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Pre- and post-infection activity of new fungicides against *Botrytis cinerea* and other fungi causing decay of table grapes

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Abstract

R.A. Serey, R. Torres, and B.A. Latorre. 2007. Pre- and post-infection activity of new fungicides against *Botrytis cinerea* and other fungi causing decay of table grapes. Cien. Inv. Agr. 34(3):215-224. Pre- and post-harvest diseases restrict table grape production and exports (*Vitis vinifera* L.) in Chile, with the most important disease being grey mold (*Botrytis cinerea*). In addition, rot due to *Aspergillus niger, Cladosporium herbarum, Penicillium expansum,* and *Rhizopus stolonifer* frequently occurs. The pre- and post-infection activity of fungicides against these pathogens was studied on Thompson Seedless table grapes. Detached, mature, berries were used, and inoculations were performed with 20 μL of a 10^6 spores·mL^{-1} suspension placed on three punctures aseptically made at the calyx end of each berry. Fungicides used (per liter) were boscalid (600 mg), boscalid (200 mg) + pyraclostrobin (100 mg), boscalid (200 mg) + kresoxim methyl (100 mg), cyprodinil (60 mg) + fludioxonil (40 mg), BAS 600 KBF (100 mg) + metrafenone (150 mg), BAS 600 KBF (200 mg) + boscalid (300 mg), BAS 600 KBF (100 mg) + pyraclostrobin (100 mg), and captan (400 mg). Each fungicide was applied either by drop (12 µL·berry^{-1}) placed on three punctures made with a sterile hypodermic needle or by 60 s immersion. Berries were then incubated in humid chambers at 20ºC. The pre-infection (protection) activity of the fungicides varied considerably among the pathogens tested and was found to be significant ($p < 0.001$) and, with one exception (*A. niger*), it was significantly ($p < 0.002$) affected by the application method. The interaction between fungicide and application method was only significant ($p < 0.001$) for *R. stolonifer* at 48 h post treatment. In general, pre-infection activity gave 0 to 4 days protection after drop applications and 0 to 21 days after immersion treatments. The post-infection (curative) activity varied among pathogens and fungicide treatments. However, it was always below 24 h.

Key words: Blue mold, curative activity, gray mold, protection activity, sour rot, *Vitis vinifera*.

Introduction

Decays are one of the main factors restricting the production and commercialization of table grapes (*Vitis vinifera* L.) in Chile and other countries (Franck *et al*., 2005; Lydakis and Aked; 2003; Lichter *et al*., 2002). Important economic losses usually occur during harvest, cold storage, and transportation of Chilean table grapes to markets in North America, Europe and Asia (Franck *et al*., 2005; Donoso and Latorre, 2006).

Botrytis cinerea Pers. is the most important pathogen affecting table grape production in Chile. Infections start from the inoculum present in the vineyard which can develop into latent infections with disease appearing later in packed table grapes during storage and transportation. Additionally, pre and post harvest decay caused by *Aspergillus niger* Tiegh, *Cladosporium herbarum* (Pers.) Link, *Penicillium expansum* Link and *Rhizopus stolonifer* (Ehrenb.) Vuill, has been reported in Chile and elsewhere (Hewitt, 1988; Zahavi *et al*., 2000; Latorre *et al*., 2002b).

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Fungicide treatments applied in the vineyard are important to prevent decay development at harvest or during post-harvest (Latorre *et al*., 2001; Franck *et al*., 2005). However, registration restrictions, tolerances established by import countries, and the development of resistant strains limit their use in table grapes and other fruit crops (Latorre *et al*., 1994; Latorre *et al*., 2002a; Errampalli and Crnko, 2004; Sallato and Latorre, 2006). Therefore, new, effective fungicide treatments with the lowest possible toxicological risk are required, as has been proposed elsewhere (Adaskaveg *et al*., 2005; Förster *et al*., 2007; McGrath, 2004).

Fungicides can provide disease control through both pre- and post-infection activity. Pre-infection activity is commonly known as protectant (preventive) activity and post-infection activity comprises a curative action that can involve both pre- and post-symptom expression activities.

