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Effect of pre and postharvest application of fungicides on postharvest decay of Bosc pear caused by *Alternaria—Cladosporium* complex in North Patagonia, Argentina

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ABSTRACT

In recent years, decays by *Alternaria spp.* and *Cladosporium spp.* constituted a hazard to "Bosc" pear fruit during cold storage with significant economic losses (about 50%) in the Rio Negro and Neuquen Valleys. Studies with new fungicides that potentially have good activity against these key pathogens of "Bosc" pears were carried out. During the years 2011, 2012 and 2013, commercial formulations of pyraclostrobin + boscalid (Pyr + Bosc), cyprodinil + fludioxonil (Cyp + Flu), fludioxonil (Flu), pyrimetanil (PyrM) and myclobutanil (Myc) were studied by *in vitro* and *in vivo* tests. Commercial formulations of Flu, PyrM and Myc were added to the study to be used and have a use register for pome fruits in Argentina. *In vivo* tests were applied postharvest on laboratory and semicommercial scale, and, preharvest, to orchard. *In vitro* tests showed high percentages of inhibition of mycelial growth and conidia germination of both Alternaria and *Cladosporium*, however Flu was highlighted in the inhibition of Alternaria (EC50 = 0.0014) and Pyr + Bosc to *Cladosporium* (EC50 = 1.14×10^{-8}). *In vivo* assays on semi-commercial scale, showed that fungicides evaluated (Pyr + Bosc, Cyp + Flu, Flu and Pyr) applied postharvest only reduced the natural incidence of *Alternaria-Cladosporium* complex (4 months at −1/0 °C), between 24 and 35%, while on wounds artificially inoculated, the reduction achieved was between 91 and 98%. These results suggest that for the control of *Alternaria-Cladosporium* complex in Bosc pears, postharvest interventions are insufficient. In this sense, the results obtained of preharvest application of Pyr + Bosc showed that the control of the incidence of this decay after 4 months of cold storage was 44.6% of *Alternaria-Cladosporium* and 95% of *B. cinerea.* The results of this study indicate that preharvest applications, with the fungicide Pyr + Bosc, reduce the incidence of decay in postharvest, so this might be an important strategy to reduce postharvest losses in the irrigated valleys of Rio Negro y Neuquen.

1. Introduction

Argentina is the largest pear producer country and the major exporter in the Southern Hemisphere. The main pear-growing area is situated in the provinces of Rio Negro and Neuquen, Patagonia. The production of Bosc pears represents approximately 30.000.000 million of Kg, it results in approximately of 1.318.682 exportable boxes.

The postharvest diseases limit the storage period and marketing life of pears. Although it is true that in Patagonia (Argentina), *Penicillium expansum* is the main pathogen of fruit in storage conditions (-1/0 °C) (Dobra et al., 1995) and *Botrytis cinerea* is the second in significance (Dobra et al., 2008), other pathogens are becoming really important in the postharvest of pear fruit. Until 2008, the pathogens *Alternaria* and *Cladosporium* did not constitute a danger for the storage of pears in the Rio Negro and Neuquen Valleys. However, in recent years, Beurre Golden Bosc pear fruit exhibited severe symptoms of disease, causing significant economic losses (approximately 50%).The diseases were diagnosed as "*Alternaria* rot" caused by *Alternaria* spp. and "*Cladosporium* rot" caused by *Cladosporium* spp. Our studies (unpublished data) indicate that these pathogens would be associated, originating together rots by *Alternaria-Cladosporium* complex.

For some fruits, such as pome, the wounds are the main way for the entry of fungus causing postharvest decays (Jones and Aldwinckle, 2002; Dobra et al., 2008). However, *Alternaria* decay of fruit frequently develops in storage from latent infections occurring in the orchard (Li et al., 2007). In these cases, the infected unripe fruit does not show

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symptoms of rot, but they appear during the months of storage at low temperature (Prusky et al., 1981; Prusky, 1996; Biggs, 1995; Thomma, 2003; Kobiler et al., 2011).

According to different authors (Roberts, 2005; Harteveld et al., 2013; Narian et al., 2016), the diseases caused by *Alternaria* species have been considered of minor importance in the past. Nonetheless, currently, due to newer agriculture technology and extensive culture of new varieties, a number of *Alternaria* species are coming up more prominent and responsible for enormous losses due to leaf spots and blights, lesions on stem, blossom blights, and decays of fruit to field, during storage and shipment. There is a paucity of information on the diseases of *Alternaria* apple and pear fruit (Harteveld et al., 2013; 2014). Currently, most of the commercial packaging in our region has modified the postharvest management practices of pear fruit: i) avoiding wounds and bruises on the fruit during harvesting and subsequent handling; ii) controlling sanitation practices, and iii) storing to conventional cold or modified atmosphere. However, these beneficial practices are usually not enough to fully protect the fruits from losses caused by increased incidence of *Alternaria* and *Cladosporium* rots. On the other hand, there is no registered synthetic fungicide for use in pear fruit against *Alternaria* and *Cladosporium*.

