Relationship between Postharvest Diseases Resistance and Mineral Composition of Citrus Fruit

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Abstract

Green and blue moulds, due to the pathogenic action of *Penicillium digitatum* and *Penicillium italicum* respectively are the main cause of orange losses during postharvest. Under Mediterranean climate conditions, both together are responsible for 80% of total postharvest citrus fruit decay. The type of orchard production system, field location with different types of climate and soil has a main influence on mineral composition of fruits. The mineral composition of fruits can have a significant impact on fruit quality and shelf life during postharvest period. These include effects on fruit colour, texture, disease susceptibility, juice composition and development of physiological disorders. Oranges from different regions from South of Spain and Portugal and from three different production systems (conventional, integrated and organic) were studied to evaluate whether both factors (origin and production system) affected the degree of fruit sensitivity to decay. Results indicate that the sensitivity to green or blue mould is determined better by the origin of fruit than by the system of production.

INTRODUCTION

Penicillium italicum and *Penicillium digitatum* are the most common postharvest pathogens of citrus fruits. *P. digitatum* works by producing ethylene to accelerate ripening. It covers the fruit with green conidia, causing the fruit to shrivel and dry out. *P. italicum* causes slimy rot and produces blue-green conidia. This species likes cooler temperatures, which explains why they are usually found on foods left too long in the refrigerator. Both diseases resemble each other in colour characteristics, style of decay, and infection symptoms; they fall under a general category called green and blue mould, when caused by *P. digitatum* and *P. italicum* respectively. These fungi live a long time and are quite durable, including even stable adverse conditions. Sometimes, fruits infected by *P. italicum* will adhere to each other to create synnemata. *Penicillium* growth typically occurs as a result of wound infections in produce. To prevent the development of these pathogens and to limit losses in commercial fruit shipments, treatment with chemical fungicides is a widely used procedure. However, such treatment may produce serious problems, with residues on the fruit (Cabras et al., 1999; Palou et al., 2008), appearance of fungicide-resistant strains of *P. digitatum* (Ben-Yehoshua et al., 1994), and their possible accumulation in human adipose tissue constituting an additional health threat (Suwalsky et al., 1999). As alternative to these fungicides some treatments have obtained successful results controlling postharvest decay. Thus, curing (holding fruits at relatively high temperature and humidity for 24 to 72 h), dipping fruit in CO_3HNa, solutions at 40 to 50ºC for 1 to 3 min or the use of antagonist microorganisms have been demonstrated to be a very effective alternative treatments to avoid *Penicillium* spp. proliferation on citrus fruit (Nunes et al., 2007; Torres et al., 2007; Pérez et al., 2008a).

However, these non-contaminant systems habitually exhibit a poorer

reproducibility than fungicides (Palou et al., 2008). Recently, Pérez et al. (2008b) observed that fruit obtained from integrated or organic production exhibited a significant higher sensibility to *Penicillium* infection than the conventional ones. It means that the reduction or elimination of chemical synthesis compounds in the citrus production would cause a significant increase in sensitivity of fruits towards *Penicillium*. It is well known than the principal factor that impacts the preservation of harvested commodities is the physiological status of the tissue and its susceptibility to pathogen attack increases due to weakened natural defences mechanisms, as well as partial degradation of cell walls and subsequent increase leakage of solutes (Droby et al., 2001). Also some studies have looked at the possible role that phenolic compounds might play as phytoalexins in some *Citrus* species (Ortuño et al., 1997; Del Río, 1998; Arcas et al., 2000; Del Río et al., 2004). The level of presence of these compounds should be determinant for the progress of fungal infection and for the efficiency of the alternative system to control it. However, the reason why this presence differs with seasons and locations has not been yet explained. In this paper a possible relationship between chemical peel components (N, P, K, Ca, Mg, Fe, Cu, Zn, Mn) of citrus fruit obtained by different production systems from different origin and the level of sensibility to *P. digitatum* and *P. italicum* infection has been studied to explain this variability.

MATERIAL AND METHODS

Plant Material

Oranges (*Citrus sinensis* 'Valencia Late') were harvest randomly from conventional, integrated and organic production systems and different regions of Southwest of Spain (Lepe - seaside of Huelva - and Río Tinto - range of Huelva) and South of Portugal (Silves - west Algarve - and Tavira - east Algarve) and were harvested in the beginning of May, June and July.

Fungal Inoculation and Fruit Decay Incidence Evaluation

P. digitatum and *P. italicum* isolated from decayed oranges from 3 locations (Lepe, Rio Tinto and South of Portugal) and maintained on potato dextrose agar medium (PDA), periodically transfer to fruit. Conidia of a 7-12 days culture grown at 25ºC were suspended in sterile distilled water with Tween 80. The suspension was adjusted to 10^6 conidia/ml using a haemacitometer. Oranges in sets of 4 replicates of 60 fruits from each origin and each production system were dipped in the suspensions of *P. digitatum* or *P. italicum* for 30 s. Decay incidence of each treatment was monitored after 15 days of storage at 20ºC and 80% RH.

