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Citrus postharvest diseases and injuries related to impact on packing lines

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ABSTRACT: Brazilian exports of fresh citrus represent less than 1% of the overall Brazilian production. Phyto sanitary barriers and poor appearance stand out among the several reasons contributing to such low exporting/production ratio. The purpose of this work was to characterize postharvest injuries in ‘Valencia’ sweet oranges and ‘Murcott’ tangors produced for foreign markets after different processing stages in a packinghouse, as well as to identify critical points and impact extent on packing lines. Sampling was performed both after pre-washing and degreening the fruits, and also at the arrival on the packing table and in the pallet. They were stored for 21 days at 25°C and 85% RH. The incidence of injuries was visually assessed every three days. An instrumented sphere with acceleration register was used to evaluate the extent of impacts (G, m/s²) at the 19 transference points of the citrus processing line. There was low rot incidence (under 3.5%) at the different stages of fruit processing, with slight increase after degreening in ‘Valencia’ orange and a decrease after fungicides treatment in ‘Murcott’ tangor. The main pathogens found in the oranges were *Lasiodiplodia theobromae* and *Penicillium digitatum*, which were surpassed by *Colletotrichum gloeosporioides* in the tangors. Impacts in the processing line were caused mainly by drops on hard surfaces, with 94.7% of them varying from 30 to 95 G. The greatest impacts were observed when fruits were transferred from the processing line to bins destined to degreening. Mechanical injuries related to oleocellosis increased until the arrival of fruits at the packing table.

Key words: Brazil, ‘Valencia’ sweet orange, ‘Murcott’ tangor, fungal rots, mechanical damages

DOENÇAS PÓS-COLHEITA EM CITROS E INJÚRIAS RELACIONADAS AO IMPACTO EM LINHAS DE BENEFICIAMENTO

RESUMO: A exportação de frutos cítricos frescos representa menos que 1% da produção brasileira. Dentre os fatores atribuídos a esta baixa relação entre exportação e produção destacam-se as barreiras sanitárias e o aspecto visual de qualidade inferior. Este trabalho objetivou caracterizar os danos pós-colheita de frutos de laranja ‘Valencia’ e tangor ‘Murcott’, destinados à exportação, após diferentes etapas do beneficiamento em packinghouse e identificar os pontos críticos e a magnitude de impacto em linhas de beneficiamento. Os frutos foram coletados na chegada ao packinghouse, tanto após a pré-lavagem como após o desverdecimento, na banca de embalagem e no palete; e armazenados individualmente durante 21 dias a 25°C e 85% de UR. A incidência de injúrias foi avaliada visualmente a cada três dias. Para a avaliação da magnitude de impactos (G, m/s²) nos 19 pontos de transferência da linha de beneficiamento de citros empregou-se uma esfera instrumentada com registrador de aceleração. Observou-se uma baixa incidência de podridões nas diferentes etapas do beneficiamento dos frutos, com valores abaixo de 3,5% e levemente superior após o desverdecimento em laranja ‘Valencia’ e também redução de podridões após aplicação de fungicidas em tangor ‘Murcott’. Os principais patógenos encontrados em laranja foram *Lasiodiplodia theobromae* e *Penicillium digitatum*, sendo superados em tangor por *Colletotrichum gloeosporioides*. Na avaliação dos impactos na linha de beneficiamento, 94,7% dos impactos variaram entre a faixa de 30-95 G, causados principalmente por quedas em superfícies rígidas. Os maiores impactos foram observados quando os frutos foram transferidos da linha de beneficiamento para bins, destinados ao desverdecimento. Os danos mecânicos de oleocellosa foram crescentes até a banca de embalagem.

