Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar

Spanish Journal of Agricultural Research 2007 5(1), 67-78
ISSN: 1695-971X

Effect of boscalid on postharvest decay of strawberry caused by *Botrytis cinerea* and *Rhizopus stolonifer*

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Abstract

In Chile, gray mold (*Botrytis cinerea*) and leak (*Rhizopus stolonifer*) are the major storage diseases of strawberry (*Fragaria* x *ananassa* Duch.) that considerably reduce yields and quality, limiting its international commercialization. The effect of preharvest fungicide treatments and postharvest treatments against storage decays was studied. Based on the results obtained, the incidence of *B. cinerea* and *R. stolonifer* was significantly lower on 'Camarosa' strawberry fruits treated with boscalid or boscalid + pyraclostrobin between blossom and harvest. Similarly, incidence of *B. cinerea* and *R. stolonifer* significantly decreased at 5ºC and 20ºC after immersion treatments with boscalid (600 to 700 mg L^{-1}) or cyprodinil + fludioxonil (371 + 250 mg L^{-1}). These postharvest treatments provided 5 and 15 days protection against these molds when strawberry fruits were stored at 20ºC and 5ºC, respectively. Fenhexamid (750 mg L^{-1}) arrested *B. cinerea,* but it was ineffective against *R. stolonifer*. Variable results were obtained with iprodione (750 mg L^{-1}). Fungicide treatments had no adverse effect on total soluble content, titrable acidity and firmness. Only boscalid + pyraclostrobin affected the external color of the treated fruits. In conclusion, fungicide treatments using new reduced-risk fungicides can be useful to extend strawberry shelf life for over a 15 day period. However, further research is needed to establish a commercial recommendation.

Additional key words*:* boscalid, cyprodinil, fenhexamid, *Fragaria,* fungicides, pyraclostrobin.

Resumen

Efecto de boscalid sobre las podredumbres de la fresa en postcosecha causadas por *Botrytis cinerea* y *Rhizopus stolonifer*

En Chile, el moho gris (*Botrytis cinerea*) y el moho negro algodonoso (*Rhizopus stolonifer*) reducen los rendimientos y la calidad de la fresa (*Fragaria* x *ananassa* Duch.) en pre y postcosecha, limitando su comercialización internacional. En este trabajo se estudió la efectividad de tratamientos de campo y postcosecha sobre el desarrollo de podredumbres, vida útil y cualidades de la fresa conservada a 5ºC y 20ºC. De acuerdo con los resultados obtenidos, la incidencia de *B. cinerea* y *R. stolonifer* en postcosecha fue significativamente menor en fruta proveniente de fresas 'Camarosa' tratadas, entre floración y cosecha, con boscalid o boscalid + pyraclostrobin. Del mismo modo, fresas 'Camarosa' tratadas por inmersión en boscalid (600 a 700 mg L^{-1}) o cyprodinil + fludioxonil (375 + 250 mg L^{-1}), presentaron menor incidencia de *B. cinerea* y *R. stolonifer* tanto a 5ºC como a 20ºC. Este tratamiento protegió la fruta durante 5 y 15 días a 20ºC y 5ºC, respectivamente. Fenhexamid (750 mg L^{-1}) controló *B. cinerea,* pero fue inefectivo contra *R. stolonifer* y con iprodione (750 mg L^{-1}) se obtuvieron resultados variables. Ningún tratamiento afectó negativamente al contenido de sólidos solubles totales, acidez titulable y firmeza. Sólo boscalid + pyraclostrobin alteró el color externo de la fruta. En conclusión, tratamientos con fungicidas con bajo riesgo toxicológico y ambiental permitirían extender la vida útil de fresas almacenadas. No obstante, se requiere información adicional antes de establecer una recomendación comercial de uso.

Palabras clave adicionales: boscalid, cyprodinil, fenhexamid, *Fragaria,* fungicidas, pyraclostrobin.

Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) is a nonclimateric fruit characterized by a short postharvest life, often estimated in less than 5 days. It is very prone to rapid dehydration, physiological disorders, bruising and other mechanical injuries, and to infections caused by several pathogens that can rapidly reduce quality of ripe fruits. These factors hinder to achieve export market and have limited strawberry production in Chile.

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Received: 06-11-06; Accepted: 31-01-07.

Gray mold (*Botrytis cinerea* Pers.) and leak [*Rhizopus stolonifer* (Ehrenb.) Vuill.] are the most frequently postharvest diseases affecting strawberries in Chile and worldwide (Ceponis *et al.,* 1987; Maas, 1998). Gray mold often occur in the field and postharvest decay caused by *B. cinerea* may develop from conidia contaminating berry surfaces, from incipient infections or from latent (quiescent) field infections. Leak is primarily a storage rot; however, it may also occur in the field on ripe fruit. Disease incidence and severity are often dependent on crop and postharvest management and weather conditions. For instance, sporulation of *B. cinerea* on plant tissues and infection are favored by cool and wet weather conditions (Sosa-Álvarez *et al.,* 1995). Plant spacing along with sanitation practices considerably reduced gray mold incidence (Legard *et al.*, 2000; Mertely *et al.*, 2000), and storage rot can be reduced by rapidly cooling harvested berries to temperatures below 5ºC. However, it is common for strawberries to encounter an average 5°C temperature during transport (Mitchell, 1992).

In addition to refrigeration, several control strategies have been evaluated to extend the shelf life of strawberries. These include hot water treatments (García *et al.*, 1995, 1996b; Vicent *et al.*, 2002), control atmospheres (Mitchell, 1992; Moyls *et al.*, 1996; Holcroft and Kader, 1999; Wszelaki and Mitcham, 2003), ultraviolet light (Nigro *et al.*, 2000), biological natural active products (Bhaskara Reddy *et al.*, 2000; Spadaro and Gullino, 2004; Hernández-Muñoz *et al.*, 2006), biological control (Wszelaki and Mitcham, 2003) and chemical control (Chéour *et al.*, 1990; García *et al.*, 1996a; Lattanzio *et al.*, 1996; Blacharski *et al.*, 2001). Most of these treatments can, however, adversely affect color, flavor, aroma or texture (Li and Kader, 1989; Ke *et al.*, 1994; García *et al.*, 1995; Holcroft and Kader, 1999; Pelayo *et al.*, 2003).

