Postharvest biological control of brown rot in peaches after cold storage preceded by preharvest chemical control[1]

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RESUMO

Pathogenic fungi cause skin darkening and peach quality depreciation in post harvest. Therefore, alternative techniques to chemical treatment are necessary in order to reduce risks to human health. The aim of this study was to evaluate the effect of the application of *Trichoderma harzianum* in association with different fungicides applied before harvest to 'Eldorado' peaches for brown rot control and other quality parameters during storage. The treatments consisted of five preharvest fungicide applications (control, captan, iprodione, iminoctadine and tebuconazole) associated with postharvest application of *T. harzianum*, after cold storage (with and without application), in three evaluation times (zero, two and four days at 20 °C), resulting in a 5x2x3 factorial design. The application of *T. harzianum* only brought benefits to the control of brown rot when combined with the fungicide captan, at zero day shelf life. After two days, there was a greater skin darkening in peaches treated with *T. harzianum* compared with peaches without the treatment, except for peaches treated with the fungicide iprodione and *T. harzianum*. The application of T. *harzianum* during postharvest showed no benefits for the control of brown rot, however, the association with fungicides reduced the incidence of *Rhizopus stolonifer* during the shelf life.

Key words: *Monilinia fructicola, Trichoderma harzianum,* quality, skin browning.

RESUMO

Controle biológico pós-colheita da podridão parda em pêssego, após armazenamento refrigerado, antecedido de controle químico pré-colheita

Fungos fitopatogênicos causam escurecimento da epiderme e depreciação da qualidade de pêssegos em pós-colheita. Dessa forma, é preciso técnicas alternativas ao tratamento químico visando a reduzir os riscos para a saúde humana. O objetivo deste trabalho foi avaliar o efeito da aplicação de *Trichoderma harzianum* e sua associação com diferentes fungicidas aplicados pré-colheita em pêssegos 'Eldorado' para o controle da podridão parda e manutenção dos parâmetros de qualidade durante o armazenamento. Os tratamentos foram compostos por cinco aplicações pré-colheita de fungicidas (testemunha, captana, iprodiona, iminoctadina e tebuconazol) associado à aplicação pós-colheita de *T.harzianum*, após o armazenamento refrigerado (com e sem aplicação), mais três épocas de avaliações (zero, dois e quatro dias a 20 ºC), resultando em um esquema fatorial (5x2x3). A aplicação de *T. harzianum* só trouxe benefícios no controle de podridão parda quando associado ao fungicida captam a zero dias de

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vida de prateleira. Exceto quando os pêssegos foram submetidos à iprodione e *T. harzianum*, observou-se, após dois dias, maior escurecimento da epiderme em pêssegos com *T. harzianum* em comparação com pêssegos sem aplicação. A aplicação de *T. harzianum* durante a pós-colheita não mostrou benefícios no controle da podridão parda, no entanto, a associação com fungicidas, reduz a incidência de *Rhizopus stolonifer* durante a vida de prateleira.

Palavras-chave: *Monilinia fructicola*, *Trichoderma harzianum*, qualidade, escurecimento da epiderme.

INTRODUCTION

In the year 2012, Brazil ranked 14th in the global production of peaches, producing 220.000 tonnes, which correspond to 1% of the world's total production (FAO, 2012). In Brazil, the state of Rio Grande do Sul is the leading producer of peaches, with 86% of the total cultivated area (Agrianual, 2011). Nevertheless, some problems decrease the fruit quality during the development and postharvest life of peach, especially brown rot incidence.

Brown rot is caused by the fungus *Monilinia fructicola* (G. Wint.) Honey and is one of the most important diseases affecting peaches. The most typical symptoms observed in peach flowers are the necrosis of anthers, peduncle and ovary (May-De Mio *et al*., 2004). In the fruits, small brown lesions are observed at first, which later change into extended brown lesions. When the infection is severe, an intervention has to be made to control this disease.