According to Harteveld et al. (2014) *Alternaria* fruit spot infection took place about 100 days after bloom (DAB) in the apple orchard and the fruit inoculations in plant did not show specific stage of fruit susceptibility. Sharma and Sharma (1991) reported that an effective spray programme for the control of apple scab or black spot (caused by *Venturia inaequalis*) also reduced *A. mali* symptoms significantly.

In the last years, new active ingredients as boscalid, pyraclostrobin, fludioxonil and cyprodinil, have been tested for the control of postharvest pathogens in pome fruits, table grapes, blueberries and strawberries (Everet et al., 2007; Serey et al., 2007; Sallato et al., 2007; Sugar, 2011).

In other countries, it has been shown that the treatments applied to fruit in the orchard affect the incidence of rots during medium − long term storage. Preharvest applications with cyprodinil and pyraclostrobin with boscalid reduced the incidence of decay caused by various fungi in pome fruits (Sholberg et al., 2003; Sugar et al., 2003; Xiao and Boal, 2009). The preharvest applications of fungicides could protect the wounds and cuticle of fruit, and reduce the inoculum on the surface of the fruit (Sugar and Basile, 2011).

Commercial formulates with these active ingredients are currently available for use in different crops in other countries. These fungicides have proved efficacy in the control of postharvest pome fruits diseases (apples and pears) caused by *Gloeosporium*, *Alternaria*, *Botrytis* and *Penicillium* (Xiao and Boal, 2009; Sugar and Basile, 2011). The active ingredients of these fungicides represent distinct classes with respect to resistance management: quinone outside inhibitors (pyraclostrobin) and inhibition of complex II of succinate-dehydrogenase (boscalid) (FRAC, 2017). Also, the possibility of preharvest applications of these substances to mitigate the incidence of *Alternaria* and *Cladosporium* decays on Bosc pears, would constitute an answer to the problem. In Argentina, the effectiveness of these active ingredients against postharvest pathogens of pome fruits has not been previously demonstrated.

The aim of this study was to evaluate the efficacy of fungicides with new active substances for the region of Alto Valle de Rio Negro and Neuquen in the control of diseases caused by *Alternaria-Cladosporium* complex on Beurre Bosc pears. The efficacy of new fungicides was evaluated by postharvest and preharvest applications, and was compared with fungicides used in the region to mitigate the incidence of diseases caused by these pathogens.

2. Materials and methods

This study was conducted in three phases: (I) Preliminary experiments were conducted *in vitro* to determinate the efficacy of chemical fungicides in controlling *Alternaria* and *Cladosporium*. (II) Evaluation *in vivo* on fruit with both pathogens, at laboratory and semicommercial scale. (III) Orchard trials to determine the curative and protective efficacy of chemical fungicides in fruit decay control at postharvest.

2.1. Pathogens

Isolates of *Alternaria alternata* (1083) and *Cladosporium herbarum* (1084) were obtained of rotten Golden Russet Bosc cv. pear fruit. Fruits showing the typical symptoms of disease were removed from storage and used for fungi isolation. Each isolate was grown at 24 °C as a monoconidial culture on potato dextrose agar (PDA) and kept into glycerol 20% in tubes at −20 °C until their use. Fungal virulence was assured by periodic inoculations of pear fruits and re-isolations, and these were stored in the collection of Laboratory of Phytopathology (Instituto de Biotecnologia Agropecuaria del Comahue, Facultad Ciencias Agrarias, CITAAC − UNCo). The isolates of high virulence in fruit were selected and used in the experiments.

Briefly, for the production of inoculum from stored cultures 10 μL were transferred into Petri plates with potato dextrose agar (PDA) for *Alternaria* or malt extract agar (MEA) for *Cladosporium,* and were incubated at 24 °C. *Alternaria* conidia were obtained from 10-days-old cultures in darkness, while *Cladosporium* conidia were obtained from 14-days-old cultures under a 12:12 h (light:dark) photo regime. In both cases, conidial suspensions were obtained by scraping the colony surface with a sterile scalpel, suspended in sterile water and filtered to remove fungal mycelium. Conidia concentrations were adjusted by direct count with a Neubauer chamber at 1×10^6 conidia/mL.

2.2. Sensitivity of Alternaria and Cladosporium to fungicides

Sensitivity to the fungicides: pyraclostrobin 12.8% w/w and boscalid 25.2% w/w (WG) (Pyr + Bosc) (Bellis, Basf), cyprodinil 37.5% w/w and fludioxonil 25% w/w (WG) (Cyp + Flud) (Switch, Syngenta) and fludioxonil 23% v/v (SC) (Flu) (Scholar, Syngenta) was evaluated for each fungus by two techniques. Commercial formulates of fungicides were used.