The introduction of new reduced-risk fungicides, as low mammalian toxicity, low toxicity to nontarget organisms, low potential for groundwater contamination, compatible with integrated pest management practices (Adaskaveg *et al.*, 2005), opens the possibility to use chemical control strategies to protect strawberries, prolonging their shelf life. Among these fungicides, boscalid (= nicobifen) has been suggested to control *B. cinerea* and *R. stolonifer* on strawberry and other fruit crops (Latorre *et al.*, 2002b; Adaskaveg *et al.*, 2005). Boscalid is a carboxamide compound that inhibits succinate ubiquinone reductase in the mitochondrial electron transport chain. It is a very stable compound that appears to provide a long protection effect without affecting fruit quality. The aims of this research study were to determine the effect of boscalid and other fungicide treatments against *B. cinerea* and *R. stolonifer*, and to study the effect of infield treatments to control postharvest decays of strawberry during storage.

Material and Methods

Inoculum and fungicides

Isolates of *B. cinerea* and *R. stolonifer* were obtained from rotten strawberry fruits on potato dextrose agar acidulated with 0.5 ml L^{-1} 1 N lactic acid (APDA), incubated at 20ºC for 7 days. The inoculum suspension was prepared with spores obtained from 15-day-old cultures on APDA, adjusted to 10^6 spores ml^{-1} using a hematocymeter.

The following formulated fungicides were used in these studies: boscalid (Cantus 50 WG, BASF), boscalid combined with pyraclostrobin (Bellis, 25.2 + 12.8 WG, BASF), cyprodinil (Vangard 50 WG, Syngenta Agribusiness), cyprodinil combined with fludioxonil (Switch, 37.5 + 25.0 WG, Syngenta Agribusiness), fenhexamid (Teldor 50 WP, Bayer CropScience), fludioxonil (Acholar 50 WP, Syngenta Agribusiness), iprodione (Rovral 50 WP, Bayer CropScience), and pyraclostrobin (Comet 25 EC, BASF).

Fungicide effectiveness *in vitro*

To assess the effect of each fungicide on mycelial growth *in vitro* 10 isolates of *B. cinerea* and one of *R. stolonifer* were used. A 6-mm-diameter plug of mycelium were taken from the margin of actively growing colonies of each isolate on APDA and placed on Petri dishes (9 cm diameter) containing APDA, except cyprodinil that was tested on gelatine, glucose agar (GGA) medium (Latorre *et al.*, 2002a), amended with the test fungicides at the concentrations of 0.5, 1.0, 5.0 or 10.0 μg ml^{-1}. Fungicides were added after autoclaving to melted agar medium cooled approximately to 50ºC. Each isolate was seeded in duplicate in each fungicide concentration, including nonamended controls. The mycelial growth diameter was determined after 7 and 14 days period of incubation at 20ºC in darkness for *B. cinerea* and *R. stolonifer*, respectively. Data were expressed as % growth inhibition and the effective median concentration (EC_{50}) was graphically estimated. These tests were performed twice.

Field experiment

To assess the postharvest protection provided by preharvest fungicide applications against *B. cinerea* and *R. stolonifer*, one-year-old strawberry cv. Camarosa were sprayed at the beginning of flowering (February 1), 7 and 14 days later. Fungicide treatments and the active ingredient per hectare used were boscalid (500, 600 and 700 g ha^{-1}), boscalid + pyraclostrobin (102 + 77, 207 + 102, and 252 + 128 g ha^{-1}), cyprodinil + fludioxonil (375 + 250 g ha^{-1}), fenhexamid (750 g ha^{-1}), and iprodione (750 g ha^{-1}). All fungicide applications were performed with a 15 L manual sprayer (Solo Model 424, Santiago, Chile) delivering 1,000 L ha^{-1} at 4 bar of pressure.

Disease incidence was evaluated by recording the number of decay berries in a 25 berry sample per replicate, 3, 7 and 15 days after the last fungicide spray. Additionally, 25 ripe healthy berries were harvested from each replicate 3 days after the last spray. They were placed in one liter humid chambers (relative humidity > 90%) and separately inoculated by spraying them with 0.5 ml of a spore suspension of *B. cinerea* (2×10^5 conidia ml^{-1}) and *R. stolonifer* (4.4×10^5 sporangiospores ml^{-1}). Berries were incubated in darkness for 5 and 15 days at 20ºC and 5ºC, respectively, then disease incidence was assessed. Berries stored at 5ºC were evaluated again after being exposed two additional days at 20ºC to simulate a commercialization period. Considering that growth of these pathogens is almost completely arrested at < 5ºC, this assay was performed at 5ºC for a better evaluation of the residual fungicide activity against these pathogens. An equal number of not inoculated berries served as controls.

Postharvest trial

The effectiveness of boscalid to control *B. cinerea* was studied first on detached strawberries 'Camarosa'. Berries were surface disinfected (0.35% NaOCl for 30 s, rinsed twice in sterile distilled water), and immersed for 10 s in an aqueous suspension of boscalid at the concentration of 0.0, 150, 300 or 600 mg L^{-1}. Berries were allowed to dry for 2 h at room temperature (20-22ºC). After drying, berries were inoculated with a 20 µl drop of a conidial suspension (1.7×10^6 conidia ml^{-1}). Two *B. cinerea* isolates, F39 and F23 (EC_{50} equal to 2.0 µg ml^{-1}) were selected. Berries were placed in humid chambers (> 90% relative humidity) and incubated in darkness for 3 days at 20ºC before to determine the necrotic lesion developed.