Chemical control is the most widely adopted method, with sprays of fungicides from flowering to pre harvest, and the iprodione products triforine, procymidone, captan, mancozeb are recommended for disease control (May-De Mio *et al*., 2004). May-De Mio *et al*. (2011) attributes the preference for the chemical control because of its easiness of use and efficiency to control brown rot, mainly tebuconazole and azoxystrobin. However, the use of the same active ingredient for a few cultivations can promote resistance (Luo & Schnabel, 2008). In spite of all benefits that the fungicide bring to brown rot control, its use is very controversial and it is not allowed during postharvest life because it can harm consumers' health (Adaskaveg & Förster, 2010). During postharvest (storage time), the fungi have an appropriate environment for development and increase brown rot incidence. This fact highlights the need to develop new techniques for brown rot control throughout postharvest.

One technique that requires more studies is the biological control with *Trichoderma* spp., basically because its use is allowed during the postharvest (storage time). It is an anamorphic fungus found in most types of soil (Harman *et al*., 2004), the fungus has an antagonistic effect on *M. fructicola* and can help brown rot control (Hong *et al*., 1998). Fact that make the use of this fungus successful is its ability to survive within a wide range of temperatures and its broad spectrum action, such as antibiosis, competition and hyperparasitism (Janisiewicz *et al*., 2000; Howell, 2003; Harman *et al*., 2004, Brunner *et al*., 2005). Hong *et al*., (1998) demonstrated that *Trichoderma atroviride* and *Trichoderma viride* have great potential for the control of brown rot in postharvest and reduced between 63-98% incidence in peaches and 67-100% in plums. Nevertheless, the optimal concentration of *Trichoderma* and its interaction with the fungicides applied before harvest are not clear yet.

Thus, the aim of this research was to evaluate the effect of the application of *Trichoderma harzianum* Rifai and its association with different fungicides applied during the cultivation (before harvest) of 'Eldorado' peaches on brown rot incidence and other quality parameters during storage.

MATERIAL AND METHODS

This study was conducted in a commercial orchard located in the municipality of Santiago, Rio Grande do Sul, Brazil, and at the Postharvest Research Center of the Federal University of Santa Maria (UFSM). The treatments consisted of five preharvest fungicide applications (control, captan, iprodione, iminoctadine and tebuconazole) associated with postharvest application of *T. harzianum* after cold storage (with and without application), three evaluation times (zero, two and four days at 20 °C), resulting in a 5x2x3 factorial arrangement (Table 1). The antagonist used was *T. harzianum* (ETSR 20), obtained from the Phytopathology Laboratory (UFSM). The fungus development was stimulated on rice seeds (Ethur, 2006). To apply the antagonist, a suspension was prepared by washing the rice grain with distillated water. The fungus concentration in this suspension was adjusted to 9 x 10^7 conidia mL^{-1}. The peaches treated with *T. harzianum* were immersed in the antagonist solution for one minute before storage.

After storage, the parameters evaluated were: a) occurrence of *Monilinia fructicola (Brown rot)*; b) occurrence of *Rhizopus stolonifer* (Ehrenb.) Vuill*;* and c) occurrence of *Penicillium* spp. evaluated by counting the peaches that showed typical fungal lesion and data expressed as percentage of total fruits in the sample; d) *Skin browning* severity: assessed on a scale of 0 – 3 according to the amount of browning on the surface of the peaches, where 0 = 0% of darkened surface; 1 = > 0% up to 10% of darkened surface; 2 = > 10% up to 30% of darkened surface; 3 = > 30% of darkened surface, according to Brackmann *et al*. (2009a). The mean was obtained by the total number of peaches multiplied by their respective level of skin browning; this sum was then divided by the total number of peaches in the sample; e) *Ethylene production:* obtained by gas chromatography. A sample of one kilogram of peaches was hermetically sealed in a 5-L container. After one hour, 1mL of the head space was withdrawn and injected into a chromatograph. Ethylene production was calculated by taking into account ethylene concentration, fruit mass, free room inside the container and time and expressed as µL C_2H_4 kg^{-1} h^{-1}; f) *Respiratory rate*: obtained by circulating the air of the same container of ethylene production through an electronic gas analyzer: the respiration rate was calculated and expressed as mL CO_2 kg^{-1} h^{-1}.