INTRODUCTION

Brazil is the first world producer of citrus fruit, with 20 million tons for each year (FNP Consultoria & Comércio, 2007). São Paulo State is responsible for 81% of the Brazilian production, from which 82% goes to juice production, 17% goes to the domestic market and only 1% is destined to fresh fruit exports (Neves & Lopes, 2005). This low exporting/production ratio is due to the cultivars, taxes, phytosanitary barriers, and quality demands by the international market (Spósito & Bassanezi, 2002). European Union, for example, classifies citrus black spot as a quarantine disease (OEPP/EPPO, 2003). Therefore, a careful selection process is necessary at the packinghouse in order to avoid exports of injured fruits. Other typical postharvest diseases such as green mold and stem-end rot also reduce fruit quality. Therefore, fruit has to be treated in order to minimize problems with the importer.

In Spain, the percentage of the fruit rotting after harvest in a typical season is 3 to 6% (Tuset, 1987). However, under favorable disease conditions, losses up to 50% can occur during marketing (Eckert, 1993; Abd-El-Aziz & Mansour, 2006). In Northeastern Brazil, a survey of postharvest diseases in citrus indicated a 21.9% incidence of fungal rots (Dantas et al., 2003). Several kinds of injuries may affect fruit’s quality after harvest. The damaged tissue becomes susceptible to infections caused by pathogenic microorganisms, besides showing scars visually detrimental to the fruits, reducing their market value (Golomb et al., 1984). Miller & Wagner (1991) found that 80% of impacts on fruits along a citrus sorting line varied from 25 and 150 G. These impacts can injure the fruits.

The purposes of this work were to characterize injuries in ‘Valencia’ orange and ‘Murcott’ tangor, destined for foreign markets, at different stages in the packinghouse and to identify critical points, as well as the extent of impact on fruits along the citrus processing lines in packinghouse.

MATERIAL AND METHODS

The terminology proposed by Zadoks (1985) was adopted in this paper: any visible and measurable symptoms caused by a harmful organism or mechanical impact are called an injury; any reduction in the quantity and/or quality of yield is called damage; any reduction in financial return due to harmful organisms is called loss.

Characterization of injuries and quantification of postharvest damages in citrus

Samples were collected at five points in the processing line of a packinghouse located in Matão, São Paulo State, Brazil, every 14 day. Fruits of ‘Valencia’ sweet orange (Citrus sinensis L. Osbeck) were destined to the European fresh market and came from Gavião Peixoto, São Paulo State (21°50’ S; 48°29’ W, 515 m altitude), harvested in July-October 2004 (eight sampling dates); from Matão, São Paulo State (21°60’ S; 48°36’ W, 585 m altitude), harvested in August 2005 (one sampling date), and from Uberlândia, Minas Gerais State (18°53’ S; 48°17’ W, 890 m altitude), harvested in August-October 2005 (five sampling dates). The ‘Murcott’ tangor [Citrus reticulata Blanco × C. sinensis (L.) Osb.] fruits were destined to the Asian fresh market and came from Pratânia, São Paulo State (22°48’ S; 48°39’ W, 685 m altitude), harvested in October-November 2004 (four sampling dates).

One hundred fruits were collected at each of the following stages commonly found in the packinghouses processing fruits to foreign markets: i) from plastic field bins at the arrival at the packinghouse; ii) after pre-washing with sodium hypochlorite at 200 mg L⁻¹ active chlorine and neutral detergent; iii) after degreening with ethylene at 1-5 µL L⁻¹ (from three to five days); iv) at the packing table (after another washing with sodium hypochlorite and detergent and application of wax + thiabendazole and imazalil fungicides, both at 1000 mg L⁻¹); v) at the fiberboard cartons (19 kg) on pallets. Because there was no need to degreened ‘Murcott’ tangor, the stages assessed for then were (i), (iv), and (v) only. Fruits were placed separately inside trays and stored at 25°C and 85% RH for 21 days. During the first 24 hours of storage, fruits were stored in a humid chamber in a plastic bag to induce rots. The incidence of injuries was visually assessed at the retrieval of fruits from the humid chambers and every three days. The fungi pathogenicity was confirmed by the inoculation of healthy fruits.

The incidences of postharvest diseases were compared at different processing stages by a non-parametric test of multiple proportions comparison at 5% probability, according to Zar (1999). This non-parametric test uses untransformed data.

