Effect of ethylene degreening on the development of postharvest penicillium molds and fruit quality of early season citrus fruits

Pedro A. Moscoso-Ramírez,[1,2] and Lluís Palou[1*]

[1]Laboratori de Patologia, Centre de Tecnologia Postcollita (CTP), Institut Valencià d´Investigacions Agràries (IVIA), 46113 Montcada, Valencia, Spain.

[2]Campus Tabasco, Colegio de Postgraduados, 86500 H. Cárdenas, Tabasco, México.

*** Corresponding author:** palou_llu@gva.es;

Tel. +34 963424117; Fax: +34 963424001

Abstract

The effect of commercial degreening with ethylene gas on fruit susceptibility and quality and development of postharvest green (GM) and blue (BM) molds on early season citrus fruits was investigated. Each cultivar was harvested with different peel color indexes (CI). Fruit were exposed for 3 d to 2 μL L^{-1} ethylene at 21ºC and 95-100% RH before or after artificial inoculation with *Penicillium digitatum* or *P. italicum*. Control fruit were kept at the same environmental conditions without ethylene. Fruit were stored at either 20 ºC for 7 d or 5 ºC for 14 d and disease incidence (%) and severity (lesion diameter) were assessed. No significant effect of commercial degreening was observed on fruit susceptibility to both GM and BM on citrus cultivars inoculated after degreening. Likewise, no significant effect was observed on disease incidence on citrus cultivars inoculated before degreening and stored at either 20 °C for 7 d or 5 °C for 14 d. In contrast, in cultivars like 'Clemenules' mandarins and 'Navelina' oranges, degreening significantly increased the severity on fruit with higher initial CI (-3.6 and 1.7, respectively). GM and BM severity on degreened and control 'Clemenules' mandarins incubated at 20 °C for 7 d was 146 and 118 mm and 56 and 46 mm, respectively. In general, commercial degreening did not significantly affect external and internal quality attributes of citrus cultivars. Commercial degreening after inoculation of less green (more mature) fruit showed a trend to increase mold severity, presumably through an aging effect (acceleration of peel senescence).

Keywords: Orange, mandarin, postharvest disease, *Penicillium digitatum*, *P. italicum*, peel color index, fruit quality

1. Introduction

Postharvest green mold (GM), caused by *Penicillium digitatum* (Pers.:Fr.) Sacc., and postharvest blue mold (BM), caused by *Penicillium italicum* Wehmer, are the most economically important postharvest diseases of citrus in Spain and all production areas characterized by low summer rainfall (Eckert and Eaks, 1989). Blue mold is especially important on citrus fruits kept under cold storage (Palou et al., 2001).

External color of citrus fruits is more related to climatic conditions than to internal maturity. Degreening with ethylene (C_2H_4) is a common commercial practice employed in many parts of the world to accelerate artificially the natural color break in which fruit turns from green to orange/yellow (Porat, 2008; Peng et al., 2013). Particularly, it is extensively implemented in Spain from September to December to market early season mandarins and oranges. Since 1970's decade to date, several research works have been conducted to study the relationship between ethylene applications and postharvest decay by phytopathogenic fungi on citrus fruit, finding a non-well-determined trend. Discrepancies observed in research results seem to be related to different environmental conditions, the amount of ethylene applied and/or different host-pathogen interactions. On the one hand, fruit degreening has been reported to induce resistance to penicillium molds or anthracnose caused by *Colletotrichum gloeosporioides*, especially when applied at 30 °C and relative humidity (RH) higher than 90% (Brown, 1973, 1975, El-Kazzaz et al., 1983a; Porat et al., 1999). On the other hand, other research results have suggested an increase in the incidence of citrus postharvest diseases such as stem-end rots or penicillium molds following ethylene exposure (McCornack, 1971; Barmore and Brown, 1985; Brown, 1986; Zhang, 2004). It was reported in another work that degreening in standard conditions at 20 ºC after fungal

69inoculation had no effect on the incidence of both GM and BM on 'Clemenules' mandarin 70fruit (Plaza et al., 2004). Given these different degreening effects depending on specific 71handling and environmental conditions, it is important to establish them for each particular 72situation. Standard commercial degreening practices for early season mandarins and 73oranges in Spain, California and other Mediterranean-climate areas consist of fruit exposure 74for 2-4 d to 2-5 μL L^{-1} ethylene at 20-22 ºC and RH > 90% (Sdiri et al., 2012a), while in 75other citrus producing areas like Florida or Brazil, commercial degreening is typically 76performed at temperatures surrounding 30 ºC (Brown, 1973, 1975).

77 Regarding the effects of ethylene exposure on the quality of harvested horticultural 78produce, the responses are numerous and varied, and they can be beneficial or detrimental 79depending on each case (Saltveit, 1999; Palou et al., 2003). In the case of citrus fruit, it is 80important to examine the effects of ethylene degreening on fruit quality attributes other 81than peel color, especially on fruit destined to prolonged storage or long-distance markets. 82Thus, the effects of ethylene or 1-methylcyclopropene (1-MCP), an ethylene action 83inhibitor, on fruit weight loss, firmness, total soluble solids content (SSC), titratable acidity 84(TA), or consumer acceptance for different citrus cultivars have been assessed with 85different results (Porat et al., 1999; Plaza et al., 2004; Tietel et al., 2010; Mayuoni et al., 862011a). Furthermore, the knowledge of the effects on nutritional quality and content of 87bioactive compounds is increasingly gaining importance (Mayuoni et al., 2011b; Sdiri et 88al., 2012b).

89 Before the interest of the Spanish citrus industry to optimize degreening treatments and 90overall fruit handling in the packinghouses for the most representative commercial 91cultivars, it is important to determine the influence of ethylene degreening in our particular

92conditions on the development of GM and BM, the most economically important cause of 93postharvest decay, and also on fruit susceptibility to these diseases. Therefore, the aims of 94this research were to: (i) determine the effect of commercial degreening with ethylene gas 95on the susceptibility to GM and BM of intact early season mandarins and oranges (ii) assess 96the effect of degreening on the incidence and development of GM and BM on fruit 97previously inoculated with *P. digitatum* or *P. italicum* and incubated at 20 ºC or stored at 5 98ºC, and (iii) study the effect of degreening at standard commercial conditions on internal 99and external fruit quality attributes. Preliminary results from this research have been 100recently published (Moscoso-Ramírez and Palou, 2013).