2.2.1. Inhibition of mycelial growth

The fungicide suspensions were added to potato dextrose agar (PDA) or malt extract agar (MEA) at 4 concentrations 0.1, 1.0, 10 and 100 μg/mL. Discs (6 mm diameter) containing mycelium of *Alternaria* or *Cladosporium* were obtained from the perimeter of a growing active colony and were placed at the centre of a 90 mm Petri plate containing PDA or MEA, with or without fungicide. Plates with media of culture without fungicide were used as the negative controls (FAO, 1982). The effect of each fungicide concentration was assessed three times for each isolate. Colony diameter was measured after 7–10 days of incubation at 24 °C. Data were obtained as the percentage of growth on fungicide-amended medium relative to growth on the no-fungicide control. The efficacy of each fungicide in inhibition of pathogen was calculated using the following formula: $\%MI = 100 \times [(NMD - TMD)/NMD]$, where NMD was the average diameter (in centimetres) from 3 non-treated controls and TMD from treated controls. The experiment was replicated twice per concentration of the fungicide for pathogen. The fungicide concentration that inhibited growth by 50% (EC50) was estimated for each isolate. The fungicide that obtained the lowest value of EC50 was considered as of highest efficiency.

2.2.2. Inhibition of spore germination

The effects of each fungicide on spore germination of *Alternaria* and *Cladosporium* were tested on sterile water (SW). Conidial suspensions (1×10^6 conidia/mL) of each pathogen were mixed with the aqueous solution of each test compound to obtain final concentrations of 0.1, 1.0, 10 and 100 μg/mL. The percentage of spore germination (100 spores for each treatment) was estimated after incubation at 24 °C in darkness for 16 h on a rotary shaker (150 rpm) using an optic

microscope. Conidia were considered germinated when the germ tube length was equal to or longer than the conidia length. Three replications were evaluated for each treatment and experiment was repeated twice. EC50 values were calculated for individual isolates by regression of the inhibition of spore germination against the logarithm of the fungicide concentration.

2.3. Effect of fungicides in postharvest assay in vivo

2.3.1. Fruits

In 2011 and 2012, Golden Russet Bosc pears were harvested at commercial maturity with flesh firmness under of 11.42 Lb and 16.1% of soluble solids. Fruits were put in fiberboard boxes (20 kg) and were stored in commercial cold storage at −1/0 °C during two months previous to use.

2.3.2. Laboratory scale

2.3.2.1. Fungicides. Pyr + Bosc (pyraclostrobin 12.8% w/w and boscalid 25.2% w/w), Cyp + Flud (cyprodinil 37.5% w/w + fludioxonil 25% w/w) and Flu (fludioxonil 23% v/v) were evaluated in the experiment.

2.3.3. Treatments

Healthy pear fruits were washed with water and detergent, dried with a towel paper, sprayed with ethanol (70% v/v), and placed at room temperature. The fruits were immersed for 1 min in each solution of fungicide at 0, 0.1, 10 and 100 μg/mL and then, a plug of mycelia was inoculated in each wound made in equatorial zone (3 × 3 mm). Ten fruits were used per treatment and each treatment was repeated three times; the whole experiment described above was conducted twice. The incidence and severity of decays in inoculated wounds were evaluated after 7–10 days of incubation into polyethylene bags at 24 °C. The incidence percentage (I%) was calculated as = 100 × [(number of inoculated wounds − number of disease wounds)/number of inoculated wounds] and the severity was recorded as rot diameter (mm).

2.3.4. Semicommercial scale

2.3.4.1. Fungicides. Pyr + Bosc (pyraclostrobin 12.8% w/w and boscalid 25.2% w/w), Cyp + Flud (cyprodinil 37.5% w/w + fludioxonil 25% w/w), Flud (fludioxonil 23% w/w) and PyrM (pyrimethanil 40% w/v) (Penbotec, Janssen) were evaluated. Flu and PyrM were added to the assay because they are registered for pome fruit in Argentina.

2.3.4.2. Treatments. Postharvest treatments of fungicides were applied in experimental packing line, by spraying of the healthy pear fruit while travelling across a series of rotating stripes, simulating a common packinghouse treatment method. Fungicides were prepared (in water) of concentration of 0.5 g/L of Pyr + Bosc (Sugar and Basile, 2011) and 1 g/L of Cyp + Flud (commercial recommendation for pears and apples). Commercial dose of Flud (2.25 mL/L) and PyrM (2.5 mL/L) were utilized. Water application was used as control.

2.3.4.3. Postharvest efficacy on natural incidence. Sets of 40 fruits treated were placed on trays packs in bags and into fiberboard boxes. Control activity of the treatments against naturally existing pathogens on fruit was evaluated after 4 months of storage in cold storage at −1/0 °C. The incidence (%) of each pathogen in fruit was evaluated by visualization of symptoms. In case of confusing identification, isolations of pathogens in PDA were carried out. There were three repetitions of each treatment.

