ALTERNATIVE SPRAYING PROGRAMS TO CONTROL BROWN ROT ON INTEGRATED PRODUCTION SYSTEMS

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SUMMARY

Four management programs for the control of peach brown rot to be recommended for integrated production systems in Brazil were tested. The control of Monilinia fructicola *was assessed for programs using biological control with the plant pathogenic fungus* Trichothecium roseum*, fungicides and phosphites. The latent infection rates of* M. fructicola *on flowers and immature fruit, in addition to the brown rot incidence at harvest and postharvest, were quantified for two experimental years. All of the tested treatments reduced the incidence of peach brown rot in the 2003/04 season. However, during the following season, when the inoculum pressure was greater, the chemical treatment (alternating iprodione, azoxystrobin, tebuconazole, mancozeb and captan) and the integrated 1 treatment (alternating* T. roseum *and Ca and K phosphites plus captan) were more efficient at reducing disease incidence in comparison with the biological treatment alone (*T. roseum *at 10^6 conidia/ml) and integrated 2 treatment (*T. roseum *and captan). In conclusion,* T. roseum *applied at blooming and preharvest, when combined with captan plus phosphites applied during the fruit development period provide brown rot control at a level equivalent to fungicidal program and can be recommended to integrated production systems.*

Introduction

Monilinia fructicola (G. Wint) Honey is the main species in Brazilian stone fruit orchards and causes both blossom blight and brown rot. The main sources of *M. fructicola* inoculum are cankers on branches or new twigs formed from infected flowers, infected fruits and mummified fruits that remain attached to the plant. The pathogen can infect fruits in both the early and late stages of development, remaining latent until the beginning of fruit maturation (Keske *et al*., 2011). In pre or postharvest periods and during storage, healthy fruits may be carriers of latent brown rot infections; when these latent infections develop into disease, the fruits show symptoms, affecting the economics of production. Pesticides are used in agriculture to increase crop yield through control of diseases and pests, and also by preservation after harvesting. However, the pesticide residues have been found in fruits and vegetables can have negative health effects on consumers (Keikotlhaile *et al*., 2010) and resistant fungal isolates develop due to continuous use (May-De Mio *et al*., 2011). Despite these risks, the use of fungicides is vital to the control of brown rot. Moreira and May-De Mio (2009) reported that 90% of the brown rot incidence caused by *M. fructicola* in a late cultivar of peach occurred during the preharvest period, during which no fungicide was applied in the South Brazilian orchard.

Fungicides, such as dicarboximides, benzimidazoles, triazoles, captan, mancozeb, methiram, propineb, thiram, folpet, chlorotalonil and ziram, can suppress the sporulation of *M. fructicola* on infected tissues (Melgarejo and De Cal, 2010). Benzimidazole, dicarboximide and triazole fungicides have been recommended for the chemical control of brown rot disease in the field during bloom and preharvest periods (Yoshimura *et al*., *2004*). Captan has shown efficient control of *M. fructicola* in peach and has been widely recommended for different diseases in temperate fruits (Guerra *et al*., 2007; May-De Mio *et al*., 2008; Adaskaveg *et al*., 2009; Peres *et al*., 2010); it is used on a regular schedule in integrated production systems for several fruit species in Brazil (MAPA, 2007).

The control of brown rot is a major challenge to the production of high quality peaches in accordance with the requirements of environmental sustainability, food safety and economic viability, and it requires the use of technologies that are not harmful to the environment or to human health. Integrated fruit production (IFP) programs have become a requirement for import markets. The European Community, for example, has very stringent requirements with regard to quality and sustainability (Moraes, 2002). To respond to these market demands, some peach growers

KEYWORDS / Biological Control / Brown Rot / Chemical Control / *Monilinia fructicola* / Postharvest / *Prunus persica* /

Received: 09/02/2012. Modified: 04/15/2014. Accepted: 04/21/2014.

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PROGRAMAS ALTERNATIVOS DE FUMIGACIÓN PARA EL CONTROL DE LA PODREDUMBRE PARDA EN SISTEMAS INTEGRADOS DE PRODUCCIÓN

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RESUMEN

*Cuatro programas de manejo basados en el uso de fungicidas, agentes biológicos y fosfitos para el control de la podredumbre parda del durazno (*Monilia fructicola*) fueron probados con el objetivo de ser incorporados en sistemas de producción integrada en Brasil, buscando atender las exigencias de cualidad y sustentabilidad del mercado mundial. Las tasas de infección latente de* M. fructicola *en flores y frutos inmaduros, y la incidencia de la enfermedad en la cosecha y poscosecha fueron cuantificados durante dos años experimentales. Todos los tratamientos ensayados redujeron la incidencia de la podredumbre parda del durazno en la cosecha 2003/04. Sin embargo, durante 2004/05, en que la presión del inóculo fue mayor, el tratamiento químico (alternando iprodione, azoxistrobina, tebuconazole, mancozeb y captan) y el tratamiento integrado 1 (alternando el antagonista* T. roseum *y fosfitos de Ca y K más captan) fueron más eficientes en la reducción de la incidencia de la enfermedad, en comparación con el tratamiento biológico solo (*T. roseum *a 10^6 conidios/ml) y el tratamiento integrado 2 (*T. roseum *y captan). En conclusión,* T. roseum *aplicado en la floración y precosecha, en conjunto con la aplicación de captan más fosfitos durante el periodo de desarrollo del fruto, promueve el control de la enfermedad en un nivel semejante al alcanzado por los fungicidas y puede ser recomendado en el sistema de producción integrado.*