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102**2. Materials and methods**

1032.1. Fruit

104 The trials were conducted from 2008 to 2011 with 'Clemenpons' and 'Clemenules' 105clementine mandarins (*Citrus reticulata* Blanco), 'Navelina' oranges (*Citrus sinensis* (L.) 106Osbeck), and 'Nova' [*C. reticulata* x (*Citrus reticulata* x (*Citrus reticulata* x *Citrus* 107*paradisi*)), synonym: 'Clemenvilla'] hybrid mandarins. Fruit were collected from 108commercial orchards in the Valencia area (Spain) and used the same day or stored up to 1 109week at 5 °C and 90% RH before use. Before each experiment, fruit were selected, 110randomized, washed, disinfected superficially by immersion for 2 min in a 0.5% sodium 111hypochlorite solution, rinsed with tap water to eliminate residual chorine, and allowed to air 112dry at room temperature. Depending on the experiment, fruit of each cultivar were 113harvested at different rind color indexes (CI = -0.07, 0.9 for 'Clemenpons' mandarins; CI = 114-6.5, -3.6, 2.2 for 'Clemenules' mandarins; CI = -5.3, 1.1, 1.7 for 'Navelina' oranges; CI =

11512.3 for ‘Nova’ hybrid mandarins), in some cases during the same season and in other cases 116in different seasons.

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1182.2. Commercial degreening procedure

119 Fruit were transported to a 1000 t commercial degreening room in a local citrus 120packinghouse (Fontestad S.A., Montcada, Valencia). Fruit were exposed to 2 μL L^{-1} 121ethylene (± 0.5 μL L^{-1}) at a constant temperature of 21 ºC (± 0.5 ºC) and RH > 95% for 72 122h, with the exception of fruit from one of the experiments with ‘Clemenules’ mandarins 123that needed 24 h of additional exposure to develop acceptable commercial color. At the 124same time, non-degreened (control) fruit were exposed to the same environmental 125conditions but without exposure to exogenous ethylene in a storage room located in the 126IVIA CTP pilot plant.

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1282.3. Fungal inoculation

129 *Penicillium digitatum* and *P. italicum*, isolates NAV-7 and MAV-1, respectively, 130from the fungal culture collection of the IVIA CTP, were cultured on potato dextrose agar 131(PDA, Sigma-Aldrich Chemical Co., St. Louis, MA, USA) plates at 25 ºC. Conidia of each 132fungus from 7-to 14-day-old were taken from the plate surface with a sterile glass rod and 133transferred to a sterile aqueous solution of 0.05% Tween 80® (Panreac, S.A.U., Barcelona, 134Spain). Conidial suspensions were filtered through two layers of cheesecloth to separate 135hyphal fragments and adjusted to a concentration of 1 x 10^5 spores mL^{-1} using a 136haemocytometer. Fruit were wounded and inoculated with the pathogens at the same time 137by immersing the tip of a stainless steel rod (2 mm length and 1 mm diameter) into the

conidial suspension and making a puncture on the peel in the equatorial region of the fruit. Different lots of fruit were inoculated with each fungus.

2.4. Effect on fruit susceptibility to disease

To evaluate the effect of degreening on the fruit susceptibility to GM and BM, fruit were artificially inoculated with *P. digitatum* or *P. italicum* about 2 h after ethylene degreening. This set of experiments was performed with fruit of each cultivar harvested at the lowest CI (CI of -0.07, -6.5, -5.3 and 12.3 for 'Clemenpons', 'Clemenules', 'Navelina' and 'Nova' cultivars). Each treatment consisted of 4 replications with 10 fruit each. Degreened and non-degreened inoculated fruit were incubated at 20ºC and 90% RH for 7 d. Fruit were examined after 3 and 7 d to determine the incidence (% of infected wounds) and severity (diameter of lesion in mm measured only in infected fruit) of the molds.

2.5. Effect on disease development

To evaluate the effect of degreening on fungal development, fruit were inoculated with the pathogens as previously described 2 h before ethylene degreening. For each cultivar, these experiments were performed with fruit harvested at all different CI previously mentioned. After commercial degreening, degreened and control fruit were stored under two different conditions: (i) incubation at 20 ºC and 90% RH for 7 d, and (ii) cold storage at 5 ºC and 90% RH for 2 weeks. Stored fruit were periodically examined to determine disease incidence and severity. For each pathogen, treatment and storage condition, 4 replications of 10 fruit each were used.

2.6. Effect on citrus fruit quality

Non-inoculated oranges and mandarins were used for quality assessment. Fruit external and internal quality were determined just after harvest (initial quality) and 2-3 d after degreening on degreened and non-degreened (control) fruit (final quality).

2.6.1. External quality

Peel color was measured using Hunter parameters (L, a, b) with a colorimeter (Model Minolta CR-300, Konica Minolta Business Technologies, Inc., Tokio, Japan). A color index (CI) was calculated: CI = 1000.a/L.b (Jiménez-Cuesta et al., 1981). For each treatment, three measurements on the equatorial area of 25 fruit were performed.

Firmness of 20 fruit per treatment was determined using an Instron Universal Testing Machine (Model 4301, Instron Corp., Norwood, MA, USA). Each fruit was compressed between two flat surfaces closing together at the rate of 5 mm min^{-1}. The machine gave the deformation (mm) after application of a load of 1 kg to the equatorial region of the fruit. Results were expressed as percentage of deformation related to initial diameter. Peel break resistance was measured on 30 fruit per treatment using the same machine. Each fruit was compressed with a 5 mm diameter steel rod until the fruit rind was broken, and the necessary pressure (kg) was measured.

