compared to the untreated control and DPA-treated fruit after 140 and 180  
367 d in cold storage (**Table 4**). Similar results have been reported on 'd'Anjou' (Chen and Spotts, 2005;  
368 Gapper et al., 2006; Zhi and Dong, 2018), 'Bartlett' (Wang & Sugar, 2015), Gem (Dong et al., 2018),  
369 and 'Packham's Triumph' (Calvo & Candan, 2015) pears. Nevertheless, ethylene inhibition did not  
370 translate in firmer fruit (**Table 4**), as previously reported in pears (Calvo, 2003; Gapper et al., 2006;  
371 Spotts et al., 2007; Zhi & Dong, 2018). These results are consistent to those obtained in 'Bartlett'  
372 pears (Wang & Sugar, 2015). The effect of this treatment on color retention was not observed in  
373 any of the orchards or harvest maturity after 140 or 180 d in storage (**Table 4**). Similar results have  
374 been previously reported in 'd'Anjou' pears (Xie et al., 2014), although generally, 1-MCP applications  
375 at harvest have shown to maintain pears greener during storage and most dramatically during shelf-  
376 life (Argenta et al., 2003; Calvo, 2003; Mitcham et al., 2001; Zhi & Dong, 2018). In fact, although 1-  
377 MCP treatment may halt normal ripening (Calvo & Candan, 2015; Chen & Spotts, 2005; Ekman et  
378 al., 2004), treated fruit in this work were able to ripen normally after 7 d at 20 °C (shelf-life) reaching,  
379 in average, 32 N of flesh firmness and yellow-green skin color (data not shown).

380

#### 381 *4.4. Effect of CA storage and SWC on superficial scald development*

382 The use of CA storage with or without the use of DPA or 1-MCP has shown to reduce SS incidence  
383 on apples and pears (Isidoro and Almeida, 2006; Smock, 1979). Overall, we found a significant

384 reduction (77 % and 70 % in average) in SS development compared to fruit stored in air or  
385 conditioned using SWC (**Table 5**), with variations between orchards. “Pirhuin 3” site showed the  
386 least benefit from CA storage (**Table 5**), most probably due to environmental growing conditions in  
387 the orchard and different metabolic make-up at harvest. In fact, this site also showed the highest  $\alpha$ -  
388 farnesene accumulation (**Fig. 4**) and later CTols accumulation rates (**Table 5**) in CA-stored fruit  
389 compared to the other sites. Fruit from “Agrofruta” and “Talcahue” sites showed significantly  
390 lower levels of  $\alpha$ -farnesene when stored in CA compared to that stored in air or conditioned after  
391 harvest (SWC) (**Fig. 4**). Similar results have been reported on apples with variations between  
392 cultivars and growing conditions (Whitaker et al., 1997). In pears, CA (and ULO in some instances)  
393 can reduce SS development but not completely prevent it from occurring (Larrigaudière et al., 2019;  
394 Truter et al., 1994).

395 Although warming treatments have shown good effect in reducing  $\alpha$ -farnesene, CT281  
396 accumulation and SS on apples (Moggia et al., 2009; Watkins et al., 2000), an increase in storage  
397 temperature on pears have been shown to be detrimental for overall fruit quality and SS incidence  
398 (Bower et al., 2003). In agreement with these results, in our study SWC not only did not reduce SS  
399 (**Table 5**), but also accelerated fruit maturity by reducing fruit firmness and green skin color, mainly  
400 in fruit from H3 (**Table 6**). Fruit with SWC treatment produced as much  $\alpha$ -farnesene (and an earlier  
401 peak in “Talcahue” and “Agrofruta” sites) as it did the one from RA storage (**Fig. 4**), and both  
402 treatments led to similar CTols accumulation dynamics (**Table 5**).

403

## 404 **5. Conclusions**

405 Superficial scald is an important physiological disorder in pears around the world. Unlike apples,  
406 fruit susceptibility is much less understood and unpredictable due to its nature. We found that fruit  
407 maturity effect on SS susceptibility vary between seasons. Only in one season (#1) the higher SS

408 sensitivity was related to higher harvest maturity. In this case, flesh firmness used as the main  
409 maturity index in this cv., showed a good correlation with the disorder's incidence. Nevertheless,  
410 and although CTols dynamics were well correlated with SS development when all seasons were  
411 combined, this parameter was not correlated with SS incidence in season #1. Warm-growing  
412 climates, such as those prevalent in Chile, although predisposing to SS development, may influence  
413 fruit biochemistry in different ways not yet elucidated or predictable using the bioclimatic indexes  
414 GDH or HL, modulating then fruit susceptibility. Nevertheless, DPA and 1-MCP treatments  
415 significantly reduced SS development; CA storage also, but with variations between orchards.  
416 Further research is needed to understand the influence of preharvest climate on fruit biochemistry  
417 and its consequence in SS development on pears.

418

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430

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647 **Table 1.** Bioclimatic indexes and SS average incidence after 140 (SS 140) and 180 d (SS 180) in  
 648 Regular Atmosphere storage (RA, -0.5 °C and >90 %RH, plus 14 d shelf life, 20 °C), and  
 649 Spearman correlation coefficients ( $r_s$ ) of harvest maturity (H1, H2, H3), GDA, HL10, and DAFB  
 650 with SS 140 and SS 180. Spearman Rank Correlation ( $r_s$ ; \*: significant at 0.05 level).