Impact critical points in citrus processing lines

An instrumented sphere (70 mm) (Techmark, Inc., Lansing, MI, USA) was used to assess the extent of impacts at transference points along the citrus processing line. The 110-meter long processing line (Maf-Roda), with electronic sorter (Optiscan 2000, Valencia, Spain) had 19 automated transference points. The instrumented sphere, with an impact range between 15 and 500 G, was placed with the fruits at the reception stage and followed the flow of the fruit until the sorting stage. The time of residence of the instrumented sphere in each stage, as well as the time...
to complete the entire cycle, was monitored by a precision chronometer. Assessments were repeated five times in the total cycle of the processing line.

The acceleration force (G) in the processing line at different transference points was compared using ANOVA followed by multiple comparisons by Tukey’s test ($p < 0.05$). The incidence of oleocellosis in three stages of processing fruits (reception, after pre-washing with sodium hypochlorite/detergent and table) was correlated to the impact (sum of acceleration in different transference points) in the respective stages and analyzed by F test ($p < 0.05$).

**RESULTS AND DISCUSSION**

**Postharvest diseases**

Mean rot incidences (2004/05) at fruit arrivals, pre-washing, degreening, packing table, and pallet stages for ‘Valencia’ sweet orange, destined to the European fresh market, were 1.8, 1.6, 2.6, 2.1 and 1.9%, respectively (Tables 1 and 2), which were considered lower in comparison to the incidences in varieties of citrus destined to the domestic market: 21% higher under similar experimental storage conditions (Fischer et al., 2007). This lower rotting incidence is probably due to the intensive management of diseases in those orchards. The most frequent pathogens identified from lesions on symptomatic fruit were *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Penicillium digitatum* (Pers.:Fr.) Sacc., *Alternaria citri* Ellis & N. Pierce, and *Phomopsis citri* Fawcett (Figure 1A). *Colletotrichum gloeosporioides* Penz., *Fusarium* spp, *Geotrichum citri-aurantii* (Ferraris) E.E. Butler, *Penicillium italicum* Wehmer and *Rhizopus stolonifer* (Ehrenb.) Vuill were also detected but low in frequency.

| Pathogen                     | Stages of processing line | Mean | |
|------------------------------|---------------------------|------|--
|                              | Bin | Washing | Degreening | Table | Pallet | |
| *Lasiodiplodia theobromae*   | 0.7 aA* | 0.3 aA | 1.3 aA | 0.3 aA | 0.7 aA | 0.7 |
| *Penicillium digitatum*      | 0.7 aA | 0.3 aA | 0.5 aAB | 0.3 aA | 0.0 aA | 0.4 |
| *Alternaria citri*           | 0.1 aA | 0.3 aA | 0.5 aAB | 0.0 aA | 0.0 aA | 0.2 |
| *Phomopsis citri*            | 0.3 aA | 0.1 aA | 0.1 aB | 0.1 aA | 0.0 aA | 0.1 |
| *Colletotrichum gloeosporioides* | 0.1 aA | 0.1 aA | 0.1 aB | 0.3 aA | 0.0 aA | 0.1 |
| *Fusarium* spp.              | 0.0 aA | 0.0 aA | 0.1 aB | 0.0 aA | 0.0 aA | 0.0 |
| **Total**                    | 1.9 a | 1.1 a | 2.6 a | 1.0 a | 0.7 a | 1.5 |

*Values represent the mean of eight replicates of 100 fruits from Gavião Peixoto, SP. Values followed by the same capital letter within columns and by the same small letter within rows are not different, according to the nonparametric test for comparisons of multiple proportions at ($p < 0.05$).*
Rot incidences among ‘Murcott’ tangors, destined to the Asian fresh market, tended to decrease with processing from 2.6% at arrival of fruits in the packinghouse to 1.6% at the packing table and 1.1% at pallet stage (Table 3). The most frequent pathogens were C. gloeosporioides, L. theobromae, and P. digitatum. P. citri., G. citri-aurantii and Fusarium spp. were also found.