PROGRAMAS ALTERNATIVOS DE PULVERIZAÇÃO PARA CONTROLE DA PODRIDÃO MARROM EM SISTEMAS INTEGRADOS DE PRODUÇÃO

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RESUMO

Quatro programas de manejo baseados no uso de fungicidas, agentes biológicos e fosfitos para o controle de Monilinia fructicola*, foram testeados na podridão parda do pêssego, visando recomendação em sistemas de produção integrada no Brasil para atender as exigências de qualidade e sustentabilidade do mercado mundial. As taxas de infecção latente de* M. fructicola *em flores e frutos imaturos e, a incidência da podridão parda na colheita e pós-colheita foram quantificados por dois anos experimentais. Todos os tratamentos testados reduziram a incidência de podridão parda do pêssego na safra 2003/04. No entanto, durante 2004/05, em que a pressão de inóculo foi maior, o tratamento químico (alternando iprodione, azoxistrobina, tebuconazole, mancozeb e captan) e o tratamento integrado 1 (alternando o antagonista* T. roseum *e fosfitos de Ca e K mais captan) foram mais eficientes na redução da incidência da doença em comparação com o tratamento biológico (apenas* T. roseum *a 10^6 conídios/ml) e o tratamento integrado 2 (*T. roseum *e captan). Em conclusão,* T. roseum *aplicado em floração e pré-colheita, quando combinado com captan mais fosfitos aplicados durante o período de desenvolvimento do fruto, promoveu o controle de podridão parda em um nível equivalente ao programa de fungicidas e pode ser recomendado no sistema de produção integrada.*

in Southern Brazil have begun to search for alternatives to the replace traditional chemical control of the disease by fungicides, as is recommended by the integrated peach production rules (Fachinello *et al.,* 2003).

Several alternative technologies, such as antagonistic microorganisms and phosphites, represent a promising approach for replacing or reducing the application of synthetic fungicides. Control by biofungicides, such as the antagonistic microorganisms *Muscodor albus* (applied by fumigation during the postharvest period; Mercier and Jimenez, 2004; Schnabel and Mercier, 2006), *Epicoccum nigrum* (applied by spraying in the field; Larena *et al*., 2005), and *Penicillium frequentans* (applied by spraying in the field and postharvest; Guijarro *et al.,* 2007), has been reported. In Brazil, *Trichothecium roseum* (Pers.: Fr.) Link, applied during both the bloom and preharvest periods (Moreira *et al*., 2008) and postharvest treatments (Moreira *et al*., 2002) has demonstrated efficient inhibition of *M. fructicola* in peach. Additionally, Moreira and May-De Mio (2007) have observed that captan and phosphites have minor effects on this antagonistic action on *T. roseum*. Phosphites have been sprayed in apple trees to control *Phytophthora* spp. (Smith and Smith), *Venturia inaequalis* (Cke.) Wint. and *Colletotrichum* spp. (Penz and Sacc.) (Boneti and Katsurayama, 2005). Despite the efficiency of several of these alternative methods, none of them have been tested in management programs for commercial orchards and for consecutive seasons.

The objective of this study was to test four management programs using fungicides, *T. roseum* antagonist, and Ca and K phosphites for the control of peach brown rot. This objective was accomplished through the assessment of the incidence of *M. fructicola* on flowers and fruit, as well as the rates of peach brown rot at harvest and postharvest. The tested treatments have been recommended to Brazilian growers who are adopting integrated production systems.

Materials and Methods

The monitoring of *M. fructicola* and the application of biological, chemical and integrated treatments were conducted in an orchard with four-years-old peach trees (cv. BR-1) spaced at 1.5 and 6.0m between trees and rows, respectively, located in the Lapa Municipality, Paraná State, in Southern Brazil, during the 2003/04 and 2004/05 growing seasons. The cv. BR-1 is a late maturing and very susceptible to brown rot cultivar.

The experimental design was randomized blocks, with five treatments, six replications and three plants per parcel. Two trees from each parcel were used as side borders, and only the data from the central tree (experimental unit) were assessed to eliminate the effect of spray drift. Untreated trees were used as control. The treatments and timing of applications for both seasons are summarized in Tables I and II. For the two seasons the same orchard was used and the treatments applied to the same trees. Although, on the second season the control was used only as a reference, no repetition was performed for this treatment. This occurred due a problem with the producer, who was worried about the high amount of inoculum in the area.

The antagonist *T. roseum* was sprayed at 10^6 conidia/ml. The fungicides iprodione (1.5 $ml{\cdot}l^{-1}$), azoxystrobin ($0.2g{\cdot}l^{-1}$), tebuconazole ($1.0ml{\cdot}l^{-1}$) and mancozeb ($2.0g{\cdot}l^{-1}$), which were recommended by the integrated production of peaches system of Paraná (IPP-PR), were tested at the recommended concentrations of the active ingredient in accordance with Brazilian rules (MAPA, 2007), and captan was applied at the label rate ($2.4ml{\cdot}l^{-1}$). Ca phosphite (20.8% P_2O_5 - 4% Ca) and K phosphite (40% P_2O_5 - 20% K_2O) from Agro Commercial Wiser Ltda., Diadema, São Paulo, Brazil, were used at 4.0 and $2.0ml{\cdot}l^{-1}$, respectively, in accordance with recommendations of the industry. All treatments were applied until runoff, in the morning, using a Jacto backpack sprayer with 16 liters of solution, operating pressure of 10^6 Pa, and a conical cone nozzle.