2.3.4.4. Efficacy of postharvest treatments on inoculated fruit. Pear fruits stored for 2 months at −1/0 °C were artificially wounded and treated with fungicides to determine their protective effectiveness against *Alternaria* and *Cladosporium*. Five sets of 20 fruits were wounded (3 × 3 mm) and treated with fungicides, and then fruit wounds were inoculated with a conidia suspension (1×10^6 conidia/mL, 10 μL) of each pathogen. Fruits without treatment were used as control. Fruits were placed in polyethylene bags and kept in fiberboard boxes. Boxes were stored for 4 months in cold storage at −1/0 °C. Incidence (%) and severity as lesion diameter (mm) were evaluated for each pathogen and treatment.

2.4. Effect of preharvest treatments on stored fruit

The effect of chemical fungicides sprayed at preharvest was evaluated on the control of *Alternaria* and *Cladosporium* rot.

2.4.1. Orchard treatments

Orchard experiments were conducted in 2012–2013 in 1 ha block of 'Golden Russet Bosc' pear trees planted in 2004 at a spacing of 3.85 m × 2 m in the Valle Medio of Rio Negro, Patagonia Argentina. Fertilization, irrigation and other cultural practices were realized by the commercial growers.

The evaluated treatments of fungicides were Pyr + Bosc (12.8% + 25.2%, dose 0.5 g/L) (Sugar and Basile, 2011) and myclobutanil (Myc) (40% w/w; Systhane, Down AgroScience; commercial dose 0.15 g/L). Sprayed trees with water were used as control. The applications were done with sprayer machine (Pazima, 2300 L). Myclobutanil (Myc) fungicide is usually used in the orchard and has registration for pome fruit in the region. Pyr + Bosc fungicide was applied 14 days before harvest (Xiao and Boal, 2009). Myc fungicide was applied 21 days before harvest (according to commercial recommendation). Each treatment was applied at seven rows of 53 plants per row.

2.4.2. Effect on natural incidence of diseases

At the opportune moment of harvest, fruits from 5 central rows were collected and placed in wooden boxes of 360 kg. Fruit boxes were stored in cold storage at −1/0 °C, 95% RH. After 4 months of storage, the natural incidence (%) of diseases caused by pathogens was determined. Isolates on PDA were done when the etiology was doubtful.

2.4.3. Protective effect on wounds of harvest

Each fruit was wounded in five points with a sterile nail to simulate stem punctures, which are common entry points for decay pathogens. The fruit was not washed or surface sterilized, so that naturally occurring pathogens and field-applied treatments on the fruit surface were not intentionally removed. There were five repetitions by treatment. All wounded fruits were stored for 4 months in fiberboard boxes in cold storage at −1/0 °C.

The fruit was removed from storage and the effect of the treatments on postharvest decay was evaluated visually. Incidence of decay was recorded as the percentage of wounds infected by each decay type.

3. Data analysis

The efficacy of fungicides was analyzed by Probit. Analysis of variation of EC50 values from two experiments were conducted using ANOVA followed by mean separation using Tukey. Each laboratory experiment was conducted at least twice. Data from repeated experiments were combined for analysis when variance between experiments was homogeneous (Lilliefors and Bartlet). Severity of disease was determined as diameter of lesion in mm and data were analyzed by one way variance analysis (ANOVA) followed by Tukey. Percentage values of disease incidence were calculated and were subjected to analysis using the General Linear Model.

4. Results

4.1. Sensibility of Alternaria and Cladosporium in vitro test

Among all evaluated fungicides, Flu was more effective in inhibiting both the mycelial growth as the conidial germination of *Alternaria* from

Table 1
Sensitivity of mycelia growth and conidial germination of *Alternaria* and *Cladosporium* to different concentrations of fungicides.

Assays	Fungicide concentration (ug/mL)	Inhibition percentage (%)					
		Alternaria			*Cladosporium*		
		Pyr + Bosc	Cyp + Flud	Flud	Pyr + Bosc	Cyp + Flud	Flud
Mycelial growth	0.1	45a	77a	83a	92a	47a	47a
	1	68b	95b	100b	100a	63b	71b
	10	85c	100b	100b	100a	79c	79b
	100	96d	100b	100b	100a	84d	87c
Conidial germination	0.1	71a	79a	69a	98a	86a	89a
	1	84b	89ab	76a	99a	88a	99b
	10	97c	96b	86b	99a	94b	100b
	100	100c	100b	99c	100a	99b	100b

Values in each column for inhibition of myceliar growth or conidial germination followed by the same letter are not significantly different according toTukey test ($p < 0.05$).

1 μg/mL. The effect of highest concentrations of the fungicides significantly inhibited the mycelial growth and conidial germination (Table 1). With respect to *Cladosporium,* the fungicide Pyr + Bosc significantly inhibited mycelial growth while Cyp + Flu significantly inhibited conidial germination (Table 1).

EC50 values for inhibiting of the mycelial growth and conidia germination, showed that Pyr + Bosc was more effective in the inhibition of *Cladosporium*; while Flud was more effective for inhibition of *Alternaria* (Table 2).