The experiment was arranged in a 5x2x3 factorial arrangement, with four replications of 25 fruits. Before the analysis of variance (ANOVA), data were submitted to Lilliefors test to check normality. Those data that did not show normal distribution were transformed by the formula $((x + 0,5)/100)^{0,5}$ before the analysis of variance. After this, the means were submitted to Tukey's test with a 5% probability of error.

RESULTS AND DISCUSSION

A significant triple interaction between the application of fungicides, *T. harzianum* and the time evaluation for the parameters incidence of brown rot (Table 2), ethylene production, respiratory rate (Table 4) and skin browning (Table 5), while for the evaluation of the other fungi there was a factorial interaction (table 3).

When evaluating the association of fungicide application before harvest with *T. harzianum*in in the postharvest, the peaches of the control treatment showed higher brown rot incidence, during the whole evaluation, except for the fungicides iprodione and captan, right after the end of storage (zero days at 20°C) (Table 2). No significant difference was observed between the fungicides, up to two days of shelf life, when the peaches received *T. harzianum*. However, peaches without application of *T. harzianum* and tebuconazole before harvest had lower brown rot incidence at two days at 20°C. After four days of shelf life at 20°C, the lowest brown rot incidence was verified on peaches with te fungicide iminoctadine associated with the application of *T. harzianum* in postharvest. This result corroborates the findings of Moreira & May-De Mio (2009), who verified that the application of iminoctadine controlled 96% of brown rot incidence after 6 days of storage at 5 °C followed by 5 days at 20 °C.

Evaluation of the effects of *T. harzianum* of each treatment showed that its application did not bring benefits for the brown rot control, except for the fungicide captan right after the end of storage (Table 2). However, Hong *et al*., (1998) obtained 63 - 98% of brown rot control with four isolates of *T. harzianum* throughout the postharvest. These results differ from those obtained in the present study. This difference can be explained by the concentration of the antagonist used by those authors (10^8) in comparison with the concentration of $9x10^7$ conidia mL^{-1} used in this study.

The application of *T. harzianum* had no effect on brown rot control; however, a synergic effect with the fungicide application on *Rhizopus stolonifer* control was observed after four days of shelf life compared with the application of the fungicide alone (Table 3). In fact, *T. harzianum* showed an effective biological control of the disease during postharvest (Batta, 2007). The same author reported *Rhizopus stolonifer* control in peaches and strawberries with the application of *T. harzianum* before incubation at 20 °C. *Penicillium* spp. did not vary much between treatments; however, it is noteworthy that

Table 1: Preharvest fungicide application in association with postharvest *Trichoderma harzianum* application for brown rot incidence control in 'Eldorado', Santa Maria, RS, Brazil

Preharvest treatment		Postharvest	Evaluation time
		T.harzianum **application**	**Days 20 ºC**
Control – water application	With	Without	zero, two and four
Captan- (Orthocide - 240g 100L^{-1} -4x*)	With	Without	zero, two and four
Iprodione (Rovral – 150ml 100L^{-1} - 3x)	With	Without	zero, two and four
Iminoctadine (Belkute - 150ml 100L^{-1} - 2x)	With	Without	zero, two and four
Tebuconazole (Folicur - 100ml 100L^{-1}-3x)	With	Without	zero, two and four

* Active ingredient, commercial product, dose and number of applications in preharvest.

fruits treated with captan, with or without *T. harzianum*, had no *Penicillium* spp. incidence.