Conidial production of T. roseum and preparation of the suspension for spraying

The *T. roseum* isolate UFPR/LEMID-F4 was used due to its previously demonstrated efficacy against *M. fructicola* (Moreira *et al.*, 2002, Moreira and May-De Mio, 2007). This isolate was collected from a commercial peach orchard cv. Chimarrita in 1997 and maintained in the collection of the Epidemiology Laboratory, Department of Crop Protection, Universidade Federal de Paraná, Brazil.

Conidia were produced by growing the fungus on wheat grains used as a solid substrate. The wheat grains were boiled in water for 15min, filtered into glass pots in 200cc portions and then sterilized by autoclaving at 121ºC for 25min on two consecutive days.

Conidia from colonies of the UFPR/LEMID-F4 isolate that had been cultivated in potato dextrose agar (PDA) medium and incubated at 25ºC in the dark for 7 days were used to inoculate the wheat grains. Conidia were removed with 10ml of sterile potato dextrose broth (PDB) medium and added to the wheat grains. After homogenization, the glass pots were closed and stored in the dark at 25ºC for 10 days. Colonized grains were mixed with water, stirred and filtered, and the resulting conidia suspension was adjusted to 10^6 conidia/ml using a hemocytometer. Then, $0.5ml{\cdot}l^{-1}$ of AG-Bem adhesive (Rohm and Haas Ltda) was added to the suspension in preparation for spraying.

A 100µl aliquot of the each aqueous suspension used for spraying was spread on the surface of water agar medium (WA) and incubated at room

TABLE I
TREATMENTS APPLIED TO THE EXPERIMENTAL PEACH ORCHARD (CULTIVAR BR-1) IN LAPA MUNICIPALITY, PARANÁ STATE, IN SOUTHERN BRAZIL, DURING THE 2003/04 GROWING SEASON

2003/04		Treatments				
Stage	Timing	Control	Biological	Chemical*	Integrated 1	Integrated 2
Blooming	01 Sep	untreated	*T. roseum*	azoxystrobin	*T. roseum*	*T. roseum*
	12 Sep	untreated	*T. roseum*	untreated	*T. roseum*	*T. roseum*
	18 Sep	untreated	*T. roseum*	iprodione	*T. roseum*	*T. roseum*
	22 Sep	untreated	*T. roseum*	untreated	Ca phosphite + captan	*T. roseum*
Fruit development	29 Sep	untreated	untreated	captan	Ca phosphite + captan	captan
	13 Oct	untreated	*T. roseum*	mancozeb + Ca phosphite	Ca phosphite + captan	captan
	23 Oct	untreated	*T. roseum*	azoxystrobin	K phosphite + captan	captan
	03 Nov	untreated	*T. roseum*	untreated	K phosphite + captan	captan
	13 Nov	untreated	*T. roseum*	captan	K phosphite + captan	captan
	24 Nov	Untreated	*T. roseum*	tebuconazole	K phosphite + captan	captan
	04 Dec	untreated	*T. roseum*	untreated	K phosphite + captan	captan
Preharvest	11 Dec	untreated	*T. roseum*	azoxystrobin	*T. roseum*	*T. roseum*
	18 Dec	untreated	*T. roseum*	untreated	*T. roseum*	*T. roseum*
Total spray per season		none	12	8	13	13

TABLE II
TREATMENTS APPLIED TO THE EXPERIMENTAL PEACH ORCHARD (CULTIVAR BR-1) IN LAPA MUNICIPALITY, PARANÁ STATE, IN SOUTHERN BRAZIL, DURING THE 2004/05 GROWING SEASON

2004/05		Treatments				
Stage	Timing	Control	Biological	Chemical*	Integrated 1	Integrated 2
Blooming	12 Aug	untreated	*T. roseum*	azoxystrobin	*T. roseum*	*T. roseum*
	19 Aug	untreated	*T. roseum*	untreated	*T. roseum*	*T. roseum*
	23 Aug	untreated	untreated	iprodione	untreated	untreated
Fruit development	31 Aug	untreated	*T. roseum*	untreated	*T. roseum*	*T. roseum*
	10 Sep	untreated	*T. roseum*	untreated	Ca phosphite + captan	captan
	17 Sep	untreated	*T. roseum*	captan	Ca phosphite + captan	captan
	29 Sep	untreated	*T. roseum*	mancozeb + Ca phosphite	Ca phosphite + captan	captan
	07 Oct	untreated	*T. roseum*	untreated	K phosphite + captan	captan
	21 Oct	untreated	*T. roseum*	azoxystrobin	K phosphite + captan	captan
	27 Oct	untreated	*T. roseum*	untreated	K phosphite + captan	captan
	12 Nov	untreated	*T. roseum*	captan	K phosphite + captan	captan
	18 Nov	untreated	*T. roseum*	untreated	K phosphite + captan	captan
Preharvest	20 Nov	untreated	untreated	tebuconazole	untreated	untreated
	22 Nov	untreated	*T. roseum*	untreated	K phosphite + captan	captan
	26 Nov	untreated	*T. roseum*	untreated	*T. roseum*	*T. roseum*
Total spray per season		none	13	7	13	13