L. theobromae was the most frequent pathogen among sweet oranges, which is consistent with Dantas et al. (2003) results for ‘Pera’ variety commercialized at Distribution Centre of Recife, Brazil, where the stem-end rot incidence was 53% of total fruit rot. Lasiodiplodia stem-end rot is an important postharvest disease in warm and humid citrus growing regions such as Florida and Caribbean (Brown & Eckert, 2000). L. theobromae usually infects the fruit from its button (calyx and disk) at the stem-end of the fruit, leading to development of soft brown to black decay symptoms at both fruit ends. Decay develops rapidly during and after excessive degreening and can be observed in the fruits at the packinghouse. It is often observed at a market arrival or shortly thereafter (Brown, 1988). In this paper, the incidence of stem-end rot by Lasiodiplodia tended to be higher among degreened sweet oranges, including that receiving fungicides application in 2005.

Green mould is one of the most important fruit decays in Florida (Browning et al., 1995) and in the Mediterranean region (Tuset, 1987). In a survey with ‘Pera’, ‘Lima’ and ‘Natal’ oranges and ‘Murcott’ tangor for the São Paulo State domestic market, green mould was the most frequent disease found with a 15.9% average incidence in all these citrus varieties (Fischer et al., 2007). The extensive spores production by this pathogen, which is easily dispersed by air, ensures its presence wherever fruit is handled, including in the field, packinghouse, transit containers, and the in marketplace. Infection takes place only through wounds, where nutrients are available to stimulate spore germination, where fruit decay begins (Ismail & Zhang, 2004).

Figure 1 - Progress of the incidence (% of diseased fruit) of postharvest pathogens in fruits of sweet orange ‘Valencia’ (mean of 2004 and 2005 - A) and tangor ‘Murcott’ (B), during three weeks of storage.

Table 3 - Incidence of postharvest fungal pathogens in ‘Murcott’ tangor fruits collected at different stages of processing line in packinghouse, after three weeks of storage at 25°C with 85% RH.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Bin</th>
<th>Table</th>
<th>Pallet</th>
<th>Mean</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colletotrichum gloeosporioides</td>
<td>1.0 aAB*</td>
<td>0.0 aA</td>
<td>0.8 aA</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Penicillium digitatum</td>
<td>1.3 aA</td>
<td>0.0 bA</td>
<td>0.0 bA</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Lasiodiplodia theobromae</td>
<td>0.3 aAB</td>
<td>1.0 aA</td>
<td>0.0 aA</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Phomopsis citri</td>
<td>0.0 aB</td>
<td>0.3 aA</td>
<td>0.0 aA</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Geotrichum citri-aaurantii</td>
<td>0.0 aB</td>
<td>0.3 aA</td>
<td>0.0 aA</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>0.0 aB</td>
<td>0.0 aA</td>
<td>0.3 aA</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.6 a</td>
<td>1.6 a</td>
<td>1.1 a</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

*Values represent the mean of eight replicates of 100 fruits from Pratânia, SP. Values followed by the same capital letter within columns and by the same small letter within rows are not different, according to the nonparametric test for comparisons of multiple proportions at (p < 0.05)
The reduction of \textit{P. digitatum} inocula through washing citrus with detergent and sodium hypochlorite, as well as with additional protection by the fungicides thiabendazole and imazalil, reduced green mold incidence to below 0.5\% in orange and to zero in tangor. \textit{P. digitatum} can develop resistance to some fungicides, particularly to the benzimidazoles (Schmidt et al., 2006). Problems of resistance can be minimized using two or three fungicides with different modes of action, in addition to the monitoring of the resistant populations in air (Palou et al., 2001). Fludioxonil, a newly registered and classified as reduced-risk fungicide in the United States, effectively reduced both \textit{Lasiodiplodia} stem-end rot and green mold when applied through a simulated commercial packing line system. This fungicide was also effective for the control of \textit{P. digitatum} isolates resistant to thiabendazole on oranges (Zhang et al., 2007).