Peel oil release pressure (kg) of 20 fruit per treatment was determined using a fruit pressure tester with a 8 mm diameter tip (Model FT327, Facchini, Alfonsine, Italy). Each fruit was wrapped with filter paper and then compressed with the tester until essential oil stains appeared. The necessary pressure (kg) was annotated.

2.6.2. Internal quality

The fruit internal quality was only determined in clementine mandarins (cvs. ‘Clemenpons’ and ‘Clemenules’). The juice from 3 previously weighed samples of 8 fruit

each was extracted with a rotatory citrus squeezer and filtered through a 0.8 mm diameter sieve. The following fruit internal quality parameters were determined: SSC was measured with a digital refractometer (Model DR-101, Optic Ivymen System, Barcelona, Spain) and expressed as percentage. TA was determined from a 5 mL aliquot by titration with 0.1 N NaOH with phenolphthalein indicator and results were given as g of citric acid per 100 mL (%). MI was calculated as the SSC/TA ratio. In all cases, two replicated measures were performed with each juice sample. Juice yield was expressed as percentage of juice (mL) per fruit weight (g).

2.7. Statistical analysis

Data from disease assessment and fruit quality parameters were analyzed by analysis of variance (ANOVA) with Statgraphics software (Statgraphics Plus, version 5.1). Data on disease incidence were transformed to the arcsine of the square root of the proportion of infected fruit to assure the homogeneity of variances. Statistical significance was judged at the level $P \leq 0.05$. When appropriated, the Fisher's Protected Least Significant Difference (LSD) test was applied to separate means. Shown values are non-transformed data.

3. Results

3.1. Effect of ethylene degreening on fruit susceptibility to disease

No significant effect of commercial degreening was observed on the incidence of both GM and BM on 'Clemenpons', 'Clemenules', 'Navelina' and 'Nova' cultivars harvested with initial peel CI of -0.07, -6.5, -5.3 and 12.3, respectively, artificially inoculated 2 h after degreening with *P. digitatum* or *P. italicum* and incubated at 20 ºC and

20895-100% RH for 7 d. Similarly, the severity of the molds was not affected by commercial 209degreening (data not shown).

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211**3.2.** Effect of ethylene degreening on disease development in fruit previously inoculated 212 with *P. digitatum* or *P. italicum*

213No significant effect of commercial degreening was observed on the incidence of both GM 214and BM on 'Clemenpons' (initial CI of -0.07 and 0.9; Fig. 1), 'Clemenules' (initial CI of -2156.5, -3.6, and 2.2; Fig. 3) and 'Nova' (initial CI of 12.3; data not shown) mandarins, and 216'Navelina' oranges (initial CI of -5.3, 1.1, and 1.7; Fig. 5) inoculated with *P. digitatum* or 217*P. italicum* about 2 h before degreening and incubated at 20 ºC and 90% RH for 7 d. 218Likewise, no effect was found on the incidence of both molds on 'Clemenpons', 219'Clemenules' and 'Nova' mandarins, and 'Navelina' oranges stored at 5 ºC for 14 d, with 220the exception of 'Clemenules' mandarins with initial CI of -3.6, in which GM incidence on 221degreened and non-degreened fruit was 100 and 85%, respectively (Fig. 3); and 'Navelina' 222oranges with initial CI of -5.3 and 1.7, in which GM incidence on degreened and non-223degreened fruit was 90 and 63% and 98 and 70%, respectively (Fig. 5).

224 On 'Clemenpons' mandarins with the initial CI of -0.07 and 0.9, commercial 225degreening 2 h after fungal inoculation had no significant effect on the severity of the 226molds on fruit incubated at 20 ºC for 7 d (Fig. 2). On 'Clemenpons' mandarins cold-stored 227at 5 ºC for 14 d, commercial degreening did not significantly affect the severity of the 228molds with the exception of BM on mandarins with an initial CI of 0.9, in which lesion 229diameters were 33 and 27 mm on degreened and control fruit, respectively (Fig. 2).

230 On 'Clemenules' mandarins, the effect of commercial ethylene degreening on 231disease severity was dependent on the initial peel CI. Degreening treatment significantly

increased the severity of the molds on fruit incubated at 20 ºC for 7 d, with the exception of mandarins with an initial CI of -6.5 (Fig. 4). On mandarins cold-stored at 5 ºC for 14 d, commercial degreening significantly increased the severity of the molds on mandarins with an initial CI of -3.6, with GM and BM severity values of 49 and 34 mm and 28 and 19 mm on degreened and non-degreened fruit, respectively. No significant effect was observed on fruit with an initial CI of -6.5 (Fig. 4).

On 'Navelina' oranges, commercial degreening had a significant effect on the severity of the molds, and it was dependent on the initial peel CI. Degreening treatment significantly increased the severity of the molds on fruit incubated at 20 °C for 7 d, with the exception of mandarins with an initial CI of -5.3 for both molds and of 1.1 for BM (Fig. 6). On mandarins cold-stored at 5 ºC for 14 d, commercial degreening significantly increased the severity of the molds on mandarins with all three initial peel CI (-5.3, 1.1, 1.7), with the exception of mandarins with an initial peel CI of -5.3 for BM (Fig. 6).

On 'Nova' hybrid mandarins degreened with an initial peel CI of 12.3, ethylene degreening did not significantly affect the severity of the molds stored neither at 20 ºC for 7 d nor at 5 ºC for 14 d, with the exception of GM severity, in which, lesion diameters were 52 and 37 mm on degreened and control fruit, respectively (data not shown).