TABLE III

INCIDENCE (%) OF *Monilinia fructicola* ON FLOWER STRUCTURES, LATENT INFECTION (%) ON FLOWERS AND GREEN FRUIT AND BROWN ROT ON FRUIT AT THE HARVEST OF CV. BR-1 DURING THE 2003/04 AND 2004/05 GROWING SEASONS, LAPA MUNICIPALITY, PARANÁ STATE, BRAZIL

Stage	Blooming [x]				Fruit development		Harvest [x]
Incidence (%)	*M. fructicola* [y]		Blossom blight		Latent infection Green fruit		Brown rot
Treatments*							
2003/04	08 Sep	25 Sep	08 Sep	25 Sep	5 Nov	15 Nov	22, 26 and 27 Dec
Control	28.3 ns	26.7 ns	0.0	0.0	0.0	0.0	76.0 a
Biological	25.0	16.7	0.0	0.0	0.0	0.0	36.0 b
Chemical	36.7	16.7	0.0	0.0	0.0	0.0	14.0 b
Integrated 1	36.7	6.7	0.0	0.0	0.0	0.0	15.0 b
Integrated 2	26.7	11.7	0.0	0.0	0.0	0.0	20.0 b
Correlation with harvest	none	0.77	none	none	none	none	1.00
2004/05	19 Aug	30 Aug	19 Aug	30 Aug	10 Nov	20 Nov	26-30 Nov 3 Dec
Control [z]	100.0	100.0	2.0	6.0	0.0	40.0	68.0
Biological	100.0 ns	100.0 ns	0.7 ns	0.6 ns	6.0 ns	27.0 a	73.0 a
Chemical	100.0	100.0	0.0	0.3	0.0	8.0 b	43.0 b
Integrated 1	100.0	100.0	1.0	2.0	5.0	2.0 b	45.0 b
Integrated 2	100.0	100.0	1.6	1.6	6.0	17.0 a	62.0 a
Correlation with harvest	none	none	none	none	none	0.61	1.00

* Control: untreated trees, Biological: *Trichothecium roseum*; Chemical: iprodione, azoxystrobin, tebuconazole, mancozeb and captan (treatments of Fungicide Program recommended by the Integrated Production of Peaches); Integrated 1**:** *T. roseum*, Ca phosphite + captan and K phosphite + captan; and Integrated 2**:** *T. roseum* and captan.

[x] For statistical analysis, flowering and harvest data were transformed to arcsine $\sqrt{x}$. In each column, means with the same letter are not significantly different (p=0.05) as determined by the Tukey test.

[y] *M. fructicola* in the flowers anthers or stigma.

[z] Values of the control on the second season were used only as a reference, because no repetition was performed for this treatment.

temperature. After 24h, 100 conidia were recorded for germination. A conidium was considered germinated if the length of its germ tube was longer than the length of the conidium itself.

Incidence of M. fructicola on flowers, latent infection on immature fruit, and brown rot at harvest and postharvest

In the 2003/04 season, the incidence of *M. fructicola* on flowers and the effect of flower disinfestations on the detection of the pathogen in floral structures, mainly in the pistil (stigma and style) and stamen (anthers and filament), were verified using a stereoscopic microscope (40×). Twenty flowers per parcel were randomly collected from each sampled tree (experimental unit) one day before the first and second treatment sprayings, on Sept 1 and 18, respectively. Ten flowers were disinfested by immersion in a 3% Na hypochlorite solution for 1min and subsequently rinsed with sterile water. Ten flowers immersed only in sterile water served as a control. The flowers were incubated in a humidity chamber (plastic boxes GERBOX (11×11×5cm)) lined with damped filter paper and stored at ambient conditions (22 ±2 ºC) for one week.

In the 2004/05 season, 50 open flowers per parcel were collected from each sampled tree (experimental unit) one day before the first and second fungicide sprayings, on Aug 12 and 23, respectively. The non-disinfested flowers were incubated as described above. The presence of *M. fructicola* as an epiphyte or as a latent infection causing blossom blight on petals was assessed one week later.

The level of latent infection by *M. fructicola* was determined using the method described by Northover and Cerkauskas (1994). Samples of 10 immature fruits per parcel were collected on Oct 22, Nov 3 and Dec 12 (2003/04) and Nov 10 and 20 (2004/05) and disinfested in an aqueous solution of 70% ethanol, 2% Na hypochlorite, and 2% paraquat for 1min. Following surface sterilization, the fruits were washed with sterile water and incubated at 23 ±2ºC at 85% relative humidity. Latent infection was evaluated after 10 days of incubation.

At harvest, the incidence of brown rot was assessed three times for each sampled tree (central tree -experimental unit) for fruits collected on Dec 22, 26 and 27 of 2003 and Nov 26 and 30 and Dec 3 of 2004 considering all fruit per tree. At postharvest, five fruits/date of harvest/parcel were placed in alveoli plastic trays maintained at room temperature (25 ±2ºC). The brown rot disease incidence was evaluated on days 3 and 5 after harvest.

The data regarding disease incidence were analyzed using ANOVA, and means were separated by LSD (least significant difference) and Tukey's multiple range test ($p<0.05$) using SAS version 6.1 (SAS Institute, Cary, NC, USA). Correlations between the disease incidence for various fruit developmental stages and the incidence of brown rot assessed at both harvest and postharvest were also determined using Statistica software version 8.0 (Tulsa, OK, USA). Temperature, relative humidity and rainfall data recorded at the weather station code 2549091 of Lapa Municipality, Paraná State, Brazil, during the 2003/2004 and 2004/2005 growing seasons were provided by SIMEPAR meteorological system of Paraná.