The modes of actions of the fungicides used in this study are varied. Anilinopyrimidines inhibit methionine biosynthesis and secretion of hydrolytic enzymes. Carboximides inhibit succinate dehydrogenase in the cell respiration process. Phenylpyrrol inhibits histidine quinase. Phthalimides are multisite inhibitors. Strobilurines (Qo inhibitor fungicides, QoI) act at the quinone binding site of the cytochrome bc1 complex in the mitochondrial cell membrane. Triazolopyrimidins have an unknown mode of action (McGrath, 2004; FRAC, 2007).

Understanding a fungicide's mode of action and also whether it has activity both pre- and post-infection contributes considerably to improved control efficiency through optimizing application timing based on the host-pathogen-interactions in table grapes and other hosts (Szkolnik, 1978; Jones and Latorre, 1985; Wong and Wilcox, 2001; Rebollar-Alviter et al., 2007).

Therefore, the objectives of this study were to evaluate, under laboratory conditions, the pre-infection and post-infection activity of boscalid, captan, cyprodinil, and the new fungicide BAS600 against *B. cinerea*, *P. expansum*, *R. stolonifer*, *A. niger* and *C. herbarum,* which are the main filamentous fungi associated with pre- and post-harvest decay of table grapes in Chile.

Materials and methods

Table grapes

All the experiments were conducted on healthy and mature 'Thompson Seedless' table grapes (total soluble solids >16%). Berries with their pedicel intact and without fungicide treatments were obtained from a commercial farm. Prior to every experiment, the berries were superficially disinfected with 0.025% sodium hypochlorite for 3 min. They were washed in tap water and were aseptically distributed on grids in polyethylene chambers (34 x 25 x 13 cm) at 20ºC and 93-96% relative humidity (RH). The RH was obtained by moistening a double paper layer placed at the bottom of each chamber. Relative humidity was verified with a HOBO sensor (Bourne, Massachussets, USA), located inside the chamber.

Isolation and inoculation

Isolates of *B. cinerea, P. expansum,* and *A. niger* were obtained from 'Thompson Seedless' table grapes. *Cladosporium herbarum* was obtained from 'Red Globe' berries, and *R. stolonifer* from strawberries. Pure cultures were obtained by sub-cultivating hyphal tips in potato dextrose agar acidified with 0.5 $mL \cdot L^{-1}$ of 90% lactic acid (APDA) at 20ºC. All isolates were pathogenic to table grapes.

The inoculum was prepared with spores obtained from 7 to 15 day old cultures in APDA. To avoid spore clusters that may impede a uniform distribution of the inoculum, the spores were suspended in 0.05% of Tween 80 in sterile distilled water. The final concentration was adjusted to 10^6 $spores \cdot mL^{-1}$ with the aid of a hemacytometer. An aliquot (20 μL) from the respective spore suspension was delivered on the surface of each berry after they were aseptically wounded with a hypodermic needle (at 2-3 mm depth) at the calyx end. Wounded berries were used for drop and immersion fungicide treatments. These inoculation methods were done to mimic damage that berries may

naturally suffer in the field and should allow the spore to be attached to the surface of the berry, germinate, develop a germ tube and possible an appressorium before penetration.

Fungicides

The fungicides used were: 1. Boscalid (Cantus 50% WG, BASF, Germany), 2. Boscalid combined with pyraclostrobin (Bellis WG; 25.2 + 12.8%, respectively, BASF, Germany), 3. Boscalid combined with kresoxim methyl (BAS 517 SC, 10.0 + 20.0%, respectively, BASF, Germany), 4. Cyprodinil combined with fludioxonil (Swicth WG, 37.5 + 25.0% respectively, Syngenta Crop Protection, Switzerland), 5. BAS 600 KBF at 20% in combination with metrafenone (BAS 560 50% SC, BASF, Germany), 6. BAS 600 KBF at 20% in combination with boscalid (Cantus 50% WG, BASF, Germany), 7. BAS 600 KBF al 20% combined with pyraclostrobin (Comet 25% EC, BASF, Germany), and 8. Captan (Captan 80 WP, BASF Chile S.A.). The rates used represent current rates for pre-harvest use against *B. cinerea* on table grapes (Table 1).