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Season	Orchard	Harvest maturity	GDA	HL10	DAFB	SS 140 (%)	SS 180 (%)
# 1	"Genova"	H1	838	22	126	2.3	19.4
		H2	910	17	133	12.6	26.6
		H3	977	1	140	14.7	42.6
	"Lo Carrizo"	H1	974	10	125	2.6	37.4
		H2	1053	10	132	9.7	36.9
		H3	1125	6	139	29.1	39.4
	"El Carmen"	H1	939	13	126	3.4	41.3
		H2	1008	10	133	16.6	32.5
		H3	1088	0	140	10.2	77.9
	"Pirhuin 3"	H1	774	30	121	15.1	30.4
		H2	844	23	128	12.9	57.3
		H3	910	0	135	51.5	67.3
# 2	"Agrofruta"	H1	891	37	121	2.0	63.1
		H2	973	31	130	0.0	37.7
		H3	1069	26	135	2.8	71.5
	"Pirhuin 3"	H1	845	31	119	2.7	60.7
		H2	939	27	126	2.3	50.1
		H3	1037	27	133	2.1	82.6
	"Pirhuin 11"	H1	845	31	119	2.0	58.8
		H2	939	27	126	2.8	62.9
		H3	1037	27	133	3.7	63.7
	"Talcarehue"	H1	861	1	131	0.0	31.1
		H2	980	1	137	0.0	15.6
		H3	1047	1	144	1.0	53.2
# 3	"Agrofruta"	H1	929	20	126	57.6	79.4
		H3	1073	20	133	55.2	61.1
	"Pirhuin 3"	H1	989	18	120	91.5	100
		H3	1135	31	135	84.3	87.8
	"Talcarehue"	H1	1095	0	128	60.5	74.7
		H3	1257	0	137	27.0	65.0

All seasons	$r_s$ (SS 140)	0.153	0.336	-0.231	-0.097
Season #1	$r_s$ (SS 140)	0.621*	0.133	-0.314	0.467
Season #2	$r_s$ (SS 140)	0.164	0.019	0.301	-0.321
Season #3	$r_s$ (SS 140)	-0.488	-0.314	0.264	-0.600
All seasons	$r_s$ (SS 180)	0.279	0.406*	0.096	-0.044
Season #1	$r_s$ (SS 180)	0.620*	0.354	-0.631*	0.527
Season #2	$r_s$ (SS 180)	0.414	0.320	0.303	-0.046
Season #3	$r_s$ (SS 180)	-0.488	-0.314	0.265	-0.543

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657 **Table 2.** Effect of harvest maturity and orchard on fruit maturity (flesh firmness, Hue) and SS incidence (%) after 140 and 180 d in RA storage (-0.5  
658 °C and >90 %RH, plus 14 d shelf-life at 20°C) in Packham’s Triumph pears over three growing seasons (#1, #2, #3). Spearman Rank Correlations  
659 between maturity indexes at harvest and SS after 140 and 180 d in all seasons or each season separately are indicated at the end of the table.  
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Season	Orchard	Harvest	Maturity index at harvest			Maturity index at 140 d		Maturity index at 180 d		SS (%)	
			Firmness (N)	Color (Hue°)	Starch index	Firmness (N)	Color (Hue°)	Firmness (N)	Color (Hue°)	140 +14 d	180 +14 d
# 1	"Genova"	H1	86.3 e <sup>z</sup>	116.4	2.1 ab	73.1 f	112.3 d	68.9 d	108.9 bc	2.3 a	19.4 a
		H2	77.0 c	113.6	2.4 b	69.4 e	109.8 b	60.5 cd	108.1 bc	12.6 a	26.6 a
		H3	72.5 b	115.4	2.7 b	59.3 b	107.8 a	55.6 c	105.1 ab	14.7 a	42.6 abc
	"El Carmen"	H1	73.8 c	115.7	1.8 a	67.4 de	110.6 bc	60.9 cd	109.5 bc	3.4 a	41.3 abc
		H2	69.4 ab	115.6	2.2 ab	63.8 c	110.0 bc	39.6 a	107.4 b	16.6 a	32.5 ab
		H3	67.6 a	113.8	2.0 ab	53.7 a	107.3 a	46.7 b	113.3 d	10.2 a	77.9 c
	"Lo Carrizo"	H1	76.5 c	115.5	2.5 b	72.7 f	110.5 bc	68.5 d	108.4 bc	2.6 a	37.4 ab
		H2	78.3 d	115.1	2.7 b	66.1 cde	110.2 bc	64.5 d	105.0 ab	9.7 a	36.9 ab
		H3	71.6 b	114.5	2.3 b	64.2 cd	110.3 bc	55.2 c	104.5 a	29.1 ab	39.4 abc
	"Pirhuin 3"	H1	74.7 c	116.6	1.9 ab	68.9 e	111.5 cd	64.5 d	108.1 bc	15.1 a	30.4 ab
		H2	71.2 b	116.4	2.1 ab	63.1 c	111.6 bcd	52.0 bc	106.1 ab	12.9 a	57.3 abc
		H3	66.3 a	114.5	2.3 b	55.6 a	107.9 a	47.2 b	104.5 a	51.5 b	67.3 bc
	<i>P value</i>		0.0013	0.2467	0.0370	0.0149	0.0033	0.0003	0.0000	0.0172	0.0232
# 2	"Agrofruta"	H1	77.8	115.2	1.6 ab	71.6 f	111.3 bcd	52.9 a	106.4	2.0	63.1 c
		H2	72.9	115.1	2.5 c	65.9 cd	111.2 bcd	58.7 a	106.4	0.0	37.7 ab
		H3	69.8	114.6	1.4 a	61.5 b	109.7 bc	64.9 b	106.7	2.8	71.5 bc
	"Talcaehue"	H1	73.4	116.8	1.4 a	41.0 a	104.7 a	61.4 b	108.1	0.0	31.1 ab
		H2	68.0	116.3	1.6 ab	70.3 def	111.5 cd	56.0 a	108.2	0.0	15.6 a