Results

The treatments applied did not significantly reduce the incidence of *M. fructicola* on the pistils and stamens after two applications as compared to the control (Tukey test, $p>0.05$), for either of two seasons (Table III). The average incidence of *M. fructicola* on the flower structures was lower in disinfested flowers than in non-disinfested flowers ($p>0.05$), and the results are summarized in Figure 1 for the two collection dates of the 2003/04 season.

In the 2004/05 season, *M. fructicola* was detected in 100% of the flower structures. Additionally, typical symptoms of blossom blight that were not observed in 2003/04 season, such as necrotic lesions on the sepals and sporodochial structures of the pathogen, were now observed on the hypanthea of the flowers, but no significant differences were observed between the treatments (Tukey test, p= 0.05; Table III).

Latent infections on immature fruits were not detected during the two evaluations conducted in the 2003/04 season. In the 2004/05 season, the number of latent infec-

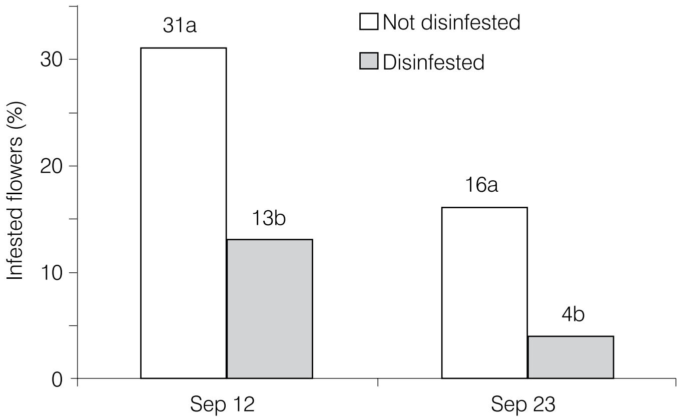

Figure 1. Incidence of *Monilinia fructicola* on disinfested (alcohol and sodium hypochlorite) and not disinfested peach flowers collected during the blooming period of the 2003/04 growing season, Lapa Municipality, Paraná State, Brazil. Columns with the same letter do not differ among themselves by the Scott-Knott test at 5% of nominal significance (p=0.05).

TABLE IV
INCIDENCE (%) OF BROWN ROT ON PEACHES (THAT HAD PREVIOUSLY RECEIVED THE APPLICATION OF THE TREATMENTS IN THE FIELD) AT THREE AND FIVE DAYS POSTHARVEST IN THE 2003/04 AND 2004/05 SEASONS, LAPA MUNICIPALITY, PARANÁ STATE, BRAZIL

Treatments *	Brown rot incidence (%) at postharvest			
	2003/04 season		2004/05 season	
	3 days	5 days	3 days	5 days
Control **	88 a	96 a	23 a	27 a
Biological	61 a	86 a	18 a	27 a
Chemical	43 b	58 b	3 b	6 b
Integrated 1	39 b	75 a	9 b	13 b
Integrated 2	41 b	62 b	12 a	13 b
Mean	54.4 A	75.4 A	13.0 B	17.2 B
Correlation with Harvest	0.82	0.69	0.63	0.66

* Treatments as in Table III. Fruits were immersed in a solution of 0.5% sodium hypochlorite before being placed in alveoli plastic trays. In each column, means with the same letter are not significantly different (p=0.05) as determined by the Scott-Knott test. Capital letters indicate different seasons on 3rd and 5th day after harvest.
** Values of the control on the second season were used only as a reference, because no repetition was performed for this treatment.

tions was not significantly different between the treatments for fruit collected on Nov 10. However, the incidence of latent infection was lower on immature fruit for the chemical and integrated 1 treatment when compared to the other treatments for the fruit collected on Nov 20 (Table III).

At the 2003/04 season harvest, all treatments differed significantly from the untreated control according to the Tukey test ($p<0.05$; Table III). The incidence of brown rot was 14, 15, 20 and 36% in the chemical, integrated 1, integrated 2 and biological treatments, respectively, compared to the control treatment, in which 76% of fruit showed the disease. A positive and significant correlation ($r= 0.77$, $p<0.05$) was observed between the incidence of *M. fructicola* on non-disinfested flowers (at the last evaluation, i.e., petal fall) and brown rot on fruit at harvest.

At the 2004/05 season harvest, a significant difference was detected between the treatments (Tukey test, $p<0.05$). The lowest incidence of brown rot was observed in the chemical and integrated 1 treatments (43 and 45%, respectively). A positive correlation was found between the incidence of latent infection (one week before the beginning of harvest) and brown rot at harvest for the 2004/05 season ($r= 0.61$, $p<0.05$). At postharvest, the chemical treatment was better than the control in both seasons and in evaluations at days 3 and 5 after harvest. The integrated 1 treatment was also better than the control on the 3rd day, but the biological treatment did not efficiently control the disease. The disease was higher in the 2003/04 season as compared to 2004/05. The correlation between harvest and postharvest disease incidence was significant across both seasons and all assessment dates (Table IV).

The viability of the *T. roseum* conidia used to spray was confirmed by a conidia germination of 88%.

Discussion

The results of the present study suggest that the application of *T. roseum* at blooming and preharvest, combined with the application of captan plus Ca and K phosphites during the fruit developmental stages, provides brown rot control at a level equivalent to that a fungicidal program under the conditions of the experiment. This management program (integrated 1) resulted in a 74.9% reduction of incidence of *M. fructicola* on flower structures in the 2003/04 season, and 80.3 and 33.8% reductions of brown rot at harvest in 2003/04 and 2004/05, respectively.