Pre-infection activity

The pre-infection (protectant) activity was the maximum period of time post-treatment where fungicides were able to protect berries from infection with mean control efficacy ≥ 75%. For this purpose, two tests were performed: 1. Berries were inoculated 0, 12, 24, 48 and 96 h after depositing a drop of 12 μL of the respective fungicide suspension over three wounds made at the calyx end of each berry. 2. Berries were wounded and inoculated 1, 2, 7, 14 and 21 days after treating berries by immersion for 60 s in the fungicide suspension. The diameter of the lesion developed was determined 2 to 3 days after inoculation.

Post-infection activity

The post-infection activity (eradication activity) was defined as the maximum post-inoculation period for applying a fungicide treatment and

Table 1. Fungicides and fungicide mixtures used in this study.
Cuadro 1. *Fungicidas y mezclas fungicidas empleadas en este estudio.*

Fungicides[1]			Concentration[3], a.i.
Active ingredient	Formulation	Family[2]	mg·L^{-1}
Boscalid	Cantus, 50% WG	Carboxamide	600
Boscalid + pyraclostrobin	Bellis, 25.2% + 12.8% WG	Carboxamide + strobilurine	200 + 100
Boscalid + kresoxim methyl	BAS 517, 20% + 10% SC	Carboxamide + strobilurine	200 + 100
BAS 600 KBF + metrafenone	BAS 600 KBF, 20% SC + BAS 560 02, 50% SC	Triazolopyrimidine + benzophenone	100 + 150
BAS 600 KBF + boscalid	BAS 600 KBF , 20% SC + Cantus, 50% WG	Triazolopyrimidine + carboxamide	200 + 300
BAS 600 KBF + pyraclostrobin	BAS 600 KBF, 20% SC + Comet, 25% EC	Triazolopyrimidine + strobilurine	100 + 100
Captan	Captan, 80% WP	Phthalimide	400
Cyprodinil + fludioxonil	Switch, 37.5% + 25.0% WG	Anilinopyrimidine + phenylpyrrol	60 + 40

[1]SC, concentrate suspension; WG, granulate dispersible; EC, emulsifiable concentrate; WP, wettable powder.
[2]The mode of action in the biosynthetic processes of fungi are: Anilinopyrimidines inhibit methionine biosynthesis and secretion of hydrolytic enzymes. Carbaximides inhibit succinate dehydrogenase in the cell respiration process. Phenylpyrrol inhibits histidine quinase. Phthalimides are multisite inhibitors. Strobilurines (Qo inhibitor fungicides, QoI) act at the quinone binding site of the cytochrome bc1 complex in the mitochondrial cell membrane. Triazolopyrimidins have an unknown mode of action (FRAC, 2007).
[3]Rates were expressed as active ingredients (a.i.) and were suspended in water before use.
[1]SC, suspensión concentrada; WG, gránulos dispersables; EC, concentrado emulsionable; WP, polvo mojable.
[2]Los modos de acción bioquímicos son: Anilinopirimdinas inhiben la biosíntesis de metionina y la secreción de enzimas hidrofíticas. Caboxamidas inhiben la enzima sucinato dehidrogenasa en el proceso de respiración celular. Fenilpirrol (Phenylpyrrol) inhiben la enzima histidina quinasa. Phthalamidas tiene múltiples sitios de accción. Estrobilurinas (Inhibidores Qo, QoI) actúan sobre el sitio de unión quinónico en el complejo del citocromo bc1 en la membrana mitocondrial. Triazolpirimidinas no tiene un modo de acción aun conocido (FRAC, 2007).
[3]Concentraciones expresadas como ingrediente activo (a.i.) y fueron suspendidos en agua antes de su uso.

obtaining 75% or higher control efficacy. Post-infection activity was only studied after drop application of each fungicide because it was assumed that immersion would have washed off the spores. Therefore, the berries were treated 0, 12, 24 and 48 h after the inoculation with a drop of 12 μL of fungicide suspension, which was placed in the inoculation site, at the calyx end of each berry. Berries were then assessed for the diameter of the lesion developed 48-72 h after the fungicide application.