	H3	66.7	115.6	1.5 ab	69.3 def	111.3 bcd	60.5 ab	108.4	1.0	53.0 b	
"Pirhuin 3"	H1	76.5	114.8	1.9 b	66.1 cd	113.0 de	72.5 c	108.7	2.7	60.7 bc	
	H2	75.1	114.9	1.4 a	64.5 bc	114.3 d	60.1 ab	108.5	2.3	50.1 bc	
	H3	68.4	114.2	1.4 a	67.0 cde	112.5 de	63.6 b	112.9	2.1	82.6 cd	
"Pirhuin 11"	H1	78.2	115.5	1.5 ab	71.3 ef	111.0 bcd	61.4 b	108.7	2.0	58.8 bc	
	H2	75.6	115.2	1.3 a	68.9 cdef	111.2 bcd	61.8 b	108.6	2.8	62.9 bc	
	H3	68.0	114.5	2.6 c	66.4 cd	109.1 b	61.4 b	108.1	3.7	63.7 bc	
	<i>P value</i>	0.1852	0.8038	0.0000	0.0000	0.0000	0.0000	0.2209	0.1443	0.0407	
# 3	"Agrofruta"	H1	69.8 c	114.8 b	1.5 a	68.3 c	104.5 a	62.5 d	105.1 ab	57.6	79.4 d
		H3	64.5 b	113.6 a	4.0 c	48.8 a	101.7 a	55.0 cb	104.5 a	55.2	61.1 a
	"Talcahue"	H1	72.5 c	115.8 b	1.6 ab	58.1 b	106.0 b	58.2 cd	108.4 b	60.5	74.7 c
		H3	57.4 a	113.4 a	1.9 ab	48.9 a	105.0 a	50.4 b	104.2 a	27.0	65.0 b
	"Pirhuin 3"	H1	78.7 d	115.5 b	2.3 b	68.3 c	110.6 c	41.7 a	105.6 ab	91.5	100.0 f
		H3	72.9 c	115.3 b	2.8 c	61.4 b	112.6 c	54.6 c	108.5 b	84.3	87.8 e
	<i>P value</i>	0.0003	0.0110	0.0000	0.0003	0.0038	0.0000	0.0018	0.1918	0.000	
All seasons	$r_s$ (SS140)	-0.199	-0.235	0.446*							
Season #1	$r_s$ (SS140)	-0.678*	-0.207	0.067							
Season #2	$r_s$ (SS140)	0.100	-0.715*	-0.135							
Season #3	$r_s$ (SS140)	0.933*	0.829*	0.086							
All seasons	$r_s$ (SS180)	-0.291	-0.432*	-0.118							
Season #1	$r_s$ (SS180)	-0.770*	-0.291	-0.067							
Season #2	$r_s$ (SS180)	0.039	-0.767*	0.129							
Season #3	$r_s$ (SS180)	0.886*	0.600	-0.143							

661 Note: <sup>z</sup>Average in the same column per season followed by the same letter indicate no statistical differences (Tukey,  $P \leq 0.05$ ). Spearman Rank Correlation ( $r_s$ ;  
662 \*: significant at 0.05 level).



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664 **Table 3.** Correlations between  $\delta\text{CTol}/\delta t$  and SS average incidence after 140 (SS 140) and 180 d (SS  
 665 180) in untreated Control, 1-MCP (0.3 mL L<sup>-1</sup>) and DPA-treated fruit (1,200 mL L<sup>-1</sup>) stored in RA (-  
 666 0.5 °C and >90 %RH, plus 14 d shelf life, 20 °C). ‘Packham’s Triumph’ pears. Season #2.

Season	Orchard (B)	Harvest	Treat (A)	$\delta\text{CTol}/\delta t$	SS 140+14 d (%)	SS 180+14 d (%)	
#2	"Agrofruta"	1	Control	0.138	2.0	63.1	
		2		0.124	0.0	37.7	
		3		0.096	2.8	71.5	
	"Pirhuin 3"	1		0.061	2.7	60.7	
		2		0.050	2.3	50.1	
		3		0.117	2.1	82.6	
	"Pirhuin 11"	1		0.062	2.0	58.8	
		2		0.055	2.8	62.9	
		3		0.118	3.7	63.7	
	"Talcahue"	1		0.075	0.0	31.1	
		2		0.071	0.0	15.6	
		3		0.227	1.0	53.0	
		"Agrofruta"	1	DPA	0.000	0.0	0.0
			2		0.000	0.0	0.0
			3		0.012	0.0	8.3
		"Pirhuin 3"	1		0.000	0.0	1.5
			2		0.010	0.4	0.0
			3		0.110	0.4	1.5
"Pirhuin 11"		1		0.071	6.2	19.0	
		2		0.038	0.4	0.0	
		3		0.110	1.2	0.0	
"Talcahue"		1		0.000	0.0	0.0	
		2		0.000	0.0	0.0	
		3		0.040	0.0	8.2	
	"Agrofruta"	1	1-MCP	0.000	0.0	0.0	
		2		0.000	0.0	0.0	
		3		0.025	0.0	0.0	
	"Pirhuin 3"	1		0.000	0.0	0.0	
		2		0.000	0.0	0.0	
		3		0.013	0.0	0.0	
	"Pirhuin 11"	1		0.013	0.0	0.0	
		2		0.023	0.0	0.0	
		3		0.036	0.0	0.0	
	"Talcahue"	1		0.000	0.0	0.0	
		2		0.000	0.0	0.0	
		3		0.021	0.0	0.0	
Spearman $r_s$ with SS 140 d				0.659*			
Spearman $r_s$ with SS 180 d				0.758*			

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Note:  $\delta\text{CTol}/\delta t$  calculated between 0 and 60 d into storage. Spearman Rank Correlation ( $r_s$ ; \*: significant at 0.05 level).