Anthracnose generally occurs on previously injured fruits by sunburn, wind, pests and phytotoxicity by pesticides. However, anthracnose symptoms can be observed even in non-injured fruits of some varieties of tangerine and their hybrids (Feichtenberger et al., 2005). This was the case of ‘Murcott’ tangor, in which anthracnose was the most prevalent disease. In Florida, anthracnose is a major cause of decay in tangerines that are harvested early in the fall, when long periods of degreening are required to enhance fruit appearance (Brown, 1988). Washing of the fruit prior to degreening removes many appressoria and reduces disease incidence (Brown, 1975). In this study, it was difficult to isolate the effect of washing due to the low field incidence of this disease.

Control of postharvest diseases in citrus is vital for maintaining quality and shelf-life in a market where transport from producer to consumer may take several weeks. Postharvest infections can be reduced by sanitation of plastic field bins and packinghouse facilities (machines, chambers, floor tiles) with chlorine and quaternary ammonium containing products. Fungicides such as thiabendazole and imazalil are important in order to minimize postharvest decay, as observed in sweet orange and tangor in 2004, although in some cases, such as in sweet orange in 2005, stem-end rots increased after fungicides application. Without using fungicides, sales of fresh Florida citrus would be reduced by at least 50\% (Mark Brown, Florida Department of Citrus, cited by Ismail & Zhang, 2004). The environment control by using cold rooms in storage and the use of refrigerated containers during transport of processed fruits delay the development of rots considerably and are important practices to reduce postharvest diseases, especially when combined with chemical treatment (Mazzuz, 1996).

Processing effectively removed fruit with sooty mold, caused by \textit{Capnodium citri} Berk. & Desm., and other biotic injuries from pathogens which infect fruits in the field to incidence below or near 1.0\% in ‘Valencia’ sweet orange (Figure 2A,C). However, in ‘Murcott’ tangor, 13.0\% of fruit collected in the pallet had alternaria brown spot (\textit{Alternaria alternata} (Fr.) Keissler) due to the high field incidence of this disease (Figure 2B). The incidences of citrus black spot (\textit{Guignardia citricarpa} Kiely) in ‘Valencia’ orange and ‘Murcott’ tangor at the initial stages of processing were 1.6 and 1.0\%, respectively. However, after packing, even after 21 days of storage, no fruit showed citrus black spot symptoms. The reduction in citrus black spot incidence results from the careful selection process at arrivals of the fruits in the packinghouse and after degreening. Waxing treatment was found to reduce postharvest development of citrus black spot in studies in Australia (Seberry et al., 1967; Wild, 1981), whereas the postharvest fungicides treatment had no effect on postharvest symptom development (Alcoba et al., 2000; Agostini et al., 2006).

Associations between titratable acidity and soluble solids content with rot incidence, both on ‘Valencia’ orange and ‘Murcott’ tangor fruits, were generally variable and not significant (dates not presented). Although associations between the increase of the infections with the rise of the soluble solids or the reduction of the acidity were found to be related in cherry fruits (Northover & Biggs, 1990), apple (Sharma & Kaul, 1988) and peach (Souza, 2007), these characteristics had not assisted in the understanding of the causes of resistance/susceptibility on the citric fruits, thus having to be considered only in the planning of the harvest. During citrus fruit maturation, drastic variations in the chemical composition do not occur (Medina et al., 2005).

Postharvest mechanical injuries

Oleocellosis increased with processing in ‘Valencia’ sweet orange, from 28.8\% at arrival in the packinghouse to 53.4\% in the packing table (Figure 2C). The reduction in mechanical injuries at the pallet (Figure 2C) is probably due to the removal of these kinds of defecting fruits during the grading process. Oleocellosis symptoms in ‘Murcott’ tangor also increased, during processing, from 7.8\% at arrival in packinghouse to 20.8\% in pallet (Figure 2D).