Design and statistical analyses

The results for both pre and post infection activity studies were expressed as % efficacy (E = 100-[(100 x treatment)·untreated control^{-1}]), but % efficacies were transformed to Probit values prior to analysis.

Considering that 48 h post-treatment disease incidence was over 50% for each pathogen, the efficacy of the pre-infection activity obtained after 48 h post-treatment were studied. For each pathogen, treatments were arranged as completely randomized design with a 2 x 8 (application methods x fungicides) factorial arrangement of treatments. Four replicates and eight berries as an experimental unit were used. Results were subjected to analysis of

Table 2. Effect of fungicide, application method, and the interaction between fungicide and application method on the efficacy of pre-infection fungicide treatments on berries with their pedicels intact of Thompson Seedless table grapes.
***Cuadro 2**. Efecto del fungicida, del método de aplicación y de la interacción entre fungicidas y métodos de aplicación en la eficacia de los tratamientos fungicidas de pr-einfección, aplicados en bayas con pedicelos de uvas Thompson Seedless.*

Source of variation	d.f.	SS	MS	F	p
			Aspergillus niger		
Method (M)	1	0.863	0.863	2.3	0.136
Fungicide (F)	7	22.665	3.238	8.633	<0.001
M x F	7	3.498	0.5	1.332	0.256
Residual	48	18.002	0.375		
Total	63	45.027	0.715		
			Botrytis cinerea		
Method (M)	1	19.25	19.25	106.898	<0.001
Fungicide (F)	7	9.057	1.294	7.185	<0.001
M x F	7	2.118	0.303	1.68	0.137
Residual	48	8.644	0.18		
Total	63	39.068	0.62		
			Cladosporium herbarum		
Method (M)	1	2.337	2.337	10.665	0.002
Fungicide (F)	7	15.466	2.209	10.082	<0.001
M x F	7	3.09	0.441	2.015	0.072
Residual	48	10.518	0.219		
Total	63	31.412	0.499		
			Penicillium expansum		
Method (M)	1	6.119	6.119	16.954	<0.001
Fungicide (F)	7	58.531	8.362	23.165	<0.001
M x F	7	3.922	0.56	1.552	0.173
Residual	48	17.326	0.361		
Total	63	85.898	1.363		
			Rhizopus stolonifer		
Method (M)	1	9.09	9.09	30.99	<0.001
Fungicide (F)	7	20.312	2.902	9.892	<0.001
M x F	7	17.874	2.553	8.705	<0.001
Residual	48	14.08	0.293		
Total	63	61.356	0.974		

[1]Treatments were drop (12 μL·berry^{-1}) applications and immersion for 60 s.
[1]Los tratamientos fueron en gotas (12µL·baya^{-1}) e inmersión por 60 s.

variance followed by a multiple comparison test according to Tukey ($p < 0.05$) using Sigmastat (Systat Software, Inc., USA). The relation between time of pre-infection activity and fungicide efficacy were study by a linear regression analysis.

The pre- and post-infection activity of the fungicide treatments were estimated, independently for each pathogen, by a linear regression analysis. Treatments were replicated four times with eight berries as experimental units.

Results

Pre-infection activity

The pre-infection activity of the fungicides varied considerably among pathogens. However, it was significantly ($p < 0.001$) affected by the fungicide used and, with one exception (*A. niger*), it was also significantly affected by the application method (immersion and localized drop applications) ($p < 0.002$) when evaluation was made 48 h after treatment. The interaction between fungicide and application method was only significant ($p < 0.001$) for *R. stolonifer* (Table 2).

In general, pre-infection activity gave 0 to 4 days protection when fungicides were tested as drops placed on the inoculation site, and varied between 0 and 21 days when berries were treated by 60 s immersion (Table 3). The maximum pre-infection activity required to obtain ≥75% control efficacy was estimated by linear regression analysis (data not presented). For example, pre-infection activity of cyprodinil plus fludioxonil protected berries for 4 days against *A. niger, B. cinerea, P. expansum* and *R. stolonifer,* and only 2 days against *C. herbarum* when this fungicide mixture was applied by drops. Using the immersion treatments, this same fungicide mixture provided 14 days protection against *B. cinerea*, 21 days against *P. expansum*, *R. stolonifer* and *A. niger* and <2 days against *C. herbarum* (Table 3).