In all of the treatments, the incidence of *M. fructicola* in the disinfested flowers was lower than that of not-disinfested flowers (Figure 1), probably because the disinfestation process kills the microflora on the floral surfaces. Therefore, floral disinfestation is not recommended for *M. fructicola* detection protocols.

The incidence rates of *M. fructicola* ranged between 28 and 26% in the first and second evaluations of the 2003/04 season, respectively, and were 100% in the following season, suggesting a high inoculum pressure during blooming in the orchard. Luo *et al.* (2001), in a study of prunes (cv. French), found that the most susceptible stage of the plant was full bloom, in which it showed a 40% incidence of blighted blossoms. However, during the late full bloom and petal fall stages, they only observed ~25 and 10% incidence of blossom blight, respectively, and no blighted blossoms were observed at the fruit set stage.

The incidence of the pathogen during full bloom in flowers of the second sampling was associated with the occurrence of brown rot in fruit at harvest in 2003/04. In the following season, however, no correlation was observed between blossom blight at full bloom and brown rot at harvest, as was also observed by Luo *et al.* (2005) in prune and May-De Mio *et al.* (2008) in peach.

A one percent incidence of *M. fructicola* is considered a high inoculum pressure according to Michailides (2005), and because of this pressure, the end of flowering can be an important source of inoculum for fruit infection in the orchard. Gell *et al.* (2008) reported that a 5-10% incidence of latent infection from blossom to preharvest periods may lead to a 16-33% incidence, or greater, of postharvest brown rot. Similar results were observed in this study.

A high correlation between the incidence of latent infection in the pit hardening stage and brown rot at harvest was also observed by Emery *et al.* (2000). Villarino *et al.* (2011) proposed that the high concentrations of chlorogenic and neochloro-

TABLE V
AVERAGE MAXIMUM AND MINIMUM TEMPERATURES, RELATIVE HUMIDITY AND RAINFALL OBSERVED AT THE WEATHER STATION* OF LAPA MUNICIPALITY, PARANÁ STATE, BRAZIL DURING 2003/2004 AND 2004/2005 GROWING SEASONS IN THE BLOOMING, FRUIT DEVELOPMENT AND PREHARVEST/HARVEST PERIODS

Seasons Stage	Period	Temperature (ºC)			Relative Humidity (%)	Rainfall (mm)
		Max.	Min.	Average		
2003/2004						
Whole season	Sep 1 - Dec 27	24.03	13.36	17.44	79.82	447.7
Blooming	Sep 1 - Sep 22	22.61	9.90	14.68	74.27	49.1
Fruit development	Sep 23 - Nov 24	23.93	13.35	17.37	80.97	200.2
Preharvest/Harvest	Nov 24 - Dec 25	25.16	15.70	19.43	81.33	198.4
2004/2005						
Whole season	Aug 12 - Dec 3	23.54	12.86	17.00	77.16	479.2
Blooming	Aug 12 - Aug 23	23.34	10.43	15.66	70.45	0.0
Fruit development	Aug 24 - Nov 20	23.25	12.89	16.90	78.16	442.3
Preharvest/Harvest	Nov 21 - Dec 3	25.72	14.92	18.92	76.56	36.9

* SIMEPAR Meteorological System of Paraná - Station code 2549091

genic acid levels in immature fruits might contribute to their increased resistance to brown rot infection by interfering with fungal melanin production. Additionally, the high concentrations of chlorogenic acid that are present in immature fruit and in fruit from genotypes with high levels of disease resistance, may contribute to the brown rot resistance of the tissue by interfering with the production of factors involved in the degradation of host polymers rather than by direct toxicity to the pathogen (Bostock *et al*., 1999). In the 2004/05 season, it was observed that the early development stage had lower latent infection rates in comparison with the preharvest stage. This information corroborated the results of Keske *et al.* (2011), who assessed seven different stages of infections under an organic production system in Southern Brazil.

It should be noted that all of the developmental stages occurred at different times during each growing season, probably due to differences in weather conditions (Table V). During the fruit development period of 2004/05, 242.1mm more rain was observed than during the 2003/04 development period.

At postharvest of the first season, the disease incidence was higher (54.4%) than at the postharvest of the second season (13%; Table IV), probably due to differences in the amounts of rainfall during the preharvest to harvest period, which were 198.4 and 36.9 mm, respectively (Table V). According to Gell *et al.* (2008), the most important economic losses due to *M. fructicola* occur in the fruit, and these losses can reach up to 80% of the harvest in years with climatologic conditions that are favorable for the development of the disease, especially in orchards with late maturing cultivars. Gell *et al.* (2008) also reported that the temperature and period of humidification are the most influential climatic factors on the penetration and infection of the fruits by *Monilinia* spp. Temperature and precipitation, as well solar radiation and wind speed, can affect the density of conidia of *Monilinia* spp., as observed by Gell *et al.* (2009) in peach tree orchards in Spain. Luo *et al.* (2007) related the incidence of brown rot in the fruit to spore density in the air. Gell *et al.* (2008) observed that the incidence of brown rot on nectarines sprayed with 10^4 conidia/ml of *M. laxa* was lower than that on fruit sprayed with 10^6 conidia/ml. The percentage of brown rot was up to 50% higher on fruit sprayed with 10^6 conidia/ml suspensions of *Monilinia laxa* and incubated at 23ºC, and the differences were especially pronounced for fruit collected 7 days before harvest and exposed to wet conditions for more than 5h.