4.2. Effect of fungicides on in vivo postharvest assays

4.2.1. Laboratory scale

The protective effect of the fungicides was evaluated on inoculated wounds. All three fungicides at 100 μg/mL, significantly reduced the incidence of *Alternaria* (Fig. 1A). Flu reduced 90% of incidence with respect to control, while another two fungicides reduced approximately 50%. Disease severity was drastically reduced with the three fungicides to 100 μg/mL (Fig. 1B).

The three evaluated concentrations of fungicides reduced the incidence of *Cladosporium* rot, but the highest effect was observed with Pyr + Bosc, however all three fungicides at 100 μg/mL showed the same reduction (90%) on the pathogen incidence (Fig. 1C). Besides, Pyr + Bosc and Cyp + Flu at 100 μg/mL, reduced the severity of pathogen in more than 65%, with respect to control (Fig. 1D).

4.2.2. Semicommercial scale

4.2.2.1. Effect of fungicides on natural incidence of postharvest diseases. The most important postharvest diseases in stored fruit after 4 months were *Alternaria* − *Cladosporium* complex rots (85.83%), while the rots caused by *B. cinerea* and *P. expansum* did not exceed 10% (Fig. 2). All fungicides reduced *Alternaria* − *Cladosporium* incidence between 35% and 24%, while *Botrytis* and *Penicillium* rots on treated fruits did not exceed 2% of incidence (Fig. 2).

Table 2
Effective concentration (EC50 values) of fungicides *in vitro* mycelial growth and conidial germination of *Alternaria* and *Cladoporium*.

Fungicide	Alternaria		Cladosporium	
	Mycelial growth*	Conidial germination*	Mycelial growth*	Conidial germination*
Pyr + Bosc	0.1822c	0.09031c	0.00049a	1.14×10^{-8}a
Cyp + Flud	0.0347b	0.02816b	0.04124b	0.01516b
Flud	0.0014a	0.00357a	0.08542b	0.07937c

* EC 50: concentration in μg/mL of each fungicide that inhibited 50% of pathogen. Values in each column for myceliar growth inhibition and conidial germination inhibition followed by the same letter are not significantly different according toTukey test ($p < 0.05$).

4.2.2.2. Effect of fungicides on inoculated wounds. The fungicides significantly reduced the incidence of *Alternaria* rot and *Cladosporium* rot on wounds inoculated with each pathogen in relation to the control. Pyr + Bosc, Cyp + Flud and Flud reduced the incidence between 91 and 98% of *Alternaria* and *Cladosporium,* respectively. The protective effect of these fungicides was demonstrated. Although the PyrM reduced the rots caused by *Alternaria* and *Cladosporium,* it was less effective than other active ingredients (Fig. 3A).

In relation to effect on disease severity, Flud and Cyp + Flud significantly reduced the lesion diameter of *Cladosporium* rot to 49.42%, while Flud and PyrM reduced *Alternaria* lesion diameter to 32.48% and 39.92%, respectively (Fig. 3B).

4.3. Effect of preharvest treatments on stored fruit

Preharvest applications of Pyr + Bosc and Myc treatments and their effect on stored fruit were evaluated.

4.3.1. Natural incidence of diseases

The postharvest diseases observed with highest incidence in stored Bosc pear fruit were *Alternaria* − *Cladosporium* complex rot and gray mold by *B. cinerea.* A single preharvest application of Pyr + Bosc reduced the incidence of *Alternaria* − *Cladosporium* complex rot by 44.6% and 95% gray mould. In addition, Myc was effective in controlling 75% of the gray mold (Fig. 4).

4.3.2. Protective effect on artificial wounds

The protective effect of preharvest spray was evaluated on wounds at harvest of fruit in the presence of natural inoculums. Pyr + Bosc reduced incidence of *Alternaria* − *Cladosporium* rot and gray mould by *B. cinerea* (Fig. 5), by 91% and 95% respectively. No appreciable blue mould incidence was observed in any treatment. Myc sprays reduced incidence of gray mould to 85.71% and rot by *Alternaria* − *Cladosporium* to 80% (Fig. 5).

5. Discussion

In this study the effectiveness of commercial formulations of fungicides on pathogens that generate the most important post-harvest rots at Golden Russet Bosc pears was evaluated. Currently, Argentina has no register of fungicides for the effective control of *Alternaria-Cladosporium* decays. Our study demonstrated differences in effectiveness of the fungicides evaluated *in vitro* and *in vivo* tests, also in post and preharvest applications. According to the results of different assays and our observations about *Alternaria* − *Cladosporium* rot, a preharvest management is necessary to reduce the incidence of this disease. Fungicides