670 **Table 4.** Effect of 1-MCP (0.3  $\mu\text{L L}^{-1}$ ) and DPA (1,200  $\mu\text{L L}^{-1}$ ) and harvest maturity (H1, H2, H3) on fruit  
 671 maturity (flesh firmness, Hue $^{\circ}$ , and IEC) after 140 and 180 d in RA storage (RA, -0.5  $^{\circ}\text{C}$  and >90 %RH).  
 672 ‘Packham’s Triumph’ pears, season #2.

Factor	Firmness (N)				Hue ( $^{\circ}$ )				IEC ( $\text{mL L}^{-1}$ )				
	“Agrofruta”	“Pirhuin 3”	“Pirhuin 11”	“Talcarehue”	“Agrofruta”	“Pirhuin 3”	“Pirhuin 11”	“Talcarehue”	“Agrofruta”	Pirhuin 3	“Pirhuin 11”	“Talcarehue”	
<b>140 d</b>													
<b>Harvest: A</b>	H1	62.9	68.9	70.7	60.4	110.4	113.5	112.1 b	109.4	57.3	95.3	74.9	91.0
	H2	64.1	72.0	68.0	67.2	112.1	113.2	111.0 ab	111.9	27.9	63.0	59.9	10.1
	H3	63.0	67.3	65.4	65.1	110.7	112.1	110.3 a	111.0	34.0	78.5	39.6	40.2
	<i>P</i> value	0.0000	0.0000	0.1526	0.0000	0.0038	0.0552	0.0378	0.0053	0.5076	0.0002	0.0000	0.0000
<b>Treatment: B</b>	Control	66.3	65.9	68.9 b	60.2	110.7	113.2	110.5	109.2	74.7 b	149.2	120.5	74.5
	DPA	56.1	72.2	70.1 b	65.9	111.6	112.7	111.1	111.0	41.3 b	86.9	53.7	66.2
	1-MCP	67.6	70.3	65.1 a	66.7	110.8	112.8	111.9	112.3	3.2 a	0.7	0.3	0.5
	<i>P</i> value	0.000	0.0000	0.0000	0.0738	0.1382	0.5816	0.0963	0.0008	0.0000	0.0000	0.0000	0.0000
<b>A x B</b>	H1-Control	71.6 a <sup>2</sup>	66.1 bc	71.3	41.0 d	111.2 ab	112.9	111.0	104.7 a	101.2	169.3 d	151.5 c	154.2 d
	H1-DPA	48.1 b	72.1 a	72.1	67.2 bc	109.1 a	113.5	111.5	111.5 bc	68.8	115.9 cd	73.2 b	118.1 d
	H1-1-MCP	69.2 abc	68.6 bc	68.6	73.0 a	110.7 ab	113.8	113.6	112.1 bc	1.9	0.73 a	0.12 a	0.6 a
	H2-Control	65.9 cd	64.5 c	68.9	70.3 ab	111.2 ab	114.3	111.2	111.5 bc	69.0	98.3 c	131.9 c	0.0 a
	H2-DPA	57.7 cd	75.1 a	69.1	68.2 ab	113.2 bc	112.7	110.5	111.2 b	11.6	90.1 bc	47.7 b	29.7 b
	H2-1-MCP	68.9 ab	76.6 a	65.9	63.1 cd	111.8 b	112.4	111.4	113.2 bc	3.3	0.71 a	0.33 a	0.5 a
	H3-Control	61.5 d	67.0 bc	66.4	69.3 ab	109.7 ab	112.5	109.1	111.3 bc	54.0	180.1 d	78.2 bc	69.4 c
	H3-DPA	62.5 c	69.4 ab	69.1	62.1 cd	112.5 bc	111.8	111.2	110.4 b	43.5	54.9 b	40.3 b	50.8 bc
	H3-1-MCP	64.9 cd	65.6 bc	60.8	63.9 cd	110.1 ab	112.1	110.6	111.5 bc	4.5	0.60 a	0.29 a	0.51 a
<i>P</i> value	0.0000	0.0000	0.1536	0.0009	0.0012	0.3079	0.2363	0.0004	0.0970	0.0317	0.0000	0.0003	
<b>180 d</b>													
<b>Harvest: A</b>	H1	44.4	51.7	66.6	71.2	106.5 b	107.2	110.9 b	112.7 b	124.4	241.4 b	177.7	139.3
	H2	51.2	58.2	64.3	65.7	105.0 a	108.2	108.5 a	110.2 a	158.9	142.4 a	312.5	286.2
	H3	60.5	60.5	64.3	64.0	107.2 b	107.9	109.4 a	112.2 b	140.9	156.3 a	173.9	202.4
	<i>P</i> value	0.0000	0.0000	0.1505	0.0000	0.0019	0.5399	0.0000	0.0000	0.5953	0.0007	0.0000	0.0000
<b>Treatment: B</b>	Control	58.8	59.2	61.5 a	65.4	106.4 b	108.2 ab	108.5 a	112.5 b	297.0 c	282.5 b	343.4	347.2
	DPA	52.3	64.6	65.4 b	67	108.8 c	108.6 b	109.5 a	110.6 a	123.9 b	251.3 b	320.2	276.8
	1-MCP	44.8	46.9	68.3 b	68.5	103.5 a	106.5 a	110.9 b	112.0 b	3.2 a	6.4 a	0.6	3.9
	<i>P</i> value	0.0000	0.0000	0.0000	0.0207	0.0000	0.0395	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<b>A x B</b>	H1-Control	52.8 c	61.2 cd	61.2	72.5 bc	106.4 bc	108	108.7 ab	114.3 d	262.6	382.8	299.1 bc	246.5 bc
	H1-DPA	44.1 b	66.9 de	69.5	71.6 bc	109.1 cd	109.4	110.7 b	111.2 bc	108.8	338.1	233.6 b	168.8 b
	H1-1-MCP	36.2 a	46.8 a	69.1	69.3 b	104.2 b	104.3	113.4 c	112.6 c	1.9	3.48	0.6 a	2.5 a
	H2-Control	58.8 cd	55.9 bc	61.8	60.3 a	106.3 bc	108.2	108.5 ab	110.3 b	383.5	238.8	466.7 d	466.2 d
	H2-DPA	56.9 cd	63.7 d	62.5	64.7 ab	109.4 cd	108.7	108.6 ab	108.3 a	89.7	179.4	470.2 d	389.4 cd

