NOTAS CIENTÍFICAS

Chitosan and fungicides on postharvest control of *Guignardia citricarpa* and on quality of 'Pêra Rio' oranges

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ABSTRACT

Rappussi, M.C.C.; Benato, E.A.; Cia, P. & Pascholati, S.F. Chitosan and fungicides on postharvest control of *Guignardia citricarpa* and on quality of 'Pêra Rio' oranges. ***Summa Phytopathologica,*** v.37, n.3, p.142-144, 2011.

Citrus fruits are affected by the black spot disease caused by the fungus *Guignardia citricarpa*. Chitosan can be used as covering for fruits and may delay the ripening process and inhibit the growth of some fungi. Thus, the control of citrus black spot using chitosan and the fungicides thiabendazole and imazalil was assessed in addition to the physicochemical quality of 'Pêra Rio' oranges. The oranges were immersed into chitosan, thiabendazole or imazalil, and in chitosan mixed with both fungicides. The fruits were then stored at 25 °C, 80% RH, for 7 days and, after this storage period, subjected to physicochemical analyses. Chitosan in association with the fungicides reduced black spot in 'Pêra Rio' oranges and delayed the change in the orange skin colour from green to yellow during the postharvest storage. Total soluble solids, titratable acidity, pH, ascorbic acid content and ratio were not influenced by the treatments. Thus, chitosan applied with the fungicides thiabendazole and imazalil showed potential to control the development of black spot lesions on 'Pêra Rio' oranges during the postharvest period.

Keywords: black spot, thiabendazole, imazalil, alternative control, physicochemical analysis

RESUMO

Rappussi, M.C.C.; Benato, E.A.; Cia, P. & Pascholati, S.F. Quitosana e fungicidas no controle pós-colheita de *Guignardia citricarpa* e na qualidade de laranjas 'Pêra Rio'. ***Summa Phytopathologica,*** v.37, n.3, p.142-144, 2011.

Os frutos cítricos são afetados pela pinta preta dos citros, causada pelo fungo *Guignardia citricarpa*. A quitosana pode ser utilizada como revestimento de frutos, sendo capaz de atrasar o processo de maturação e inibir o crescimento de fungos. Dessa maneira, o controle da pinta preta utilizando quitosana e os fungicidas tiabendazole e imazalil foi avaliado, assim como a qualidade físico-química de laranjas 'Pêra Rio'. Frutos de laranja foram imersos em quitosana, tiabendazole ou imazalil, e em quitosana em mistura com ambos os fungicidas. Os frutos foram armazenados a 25°C, 80% UR, por 7 dias e após o período de armazenamento foram submetidos a análises fisico-químicas. Quitosana em associação com os fungicidas reduziu o aparecimento da pinta preta em laranjas 'Pêra Rio' e atrasou a alteração de verde para amarelo da cor da casca das laranjas durante o armazenamento pós-colheita. Os sólidos solúveis totais, a acidez titulável, o pH, o ácido ascórbico e o *ratio* não sofreram influência dos tratamentos. Deste modo, a quitosana em aplicação com os fungicidas tiabendazol e imazalil aumentou o potencial de controle da pinta preta em laranjas 'Pêra Rio' em pós-colheita, mantendo a qualidade dos frutos cítricos.

Palavras-chave adicionais: pinta preta, tiabendazol, imazalil, controle alternativo, análise fisico-química.

Brazil is the largest citrus fruit producer in the world and this crop production is affected by citrus black spot disease, caused by *Guignardia citricarpa* Kiely [anamorphic *Phyllosticta citricarpa* (McAlpine) Aa], classified as a quarantine disease by the European Union and Mediterranean PPO (4). Symptoms, characterized by dark speckled spots or blotches on the rinds of fruit, develop late in the season or during postharvest if the fruit is not stored or transported properly, complicating its control (7). Alternative control measures beside the conventional chemical control require further investigation targeting black spot disease. Chitosan is a chitin deacetilated polymer, soluble in organic acids that can be used as fruit covering and exhibit microbial activity and potential as resistance inducer (3, 8, 9). Chitosan inhibited the development of new lesions of *G. citricarpa* in 'Valência' oranges at room condition or under refrigeration and stimulated defence responses in citrus skin (6). Fungicides benzimidazole and imazalil are usually used for fungal disease control on postharvest oranges (5) and treatments must not modify fruit visual and nutritional quality. Thus, the aim of this work was to evaluate the effect of chitosan for the control of *G. citricarpa* on 'Pêra Rio' oranges and also the fungicides thiabendazole and imazalil. The effects of chitosan on the postharvest quality of 'Pêra Rio' oranges were also investigated.