3.3. Effect of ethylene degreening on citrus fruit quality

In general, the external quality attributes of citrus cultivars with different initial peel CI were not influenced by ethylene degreening. Although significant differences between degreened and control fruit were found in few cases, the practical impact of such differences was minimal (Table 1). For instance, 'Clemenules' mandarin fruit degreened with an initial peel CI of -6.5 were significantly more firm (lower deformation) than non-

256degreened fruit, but the deformation values were 5.48 and 6.02%, respectively. Peel break
257resistance was significantly higher on degreened 'Clemenules' mandarins with an initial
258peel color index of -6.5 (1.7 kg) than on control fruit (1.3 kg), but the force difference was
259only of 0.4 kg (Table 1). Peel oil release pressure was significantly lower on degreened
260'Clemenpons' mandarins and 'Navelina' oranges, with the initial peel CI of 0.9 (3.99 kg)
261and -5.3 (5.40 kg), respectively, than on control fruit (4.93 and 5.40 kg, respectively).
262No significant effect of commercial degreening was observed on internal quality attributes
263(TA, SSC, MI, and juice yield) of 'Clemenpons' and 'Clemenules' clementine mandarins
264(Table 2).
265
266**4. Discussion**
267This research work has determined the effect of commercial degreening with ethylene gas
268at the standard Spanish conditions on fruit susceptibility and development of GM and BM
269in early season commercially valuable mandarins and oranges, as well as on the most
270important citrus fruit quality attributes. In general, commercial degreening before fungal
271inoculation with *P. digitatum* or *P. italicum* had no significant effect on the susceptibility to
272postharvest GM and BM on mandarins and oranges harvested with low CI and incubated at
27320 ºC for 7 d. The implications of these results for the Spanish citrus industry are positive
274because, not being more susceptible, degreened fruit will not require special or additional
275preventive antifungal treatments to protect the fruit from infections by *Penicillium* spp. that
276may take place after degreening in the packinghouse facilities or storage rooms. According
277to previous literature, not only the cultivar, but also the degreening treatment conditions
278and the type of pathosystem clearly affect the influence of degreening on citrus fruit
279susceptibility to postharvest disease. Thus, a decrease in fruit susceptibility to GM was

observed when oranges were degreened with 5 μL L^{-1} of ethylene at 30 ºC and 90-96% RH for 3 d (Brown, 1973). In contrast, these degreening conditions significantly increased the fruit susceptibility of 'Robinson' tangerines to anthracnose caused by *C. gloeosporioides* (Brown, 1975). In work by El-Kazzaz et al. (1983a) with oranges, the application of exogenous ethylene before fungal inoculation significantly decreased BM severity on 'Valencia' oranges. These authors suggested that longer degreening times reduced glucosamine content, and consequently more resistance to the disease, considering that glucosamine is an indicator of fungal growth. Likewise, these results showed no polyphenol oxidase (PPO) activity in both inoculated and non-inoculated fruit, but phenylalanine ammonia-lyase (PAL) activity increased significantly only in the peel of control fruit exposed to 1000 μL L^{-1} of ethylene for 6 d.

Results from this research work showed that the influence of commercial degreening on the development of GM and BM on previously inoculated fruit was dependent not only on the cultivar but also on the initial fruit peel CI and the storage conditions in which the fruit were held after the 72-h degreening period. Furthermore, the effect on the incidence of the molds (% of infected fruit) was generally different than that on the severity of the molds (lesion diameter). In general, no significant effect on the incidence of both molds was noticed on mandarins and oranges with different initial CI artificially inoculated with *P. digitatum* or *P. italicum* before degreening and incubated at 20 ºC for 7 d after degreening. These findings are similar to those obtained by Plaza et al. (2004), who observed that degreening with 5-10 μL L^{-1} of ethylene at 20 ºC and 90-95% RH had no effect on the incidence of BM or GM on 'Clemenules' mandarins inoculated before degreening. Also, degreening with ethylene gas (5 μL L^{-1} of ethylene for 2 d) did not

303significantly affect the incidence of GM on ‘Valencia’ oranges (McCornack, 1971). Since 304the variable disease incidence measures the amount of infections that take place from free 305conidia deposited in the infection courts (rind wounds) during the artificial inoculation 306procedure, it is clear that ethylene exposure did not affect the germination ability of these 307spores, which remained very high during incubation at 20 ºC (Figs. 1, 3, 5). This is in 308agreement with previous results in which the presence or absence of ethylene had no effect 309on the pathogenicity or virulence of the fungus on green-mold-infected citrus fruit (Chalutz, 3101979; Mullins et al., 2000). However, in some of our trials the effect of commercial 311degreening was different on fruit stored at 5 ºC for 14 d. Particularly, the treatment favored 312GM incidence on ‘Clemenules’ mandarins with an initial CI of -3.6 and ‘Navelina’ oranges 313with initial CI of -5.3 and 1.7, while it did not affect the incidence of GM or BM in the rest 314of cases. Hence, it seems that in these particular cases, the interaction between previous 315ethylene exposure and prolonged storage at low temperature somehow affected the 316susceptibility of the fruit host allowing a higher amount of spores to germinate and cause 317viable infections. In any case, none of our trials showed a noticeable induction of fruit 318resistance to disease following commercial ethylene degreening of previously inoculated 319fruit. These results differ from those by Porat et al. (1999), who found a 10% decrease in 320the incidence of molds caused by *P. digitatum* and *P. italicum* in response to the application 321of 10 μL L^{-1} ethylene for 60 h on ‘Shamouti’ oranges naturally infected and stored for 4 322weeks at 20 ºC. Further, the incidence of GM was reduced by degreening oranges (5 μL L^{-1} 323of ethylene at 30 ºC and 90-96% RH) for 3 d (Brown, 1973). This dissimilar behavior can 324be logically attributed to important differences in the degreening treatment conditions, 325specifically to different amounts of ethylene and length and temperature of exposure. In

326fact, some research established an involvement of ethylene perception in promoting defense
327responses in citrus fruit infected by *P. digitatum* through the accumulation of defense-
328related mRNAs (Marcos et al., 2005) and global results of transcriptomic analysis of citrus
329fruit peel tissue reveal fundamental effects of phenylpropanoids and ethylene on induced
330resistance (Ballester et al., 2011).