In a second experiment, the protectant effect of boscalid at the concentration of 500, 600 and 700 mg L^{-1} was compared with the premixed formulation of boscalid plus pyraclostrobin, at the rates of 102 and 77, 207 and 102, and 252 and 128 mg L^{-1}, respectively. The fungicides cyprodinil + fludioxonil (375 + 250 mg L^{-1}), fenhexamid (750 mg L^{-1}), and iprodione (750 mg L^{-1}) were used as reference protectant. Surface disinfected strawberries 'Camarosa', uniform in size and maturity, were used. Berries were immersed for 10 min in an aqueous suspension of each of the tested fungicides and allowed to dry for 2 h at room temperature before to spray them to runoff with a spore suspension of *B. cinerea* (2.2×10^5 conidia ml^{-1}) or *R. stolonifer* (6.3×10^5 sporangiospores ml^{-1}). Disease incidence was determined after 5 days at 20ºC in humid chambers. This experiment was equally repeated storing berries at 5ºC. Berries were inoculated as above mentioned and disease incidence was recorded over 15 and 20 days of storage for berries inoculated with *R. stolonifer* and *B. cinerea*, respectively. An equal number of treated and inoculated berries and non treated and non inoculated berries were left as controls. All treatments were reevaluated after two additional days of incubation at 20ºC.

Effect of fungicide treatments on fruit quality

The effect of immersion fungicide treatments on berry quality was assessed on 5 berries samples, selected for uniformity from each of four replicates. Selected berries have approximately 2/3 of their red color, were uniform in size, and free of physiological disorders and fungal infections.

The impacts of fungicide treatments on inoculated berries and controls was assessed before and after stored them for 20 days at 5ºC plus 2 days at 20ºC. The following parameters were assessed: total soluble solid (TSS) contents, external color (C), firmness, and titratable acidity (TA). Total soluble solids content (%) was determined in the juice of ground strawberries by means of an Atago RX-1000 refractometer (Atago Co Ltd., Tokyo, Japan). The external color was determined with a Minolta colorimeter by measuring lightness (L*), hue angle and chroma. Firmness was determined with a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, UK), as the maximum penetration force reached during penetration to 6 mm in depth in 2 s, using a 3 mm diameter probe. Titratable acidity was determined by titrating 5 ml of juice to end point of pH 8.2 with 0.1 N NaOH and it was expressed as percentage (v/v) of citric acid. An equal number of non inoculated and non treated berries served as controls. All measurements were done in duplicate.

Design and statistical analyses

In field test, treatments were replicated four times in a randomized complete block design with each replicate consisting of 28 strawberry plants distributed on 3.8 m^2. Blocks were placed north to south, perpendicular to prevailing winds. Significant differences among the main effects were determined by analysis of variance (ANOVA), and treatment means were separated according to Waller-Duncan k ratio test (LSD) ($P = 0.05$). Data were processed using Multstat (Shane *et al.*, 1990). The relationship between doses of boscalid and disease incidence was studied by linear regression analysis.

Treatments of the first postharvest fungicide evaluation were randomly distributed as complete randomized design with a 2×4 (isolates and rates of boscalid) factorial structure, with four replicates each composed of a set of 5 ripe berries, uniform in size and color. Results were subjected to two ways ANOVA (Sigmastat, Systat Software, Inc., USA). Means were separated according to Waller-Duncan k ratio test (LSD) ($P = 0.05$) using Mulstat.

Treatments of the second postharvest fungicide evaluation were randomly distributed as a complete randomized design with four replicates each of 5 berries uniform in size and maturity. Data were subjected to ANOVA and means were separated according to Waller-Duncan k ratio test (LSD) ($P = 0.05$) using Multstat.

The effect of fungicide treatments on fruit quality was analyzed for variance following a complete randomized design and means were separated according to Waller-Duncan k ratio test (LSD) ($P = 0.05$) using Multstat.

Results

Fungicide sensibility *in vitro*

At a concentration of 0.5 μg ml^{-1}, inhibition of the mycelial growth of *B. cinerea* among tested fungicides exhibited a considerably variation, ranged boscalid from 0 to 71.4%, cyprodinil from 66.2 to 96.9%, fenhexamid from 3.2 to 89.4%, fludioxonil from 71.1 to 99.4%, iprodione from 0 to 67.7%, and pyraclostrobin from 21.4 to 74.9%. The mean EC_{50} values estimated for isolates of *B. cinerea* were 0.5 μg ml^{-1} for boscalid, 0.3 μg ml^{-1} for cyprodinil, 0.4 μg ml^{-1} for fludioxonil, 0.7 μg ml^{-1} for fenhexamid, 1.0 μg ml^{-1} for pyraclostrobin and 2.0 mg l^{-1} for iprodione.

At a concentration of 0.5 μg ml^{-1}, inhibition of the mycelial growth of *R. stolonifer* was higher than 95% with cyprodinil and fludioxonil, it was slightly inhibited (< 3%) with boscalid and no mycelial growth inhibition was obtained with other fungicides. At a concentration of 5 μg ml^{-1} inhibition of the mycelial growth was 93.7, 55.7, 37.1, and 0.0% with iprodione, pyraclostrobin, boscalid, and fenhexamid, respectively. The EC_{50} values estimated for *R. stolonifer* were < 0.5 μg ml^{-1} for cyprodinil and fludioxonil, 2.0 μg ml^{-1} for iprodione, 5.0 μg ml^{-1} for pyraclostrobin and 8.5 μg $ml^{-1}$for boscalid. Fenhexamid was completely ineffective to control *R. stolonifer in vitro*.

Field tests

At harvest, a moderate incidence of gray mold was obtained; however, leak was undetectable. Mean gray mold incidences were 24.0, 27.2, and 20.0% on untreated controls, after 3 h, and 7 and 14 days of the last fungicide application, respectively. Results became significantly different ($P \leq 0.01$) only 7 and 14 days after finishing fungicide treatments. Under these conditions, the best control was obtained with 700 g ha^{-1} of boscalid, significantly ($P \leq 0.05$) reducing gray mold from 5 and 6.8% obtained on untreated controls to less than 2.3% incidences. The highest doses of boscalid and boscalid mixed with pyraclostrobin provided a significant ($P \leq 0.05$) protection against *B. cinerea* after 14 days of the last spray (Table 1). At harvest, the relationship between dose of boscalid and gray mold incidence, 7 and 14 days after the last spray were best explained by a linear regression analysis with $y = -0.0317x + 26.68$ ($r^2 = 0.98$) and $y = -0.0125x + 20.72$ ($r^2 = 0.76$), respectively (Fig. 1A).