Concerning the biological control method used in this study, *T. roseum* applied alone was less effective than the other treatments in reducing brown rot disease, although it may be tested as a strategy to reduce the primary inoculum by colonizing mummies. Interestingly, *T. roseum* was recovered from the external tissues of the mummies from the experiment orchard during the next winter and was never pathogenic to fruits during these experiments. It also seems that captan and Ca and K phosphites had low or no effect on the survival of *T. roseum*, because they have little effect on the mycelium growth of the antagonist, as was previously demonstrated by Moreira and May-De Mio (2007). Hong *et al.* (2000) reported that *M. fructicola* was not recovered from mummies that were overgrown with species of *B. cinerea, C. herbarum, M. racemosus, Penicillium* spp., *R. stolonifer, T. atroviride* or *T. roseum* in commercial orchards. Some of these commonly recovered fungi may have contributed to the decline in the recovery of *M. fructicola* from mummies during the winter and subsequently to the reduction of primary inoculum in California stone fruit orchards. Among the challenges of biological control agents for application on a scale similar to that of fungicides are the inoculum production of the antagonist, the preparation of dense spore suspensions, the monitoring of the viability of spores prior to application in the orchard, and survival of the antagonist under different weather conditions and production systems in commercial fields.

Phosphites can affect the survival of the pathogen, but they may have fewer residual effects because they are quickly absorbed. When associated with captan, however, the Ca and K phosphites provide more consistent control of the pathogen and protect the fruits from new infections. Both captan and phosphites seem to reduce the amount of pathogen inoculum and improve the efficacy of biological control agents. The phosphites may act directly and/or indirectly by inhibiting the development of fungi and/or activating the defense system of the host plant, respectively (Speiser *et al*., 1999). Phosphites have both antifungal and nutritional actions and have been used against several pathogens. The use of phosphite for control of *M. fructicola* in peach trees was reported by Moreira and May-De Mio (2009).

In summary, the results of this study demonstrated that the control of brown rot is not an simple task, due to the complexity of host-pathogen interactions and the influence of weather conditions on the incidence of latent infection and on disease development. Therefore, further studies are necessary to develop successful methods for controlling brown rot that are based on good agricultural practices. Biological treatment might be further tested by applying it during harvest, when fewer fungicides are available, and after the harvest to reduce overwintering survival of inoculum produced in the mummies.

Conclusion

T. roseum applied at blooming and preharvest, when combined with captan plus Ca and K phosphites (integrated 1 treatment) applied during the fruit development period, provides, under the condition of the experiment, brown rot control at a level equivalent to that of the chemical fungicidal program tested.

ACKNOWLEDGMENTS

The authors thank Edir Buske for his support and collaboration (*in memoriam*). This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES - Brazil) and Grupo de Ensino Extensão em Pesquisa de Produção Integrada de Frutas (GEEPPI).

REFERENCES

Adaskaveg J, Gubler D, Michailides T, Holtz B (2009) Efficacy and timing of fungicides, bactericides, and biologicals for deciduous tree fruit, nut, strawberry, and vine crops. http://www2.uckac.edu/plantpath/FungicideEfficacy/ (Cons. 10/11/2010.

Boneti JI, Katsurayama Y (2005) Viabilidade do uso de fosfitos no controle da sarna da macieira. *Agropec. Catarin. 18*: 51-54.

Bostock RM, Wilcox SM, Wang G, Adaskaveg JE (1999) Suppression of *Monilinia fructicola* cutinase production by peach fruit surface phenolic acids. *Physiol. Mol. Plant Pathol. 54*: 37-50.

Emery KM, Michailides TJ, Scherm H (2000) Incidence of latent infection of immature peach fruit by *Monilinia fructicola* and relationship to brown rot in Georgia. *Plant Dis. 84*: 853-857.

Fachinello CJ, Coutinho EF, Marodin GAB, Botton M, May-De Mio LL (2003) *Normas Técnicas e Documentos de Acompanhamento da Produção Integrada de Pêssego*. Universidade Federal de Pelotas. Brazil. 92 pp.

Gell I, De Cal A, Torres R, Usall J, Melgarejo P (2008) Relationship between the incidence of latent infections caused by *Monilinia* spp. and the incidence of brown rot of peach fruit: factors affecting latent infection. *Eur. J. Plant Pathol. 121*: 487-498.

Gell I, De Cal A, Torres R, Usall J, Melgarejo P (2009) Conidial density of *Monilinia* sp. on peach fruit surfaces in relation to the incidences of latent infections and brown rot. *Eur. J. Plant Pathol. 123*: 415-424.

Guerra DS, Marodin GAB, Zanini CLD, Argenta F, Graselli V, Nunes JL da S (2007) Utilização de pesticidas na produção de pêssegos "Marli", nos sistemas de produção integrada e convencional. *Rev. Bras. Frutic. 29*: 91-95.

Guijarro B, Melgarejo P, Torres R, Lamarca N, Usall J, De Cal A (2007) Effects of different biological formulations of *Penicillium frequentans* on brown rot of peaches. *Biol. Contr. 42*: 86-96.

Hong CX, Michailides TJ, Holtz B (2000) Mycoflora of stone fruit mummies in California orchards. *Plant Dis. 84*: 417-422.