331 Regarding the effect of commercial degreening on disease severity, it was observed
332in these experiments that it highly depended on the cultivar and particularly on initial peel
333CI, while the effect of storage conditions (incubation at 20 ºC or storage at 5 ºC) was less
334relevant (Figs. 2, 4, 6). The variable disease severity quantifies the growth rate of the
335pathogen once the infection has been initiated. Hence, only the fruit with values of disease
336incidence different than zero were considered for its calculation. Therefore, the effect of
337ethylene degreening on this variable reflects the effect of the treatment not on free spores
338and their capacity to germinate and initiate infection in rind wounds, but on the ability of
339fungal hyphae to grow and multiply in the infection court. The fact that this mycelial
340growth chiefly depends on the fruit host natural resistance to disease, defined basically by
341the genotype and the physical and physiological condition (Palou et al., 2007, 2008), can
342explain the differences in disease severity that were observed in this work. On
343'Clemenpons' clementines with different initial peel CI, degreening treatment did not affect
344the severity of the molds, but it significantly increased that of fruit with higher initial CI of
3450.9 for BM on fruit stored at 5 ºC (Fig. 2). On 'Clemenules' mandarins with lower initial
346CI of -6.5, ethylene degreening did not affect the severity of both GM and BM, while it
347significantly increased that of fruit with higher initial CI of -3.6 or 2.2 (Fig. 4). On
348'Navelina' oranges with initial CI of -5.3, degreening exposure did not affect mold severity,
349but the treatment significantly increased the severity of the molds on fruit with higher initial

350CI of 1.1 and 1.7 (Fig. 6). It may be therefore concluded from these observations, the 351results obtained in the previous susceptibility trials performed with fruit with low CI, and 352from the general lack of influence on the variable disease incidence that the effect of 353commercial degreening with ethylene on the development of penicillium molds relied more 354on the natural resistance of each cultivar and on particular fruit condition than on a primary 355influence of the gas on the pathogenic fungus itself. While the peel condition of the 356greenest mandarins and oranges (more immature, lower initial peel CI) was not affected or 357positively affected by commercial degreening in terms of susceptibility to decay, exposure 358to exogenous ethylene of less green citrus fruit (more mature, higher initial CI) significantly 359increased peel susceptibility to disease, presumably through an acceleration of the 360degradation of peel constitutive compounds associated with natural resistance (Porat, 3612008). It is known that the content of major compounds with antifungal activity naturally 362present in the peel of citrus fruit, mostly phenolic compounds such as flavanones, flavones, 363etc. that act as the first line of defense against pathogens, is higher in immature fruit and 364decreases as the fruit ripen and age to senescence, either while remaining in the tree or after 365harvest (Ortuño et al., 2006; Palou et al., 2007). Some postharvest treatments of different 366nature have shown the ability to maintain for longer the effective levels of these 367constitutive antifungals, typically by an enhancement of the activity of defense-related 368enzymes such as PAL, *β*-1,3-glucanase (GLU), peroxidase (POD), or PPO (Ben-Yehoshua 369et al., 1992; Lu et al., 2013). Since the application of exogenous ethylene can alter or 370accelerate the natural processes of fruit development, ripening and senescence (Kader, 3711985), it could be possible that ethylene degreening contributed to a significant loss of 372bioactive compounds in the rind of more mature fruit (El-Kazzaz et al., 1983b; Sdiri et al., 3732012b). The influence of the genotype, fruit condition and treatment conditions on the

effect of ethylene exposure on disease severity can further be explained by contradictory results in the literature. In contrast to our findings, work reported by El-Kazzaz et al. (1983b), showed that exposure to ethylene at concentrations of 1, 10, 100 or 1000 μL L^{-1} for 6 d did not affect lesion diameters on 'Navel' oranges inoculated with *P. italicum* and incubated at 20 ºC for 6 d. These authors, however, did not characterize the initial maturity of the oranges used for the trials. According to our results, further research should focus on the relations between decay severity and initial CI on fruit from each cultivar inoculated after degreening. Moreover, future studies might consider the determination of threshold values of initial CI for particular cultivars such as 'Clemenules' mandarins or 'Navelina' oranges for the significant shift of peel natural resistance to molds under Spanish degreening conditions.

In this work, ethylene degreening treatment had, in general, no practical effect on external quality attributes of citrus fruit, which is in agreement with results reported in other research works (Porat et al., 1999; Sdiri et al., 2012b). One exception was the fruit firmness of 'Clemenules' mandarins with an initial peel CI of -6.5, which was slightly increased by ethylene degreening. This result coincided with the findings reported by Plaza et al. (2004) on the same cultivar. Likewise, we observed that ethylene degreening had no significant effect on internal quality attributes of 'Clemenpons' and 'Clemenules' mandarin fruit. These findings are similar to those obtained on fruits of these cultivars degreened at 2 μL L^{-1} for 5 d followed by a cold-quarantine at 1 ºC for 16 d (Sdiri et al., 2012b) or on 'Satsuma' mandarin degreened at 4 μL L^{-1} for 5 d (Tietel et al., 2010). In addition, a variety of results obtained with different citrus species and cultivar also support our findings (Porat

et al., 1999; Plaza et al., 2004; Mayuoni et al., 2011a). As one exception, ethylene degreening decreased acidity levels in 'Mosambi' oranges (Ladaniya and Singh, 2001).

We concluded that the effect of commercial degreening with exogenous ethylene on the development of citrus penicillium molds relied more on fruit condition than on a direct effect of ethylene on the growth of the pathogenic fungi. While the peel condition of the greenest (more immature) mandarins was not affected by commercial degreening, exposure to exogenous ethylene of less green (more mature) fruit significantly increased peel disease severity in some cultivars, presumably through a senescence effect on the fruit peel.