Table 1. Gray mold (*Botrytis cinerea*) incidence obtained on strawberry cv. Camarosa after three fungicide applications between flowering and harvest

Fungicides[1]	Dose (g ha^{-1})	% rotten fruits after the last fungicide application		
		3 h	7 days	14 days
Boscalid	500	20.0 ± 5.7^{ns}	9.0 ± 3.8 ab^{2}	16.0 ± 6.5 abc^{2}
	600	7.3 ± 8.5	7.0 ± 3.8 a	15.0 ± 6.0 abc
	700	18.0 ± 14.8	6.0 ± 4.0 a	9.0 ± 3.8 a
Boscalid + pyraclostrobin	102 + 77	21.0 ± 6.8	15.0 ± 6.0 bc	14.0 ± 5.2 abc
	207 + 102	16.0 ± 9.8	15.0 ± 8.9 bc	12.0 ± 3.3 ab
	252 + 128	16.0 ± 8.0	8.0 ± 3.3 ab	9.0 ± 3.8 a
Cyprodinil + fludioxonil	375 + 250	17.0 ± 8.9	15.0 ± 5.0 bc	14.0 ± 7.7 abc
Fenhexamid	750	13.0 ± 3.8	10.0 ± 2.3 ab	18.0 ± 5.2 bc
Iprodione	750	13.0 ± 6.0	18.0 ± 5.2 c	20.0 ± 3.3 c
Untreated	—	24.0 ± 3.3	27.0 ± 6.0 d	20.0 ± 5.6 c

[1] Three weekly fungicide sprays were done with approximately 1,000 L ha^{-1}. [2] Means of four replicates followed by the same letters were not statistically different according to Duncan-Waller k-ratio test ($P = 0.05$). ±: standard deviation. ns: ANOVA not significant at $P < 0.05$.

On fruits that were inoculated with *R. stolonifer* or *B. cinerea* at harvest, the protection effects obtained with preharvest fungicide applications were not significant after 10 or 15 days of cool storage, respectively. However, a significant ($P \leq 0.01$) protectant effect against *B. cinerea* and *R. stolonifer* was obtained when these same fruits were maintained for two days at 20ºC. Independently of fungicide treatments, gray mold incidence decreased from 90% on untreated controls to 25 to 65% on treated fruits. Leak decreased from 95% on untreated controls to 45 to 80% on treated fruits. Under these experimental conditions, the mixture of cyprodinil and fludioxonil, and boscalid (600, 700 mg L^{-1}) provided the best protection against *B. cinerea*. Similarly, boscalid (700 g ha^{-1}) and iprodione (750 g ha^{-1}) gave the best protection against leak (Table 2). The relationship between dose of boscalid and fruit rots incidences were best explained by a linear regression with $y = -0.09x + 89.48$ ($r^2 = 0.98$) and $y = -0.06x + 98.02$ ($r^2 = 0.81$) for *B. cinerea* and *R. stolonifer*, respectively (Fig. 1B).

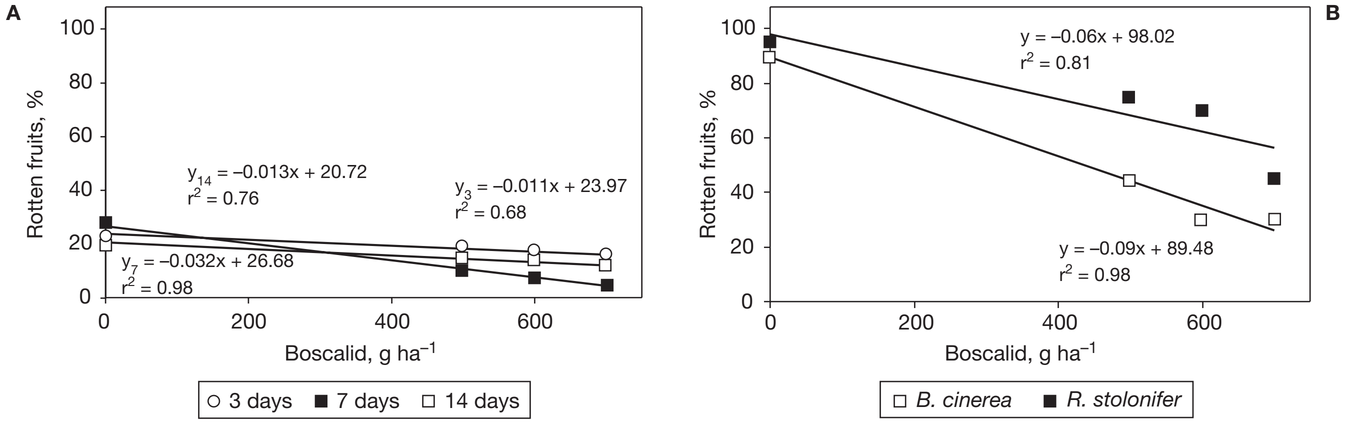

Figure 1. Relationship between rates of boscalid applied three times between flowering and harvest and fruit decay incidences caused by *Botrytis cinerea* and *Rhizopus stolonifer* on strawberries cv. Camarosa. A: Residual effect against *B. cinerea* obtained 3, 7 and 14 days after the last fungicide spray. B: Residual effect obtained on fruits harvested 3 days after the last fungicide spray, inoculated with conidia of *B. cinerea* or sporangiospores of *R. stolonifer* and then stored in humid chambers for 15 days at 5ºC and 2 additional days at 20ºC.