Keikotlhaile BM, Spanoghe P, Steurbaut W (2010) Effects of food processing on pesticide residues in fruits and vegetables: A meta-analysis approach. *Food Chem. Toxicol. 48*: 1-6.

Keske C, Amorim L, May-De Mio LL (2011) Peach brown rot incidence related to pathogen infection at different stages of fruit development in an organic peach production system. *Crop Protec. 30*: 802-806.

Larena I, Torres R, De Cal A, Liñan M, Melgarejo P, Domenichini P, Bellini A, Mandrin JF, Lichou J, De Eribe XO, Usall J (2005) Biological control of postharvest brown rot (*Monilinia* sp:) of peaches by field applications of *Epicoccum nigrum*. *Biol. Contr. 32*: 305-310.

Luo Y, Morgan DP, Michailides TJ (2001) Risk analysis of rot blossom blight of prune caused by *Monilinia fructicola*. *Phytopathology 91*: 759-768.

Luo Y, Michailides TJ, Morgan DP, Krueger WH, Buchner RP (2005) Inoculum dynamics, fruit infection, and development of brown rot in prune orchards in California. *Phytopathology 95*: 1135-1136.

Luo Y, Ma Z, Reyes HC, Morgan D, Michailides TJ (2007) Quantification of airborne spores of *Monilinia fructicola* in stone fruit orchards of California using real-time PCR. *Eur. J. Plant Pathol. 118*: 145-154.

MAPA (2007) Ministério da Agricultura, Pecuária e Abastecimento. Brazil. http://www.agricultura.gov.br (Cons. 20/09/2007).

May-De Mio LL, Moreira LM, Monteiro LB, Justiniano Júnior PR (2008) Infecção de *Monilinia fructicola* no período da floração e incidência de podridão parda em frutos de pessegueiro em dois sistemas de produção. *Trop. Plant Pathol. 33*: 173-180.

May-De Mio LL, Luo Y, Michailides TJ (2011) Sensitivity of *Monilinia fructicola* from Brazil to tebuconazole, azoxystrobin, and thiophanate-methyl and implications for disease management. *Plant Dis.* 95: 821-827.

Melgarejo P, De Cal A (2010) Podredumbre parda del melocotonero. In *EFSA Panel on Plant Health* (2011) Pest risk assessment of *Monilinia fructicola* for the EU territory and identification and evaluation of risk management options. *Eur. Food Safety Author. J.* 9(4): 2119. 155 pp.

Mercier J, Jiménez JI (2004) Control of fungal decay of apples and peaches by the biofumigant fungus *Muscodor albus*. *Postharv. Biol. Technol. 31*: 1-8.

Michailides TJ (2005) Inoculum potential at bloom (Flower Incubation Technique, FIT). http://tjm.uckac.edu/TJM-Site/blossom-infection/id-i:htm (Cons. 02/10/2005).

Moraes MVP de (2002) Apresentação. In Andrigueto JR, Kososki AR (Orgs.) *Marco Legal da Produção Integrada de Frutas do Brasil*. Ministério da Agricultura, Pecuária e Abastecimento. Brasília, Brazil.

Moreira LM, May-De Mio LL (2007) Crescimento micelial de *Monilinia fructicola e Trichothecium roseum* em diferentes temperaturas e sensibilidade a fungicidas e fosfitos. *Sci. Agr. 8*: 337-341.

Moreira LM, May-De Mio LL (2009) Controle da podridão parda do pessegueiro com fungicidas e fosfitos avaliados em pré e pós-colheita. *Ciênc. Agrotec. 33*: 405-411.

Moreira LM, May-De Mio LL, Valdebenito-Sanhueza RM, Lima MLRZC, Possamai JC (2002) Controle em pós-colheita de *Monilinia fructicola* em pêssegos. *Fitopatol. Bras. 27*: 395-398.

Moreira LM, May-De Mio LL, Valdebenito-Sanhueza RM (2008) Fungos antagonistas e efeito de produtos químicos no controle da podridão parda em pomar de pessegueiro. *Summa Phytopathol. 34*: 272-276.

Northover J, Cerkauskas RF (1994) Detection and significance of symptomless latent infections of *Monilinia fructicola* in plums. *Can. J. Plant Pathol. 16*: 30-36.

Peres NA, Mackenzie SJ, Fraisse C de W, Pavan W (2010) Strawberry advisory system for control of Anthracnose and Botrytis fruit rots. In Czermainski AB, Alves SAM. (Eds.) *Workshop de Epidemiologia de Doenças de Plantas*. 3rd ed. Bento Gonçalves, Brazil. pp. 27-31.

Schnabel G, Mercier J (2006) Use of a *Muscodor albus* pad delivery system for the management of brown rot of peach in shipping cartons. *Postharv. Biol. Technol. 42*: 121-123.

Speiser B, Berner A, Haseli A, Tamm I (1999) Control of downy mildew of grapevine with potassium phosphonate: effectivity and phosphonate residues in wine. *Biol. Agric. Hortic. 17*: 305-312.

Villarino M, Sandín-España P, Melgarejo P, De Cal A (2011) High chlorogenic and neochlorogenic acid levels in immature peaches reduce *Monilinia laxa* infection by interfering with fungal melanin biosynthesis. J. Agric. Food Chem. *59*: 3205-3213.

Yoshimura MA, Luo Y, MA Z, Michailides TJ (2004) Sensitivity of *Monilinia fructicola* from stone fruit to thiophanate-methyl, iprodione, and tebuconazole. *Plant Dis. 88*: 373-378.