Regarding ethylene production, no significant difference was observed between the fungicides, with or without *T. harzianum*, right after the end of storage (Table 4). After two days of shelf life, higher ethylene production was found in the peaches of the control treatment, probably because of the higher brown rot incidence in the same treatment (Table 2). Another study showed that higher rot incidence culminated in elevated ethylene production in peaches (Brackmann *et al.*, 2009a). After four days, higher ethylene production was

Table 2: Brown rot incidence of 'Eldorado' peaches treated before harvesting with fungicide and stored at -0.5 °C for 40 days and four days at 20 °C in association with *Trichoderma harzianum* treatment after storage

Treatments		Brown rot (%)		
		Days at 20 ºC		
		zero	Two	Four
With *T. harzianum*	Control (Water)	4.30Ca*	26.9Ba	87.4Aa
	Captan	1.12Cab	11.4Bb	47.3Ab
	Iprodione	4.50Ba	9.04Bb	23.8Ac
	Iminoctadine	0.00Bb	8.51Ab	8.51Ad
	Tebuconazole	0.00Cb	9.15Bb	21.5Ac
Without *T. harzianum*	Control (Water)	4.13Ca	12.6Ba	80.2Aa
	Captan	4.34Ca	13.0Ba	43.7Ab
	Iprodione	0.00Cb	6.72Bab	21.9Ab
	Iminoctadine	0.00Bb	5.02Ab	9.77Ac
	Tebuconazole	0.00Bb	1.04Bc	18.2Ac
Control	With *T. harzianum*	4.30a	26.9a	87.4a
	Without *T. harzianum*	4.13a	12.6b	80.2a
Captan	With *T. harzianum*	1.14b	11.4a	47.3a
	Without *T. harzianum*	4.34a	13.0a	43.7b
Iprodione	With *T. harzianum*	4.50a	9.04a	23.8a
	Without *T. harzianum*	0.00b	6.72a	21.9a
Iminoctadine	With *T. harzianum*	0.00a	8.51a	8.51a
	Without *T. harzianum*	0.00a	5.02a	9.77a
Tebuconazole	With *T. harzianum*	0.00a	9.15a	21.5a
	Without *T. harzianum*	0.00a	1.04b	18.2a
CV fungicides	**13.8**			
CV *T. harzianum*	**15.0**			
CV Days at 20 ºC	**12.7**			

*Means followed by the same small letters in the columns and capital letters in the rows are not significantly different by the Tukey's test at 5% probability.

Table 3: Incidence of *Rhizopus stolonifer* and *Penicillium* spp. in 'Eldorado' peaches treated before harvesting with fungicide and stored at -0.5 °C for 40 days and four days at 20 °C, in association with *Trichoderma harzianum* treatment after storage

Treatments	***Rhizopus stolonifer*** **(%)**			***Penicillium*** **spp. (%)**		
	With	Without	Mean	With	Without	Mean
	T. harzianum			*T. harzianum*		
Control (water)	4.26Abc	6.21Ab	5.24	0.75Acd	1.00Abc	0.88
Captan	7.86Bab	15.6Aa	11.71	0.00Ad	0.00Ac	0.00
Iprodione	2.27Bc	6.77Ab	4.52	1.75Abc	2.22Ab	1.99
Iminoctadine	10.8Ba	17.4Aa	14.10	8.00Ba	15.4Aa	11.7
Tebuconazole	6.94Bab	22.3Aa	14.62	4.25Ab	2.00Bbc	3.13
Mean	6.43	13.6		2.95	4.12	
CV(%)		13.6		21.5		

*Means followed by the same small letters in the columns and capital letters in the rows are not significantly different by the Tukey's test at 5% probability.

observed in the fruit of the control and iminoctadine treatments. Nevertheless, without *T. harzianum*, the behavior was different and a significant difference was only observed between treatments after four days of shelf life, if the treatment with captan showed higher ethylene production.

Comparing the effect of *T. harzianum*in in each fungicide treatment, it was found that the antagonist application increased ethylene production significantly in all treatments, after two days of shelf life at 20 °C. This higher ethylene production influenced the fruit negatively, because ethylene triggers a series of events that culminate in fruit ripening. Kiwi fruit infected with *Botrytis cinerea* showed faster pulp softening as a result of high ethylene production (Brook, 1991). Ethylene is a compound produced by plants and some microorganisms, such as *Verticillium* spp., *Fusarium* spp., *Colletotrichum* spp. and *Botrytis cinerea* (Tzeng & De Vay, 1984; Kader, 1992; Qadir *et al*., 1997; Cristescu *et al*., 2007; Cantu *et al*., 2009).