Table 2. Incidence of gray mold (*Botrytis cinerea*) and leak (*Rhizopus stolonifer*) obtained on strawberry cv. Camarosa stored for 15 days at 5ºC and 2 days at 20ºC. Fruits were harvested 3 h after the last of three fungicide sprays done between flowering and harvest

Fungicides[1]	Dose (g ha^{-1})	Disease incidence after cool storage[2] (%)			
		B. cinerea[3]		*B. stolonifer*[3]	
		0 days	2 days	0 days	2 days
Boscalid	500	0.0 ± 0.0^{ns}	45.0 ± 19.1 ab[4]	25.0 ± 37.9^{ns}	75.0 ± 30.0 ab[4]
	600	0.0 ± 0.0	30.0 ± 25.8 a	5.0 ± 10.0	70.0 ± 25.8 ab
	700	0.0 ± 0.0	30.0 ± 11.5 a	0.0 ± 0.0	45.0 ± 10.0 a
Boscalid + pyraclostrobin	102 + 77	0.0 ± 0.0	45.0 ± 25.2 ab	10.0 ± 20.0	80.0 ± 16.3 ab
	207 + 102	5.0 ± 10.0	65.0 ± 34.2 bc	5.0 ± 10.0	65.0 ± 25.2 ab
	252 + 128	0.0 ± 0.0	35.0 ± 30.0 ab	5.0 ± 10.0	65.0 ± 19.1 ab
Cyprodinil + fludioxonil	375 + 250	0.0 ± 0.0	25.0 ± 19.1 a	5.0 ± 10.0	70.0 ± 34.6 ab
Fenhexamid	750	5.0 ± 10.0	35.0 ± 34.2 ab	5.0 ± 10.0	60.0 ± 16.3 ab
Iprodione	750	0.0 ± 0.0	45.0 ± 19.1 ab	0.0 ± 0.0	55.0 ± 10.0 a
Controls:					
— Inoculated	—	0.0 ± 0.0	90.0 ± 11.5 c	35.0 ± 47.3	95.0 ± 10.0 b
— Non inoculated	—	10.0 ± 20.0	80.0 ± 16.3 c	nt	nt

[1] Three weekly fungicide sprays were done with approximately 1000 L ha^{-1}. [2] Fruits harvested 15 days after the last fungicide application were inoculated with a spore suspension of *R. stolonifer* or *B. cinerea,* before storing them at 5ºC for 10 and 15 days, respectively. [3] Disease incidences obtained immediately after fruits were taken from cool storage and after 2 days at 20ºC. [4] Means of four replicates followed by the same letters were not statistically different according to Duncan-Waller k-ratio test ($P + 0.05$). ±: standard deviation. ns: ANOVA were not significant at $P < 0.05$. nt, not tested.

Postharvest tests

The relative resistant and sensitive isolates of *B. cinerea* were equally controlled with boscalid obtaining over 90% control with 300 mg L^{-1}. The effect of concentration was significant ($P \leq 0.001$) but differences between these isolates and the interaction between isolates and concentration were non significant. The relationship between rate of boscalid and gray mold severity was best explained by an exponential model $y = 104.84x^{-2,246}$ ($r^2 = 0.91$).

Gray mold and leak significantly ($P \leq 0.01$) decreased on treated fruits after 5 days at 20ºC. Under these experimental conditions, all fungicide treatments, except fenhexamid and iprodione, provided a significant ($P \leq 0.05$) protection against *B. cinerea*, being boscalid, boscalid plus pyraclostrobin and cyprodinil plus fludioxonil the best treatments. Leak was significantly ($P = 0.05$) controlled with boscalid (600, 700 mg L^{-1}), and boscalid plus pyraclostrobin. However, boscalid (500 mg L^{-1}), fenhexamid, iprodione and cyprodinil plus fludioxonil were ineffective against *R. stolonifer* (Table 3). The relationship between rate of boscalid and fruit rot incidences were best explained by a linear regression with $y = -0.09x + 60.35$ ($r^2 = 0.97$) and $y = -0.09x + 101.03$ ($r^2 = 0.78$) for *B. cinerea* and *R. stolonifer*, respectively (Fig. 2A).

The development of gray mold and leak was considerably arrested on treated fruits at 5ºC. Gray mold incidence significantly ($P \leq 0.05$) decreased from 85% on untreated but inoculated controls to < 35%, and leak decreased from 55% on untreated but inoculated controls to < 25% (Table 4). Boscalid and boscalid plus pyraclostrobin provided a complete control of *B. cinerea* on fruits stored for 20 days at 5ºC while a partial control was obtained with cyprodinil plus fludioxonil, fenhexamid and iprodione. Similarly, *R. stolonifer* was completely arrested on fruits treated with boscalid or boscalid plus pyraclostrobin but, only a partial control was achieved with cyprodinil plus fludioxonil, fenhexamid, and iprodione in fruits maintained in cool storage for 15 days after treatment (Fig. 3).

Gray mold and leak considerably increased after exposing fruits for two days at 20ºC. However, rot incidences were significantly ($P \leq 0.05$) lower on treated fruits. For instance, gray mold was reduced from 100% on inoculated but untreated controls to 10 to 55% on treated fruits, and the best protection was obtained with bos-

Table 3. Effect of fungicide treatments on the development of *Botrytis cinerea* and *Rhizopus stolonifer* obtained on strawberries cv. Camarosa subjected to a 10 min immersion treatment and stored at 20ºC for 5 days

Fungicide treatments	**Rate (mg L^{-1})**	***B. cinerea*** **(%)**	***R. stolonifer*** **(%)**
Boscalid	500	20.0 ± 16.3 ab[1]	75.0 ± 19.1 de[1]
	600	0.0 ± 0.0 a	45.0 ± 19.1 bcd
	700	0.0 ± 0.0 a	20.0 ± 16.3 ab
Boscalid + pyraclostrobin	102 + 77	15.0 ± 30.0 ab	35.0 ± 47.3 abc
	207 + 102	10.0 ± 11.5 a	10.0 ± 11.5 a
	252 + 128	5.0 ± 10.0 a	5.0 ± 10.0 a
Cyprodinil + fludioxonil	375 + 250	5.0 ± 10.0 a	60.0 ± 36.5 cde
Fenhexamid	750	45.0 ± 41.2 bc	95.0 ± 10.0 e
Iprodione	750	55.0 ± 10.0 c	75.0 ± 19.1 de
Controls:			
— Inoculated	—	60.0 ± 28.3 c	95.0 ± 10.0 e
— Non inoculated	—	10.0 ± 11.5 a	20.0 ± 23.1 ab

[1] Means of four replicates followed by the same letters were not statistically different according to Duncan Waller k-ratio test ($P = 0.05$). ±: standard deviation.

calid plus pyraclostrobin (Table 4). The relationship between rate of boscalid and fruit rot incidences were best explained by a linear regression with $y = -0.11x + 95.56$ ($r^2 = 0.97$) and $y = -0.14x + 99.48$ ($r^2 = 0.94$) for *B. cinerea* and *R. stolonifer*, respectively (Fig. 2B).