All the fungicides controlled *B. cinerea* and *C. herbarum* with control efficiencies higher than 78.8 and 82.3%, respectively, when inoculations were made 48 h after drop applications of fungicides. However, the efficacy of most of these fungicides decreased considerably after immersion treatments. For example, captan had 88.6% control efficacy against *B. cinerea* after drop applications; this decreased to 45.3% after immersion treatments (Table 3).

Independent from the application method, pre-infection applications of boscalid were effective (mean control efficacy 95.3%) in control of *A. niger*, but were relatively ineffective against *P. expansum* (66.6% control efficacy) and *R. stolonifer* (63.9% control efficacy). However, the pre-infection activity of boscalid against *P. expansum* improved significantly when this fungicide was combined with pyraclostrobin or kresoxim methyl (99.1 and 91.9% control efficacy, respectively). Pre-infection applications of BAS 600 KBF in combination with boscalid, metrafenone or pyraclostrobin were weak or ineffective in control of *P. expansum* (Table 3).

Cyprodinil combined with fludioxonil efficiently controlled *B. cinerea, A. niger, P. expansum* and *R. stolonifer*, with mean efficiencies >89.4%, but it was relatively weak in control of *C. herbarum* (67.1%). Pre-infection drop applications of captan were effective in control of *B. cinerea* and arrested the development of decay caused by *A. niger, C. herbarum*, and *P. expansum*. Nevertheless, the same concentration of captan was ineffective against *B. cinerea* after immersion treatments. Regardless of the application method, captan was ineffective against *R. stolonifer* (Table 3).

Post-infection activity

The post-infection activity (curative action) varied among pathogens and fungicide treatments. Nevertheless, this was always short, below 24 h (Table 3). The maximum curative action (24 h) of cyprodinil in combination with fludioxonil was obtained against *A. niger* and *P. expansum*. However, the post-infection activity for the same treatment was estimated as 0, <12 h, and 12 h against *C. herbarum*, *B. cinerea*, and *R. stolonifer*, respectively. Similarly, captan had no curative action against *R. stolonifer*, <12 h against *B. cinerea* and *P. expansum,* and 24 h against *A. niger* and *C. herbarum*.

Table 3. Efficacy and pre and post infection activity of new fungicides against *Botrytis cinerea, Penicillium expansum, Rhizopus stolonifer, Aspergillus niger* and *Cladosporium herbarum* determined on detached mature berries of Thompson Seedless table grapes.

Cuadro 3. *Eficacia y actividad de pre y post infección de nuevos fungicidas contra* Botrytis cinerea, Penicillium expansum, Rhizopus stolonifer, Aspergillus niger *y* Cladosporium herbarum, *determinada en bayas cortadas y maduras de uvas Thompson Seedless* .

Fungicide treatments	Concentration mg·L^{-1}, a.i.	Efficacy[1]			Estimated pre-infection activity[2]		Estimated activity[3]
		Drop[4] %	Immersion[4] %	Mean[5] %	Drop days	Immersion days	Drop h
			Aspergillus niger				
BAS 600 KBF + metrafenone	100 + 150	63.9	64.6	64.3c[6]	0	0	0
BAS 600 KBF + boscalid	200 + 300	99.3	91.7	95.5a	4	21	24
BAS 600 KBF + pyraclostrobin	100 + 100	69.3	70.1	69.7bc	<2	<2	24
Boscalid	600	100	90.5	95.3a	4	21	24
Boscalid + pyraclostrobin	200 + 100	96.4	95.7	96.1a	4	21	24
Boscalid + kresoxim methyl	100 + 200	87.9	97.0	92.5a	4	21	24
Cyprodinil + fludioxonil	60 + 40	99.3	97.6	98.5a	4	20	24
Captan	400	97.1	85.3	91.2ab	4	2	24
			Botrytis cinerea				
BAS 600 KBF + metrafenone	100 + 150	78.85	30.95	54.9d[6]	4	0	< 12
BAS 600 KBF + boscalid	200 + 300	90.6	72.6	81.6ab	4	<2	< 12
BAS 600 KBF + pyraclostrobin	100 + 100	70.3	43.3	56.8cd	4	0	< 12
Boscalid	600	77.2	57.0	67.1bcd	2	< 2	< 12
Boscalid + pyraclostrobin	200 + 100	94.8	54.0	74.4abc	2-3	< 2	< 12
Boscalid + kresoxim methyl	100 + 200	91.7	60.2	76.0abcd	2-3	< 2	< 12
Cyprodinil + fludioxonil	60 + 40	96.2	82.5	89.4a	4	10-14	12-24
Captan	400	88.6	45.3	67.0bcd	3	0	< 12
			Cladosporium herbarum				
BAS 600 KBF + metrafenone	100 + 150	82.05	67.15	75.6d6	4	<2	< 12
BAS 600 KBF + boscalid	200 + 300	94.6	94.4	94.5ab	4	11	24
BAS 600 KBF + pyraclostrobin	100 + 100	80.3	84.6	82.5bcd	4	5	24
Boscalid	600	87.8	90.4	89.1abc	3-4	10	< 12
Boscalid + pyraclostrobin	200 + 100	96.9	79.0	88.0abc	4	2	24
Boscalid + kresoxim methyl	100 + 200	88.8	68.3	78.6cd	4	<2	< 12
Cyprodinil + fludioxonil	60 + 40	82.6	51.5	67.1d	2-3	<2	< 12
Captan	400	98.9	94.0	96.5a	4	12	24