H2-1-MCP	37.7 ab	55.3 b	68.4	72.4 bc	99.3 a	107.7	108.6 ab	112.1 bc	3.3	9.1	0.7 a	3.1 a
H3-Control	64.8 de	60.5 c	61.6	63.6 ab	106.6 bc	108.3	108.0 a	112.9 c	244.9	225.8	264.6 bc	328.8 c
H3-DPA	56.0 cd	63.0 d	64.2	64.7 ab	107.8 c	107.8	109.3 ab	112.2 c	173.3	236.3	256.7 bc	272.2 bc
H3-1-MCP	60.5 d	58.4 bc	67.3	63.8 ab	107.0 c	107.5	110.8 bc	111.4 bc	4.5	6.6	0.5 a	6.1 a
<i>P</i> value	0.0000	0.0000	0.1559	0.0000	0.0000	0.1877	0.0085	0.0000	0.1049	0.0559	0.0000	0.0003

673 Note: <sup>2</sup>Averages in the same column followed by the same letter do not statistical differences (Tukey,  
674  $P \leq 0.05$ ).

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694 **Table 5.** Correlations between  $\delta\text{CTol}/\delta t$  and SS average incidence after 140 (SS 140) and 180 d (SS  
695 180) in fruit from different orchards and harvest maturity, stored in RA (-0.5 °C and >90 %RH, plus  
696 14 d shelf life, 20 °C), CA (O<sub>2</sub>: 2.0 kPa, CO<sub>2</sub>: 1.0 kPa, -0.5 °C and >90 %RH), and conditioned (SWC; 1  
697 week at 5 °C, 1 week at 3 °C, 1 week at 2 °C and the rest at -0.5 °C and >90 %RH). ‘Packham’s Triumph’  
698 pears. Season #3.

Season	Orchard	Harvest	Treat.	dCTol/dt	SS	
					140+14 d (%)	180+14 d (%)
#3	“Agrofruta”	1	RA	0.062	58.0	79.0
		3		0.076	55.0	61.0
	“Pirhuin 3”	1		0.056	92.0	100.0
		3		0.091	84.0	87.0
	“Talcahue”	1		0.097	61.0	75.0
		3		0.079	27.0	65.0
	“Agrofruta”	1	CA	0.014	0.0	2.0
		3		0.050	0.0	5.0
	“Pirhuin 3”	1		0.035	74.0	85.0
		3		0.024	20.0	3.0
	“Talcahue”	1		0.006	0.0	4.0
		3		0.027	1.0	10.0
“Agrofruta”	1	SWC	0.135	52.0	46.0	
	3		0.110	29.0	56.0	
“Pirhuin 3”	1		0.081	90.0	100.0	
	3		0.106	89.0	94.0	
“Talcahue”	1		0.067	42.0	30.0	
	3		0.019	13.0	36.0	
Spearman $r_s$ with SS140 d				0.644*		
Spearman $r_s$ with SS180 d				0.622*		

699 Note:  $\delta\text{CTol}/\delta t$  calculated between 0 and 60 d into storage. Spearman Rank Correlation ( $r_s$ ; \*: significant at  
700 0.05 level)  
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706 **Table 6.** Effect of RA (-0.5 °C and >90 %RH), CA (O<sub>2</sub>: 2.0 kPa, CO<sub>2</sub>: 1.0 kPa, -0.5 °C and >90 %RH), and  
 707 SWC (1 week at 5 °C, 1 week at 3 °C, 1 week at 2 °C and the rest at -0.5 °C and >90 %RH) and harvest  
 708 maturity (H1, H3) on fruit maturity (flesh firmness, Hue°, and IEC) after 140 and 180 d. ‘Packham’s  
 709 Triumph’ pears, season #3.