Effect of fungicide treatments on fruit quality

Physiological deterioration of berry after 20 days of storage at 5°C and two days at 20°C was characterized by fruit softening and darkness of red skin color. Firmness reduced from 1.3 to 0.4 N and skin color varied from 50.6 to 36.8° for berry lightness, 45.1 to 35.6 for chroma and 52.1 to 33.3° for hue angle.

Fungicides significantly reduced fruit softening and skin color measured by chroma. Berry treated with boscalid + pyraclostrobin was 1.5, 1.2 and 1.1 N and was the best treatment compared with 0.8 N of boscalid alone and iprodione or 0.4 N of untreated. Chroma value of the berry color was significantly higher in fruit treated with boscalid 252 mg L^{-1} + pyraclostrobin 128 mg L^{-1} than

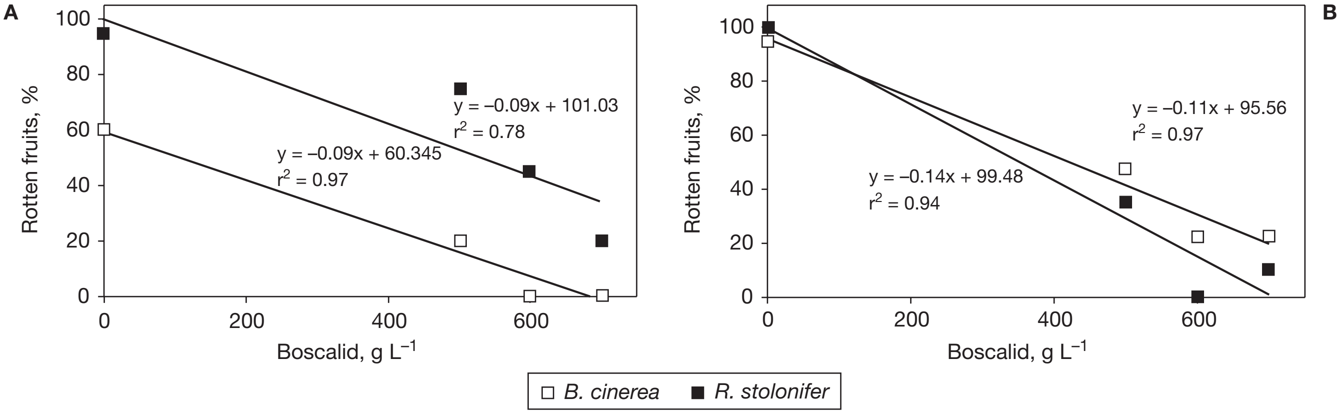

Figure 2. Relationship between rates of boscalid, applied by immersing fruits during 10 min in an aqueous suspension, and fruit decay developed on strawberry fruits cv. Camarosa treated by 10 min immersion and then inoculated with conidia of *Botrytis cinerea* or sporangiospores of *Rhizopus stolonifer*. A: On inoculated fruits stored for 5 days at 20ºC in humid chambers. B: On inoculated fruits stored at 5ºC for 15 and 20 days, fruits inoculated with *R. stolonifer* and *B. cinerea* respectively, and then exposed for 2 additional days at 20ºC, simulating a commercialization period.

Table 4. Gray mold (*Botrytis cinerea*) and leak (*Rhizopus stolonifer*) of strawberry cv. Camarosa obtained after cool storage and two additional days at 20ºC

Fungicides	Rate	*B. cinerea*[1]		*B. stolonifer*[1]	
	(mg L^{-1})	20 days	20 + 2 days	15 days	15 + 2 days
Boscalid	500	5.0 ± 10.0 a[2]	35.0 ± 30.0 abcd[2]	0.0 ± 0.0 a[2]	35.0 ± 19.1 bc[2]
	600	0.0 ± 0.0 a	25.0 ± 19.1 abc	0.0 ± 0.0 a	0.0 ± 0.0 a
	700	0.0 ± 0.0 a	15.0 ± 10.0 a	0.0 ± 0.0 a	10.0 ± 11.5 ab
Boscalid + pyraclostrobin	102 +77	10.0 ± 11.5 a	10.0 ± 11.5 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	207 +102	10.0 ± 11.5 a	20.0 ± 16.5 ab	0.0 ± 0.0 a	0.0 ± 0.0 a
	252 +128	0.0 ± 0.0 a	10.0 ± 11.5 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Cyprodinil + fludioxonil	375 +250	5.0 ± 10.0 a	45.0 ± 25.2 bcd	10.0 ± 11.5 ab	45.0 ± 34.2 cd
Fenhexamid	750	10.0 ± 11.5a	50.0 ± 11.5 cd	25.0 ± 10.0 c	65.0 ± 10.0 de
Iprodione	750	35.0 ± 10.0 b	55.0 ± 19.1 de	15.0 ± 19.1 bc	40.0 ± 36.5 cd
Controls:					
— Inoculated	—	85.0 ± 19.1 c	100.0 ± 0.0 f	55.0 ± 10.0 d	100.0 ± 0.0 f
— Non inoculated	—	40.0 ± 28.3 b	80.0 ± 23.1 ef	5.0 ± 10.0 ab	80.0 ± 40.0 ef

[1] Berries were treated by a 10 min immersion, and *R. stolonifer* and *B. cinerea* were determined after 15 and 20 days and reevaluated after two days at 20ºC. [2] Means of four replicates followed by the same letters were not statistically different according to Duncan Waller k-ratio test ($P = 0.05$). ±: standard deviation.

untreated fruit, therefore red color remained on the fruit under the combination of these two fungicides (Table 5).