Fresh fruit of 'Pêra Rio' oranges (*Citrus sinensis* L. Osbeck)

produced in Mogi Guaçu (São Paulo State, Brazil) were harvested and taken to the lab. Fruit were washed with tap water plus commercial detergent and surface-sterilized with sodium hypochlorite (200 mg L^{-1}) for 10 min. All the spots on the naturally infected oranges were marked with a pen before treatments. Chitosan was supplied by Cyrbe Ltda (Sumaré, São Paulo State, Brazil) in the concentration of 10%, diluted in citric acid. The treatments were control, chitosan 2% (v/v), thiabendazole (480 g a.i./100 L^{-1}), imazalil (100 mL a.i./100 L^{-1}), chitosan plus thiabendazole and chitosan plus imazalil, applied in the same dosage used in the other treatments. Chitosan and thiabendazole were applied by spraying and imazalil by immersion of fruit for 2 min. After treatments, the fruit were stored at 25ºC/80% RH and evaluated 7 days later by counting the new developed black spot lesions on fruit surface. The experiment was repeated three times with similar results. The experimental design was completely randomized with 40 replications and one fruit per set and results were tested for significant differences by Tukey's test, probability of 5%.

Fruit total soluble solid, pH, citric and ascorbic acids and color of orange peel were evaluated immediately upon arrival from field (harvest day) and after the treatments storage period. Ten fruit of each treatment were evaluated regarding the physical-chemical attributes according to Carvalho et al. (1990). Skin color at three points of each fruit was determined by using a Minolta Chroma meter, model CR 300, parameters L, a, b. Total soluble solid content of the juice was measured with an Atago Hand Refractometer, model ATC-1E. The evaluation was carried out in replicate; 10 mL of orange juice was diluted with 90 mL of distilled water and the pH was determined with a Micronal pH meter, model B-274; titratable acidity was calculated as percent citric acid and titration was measured with 0.1 N NaOH at pH 8.1. The ratio was determined based upon the relationship between total soluble solids and titratable acidity. Ascorbic acid content was determined through 2,6-dichlorophenol-indophenol titration (2). The results were tested for significant differences by Tukey's test at the probability level of 5%.

Chitosan and fungicides when applied alone did not reduce the development of black spot new lesions on fruit, i.e., quiescent infections continued to appear on fruit. Imazalil and thiabendazole, even though the main fungicides used to control postharvest diseases of citrus, did not reduce the incidence of black spot. This shows that the efficiency of these products applied shortly before or after harvest may not be as expected. However, chitosan applied together with thiabendazole or imazalil resulted in remarkable reduction on the development of new lesions (Figure 1). Chitosan alone may have a direct effect on the pathogen as demonstrated previously (1, 6) and it was demonstrated in this work that chitosan helped the adhesion of the synthetic fungicides to the peel, which decreased the incidence of the disease. There are many reports on chitosan applications combined with other antifungal compounds increasing the efficiency of control of postharvest diseases as reviewed by Zhang et al. (9).