Quality Studies

At harvest moment, fruit firmness was evaluated using 20 non-inoculated fruits from each treatment with a Zwick 3300 non-destructive hand densimeter. Subsequently fruits were distributed in 4 groups of 5 oranges, which were separately extracted to determine the percentage of juice and the percentage of peel in front of the total fresh weight of each group of fruits. The soluble solids concentration (SSC) with a refractometer and the titratable acidity with an automatic titrator that measured the volume of 0.1 N NaOH required by 10 ml of juice to reach pH 8.0 were also measured. Data were expressed as ºBrix and percentage of citric acid, respectively. Maturity index (MI) was evaluated by the ratio: ºBrix / % Citric acid for each extracted juice.

Mineral Composition

Peel of nine fruits, distributed in three groups of three fruits were dried at 60°C for 48 h, grounded, ashed at 450ºC, and digested in 10 cm^3 HCl 1 N. Standardized procedures (AOAC, 1990) were used to measure nutrient concentrations. Nitrogen was analysed by the Kjeldahl method, P was determined colorimetrically by the molybdo-vanadate method, K was measured by flame photometry, and Mg, Ca, Fe, Cu, Mn and Zn were

measured by atomic absorption spectrometry.

Statistical Analysis

In all the parameter studied the results presented correspond to the mean values obtained in the three different harvesting dates tested. A 2-way analysis of variance (ANOVA) procedure was used for testing the possible significant effect ($P \leq 0.05$) of the origin and the production system of the samples on the global decay incidence, independently for each fungi inoculation. A 1-way analysis of variance (ANOVA) procedure was used for testing possible significant effect ($P \leq 0.05$) of the different combinations of origins and production systems on mineral contents and quality parameters. When the effect of the treatment was significant, means were analysed by the Tukey's HSD test ($P \leq 0.05$). The performed data was statistically analysed using CoStat 5.01™ statistical software (Cohort Software Minneapolis, MN, USA).

RESULTS AND DISCUSSION

Results of fruit quality are shown in Table 1, decay incidence in Table 2 and mineral composition in Table 3. Globally considered, no significant effect on *Penicillium* decay was found as consequence of the different production systems tested (Table 2). However, conventional fruits cultured in Lepe showed a significant lower sensibility to both fungi than the oranges obtained by integrated production in the same culture location. In opposite, Silves oranges cultured under organic production exhibited significant lower incidence of *P. digitatum* decay than the fruits of the same origin produced under the other two systems. On the other hand, independently of the production system how they were cultured, Lepe oranges showed lower mean values of decay incidence than the fruits of other locations. In consequence, results seem to indicate that the sensitivity to green or blue mould is determined better by the origin of fruit than by the system of production. It is possible to conclude that the reduction or elimination of chemical synthesis compounds in the citrus production does not cause a significant increase in sensitivity of fruits towards *Penicillium*. It seems that the higher resistance to the pathogen could be bound more to the edafoclimatic factors of a certain culture area that to the provision type that is applied externally to it.

Although to identify clearly a determined element, whose presence or absence was coincident with higher or lower decay incidences, is not possible. Lepe oranges showed the highest mean values of N, Mg and Fe and the lowest of Co and Mn (Table 3). Furthermore, Lepe oranges obtained by conventional production exhibited higher values of N and Fe than the integrated ones, but these differences did not reach a statistical significance. The higher presence of N and Fe could be directly related with a higher presence of proteins associated to Fe in the peel fruit. For instance, lipoxigenase is a Fe-protein associated to natural plant defence-related compounds biosynthesis, such as hexanal ($C_6H_{12}O$ hexyl aldehyde) and it is induced by cell wall breaking in plant tissues (Royo et al., 1996). At the other hand, a higher presence of Mg suggests higher chlorophyll content in the fruit peel and, in consequence, a lower level of fruit maturity and a higher resistance to fungal infection.

The significant lower MI values showed by Lepe oranges explained both the higher resistance to decay due to *Penicillium* and the higher Mg content in comparison to the mean values exhibited by Silves and Tavira oranges (Table 1). However, Río Tinto fruits in spite of showing similar MI values to Lepe oranges exhibited a higher sensibility to fungal infection. This fact indicates that Rio Tinto oranges are especially sensible to *Penicillium* attack, since they showed similar levels of decay incidence than the riper oranges produced in Portugal. In a previous work, Nunes et al. (2007) after a treatment of curing during 18 h at 40 ºV observed that artificially inoculated with *P. digitatum* and *P. italicum* Valencia fruit of this origin presented about 5% of decay incidence, whereas similar treated fruits cultured in Tarragona (northwest Spain) or Tavira did not exhibit any decay. As Río Tinto oranges are characterised for exhibiting the highest contents of Mn (Table 3), results suggest that a special attention should be dedicated to the presence of

this element in the orange peel, because it may be a limiting factor required for *Penicillium* infection. It is well known that numerous enzymes are Mn-dependant, especially a lot of them related with nucleotide biosynthesis and replication.