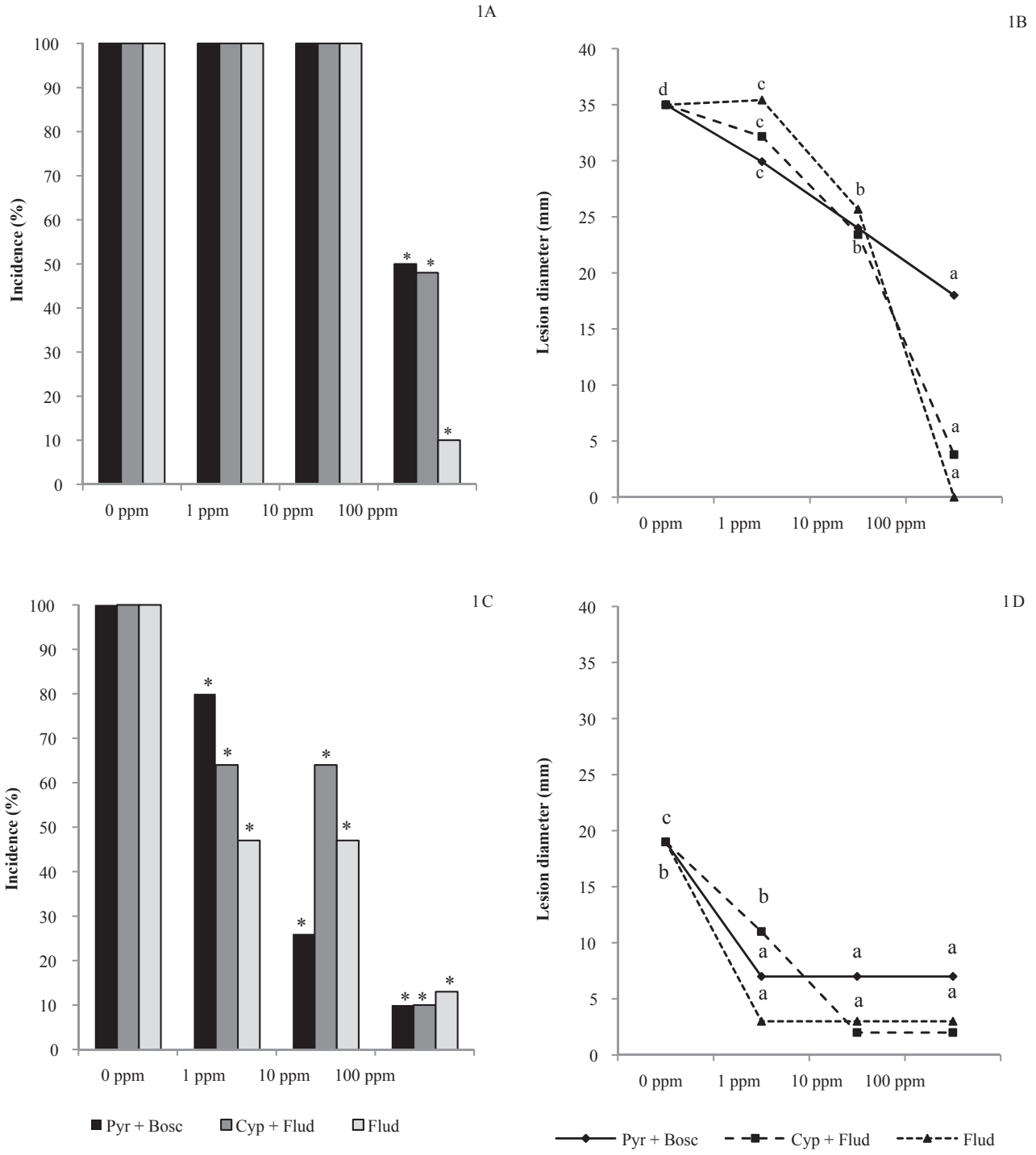

Fig. 1. Effect of different concentrations of fungicides on incidence (%) and severity (lesion diameter, mm) of *Alternaria* (1A–1B) and *Cladosporium* (1C–1D) on inoculated pear fruits and stored at 24 °C for 7 (A) and 10 (B) days. Bars correspond to the incidence (%) of the disease. Lines indicate disease severity (mm). Asterisks (*) on each bar indicate significant differences according to Contrast tests with respect to the control treatment for each pathogen. Same letters in lesion diameter of each pathogen are not statistically significantly different according to Tukey test ($p < 0.05$).

which more effectively inhibited mycelial growth and germination of *C. herbarum* were the combination of Pyr + Bosc, while Cyp + Flud for *Alternaria* sp. Both fungicides, Pyr + Bosc and Cyp + Flud, in trials on fruit, decreased the incidence and severity of diseases caused by both pathogens. In concordance with other reports, *in vitro* tests do not always accurately predict the performance of fungicides on fruits in the field (Everett and Neilson, 1996; Everett et al., 2005; Everett and Timudo Torrevilla, 2007).

Assays of fruit wounds inoculated with each pathogen showed that fungicides were effective in controlling both fungus. However, *Cladosporium* showed greater sensibility to fungicides than *Alternaria*. There are few reports in the literature on this particular pathogen affecting pear/apple fruits.