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506Table 1. Effect of commercial degreening on external quality attributes of citrus fruit

Cultivar	Initial CI	Firmness (% deformation)			Peel break resistance (kgf)			Peel oil release pressure (kgf)		
		At harvest	Control	Degreened	At harvest	Control	Degreened	At harvest	Control	Degreened
'Clemenpons'	-0.07	6.27	8.71a	8.85a	1.23	1.13a	1.09a	-	-	-
	0.9	5.17	5.89a	5.37a	1.35	1.43a	1.27a	4.22	4.93a	3.99b
'Clemenules'	-6.5	4.26	6.02a	5.48b	1.8	1.3a	1.7b	-	-	-
	-3.6	4.42	5.91a	5.87a	1.56	1.46a	1.40a	5.15	5.30a	5.20a
	-0.6	3.44	4.76a	4.53a	1.35	1.57a	1.34a	4.60	4.60a	4.90a
	2.2	3.28	4.56a	4.10a	1.49	1.46a	1.40a	3.75	4.80a	4.46a
'Navelina'	-5.3	1.09	1.51a	1.49a	2.72	2.36a	1.93b	5.90	5.87a	5.40b
	1.1	1.63	2.63a	2.40a	2.11	2.06a	1.80a	5.18	5.76a	5.53a
	1.7	1.60	2.40a	2.03a	2.33	1.86a	1.83a	5.64	5.23a	5.86a
'Nova'	12.3	1.62	2.20a	2.26a	2.17	1.62a	1.70a	5.09	5.75a	5.73a

507Firmness and peel oil release pressure were measured on 20 fruit per treatment; peel break resistance was 508measured on 30 fruit per treatment.
509CI = Color index (CI = 1000.a/L.b; Hunter parameters).
510-, not determined.
511For each cultivar and initial CI, mean values within rows followed by the different letters are significantly 512different according to Fisher's protected LSD test ($P = 0.05$) applied after an ANOVA.
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525Table 2. Effect of commercial degreening on internal quality attributes of clementine 526mandarin fruit

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Treatments	TA (% citric acid)	SSC (%)	Maturity index (MI)[a]	Juice yield (%)
'Clemenpons'				
At harvest	0.84	11.03	13.21	50.65
Control	1.31a	11.02a	8.41a	52.77a
Degreened	1.36a	11.43a	8.39a	52.24a
'Clemenules'				
At harvest	0.86	10.53	12.26	45.87
Control	1.31a	10.15a	7.75a	46.80a
Degreened	1.27a	10.47a	8.26a	48.96a

528 [a] MI = SSC/TA (SSC = soluble solids concentration; TA = titratable acidity).
529 For each cultivar, columns followed with the different letters are significantly different
530 according to Fisher's protected LSD test ($P = 0.05$) applied after an ANOVA.
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Fig. 1. Effect of commercial degreening (2 μL L^{-1} ethylene at 21 ºC and 95-100% RH for 72 h) on the incidence of green (GM) and blue (BM) molds on 'Clemenpons' mandarins harvested with different color index (CI = 1000.a/L.b; Hunter parameters), artificially inoculated 2 h before degreening, and stored at either 20 ºC and 90% RH for 7 d or 5 ºC and 90% RH for 14 d. For each disease, storage temperature and CI, columns with the same letters are not significantly different according to Fisher's protected LSD test (P = 0.05) applied to arcsine-transformed data. Non-transformed means are shown.

Fig. 2. Effect of commercial degreening (2 μL L^{-1} ethylene at 21 ºC and 95-100% RH for 72 h) on the severity of green (GM) and blue (BM) molds on 'Clemenpons' mandarins harvested with different color index (CI = 1000.a/L.b; Hunter parameters), artificially inoculated 2 h before degreening, and stored at either 20 ºC and 90% RH for 7 d or 5 ºC and 90% RH for 14 d. For each disease, storage temperature and CI, columns with the same letters are not significantly different according to Fisher's protected LSD test (P = 0.05).

Fig. 3. Effect of commercial degreening (2 μL L^{-1} ethylene at 21 ºC and 95-100% RH for 4 d) on the incidence of green (GM) and blue (BM) molds on 'Clemenules' mandarins harvested with different color index (CI = 1000.a/L.b; Hunter parameters), artificially inoculated 2 h before degreening, and stored at either 20 ºC and 90% RH for 7 d or 5 ºC and 90% RH for 14 d. For each disease, storage temperature and CI, columns with the same letters are not significantly different according to Fisher's protected LSD test (P = 0.05) applied to arcsine-transformed data. Non-transformed means are shown.

Fig. 4. Effect of commercial degreening (2 μL L^{-1} ethylene at 21 ºC and 95-100% RH for 4 d) on the severity of green (GM) and blue (BM) molds on 'Clemenules' mandarins harvested with different color index (CI = 1000.a/L.b; Hunter parameters), artificially inoculated 2 h before degreening, and stored at either 20 ºC and 90% RH for 7 d or 5 ºC and 90% RH for 14 d. For each disease, storage temperature and CI, columns with the same letters are not significantly different according to Fisher's protected LSD test ($P = 0.05$).

Fig. 5. Effect of commercial degreening (2 μL L^{-1} ethylene at 21 ºC and 95-100% RH for 3 d) on the incidence of green (GM) and blue (BM) molds on 'Navelina' oranges harvested with different color index (CI = 1000.a/L.b; Hunter parameters), artificially inoculated 2 h before degreening, and stored at either 20 ºC and 90% RH for 7 d or 5 ºC and 90% RH for 14 d. For each disease, storage temperature and CI, columns with the same letters are not significantly different according to Fisher's protected LSD test ($P = 0.05$) applied to arcsine-transformed data. Non-transformed means are shown.

Fig. 6. Effect of commercial degreening (2 μL L^{-1} ethylene at 21 ºC and 95-100% RH for 3 d) on the severity of green (GM) and blue (BM) molds on 'Navelina' oranges harvested with different color index (CI = 1000.a/L.b; Hunter parameters), artificially inoculated 2 h before degreening, and stored at either 20 ºC and 90% RH for 7 d or 5 ºC and 90% RH for 14 d. For each disease, storage temperature and CI, columns with the same letters are not significantly different according to Fisher's protected LSD test ($P = 0.05$).