			Penicillium expansum				
BAS 600 KBF + metrafenone	100 + 150	13.05	41.45	27.2c[6]	0	0	0
BAS 600 KBF + boscalid	200 + 300	43.7	75.2	59.5b	0.5	3	< 12
BAS 600 KBF + pyraclostrobin	100 + 100	55.0	86.7	70.9b	0.5	4	< 12
Boscalid	600	54.1	79.0	66.6b	0.5	3	< 12
Boscalid + pyraclostrobin	200 + 100	99.4	98.7	99.1a	4	20	24
Boscalid + kresoxim methyl	100 + 200	88.0	95.8	91.9ab	4	10	< 12
Cyprodinil + fludioxonil	60 + 40	100	98.5	99.3a	4	21	24
Captan	400	67.8	84.8	76.3b	nt	5	< 12
			Rhizopus stolonifer				
BAS 600 KBF + metrafenone	100 + 150	90.6ab[6]	18.6b[6]	54.6	4	<2	0
BAS 600 KBF + boscalid	200 + 300	92.2ab	26.9b	55.4	4	<2	0
BAS 600 KBF + pyraclostrobin	100 + 100	73.8bc	72.5a	73.15	4	<2	0
Boscalid	600	74.7bc	53.1ab	63.9	<1	<2	0
Boscalid + pyraclostrobin	200 + 100	66.6bc	84.6a	75.6	1	6	0
Boscalid + kresoxim methyl	100 + 200	63.6bc	81.9a	72.8	0	3	0
Cyprodinil + fludioxonil	60 + 40	98.5a	85.9a	92.2	4	21	12
Captan	400	43.8c	26.6b	35.2	0	0	0

[1]Efficacy (E) was defined as (E = 100-[(100 x treatment)·untreated control-1]), determined 48 h after drop and immersion treatments.
[2]Pre-infection (protectant) activity was defined as time between application and inoculation to obtain ≥ 75% efficacy. Estimations were made by linear regression analysis. Drop and immersion applications were studied up to 4 and 21 days, respectively.
[3]Post-infection (curative) activity was defined as time between inoculation and fungicide treatments to obtain ≥ 75% efficacy after drop applications. Estimations were made by linear regression analysis. Drop applications were studied up to 4 days.
[4]Treatments were drop (12 µL·berry^{-1}) applications and immersion treatment for 60 s.
[5]The interactions between application methods and fungicide treatments were only significant for *R. stolonifer* (Table 2); therefore, the average of the % efficacy obtained after drop and immersion applications were compared.
[6]Means of four replicates followed by the same letters in each column for each pathogen were not significantly different according to Tukey (p = 0.05). nt = not tested.