Factor		Firmness (N)			Hue (°)			IEC (µL L <sup>-1</sup> )		
		“Agrofruta”	“Talcarehue”	“Pirhuin 3”	“Agrofruta”	“Talcarehue”	“Pirhuin 3”	“Agrofruta”	“Talcarehue”	“Pirhuin 3”
<b>140 d</b>										
<b>Harvest: A</b>	H1	65.4	60.0	66.7 b	109.6 b	110.3 b	112.1	45.6	122.3	89.4
	H3	47.6	48.9	58.0 a	104.9 a	107.9 a	110.1	47.4	157.3	94.8
	<i>P value</i>	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6389	0.0012	0.2922
<b>Treatment: B</b>	RA	58.7	53.4	64.8 b	104.7 a	107.9 a	109.8	59.0	136.4	87.5
	CA	56.9	56.9	57.9 a	112.3 b	113.0 b	113.6	31.8	68.0	77.0
	SWC	55.2	52.9	64.2 b	104.9 a	107.4 a	109.9	48.9	215.9	111.7
	<i>P value</i>	0.0070	0.0017	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<b>A x B</b>	H1-RA	59.9 d <sup>z</sup>	66.4 c	68.1	106.7	107.8	111.6 c	67.1 c	141.8 b	92.9 ab
	H1-CA	68.3 e	58.1 b	62.7	114.3	114.4	114.8 d	24.7 a	62.6 a	75.5 a
	H1-SWC	67.9 e	55.5 b	68.9	107.9	108.7	109.8 b	44.9 b	162.6 b	99.7 b
	H3-RA	52.8 c	48.9 ab	61.4	102.7	106.1	108.0 a	50.9 bc	130.9 b	82.2 ab
	H3-CA	48.8 b	47.7 a	53.3	110.2	111.5	112.4 cd	38.9 ab	73.4 a	78.5 ab
	H3-SWC	42.8 a	50.3 b	59.2	101.8	106.1	109.9 b	52.4 bc	269.3 c	123.7 c
	<i>P value</i>	0.0000	0.0000	0.5968	0.2219	0.5877	0.0029	0.0044	0.0000	0.0243
<b>180 d</b>										
<b>Harvest: A</b>	H1	65.4	60.0	66.7 b	107.5 b	110.3 b	112.1	45.3	79.8	74.2
	H3	48.1	48.9	58.0 a	103.8 a	107.9 a	110.1	38.0	95.6	76.3
	<i>P value</i>	0.0000	0.0000	0.0000	0.0000	0.0001	0.0002	0.1221	0.0188	0.6440
<b>Treatment: B</b>	RA	58.6	53.5	64.8 b	103.1 a	107.0 a	109.8	50.5	88.6	63.5 a
	CA	56.4	57.0	57.9 a	108.0 b	113.0 b	113.6	27.5	52.5	62.8 a
	SWC	55.4	52.8	64.2 b	105.9 a	107.4 a	109.9	47.0	122.0	99.4 b
	<i>P value</i>	0.0067	0.0181	0.0000	0.0000	0.0000	0.0000	0.0011	0.0000	0.0000
<b>A x B</b>	H1-RA	68.3 e	58.1 bc	68.3	104.5	106.0	110.6 b	64.3 c	90.8 bc	67.9
	H1-CA	59.9 d	66.4 c	62.7	112.6	109.3	114.0 c	27.5 a	48.7 a	62.1
	H1-SWC	67.9 e	55.5 b	69.1	104.7	109.9	105.4 ab	43.9 ab	100.0 bc	92.6
	H3-RA	48.8 b	48.9 ab	61.4	101.7	105.0	112.6 a	36.6 ab	86.4 b	59.1
	H3-CA	52.8 c	47.6 a	53.2	103.4	105.2	110.4 bc	27.5 a	56.5 ab	63.5
	H3-SWC	42.8 a	50.3 ab	59.4	107.2	104.9	98.8 ab	50.0 ab	144.1 c	106.3
	<i>P value</i>	0.0000	0.0004	0.4263	0.2237	0.6034	0.0106	0.0144	0.0123	0.1487

710 Note: <sup>z</sup>Averages in the same column followed by the same letter do not statistical differences (Tukey,  
 711  $P \leq 0.05$ ).

712

713 **Figure 1.** Alpha-farnesene (A, B, C) content and antioxidant activity (AO; D, E, F) in peel tissue from  
714 'Packham's Triumph' pears from different growing sites ("Genova", "Pirhuin 3", "Lo Carrizo", "El  
715 Carmen", "Pirhuin 11", "Talcahue", "Agrofruta") in seasons #1 (A, D), #2 (B, E), and #3 (C, F) during  
716 RA storage (-0.5 °C, >90 %RH). Each data point is the mean of nine replicates  $\pm$  standard error.

717 **Figure 2.** Correlation analysis ( $r_s$ ) between CTols rate of accumulation (0-60 d in storage) and SS  
718 after 180 d (plus shelf-life) in pears cv. 'Packham's Triumph', and polynomial regression adjusted  
719 (red lines) to data from all seasons (A), seasons #1 (B), #2 (C), and #3 (D). Spearman Rank  
720 Correlation ( $r_s$ ; \*: significant at 0.05 level).

721 **Figura 3.** Alpha-farnesene (A, B, C, D) content and antioxidant activity (AO; E, F, G, H) in peel tissue  
722 of untreated Control (—), DPA-treated (1,200  $\mu\text{L L}^{-1}$ ; ---), and 1-MCP-treated (0.3  $\mu\text{L L}^{-1}$ ; -◆-)  
723 'Packham's Triumph' pears from "Talcahue" (A, E), "Pirhuin 11" (B, F), "Pirhuin 3" (C, G), and  
724 "Agrofruta" (C, F) sites during cold storage (RA, 0.5°C, >90 % RH). Each data point is the mean of  
725 nine replicates  $\pm$  standard error.