Discussion

Strawberries production has been primarily oriented to internal fresh market and for industrial purposes in Chile. However, there is a great potential to increase its production mainly for export to long distance markets that would imply at least a 15-day period of maritime transport. Considering that strawberries have high respiration rate during storage and that they are very susceptible to postharvest fungal diseases, the development of chemical control to protect berries during transportation is needed.

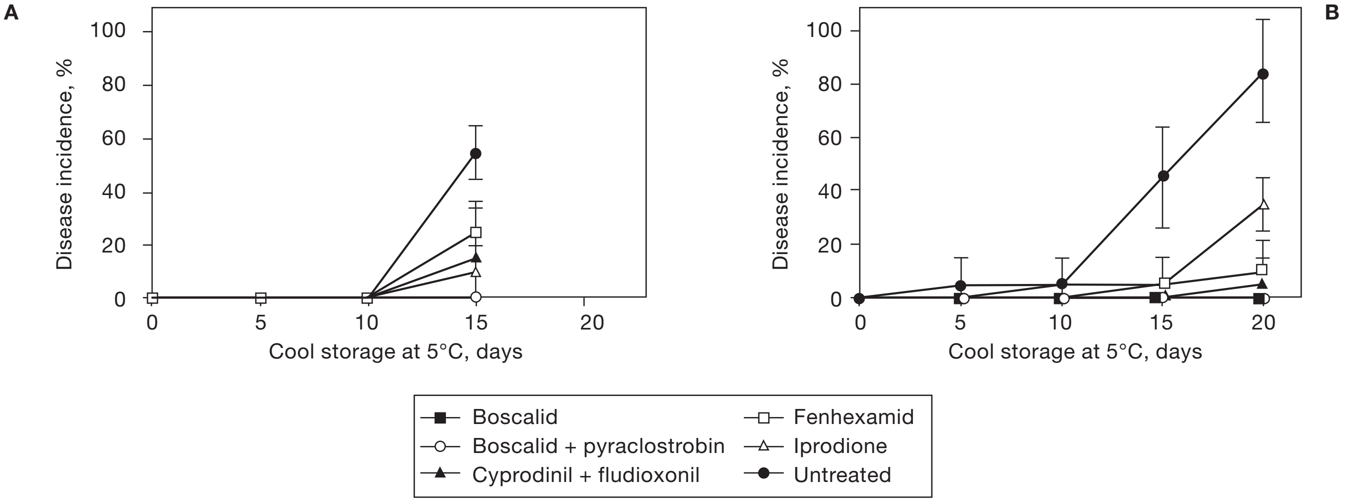

Figure 3. Disease progress curves obtained on strawberry cv. Camarosa that were treated by immersion for 10 min in an aqueous suspension of the tested fungicides, and were inoculated with *Rhizopus stolonifer* (A) and *Botrytis cinerea* (B) before to store them at 5ºC. Fungicides used were 700 mg L^{-1} boscalid, 252 mg L^{-1} boscalid premixed with 128 mg L-1 pyraclostrobin, 375 mg L^{-1} cyprodinil premixed with 250 mg L^{-1} fludioxonil, 750 mg L^{-1} fenhexamid, 750 mg L^{-1} iprodione and untreated but inoculated control. Bars = standard deviation.

This study demonstrated that reduced-risk fungicides, like boscalid and the premix formulation of cyprodinil and fludioxonil, with favorable toxicological and environmental profiles open a good possibility to use them for postharvest treatments, as it has been proposed for other fruit crops (Adaskaveg *et al.*, 2005). However, the use of registered fungicides for postharvest treatment should only be considered. At present, postharvest fungicide treatments are not available in most countries.

A significant protection against *B. cinerea* and *R. stolonifer* was obtained even 20 days after treatments which would be sufficient to protect Chilean strawberries during maritime transportation to international markets. Furthermore, exposing berries to 20ºC considerably increased disease incidence on untreated controls. However, a good protection against *B. cinerea* and *R. stolonifer* was still obtained on treated berries, particularly considering that just after fungicide treatments berries were challenged with a relatively high inoculum concentration of these pathogens. In addition, fenhexamid appeared as a good alternative against *B. cinerea* but, without sufficient fungicide activity against *R. stolonifer*. The high incidence of gray mold infection on not inoculated fruits, not different in decay incidence by inoculated ones (Table 2), was possible due to gray mold infections that occurred at bloom, remaining latent on petal, sepals and stamens on the fruit (Powelson, 1960).

In this study, cyprodinil and fludioxonil at concentrations of 0.1 mg L^{-1} were the most powerful fungicides *in vitro*, followed by boscalid and pyraclostrobin with good activity in reducing *in vitro* mycelial growth of *B. cinerea* and a partial activity against *R. stolonifer*. However, only one isolate of *R. stolonifer* was used in this study; therefore, other isolates should be tested before to conclude. Results obtained *in vivo* demonstrated a significant relationship between rates of boscalid and gray mold control, as well as between rates of boscalid and leak control when this fungicide was applied at concentrations higher than 500 mg L^{-1}. Similar to other works (Smith *et al.*, 1991; Matheron and Porchas, 2004), the relative sensitivity of mycelial growth of *B. cinerea* and *R. stolonifer* to tested active ingredients demonstrated in laboratory evaluations that did not have consistent predictive value with respect to performance of these fungicides *in vivo*. This disparity between activity *in vitro* and *in*

Table 5. Effect of fungicide treatments on quality of strawberry cv. Camarosa, treated for 10 min in an aqueous fungicide suspension, cool stored at 5ºC for 20 days and then maintained for two days at 20ºC. Berries were inoculated with a conidial suspension of *Botrytis cinerea* just after treatment