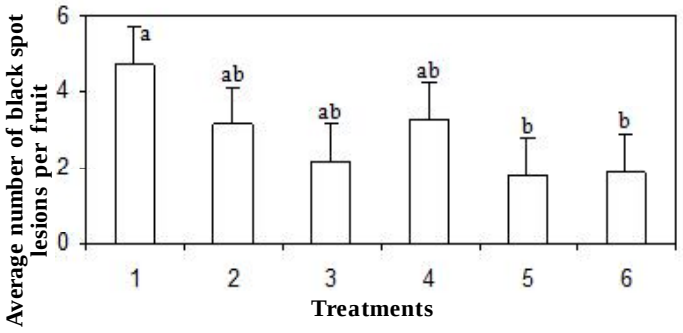

Figure 1. Effect of chitosan and the fungicides thiabendazole and imazalil on citrus black spot in 'Pêra Rio' oranges stored at 25ºC,80% RH and evaluated 7 days after treatment. Values with the same letters are not statistically different (Tukey 5%) and the bars represent the means ± SD (n=40). Treatments: 1- Control; 2- Chitosan 2% (v/v); 3- Thiabendazole (480 g a.i./100 L^{-1}); 4- Imazalil (100 mL i.a../100 L^{-1}); 5- Thiabendazole + Chitosan; 6- Imazalil + Chitosan. Chitosan and fungicides of treatment 5 and 6 were applied in the same concentrations used in treatments 2, 3 and 4.

The total soluble solids, pH and ascorbic acid of fruit submitted to the treatments remained practically unchanged after storage comparing to the fruit analysed in the harvest day, as they arrived from orchard. However, some changes were observed in titratable acidity and ratio. It was also observed an increase in fruit skin luminosity and chroma values (a and b parameters) (Table 1). Regarding to the treatments, titratable acidity showed a significant increase in fruit treated with thiabendazole and chitosan plus imazalil, compared to the control treatment. Thus, the ratio for these fruit also showed a significant reduction. The differences observed on b values for thiabendazole-treated fruit could be due the white residue on the orange surface that is removed by rotating brushes at the packing houses.

Table 1. Total soluble solids, pH, titratable acidity, ascorbic acid, ratio and skin color of 'Pêra Rio' oranges treated with chitosan and/or fungicides, stored at 25º C/80% RH and evaluated 7 days after treatment.

Treatments	Total soluble solids (%)	pH	Titratable acidity (% citric acid)	Ascorbic acid (mg 100 g^{-1})	Ratio (TSS/TA)	$L^{x,y}$	$a^{x,y}$	$b^{x,y}$
Harvest day	8.87	3.35	1.09	42.78	8.14	54.36	-20.17	39.87
Control	9.16 a	3.39 a	1.06 b	43.75 a	8.63 a	64.92 ab	-10.20 a	57.20 a
Chitosan	8.53 a	3.38 a	1.07 b	46.02 a	8.00 ab	59.15 b	-18.10 c	46.11 bc
Thiabendazole	8.84 a	3.27 a	1.29 a	48.31 a	6.83 b	66.35 a	-10.83 a	38.07 c
Imazalil	9.03 a	3.26 a	1.14 ab	46.84 a	7.90 ab	65.16 a	-11.54 ab	56.53 a
Ch + T[1]	8.52 a	3.36 a	1.09 b	45.91 a	7.79 ab	63.96 ab	-13.90 abc	50.23 ab
Ch + I[2]	8.74 a	3.28 a	1.30 a	44.22 a	6.74 b	61.86 ab	-17.66 bc	48.67 ab

Mean of ten fruit per treatment. Means followed by the same letters in the column are not significantly different from each other by Tukey test (P = 0.05).
[1] Chitosan + Thiabendazole; [2]Chitosan + Imazalil. [x] On Minolta colorimeter, L,a,b system, where L stands for luminosity (0=black to 100=white), a and b chroma (a^-=green to a^+=red and b^-=blue to b^+=yellow). [y] Mean of ten fruit per treatment. Means followed by the same letters in the column are not significantly different from each other by Tukey test (P = 0.05).

The change of the orange skin color of green to yellow was delayed by chitosan. These results show that the polymer has potential to maintain the qualities of the postharvest citrus and may prolong its storage life. It was not observed phytotoxicity symptoms caused by chitosan on orange fruit. The chitosan coating is non-toxic, safe and helps the long-term storage of food (3, 8).

Thus, the present work demonstrated that chitosan applied with the fungicides thiabendazole and imazalil enhanced the potential for controlling of black spot lesions on 'Pêra Rio' oranges. Also, chitosan delayed the change of the orange skin color during postharvest at room conditions and did not influence total soluble solids, titratable acidity, pH, ascorbic acid content and ratio. These results indicating that chitosan can be applied together with fungicides in packinghouses aiming the control of citrus black spot maintaining the quality of citrus fruit.

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