[1]Eficacia (E) se definió como E = 100-[(100 x tratamiento)·control sin tratar-1]), determinado 48 h luego de la aplicación en gotas e inmersión.
[2]Actividad de pre-infección (protectora) se definió como el tiempo entre la aplicación y la inoculación de modo de obtener una eficacia ≥ 75%. Estimaciones realizadas por análisis de regresión lineal. Las aplicaciones en gota e inmersión se estudiaron respectivamente hasta 4 y 21 días postratamiento.
[3]Actividad de post-infección (curativa) se definió como el tiempo entre la inoculación y la aplicación del fungicida, vía gota o por inmersión, de modo de obtener una eficacia ≥ 75%. Estimaciones realizadas por análisis de regresión lineal. Las aplicaciones en gota se estudiaron hasta 4 días postratamiento.
[4]Tratamientos en gotas (12 µL·baya-1) e inmersión por 60 s.
[5]La interacción entre el método de aplicación y los fungicidas fue solamente significativa en el caso de R. stolonifer *(Cuadro 2); por lo tanto, se comparó el porcentaje de eficacia promedio obtenido para tratamientos vía gota e inmersión.*
[6]Promedios de cuatro repeticiones seguidos por iguales letras en cada columna no son significativamente diferentes de acuerdo con Tukey (p = 0,05). nt = no estudiado.

A 24 h post-infection activity against *A. niger, C. herbarum*, but lack of activity against *B. cinerea*, *P. expansum* and *R. stolonifer* was obtained after drop applications of boscalid (alone or combined with BAS 600 KBF, pyraclostrobin or kresoxim methyl) and BAS 600 KBF combined with metrafenone (Table 3).

Discussion

Production of Chilean table grapes for international markets requires the use of fungicide treatments as one of the main components for disease management. This allows controlling rots caused by *B. cinerea*

and other filamentous fungi that are commonly present in the vineyards, causing decays before and after harvest (Hewitt, 1988; Pszczolkowski *et al*., 2001; Latorre, 2002b, 2004; Franck *et al*., 2005; Donoso and Latorre, 2006).

Several studies have been conducted on the efficacy and activity of various fungicides against other plant pathogenic fungi (O'Leary *et al*., 1984; Poblete and Latorre, 2001; Wong and Wilcox, 2001; Ferreira *et al*., 2006; Holb and Schnabel, 2007; Rebollar-Alviter *et al*., 2007). However, to our knowledge this is the first report regarding pre- and post-infection activity of boscalid and cyprodinil in combination with fludioxonil, BAS 600 KBF and captan against filamentous fungi affecting mature table grapes.

Our study demonstrated important differences in the pre- and post-infection activity of the fungicides evaluated. Coincident with previous reports on other fruits and pathogens, protective applications of these fungicides were more effective in reducing decay incidence than curative applications (Holb and Schnabel, 2007). All the fungicides tested had a null or low (<24 h) post-infection activity against filamentous fungi causing rots on mature Thompson Seedless table grapes. The high inoculum concentrations used in these studies and the fast development of these rots can possibly explain these results. It has been demonstrated that symptoms of gray mold, caused by *B. cinerea*, appeared 12- to 24 h post-inoculation at optimal temperature and humidity (Latorre and Rioja, 2002). Therefore, curative treatments are unlikely to be useful for control decays under field conditions. However, fungicides that do not perform well in our laboratory tests may still do better under field conditions, because in the field, post-infection activity affects a range of pathogen activities including germination of spores deposited on the surface of berry after fungicide applications. Additionally, it is unlikely that grapes would be exposed to the high inoculum concentration as it was always used in these tests.

Similar to previous reports, these results provided additional evidence regarding the high effectiveness of 600 $mg \cdot L^{-1}$ of boscalid and 60 $mg \cdot L^{-1}$ of cyprodinil in combination with 40 $mg \cdot L^{-1}$ fludioxonil against *B. cinerea* on table grapes and other fruit crops (Forster and Staub, 1996; Blacharski *et al*., 2001; Latorre *et al*., 2001; Sholberg *et al*., 2003; Sallato *et al*., 2007; Wedge *et al*., 2007). Cyprodinil plus fludioxonil effectively controlled *P. expansum, R. stolonifer* and *A. niger,* but was ineffective in controlling *C. herbarum*. It is interesting that a relatively low concentration of cyprodinil in combination with fludioxonil was used in this study. Therefore, it is possible that using higher concentrations a better control efficacy against these fungi could be obtained.