Fungicide treatments	**Rate (mg L^{-1})**	**Berry quality parameters**					
		Solid soluble (%)	**Titratable acidity (%)**	**Firmness (N)**	**Color**		
					Lightness	**Chroma***	**Hue angle**
Before fungicide treatments							
Untreated	—	7.4	1.2	1.3	50.6	45.1	52.1
After fungicide treatments							
Boscalid	500	7.5 ± 0.3 de[1]	1.05 ± 0.02 ab[1]	0.9 ± 0.2 b[1]	37.0 ± 1.0[ns]	38.4 ± 1.2 abc[1]	39.9 ± 1.8[ns]
	600	7.2 ± 0.2 cd	1.07 ± 0.03 abc	0.8 ± 0.2 b	36.8 ± 1.6	41.7 ± 2.2 bc	36.6 ± 2.7
	700	7.7 ± 0.2 ef	1.12 ± 0.03 bc	0.7 ± 0.1 b	37.0 ± 0.9	40.5 ± 3.2 abc	38.7 ± 2.9
Boscalid + pyraclostrobin	102 + 77	5.8 ± 0.3 a1	1.08 ± 0.02 abc	1.5 ± 0.5 d	38.7 ± 1.4	43.7 ± 2.1 c	37.2 ± 1.4
	207 + 102	6.4 ± 0.3 b	1.08 ± 0.02 abc	1.2 ± 0.2 c	37.7 ± 1.3	40.6 ± 2.3 abc	36.5 ± 1.6
	252 + 128	7.0 ± 0.2 c	1.05 ± 0.04 a	1.1 ± 0.1 c	39.1 ± 1.7	44.9 ± 2.1 c	36.3 ± 1.3
Cyprodinil+ fludioxonil	375 + 250	8.1 ± 0.4 fg	1.09 ± 0.01 abc	0.5 ± 0.1 a	35.9 ± 3.0	35.0 ± 8.4 a	34.7 ± 4.1
Fenhexamid	750	8.3 ± 0.2 g	1.04 ± 0.06 a	0.5 ± 0.1 a	37.0 ± 2.7	36.3 ± 5.4 ab	36.6 ± 3.5
Iprodione	750	7.5 ± 0.3 de	1.09 ± 0.01 abc	0.8 ± 0.2 b	36.8 ± 2.0	41.7 ± 3,1 bc	36.8 ± 1.8
Untreated	—	7.8 ± 0.2 ef	1.12 ± 0.02 c	0.4 ± 0.0 a	36.8 ± 2.9	35.6 ± 5.0 ab	33.3 ± 5.5

[1] Means of four replicates, each of 25 fruits, followed by the same letters were not statistically different according to Duncan Waller k-ratio test ($P = 0.05$). ns, ANOVA not significant at $P < 0.05$.

vivo were very likely explained by multiple host, pathogen and environment interactions encompassed by *in vivo* studies, whereas *in vitro* tests only assessed the interaction between the pathogen and the fungicide.

The optimum concentration of boscalid to control gray mold was estimated at rate of 150 mg L^{-1} which was coincident with a previous work done on table grapes to control *R. stolonifer* and *A. niger* (Latorre *et al.*, 2002b). However, in this study boscalid only provided good control of gray mold and leak at much higher concentrations applied as 10 min immersion treatments. This apparent discrepancy could very likely be explained by differences in testing procedures and due to the nature of strawberry fruit surfaces that may not retain enough fungicide after a 10 min immersion treatment at lower fungicide concentrations. Therefore, the relative low efficacy demonstrated by boscalid premixed with pyraclostrobin can be explained by the relative low fungicide deposits left after treating berries even at the highest commercial rate at present being suggested to control *B. cinerea* on other fruit crops.

It was interesting to establish a long term effect of boscalid, cyprodinil plus fludioxonil against *B. cinerea* and *R. stolonifer* on strawberries stored at 5ºC. Even a better effect must be expected at 0ºC which should be the storage temperature for long distance transportation. Similar to other reports using these fungicides, in field treatments few days before harvest, can considerable protect fruits after harvest (Sholberg *et al.*, 2003; Errampalli and Crnko, 2004; Franck *et al.*, 2005). However, it is well known that the environmental conditions of one year are different from another year and that the behavior of one cultivar is different from another. Therefore, this conclusion needs further confirmation considering that this study was only performed in a single locality in one growing season using one strawberry cultivar.

In contrast to cyprodinil and fludioxonil, a broad variation in sensitivity among isolates of *B. cinerea* was obtained *in vitro* with boscalid and pyraclostrobin, suggesting a pronounced genetic variation of resistance being present in the population of this pathogen. Nevertheless, the less sensitive *B. cinerea* isolate to boscalid was effectively control *in vivo*. Therefore, there was no evidence for the presence of resistant strains among isolates of *B. cinerea*.

Loss of fruit texture adversely affects quality, reducing postharvest shelf life of strawberries and other perishable fruits. With the exception of fruit treated with boscalid plus pyraclostrobin, a pronounced change in firmness occurred before and after berries were treated with boscalid and other tested fungicides. It is very possible that pyraclostrobin delayed senescence, interfering the ethylene metabolism as it has been suggested previously for other strobilurin fungicides (Ypema and Gold, 1999; Bartlett *et al.*, 2002). However, significant differences were obtained between berries treated with boscalid and untreated controls that were at least partially attributed to the control effect provided by this fungicide.

In general, TSS contents were higher on treated than on untreated berries that was possible associated to a partial water loss. Independently of the fungicide treatment, considerable differences in chroma and less acute differences were obtained with lightness and hue angle between treated and nontreated controls after cool storage plus ripening period. Therefore, based on these results there were no evidences for the presence of adverse effect of boscalid and other fungicide treatment on berry quality.

In conclusion, data presented in this study show that immersion treatments using an aqueous suspension of boscalid and other existing fungicides are a feasible strategy to prevent spoilage of strawberry during storage. This can extend shelf life for over the minimum period required to ship Chilean strawberries to foreign markets, and without adversely affect quality. However, further research is needed to establish a commercial recommendation.

Acknowledgements

The authors acknowledge BASF Chile S.A. for their financial support.

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