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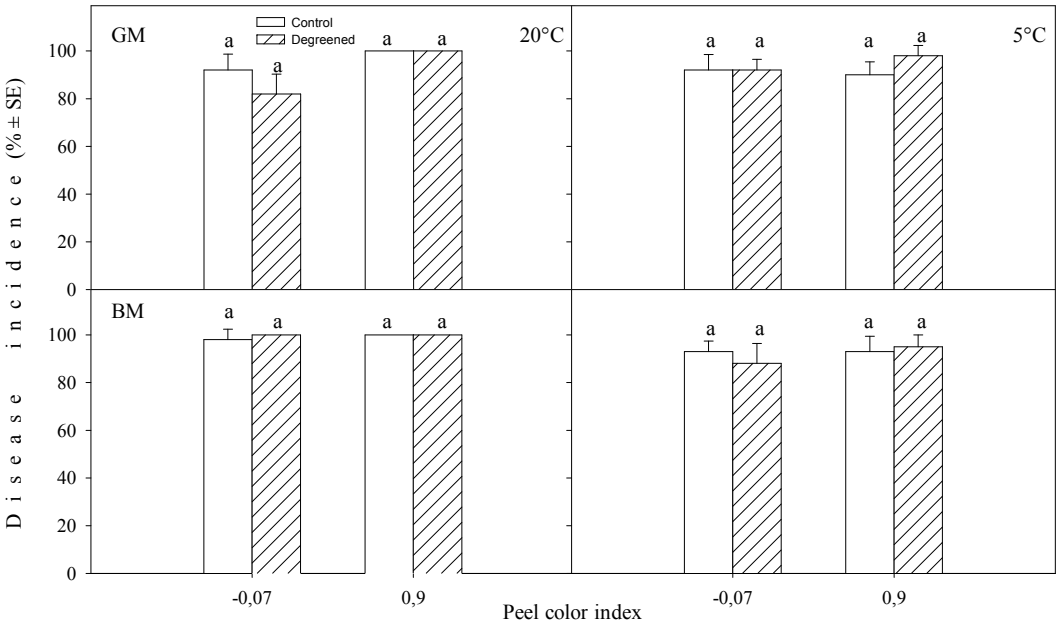

592Figure 1.

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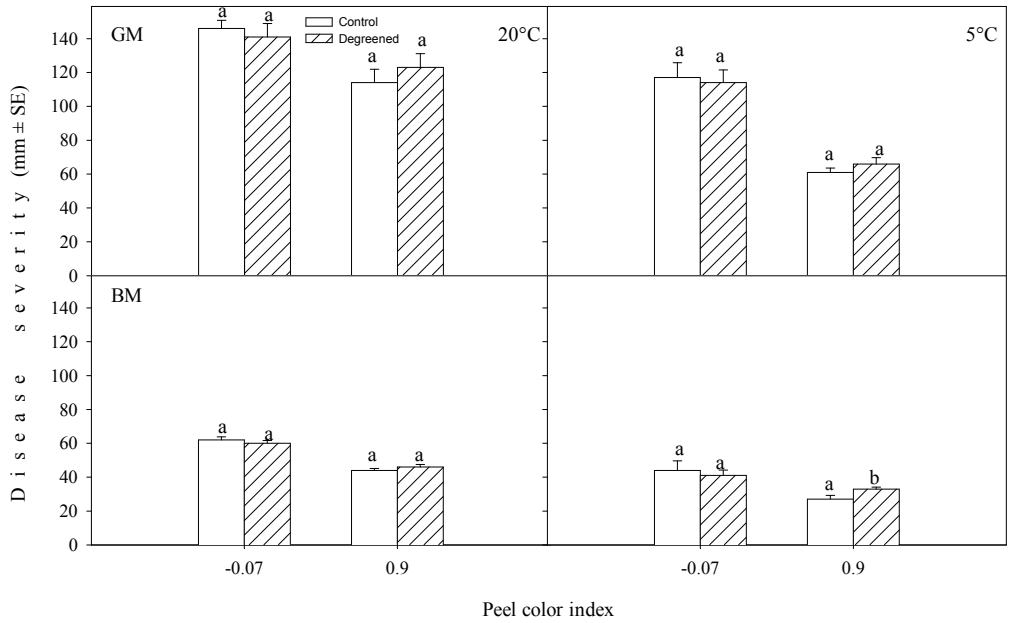

607Figure 2.

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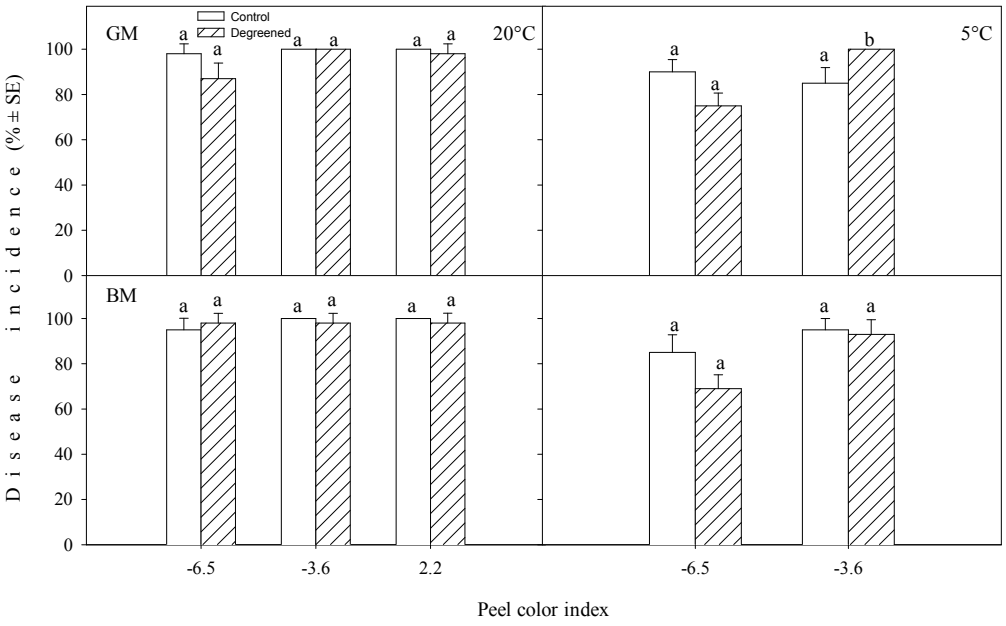

613Figure 3.