In semicommercial trials, with postharvest application of fungicides in the packinghouse line, we observed high natural incidence (not inoculated and non treated fruits) of decays by *Alternaria-Cladosporium* complex that exceeded 85%. These results show the importance of the problem for the pears storage and production in the main pear-growing

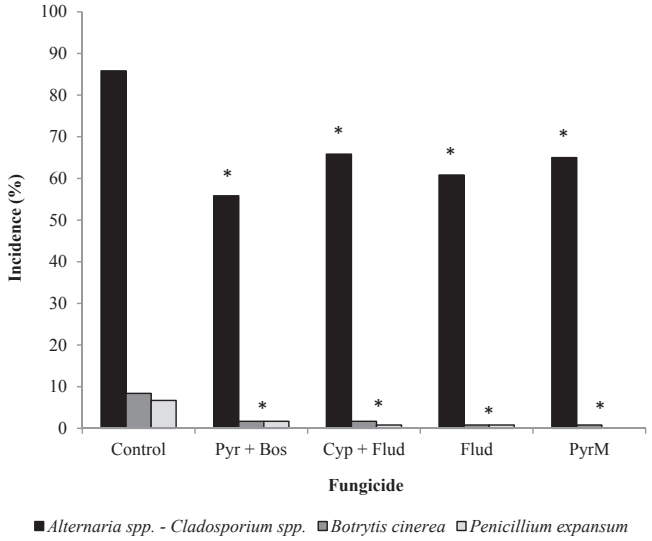

Fig. 2. Natural incidence of postharvest diseases on pear fruits treated with fungicides in experimental packing line and stored at-1/0 °C for 4 months. Fruit after 2 months stored at −1/0 °C, was treated and kept during 4 months until evaluation. Asterisks (*) on each bar indicate significant differences according to Contrast tests with respect to the control treatment for each pathogen.

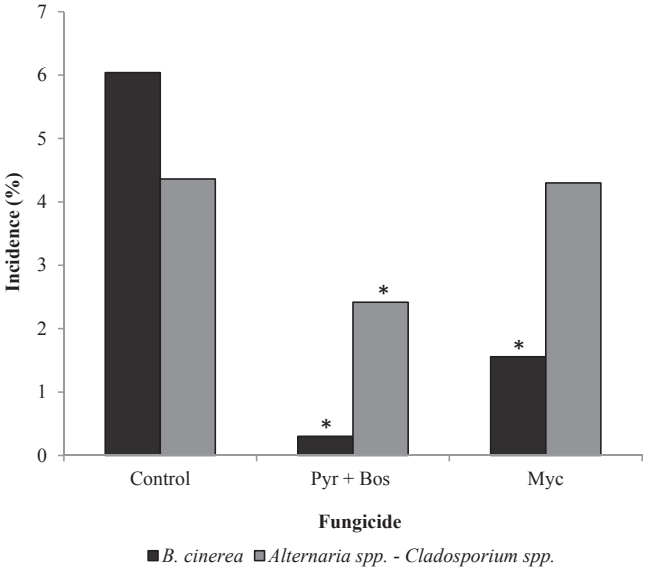

Fig. 4. Natural incidence (%) of postharvest diseases on pear fruits treated with fungicides at preharvest and stored at-1/0 °C for 4 months. Asterisks (*) on each bar indicate significant differences according to Contrast tests with respect to the control treatment for each pathogen.

region of Argentina, situation that we have already reported (Lutz and Sosa, 2015). Postharvest fungicides reduced the incidence of *Alternaria-Cladosporium* rots between 23 and 35%. These results indicate certain ineffectiveness in the postharvest use of these fungicides to control these pathogens. This may be explained by the presence of latent infections that occur during the growth period of the fruit and which are then developed in storage (Prusky et al., 1981; Prusky et al., 2002; Li et al., 2007). It must be considered that a percentage of infections, mainly by *Alternaria*, could be of latent type (Biggs, 1995; Prusky et al., 2002; Thomma, 2003; Li et al., 2007). As postharvest pathogen, it has been described that *Alternaria spp*. has complex mechanisms of infection and that it has the ability to infect fruit in different periods (Li et al., 2007). For example, *A. alternata* infects fruits of persimmon in the early development stage, while on mango fruit it appears two weeks after petal fall (Prusky et al., 1981; Prusky et al., 1983). The time and environmental conditions under which the infections occur, would affect the effectiveness of postharvest fungicides evaluated in this study. Considering aspects related to the epidemiology of pathogen and to the effectiveness of evaluated fungicides, the pre-harvest use of systemic fungicides, could protect the fruit against the occurrence of latent or other infections occurring at harvest. In pear fruits, there are few reports on the epidemiology of *Alternaria*, however on apple fruit, according to Harteveld et al. (2014), *Alternaria* fruit spot infection occurs between 95 to 110 days after bloom (about a month before harvest).

According Xiao and Kim (2004), the use of preharvest fungicides is needed to reduce decay. They suggest that the benefits of preharvest fungicide applications are (1) to reduce or control latent infections, (2) to reduce spore load on the surfaces of the fruit, and (3) to protect wounds that occur at harvest and during the handling process from infection by decay-causing pathogens.