Right after the end of storage, the respiration rate was higher in the peaches without fungicide treatment combined with *T. harzianum* (Table 4). After two and four days of shelf life, the highest respiration rate was verified in the peaches of the control treatment. Similar results were found in the peaches without the application of *T. harzianum*, where the peaches of the control treatment showed higher respiration rate at chamber opening and four days of shelf life. These results are probably associated with ethylene production of the *T. Harzianum* fungus, leading to autocatalytic ethylene production, since it is strongly stimulated by exogenous factors, such as fungus infections (Yang & Hoffman, 1984). This process accelerates fruit metabolism and increases respiration and fruit ripening (Chitarra & Chitarra, 2005).

After two days of shelf life, higher skin browning was observed in the peaches treated with *T. harzianum* as compared to with peaches without the fungus treatment, except for peaches with iprodione and *T. harzianum*

Table 4: Ethylene production and respiratory rate of 'Eldorado' peaches treated before harvesting with fungicide and stored at -0.5 °C for 40 days and four days at 20 °C, in association with *Trichoderma harzianum* treatment after storage

Treatments		**Ethylene (μL C_2H_4 kg^{-1} h^{-1})**			**Respiration (mL CO_2 kg^{-1} h^{-1})**		
		Days at 20 ºC			**Days at 20 ºC**		
		Zero	**two**	**four**	**Zero**	**two**	**Four**
With *T. harzianum*	Control (Water)	2.55Ca*	33.2Ba	86.4Aa	25.2Ca*	45.0Ba	63.2Aa
	Captan	2.39Ca	30.7Bb	83.2Ab	13.5Cc	35.3Bb	44.5Ac
	Iprodione	1.77Ca	23.5Bd	58.8Ad	16.5Cb	22.7Bc	41.6Ad
	Iminoctadine	2.42Ca	24.6Bcd	85.9Aa	23.8Ba	18.2Cd	50.8Ab
	Tebuconazole	1.96Ca	26.3Bc	70.3Ac	18.5Bb	17.4Bd	33.5Ae
Without *T. harzianum*	Control (Water)	0.74Ca	4.22Ba	11.6Ab	24.0Ca	32.6Bb	38.8Aa
	Captan	0.52Ca	3.34Ba	14.9Aa	15.6Cc	27.5Bc	32.5Acd
	Iprodione	0.53Ca	3.38Ba	7.87Ac	17.4Cc	31.7Bb	34.8Abc
	Iminoctadine	0.65Ca	3.13Ba	9.60Abc	19.9Bb	35.2Aa	36.2Ab
	Tebuconazole	0.64Ca	3.61Ba	10.5Ab	16.3Cc	24.5Bd	32.5Ad
Control	With *T. harzianum*	2.54a	33.2a	86.5a	25.3a	45.0a	63.2a
	Without *T. harzianum*	0.74b	4.21b	11.6b	24.0a	32.6b	38.8b
Captan	With *T. harzianum*	2.39a	30.8a	83.2a	13.5b	35.3a	44.5a
	Without *T. harzianum*	0.52b	3.46b	14.9b	15.6a	27.5b	32.5b
Iprodione	With *T. harzianum*	1.77a	23.5a	58.9a	16.5a	22.7b	41.6a
	Without *T. harzianum*	0.53a	3.37b	7.87b	17.3a	31.7a	34.7b
Iminoctadine	With *T. harzianum*	2.42a	24.6a	85.9a	23.7a	18.2b	50.8a
	Without *T. harzianum*	0.65b	3.13b	9.60b	20.0b	35.2a	36.2b
Tebuconazole	With *T. harzianum*	1.96a	26.3a	70.3a	18.5a	17.5b	33.5a
	Without *T. harzianum*	0.64a	3.61b	10.5b	16.3b	24.5a	32.5a
CV fungicides			4.49			4.01	
CV *T. harzianum*			4.00			2.86	
CV Days at 20 ºC			3.78			4.62	

*Means followed by the same small letters in the columns and capital letters in the rows are not significantly different by the Tukey's test at 5% probability.