Incidences of dehydrated ‘Valencia’ orange and ‘Murcott’ tangor fruits decreased with the processing. Among ‘Valencia’ oranges, 38\% were dehydrated at the arrival to the packinghouse, while only 2\% were dehydrated in the pallets (Figure 2C). Similarly, among ‘Murcott’ tangor fruits 19\%, were dehydrated at the arrival in the packinghouse, while less than 1\% were
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dehydrated in the pallet (Figure 2D). A better preservation during storage of fruits from the packing table and pallet stages, related to water loss, is due to the covering of fruits with carnauba wax. Besides reducing the dehydration, the wax sealing improved its visual quality (Kaplan, 1986). According to Waks et al. (1985) the waxes minimize stem-end rind breakdown and others collapses of rind tissue and can protect the fruit of the entrance of pathogen.

Other fruit injuries, such as healed lesions from pest attacks or wind injury, as well as pesticide spray injuries, were reduced with the sorting and culling processes. Yet, non-healed lesions from the harvest increased up to the processing table stage (Figure 2C, D). Non-healed lesions are due to fruit impacts in sorting line surfaces, and they could be related to incidence of some postharvest diseases, like Penicillium rots, that enter fruit only through wounds (Ismail & Zhang, 2004). Other imperfections observed in orange and tangor fruits were pressure bruises, chimeras, ethylene damage, and wax damage. These imperfections are not presented due to their very low incidence.

Impact critical points in citrus processing lines

Most impacts (94.7%) were within 30–95 G and caused by drops against hard surfaces. Maximum acceleration was observed at the placement of washed fruits into empty bins destined to degreening (Figure 3). Mean acceleration in this point reached 272 G and differed from other points ($p < 0.05$). A positive correlation was observed between the impacts in the processing line and the incidence of oleocellosis in ‘Valencia’ fruits ($r = 0.97$).

The 272 G acceleration at the return of washed fruits to bins destined to degreening corresponds to the most critical situation favoring fruit injuries. The first falling fruits are exposed to higher drops (80 cm) and, consequently, greater impacts when in comparison to the last falling fruits. Despite the high acceleration value observed in this study, Miller & Wagner (1991) reported that acceleration values may reach 300 G during fruit reception stages. Points that favor the occurrence of mechanical injuries along the sorting line include: fruit drops at the entrance of the processing line; transference of fruits among the line components; hard surfaces; high speed of fruits; inadequate maintenance of equipment; and excessive wax residue on the line (Sardi, 2001).

Mechanical injuries caused by processing of grapefruits (Citrus paradise Macf.), observed by Skaria et al. (2003), were followed by increased rot incidences.
Sorted fruits, even treated with thiabendazole and sodium o-phenylphenate (SOPP), presented higher incidence of green mold than carefully packed fruits in the field, without postharvest treatment with fungicides (Skaria et al., 2003). ‘Tahiti’ lime fruits submitted to impacts showed great depreciation in external appearance, with symptoms of blossom-end rots and reduction of shelf-life by six days, when in comparison to the 15-day shelf-life of non-impacted fruits (Durigan et al., 2005). Sargent et al., (1992) commented that modifications in some points of classification lines of table tomatoes, such as surface protections, reduced impact pressure by up to 50%. Timm & Brown (1991) also observed decreased impacts in classification lines of avocado, papaya and pineapple when using rubber covers. Impact decreases can be promoted by using cushioning materials, reducing rough surfaces, such as concrete or metal, which are the most likely cause of fruit damage (Miller & Wagner, 1991).

CONCLUSIONS

Fungal rot incidences among ‘Valencia’ oranges and ‘Murcott’ tangors were low at the different processing stages. Injuries caused by diseases, pests, and abiotic factors decreased along with the fruit selection process in the packinghouse, unlike oleocellosis symptoms, which increased with each process. Impact levels for the processing line machinery remained within the figures reported in the literature, with maximum acceleration observed at the return of the fruits for bins destined to degreening. Low cost alternatives, such as surface impact protection, or the decrease of drop heights to which fruits are subjected, may significantly reduce the extent of impacts with a consequent reduction in physical injuries and longer postharvest preservation of fruits.
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