726 **Figura 4.** Alpha-farnesene (A, B, C) content and antioxidant activity (AO; D, E, F) in peel tissue of  
727 'Packham's Triumph' pears stored in refrigerated air (RA, -0.5°C and >90 %RH; —), stepwise  
728 cooling (SWC; 1 week at 5 °C, 1 week at 3 °C, 1 week at 2 °C and the rest at -0.5 °C, >90 %RH; -▲-),  
729 and controlled atmosphere (CA, O<sub>2</sub>: 2.0 kPa, CO<sub>2</sub>: 1.0 kPa; ---) from "Talcahue" (A, D), "Pirhuin 3"  
730 (B, E), and "Agrofruta" (C, F) sites. Each data point is the mean of nine replicates  $\pm$  standard error.

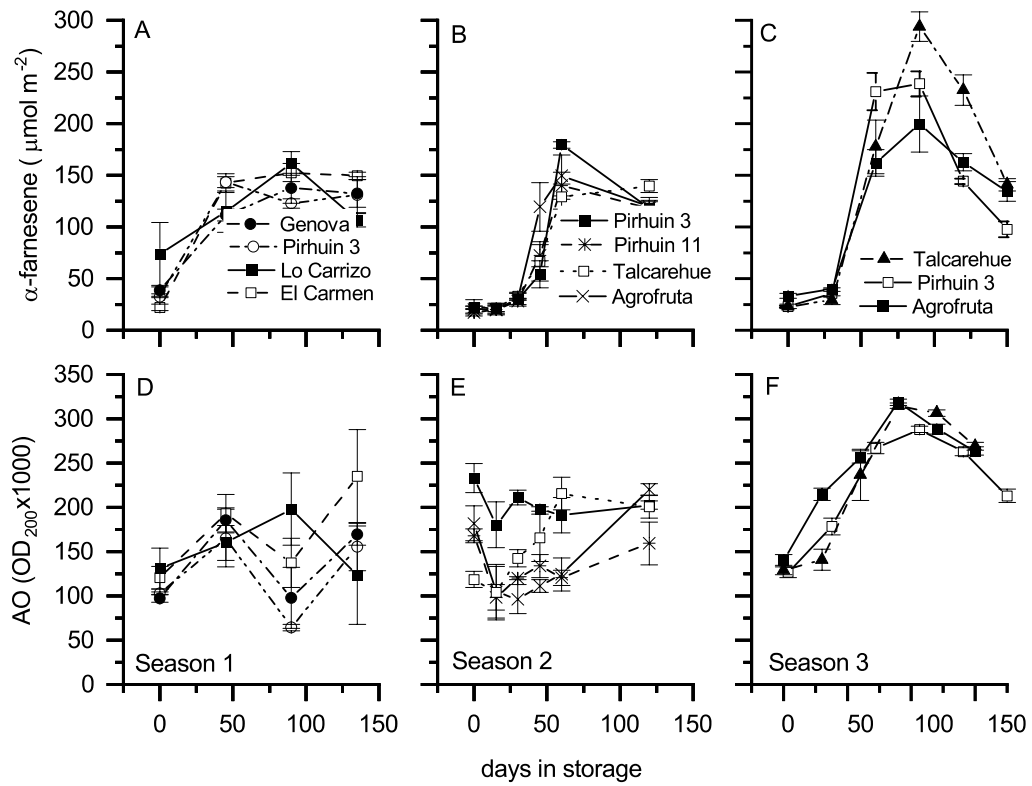
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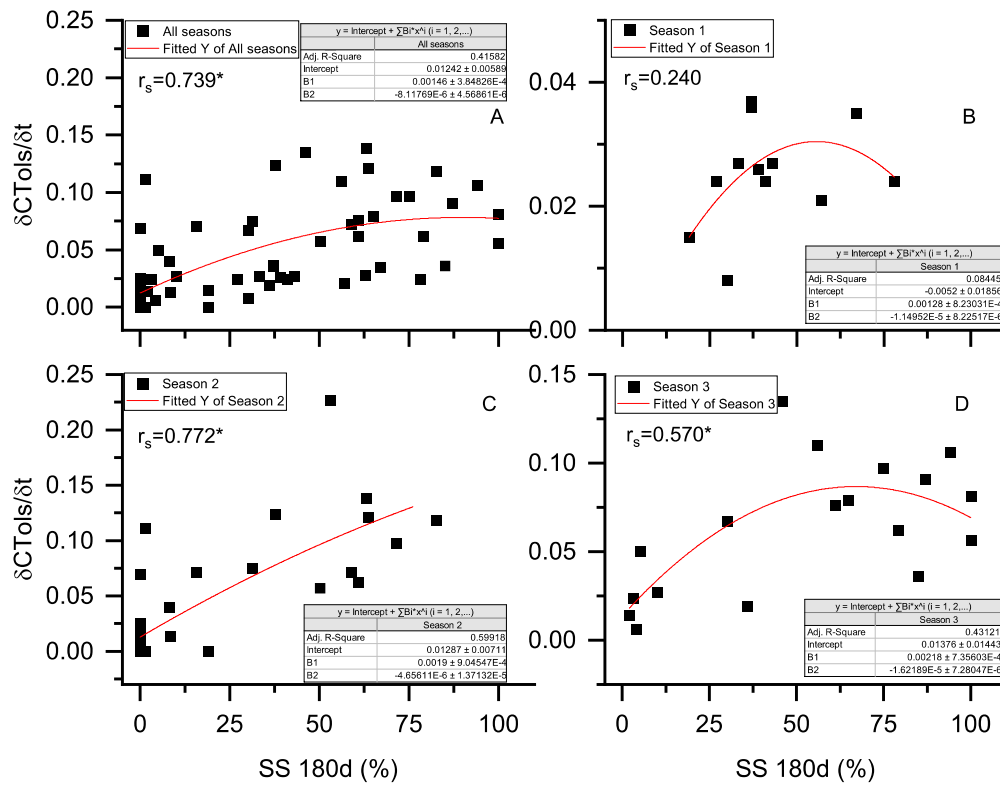