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Tables

Table 1. Quality parameters of 'Valencia Late' oranges from different origins and production systems.

Origin and Production system[x]	Weight (g)	Firmness (N)	Peel (% fruit weight)	Juice (%)	Pulp (%)	pH	Acidity (% Citric)	S. solids (ºBrix)	Maturity Index (ºBrix / % Citric)
Lepe Conventional	141.9ab	20.4b	24.9a	52.5a	37.2	3.3	23.6b	12.7c	0.54a
Lepe Integrated	149.5ab	20.7b	25.9a	55.8a	35.5	3.3	22.1b	11.9bc	0.54a
Rio Tinto Integrated	129.0a	18.5a	24.4b	57.8a	36.7	3.7	20.7b	10.2a	0.49a
Rio Tinto Organic	177.7bc	20.2b	29.1a	64.7a	36.5	4.1	22.6b	10.4ab	0.46a
Tavira Integrated	215.1cd	19.2ab	21.7a	93.7b	43.6	3.5	10.1a	12.0c	1.19b
Silves Conventional	132.9a	20.4b	26.1a	57.2a	39.2	3.6	12.0a	12.8c	1.07b
Silves Integrated	239.9d	20.3b	24.3a	88.6b	36.6	3.6	9.6a	11.7bc	1.22b

[x]Mean values of 9 determinations (3 harvest dates × 3 replicates). Within each column the same lower case letter indicate no significant difference due to origin and production system according to Tukey's HSD test ($P \leq 0.05$). No letters indicate no significant effect due to the cited factor detected by ANOVA ($P \leq 0.05$)

Table 2. Decay incidence decay of 'Valencia Late' oranges from different origins and production systems.

Decay Incidence[z] (%)	Production system Species of *Penicillium* inoculated							
Sample origin	Conventional		Integrated		Organic		Average	
	P. dig.	*P. ital.*	*P. dig.*	*P. ital.*	*P. dig.*	*P. ital.*	*P. dig.*	*P. ital.*
Lepe	82.1 bB	82.1 bB	95.7 aB	87.1 aB	-	-	88.9 B	84.6 B
Río Tinto	-	-	98.5 AB	97.0 A	100.0 A	98.5	99.2 A	97.8 A
Tavira	100.0 A	97.1 A	-	-	-	-	100 A	97.1 A
Silves	98.5 aA	100.0 A	100.0 aA	100 A	95.6 bB	97.0	98.0 A	99.0 A
Average	93.5	93.1	96.3	93.7	97.8	97,8	95.7	94.4

[z]Means of the 3 harvest dates. Within origin and fungal disease the same lower case letter indicate no significant difference between decay incidences due to production system, and within system production the same capital letters indicate no significant difference between decay incidences due to origin, according to Tukey's HSD test ($P \leq 0.05$). No letters indicate no significant effect due to these 2 factors detected by ANOVA ($P \leq 0.05$).

Table 3. Mineral composition of Valencia Late oranges from different origins and production systems.

Sample origin	Production system	Mineral composition[z] Macronutrients (g kg^{-1})[y] and Micronutrients (mg kg^{-1})[y] N	P	K	Ca	Mg	Fe	Co	Zn	Mn
Lepe	Conventional	11.5 b	0.8 ab	6.1 a	4.8 ab	1.4 b	8.3 b	4.0 b	6.0 ab	7.0 b
Lepe	Integrated	10.2 ab	0.7 a	5.4 a	6.8 bc	1.5 b	7.5 ab	5.0 b	6.0 ab	7.0 b
Río Tinto	Integrated	9.6 ab	0.9 c	9.0 b	5.1 ab	1.2 ab	7.3 ab	8.0 b	8.0 b	12.0 a
Río Tinto	Organic	10.5 ab	1.0 c	9.0 b	4.4 a	1.2 ab	7.2 ab	8.0 b	6.0 ab	14.0 a
Tavira	Conventional	10.7 ab	0.9 ab	7.1 ab	6.7 bc	0.9 a	6.6 ab	13.0 a	7.0 ab	9.0 b
Silves	Conventional	9.9 ab	0.8 ab	5.4 a	8.7 cd	0.8 a	6.0 a	7.0 b	4.0 a	7.0 b
Silves	Integrated	9.1 a	0.8 ab	5.0 a	9.2 d	1.2 ab	6.7 ab	6.0 b	4.0 a	8.0 b

[z]Means of the 3 harvest dates. Within each column the same lower case letter indicate no significant difference due to origin and production system according to Tukey's HSD test ($P \leq 0.05$).
[y]Dry weight