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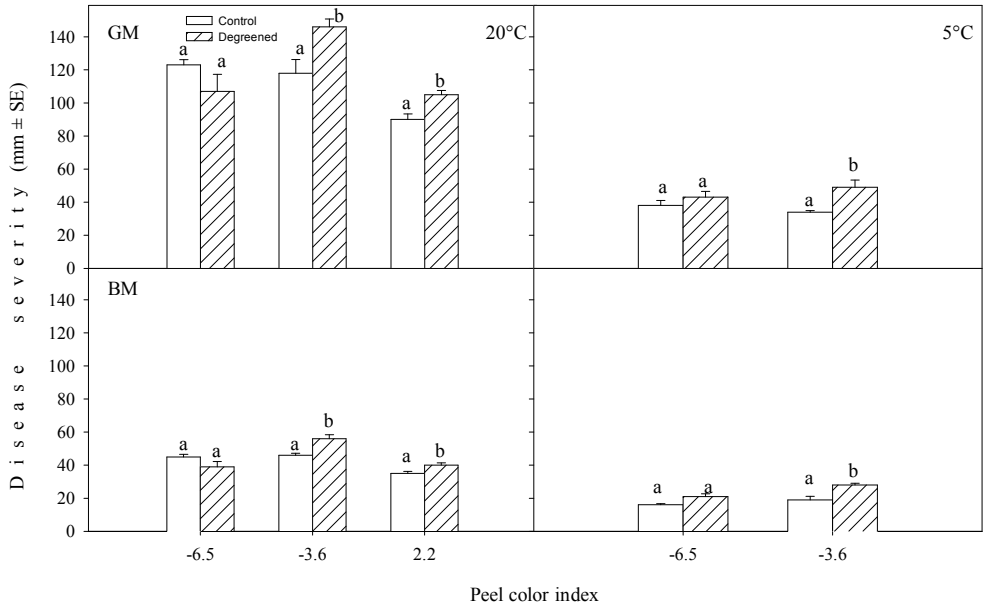

619Figure 4.

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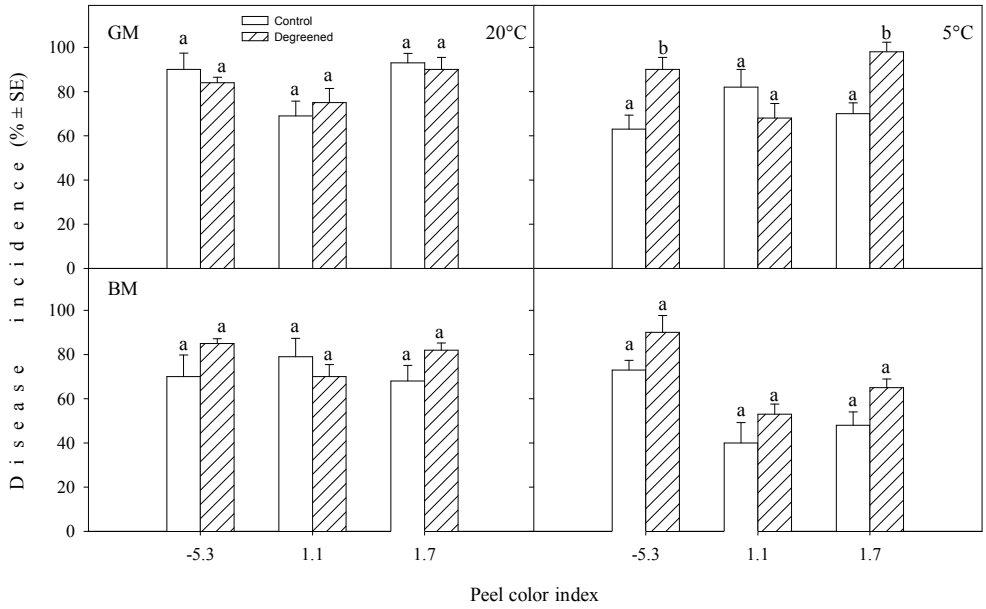

625Figure 5.

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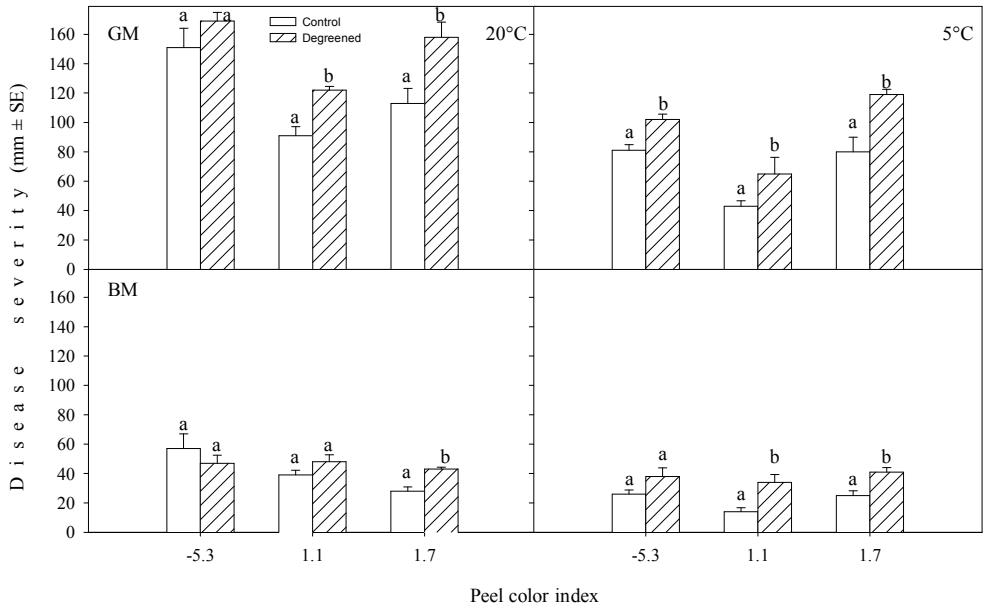

631Figure 6.

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