Table 5: Skin browning of 'Eldorado' peaches treated before harvesting with fungicide and stored at -0.5 °C for 40 days and four days at 20 ºC, in association with *Trichoderma harzianum* treatment after storage

Treatments		**Skin browning**		
		Daysat20 ºC		
		zero	**two**	**four**
With *T. harzianum*	Control (Water)	0.30Aa*	0.38Ab	0.38Ac
	Captan	0.18Cab	0.64Ba	0.95Aa
	Iprodione	0.10Bab	0.11Bc	0.56Ab
	Iminoctadine	0.12Cab	0.27Bbc	0.78Aa
	Tebuconazole	0.08Cb	0.33Bb	0.49Ab
Without *T. harzianum*	Control (Water)	0.18Aa	0.19Aab	0.23Aab
	Captan	0.19Aa	0.25Aa	0.27Aa
	Iprodione	0.15Aa	0.17Aab	0.18Ab
	Iminoctadine	0.08Ab	0.09Ab	0.15Ab
	Tebuconazole	0.02Ab	0.02Ab	0.02Ac
Control (Water)	With *T. harzianum*	0.30a	0.38a	0.38a
	Without *T. harzianum*	0.18b	0.19b	0.22b
Captan	With *T. harzianum*	0.18a	0.64a	0.96a
	Without *T. harzianum*	0.19a	0.25b	0.27b
Iprodione	With *T. harzianum*	0.10a	0.11a	0.56a
	Without*T. harzianum*	0.15a	0.17a	0.18b
Iminoctadine	With *T. harzianum*	0.12a	0.27a	0.78a
	Without *T. harzianum*	0.08a	0.09b	0.15b
Tebuconazole	With *T. harzianum*	0.08a	0.33a	0.49a
	Without *T. harzianum*	0.02a	0.02b	0.02b
CV fungicides		**40.7**		
CV *T. harzianum*		**29.80**		
CV Days at 20 ºC		**19.8**		

*Means followed by the same small letters in the columns and capital letters in the rows are not significantly different by the Tukey's test at 5% probability.

(Table 5). Between the fungicides in association with *T. harzianum*, the fruits of the control treatment showed greater skin browning at chamber opening and differ significantly from the tebuconazole treatment. The evaluation after two days of shelf life revealed greater skin browning of the peaches of the captan treatment, whereas at four days, the highest skin browning level was observed in the peaches of the captan and iminoctadine treatments. This greater skin browning of peaches treated with *T. harzianum* was probably associated with the growth of the antagonist fungus. Another study suggested that skin browning results from the contact of the skin with water during the application of postharvest fruit treatment (Brackmann *et al*., 2009b).

Lower incidence of skin browning in fruit treated with iprodione fungicide after two days of evaluation may be due to the high sensitivity of *T. harzianum* to the active ingredient of the fungicide iprodione. *In vitro* tests demonstrated the negative effect of iprodione on mycelia development and sporulation of *T. harzianum* and *T. viride* (Silva *et al*., 1999). Another study demonstrated that the same fungicide reduced the development of an antagonist of *Trichothecium roseum* (Moreira & May De Mio, 2007) by 80%. Among the peaches without *T. harzianum* treatment, the greatest skin browning was observed in fruits with the captan treatment, after four days of shelf life (Table 5). These results indicated that the *T. harzianum* and the fungicide captan negatively affected fruit quality with increased skin browning.

CONCLUSION

The application of *T. harzianum* during postharvest did not bring benefits to brown rot control, however, the association of *T. harzianum* and fungicides reduced the incidence of *Rhizopus stolonifer* during shelf life. The fungicide captan increased skin browning during shelf life, mainly when associated with the application of *T. harzianum*, but inhibited the incidence of *Penicillium* spp. The application of *T. harzianum* in postharvest increased ethylene production in all fungicide treatments of this study.

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