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738 **Fig. 1.**

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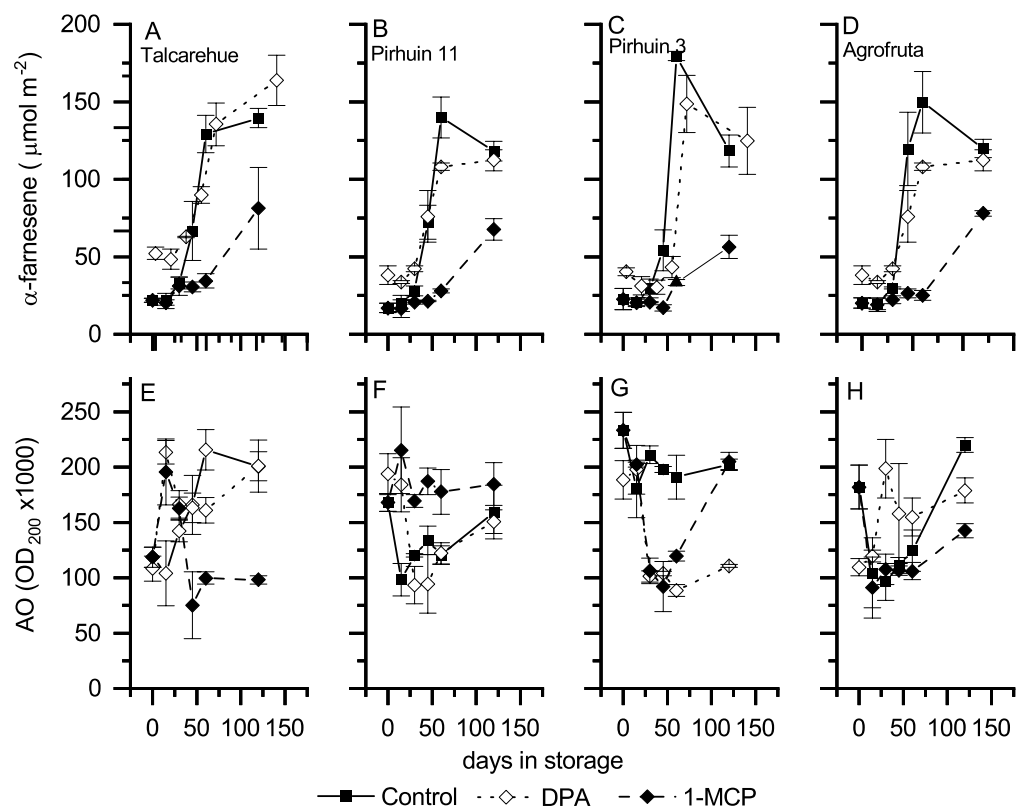
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741 **Fig. 2.**

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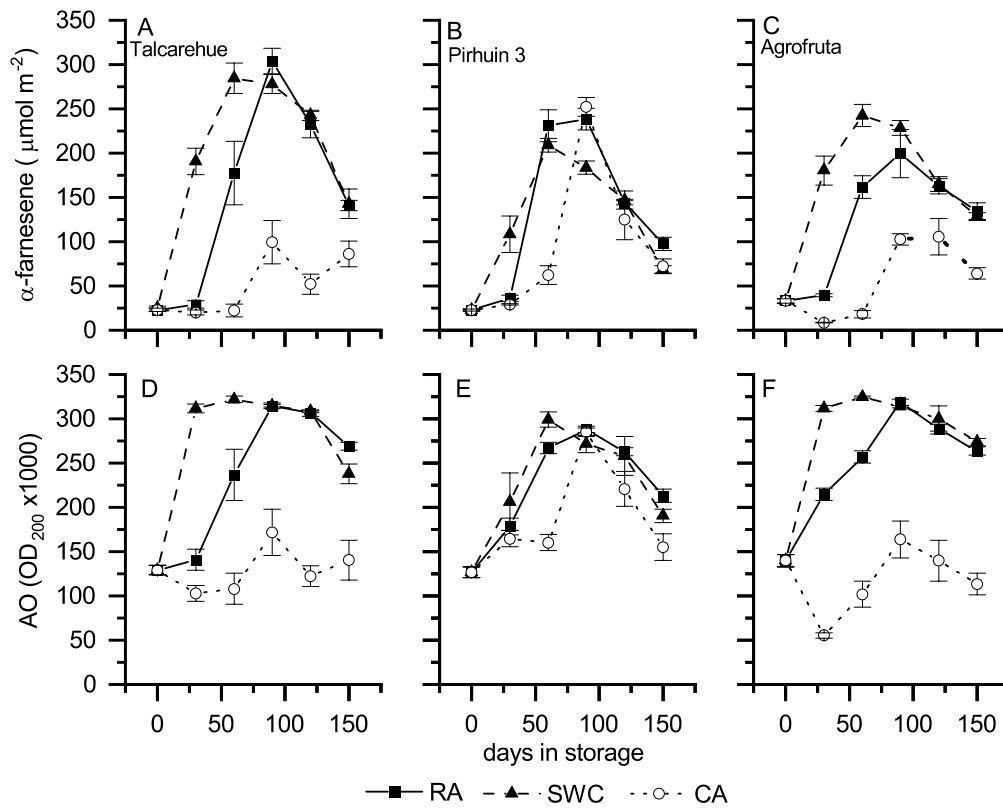
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746 **Fig. 3.**

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752 Fig. 4.

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