With one exception, *R. stolonifer*, 100 $mg \cdot L^{-1}$ of boscalid combined with 200 $mg \cdot L^{-1}$ of kresoxim methyl, and 200 $mg \cdot L^{-1}$ boscalid combined with 100 $mg \cdot L^{-1}$ of pyraclostrobin effectively protected berries against *B. cinerea* and it prevented the development of rot caused by *A. niger*, C. *herbarum, P. expansum* and *R. stolonifer* (Forster and Staub, 1996; Latorre *et al*., 2001; Franck *et al*., 2005; Rosslenbroich and Stuebler, 2000; Wedge *et al*., 2007).

Based on this research, the efficacy of the fungicide protectant activity varied with application method when this effect was tested 48 h post-treatment. In most cases, a better control was obtained with localized drop applications. These differences were mainly attributed to the imperfect coverage of the berry surface obtained by immersion applications. On the contrary, drop applications allowed direct contact of the pathogen with the fungicide deposits. At the same time, the drop applications could possibly facilitate the product absorption by the berry, improving the degree of control obtained.

Therefore, the localized drop application in detached table grape berries was an efficient and reproducible methodology which allowed us to study the *in vivo* effectiveness of new fungicides. Nevertheless, these results also suggest the need to evaluate these treatments according to the commercial application method before establishing a recommendation. A similar methodology has previously been used to study the effectiveness of these and other fungicides in table grapes and strawberries (Franck *et al*., 2005; Sallato *et al*., 2006; Holb and Schnabel, 2007).

Resumen

Las enfermedades de pre y postcosecha limitan la producción y exportación de uva de mesa (*Vitis vinifera* L.) en Chile. Especialmente importante es la pudrición gris (*Botrytis cinerea*). Además, son frecuentes las pudriciones causadas por *Aspergillus niger, Cladosporium herbarum, Penicillium expansum* y *Rhizopus stolonifer.* Este trabajo tuvo el propósito de estudiar, en bayas de uvas Thompson Seedless, la actividad de pre y post-infección de nuevos fungicidas. Con este propósito se empleó bayas maduras (<16% sólidos solubles) con pedicelos intactos. Cada baya se inoculó en el extremo calicinal, depositando 20 μL de una suspensión de 10^6 esporas·mL^{-1} sobre tres heridas practicadas asépticamente con una aguja hipodérmica estéril. Los productos y concentraciones empleadas por litro fueron: boscalid (600 mg), boscalid (200 mg) + pyraclostrobin (100 mg), boscalid (200 mg) + kresoxim metil (100 mg), cyprodinil (60 mg) + fludioxonil (40 mg), BAS 600 KBF (100 mg) + metrafenone (150 mg), BAS 600 KBF (200 mg) + boscalid (300 mg), BAS 600 KBF (100 mg) + pyraclostrobin (100 mg), y captan (400 mg). Cada fungicida se aplicó vía gota (12 µL·baya^{-1}) depositada sobre tres heridas practicadas en cada baya con una aguja hipodérmica estéril o por inmersión durante 60 s. Luego las bayas se incubaron en cámaras húmedas a 20ºC. La actividad de pre-infección varió considerablemente entre patógeno y dependió significativamente ($p < 0.001$) del fungicida usado y con sólo una excepción (*A. niger*), el método de aplicación tuvo un efecto significativo ($p < 0.002$). La relación entre fungicida y método de aplicación, determinado a las 48 h post-tratamiento, fue significativo ($p < 0.001$) sólo para *R. stolonifer.* En general, la actividad de pre-infección otorgó una protección entre 0 y 4 días al aplicar cada producto en gota y entre 0 y 21 días luego de aplicaciones por inmersión. La actividad de post-infección (acción curativa) varió entre patógenos y dependió del fungicida aplicado. Sin embargo, ésta fue siempre inferior a 24 h.

Palabras clave: Acción curativa, acción preventiva, fungicidas, moho azul, moho gris pudrición ácida,*Vitis vinifera*.

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