On the other hand, the high incidence of *B. cinerea* in fruit stored for four months in cold storage may be due to environmental conditions during season with rains during the 30 days preharvest (Spotts and Serdani, 2006; Spotts et al., 2009). In this work, all fungicides applied at postharvest diminished the incidence of gray mould by *B. cinerea.* Our results agree with other studies for different crops (Sholberg et al., 2003; Errampalli, 2004; Errampalli and Crnko, 2004; Franck et al., 2005; Sallato et al., 2007). According to Xiao and Boal (2009), Pristine (Pyr + Bosc, Basf, USA) could also reduce inoculum levels of *B. cinerea* and *P. expansum* on the surface of apple fruit, this could explain our results on pear fruit.

The two fungicides applied two or three weeks before harvest reduced the natural incidence of all pathogens. In the case of *B. cinerea*, pyraclostrobin plus boscalid showed better performance than myclobutanil. Preharvest treatments of Bartlett and Bosc pear orchards with protective fungicides were used in several cases to control postharvest

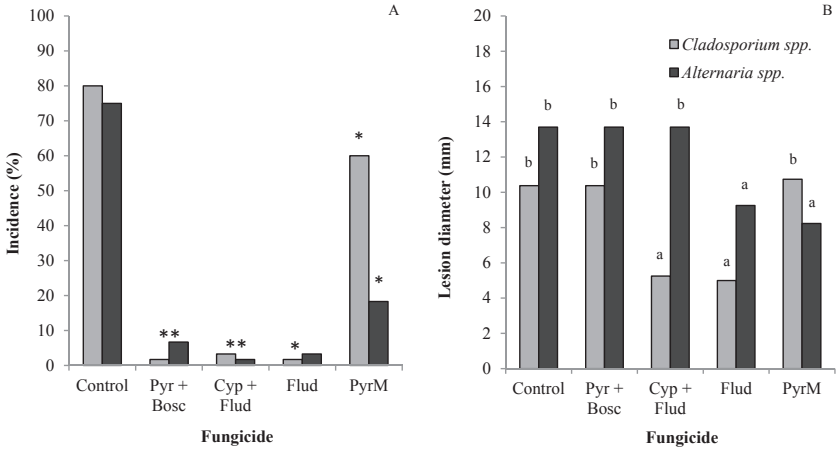

Fig. 3. Effect of several fungicides on incidence (%) (A) and severity (lesion diameter, mm) (B) of *Alternaria* and *Cladosporium* rot on inoculated pear fruits stored at −1/0 °C for 4 months. Asterisks (*, **) on each bar indicate significant differences according to Contrast tests with respect to the control treatment for each pathogen. For lesion diameter of each pathogen, same letters in each bar of same colour are not significantly different according to Tukey test ($p < 0.05$). Assay with postharvest treatments of fungicides was realized on experimental packing line. Fruits with 2 months of storage at −1/0 °C, was treated and kept during 4 months until their evaluation.

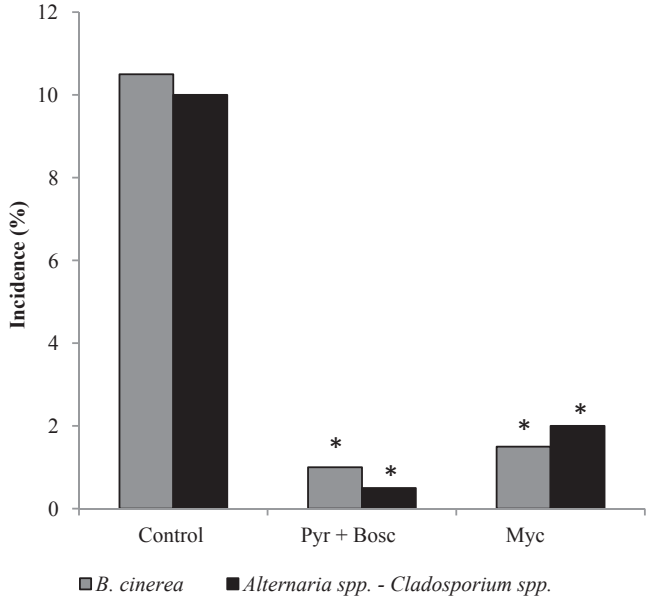

Fig. 5. Incidence (%) of postharvest diseases on pear fruits artificially wounded in harvest, and treated with fungicides at preharvest (14 or 21 previous days) after storage at-1/0 °C for 4 months. Asterisks (*) on each bar indicate significant differences according to Contrast tests with respect to the control treatment for each pathogen.

rots caused by wound pathogens such as *Penicillium* and *Botrytis* (Adaskaveg et al., 2005).

The greatest reduction of the pathogens by Pyr and Bosc agrees with the results obtained by applying Pristine (Basf, USA) in Bosc pears two weeks before harvest (Xiao et al., 2009; Kim and Xiao, 2010) to control postharvest *Alternaria* − *Cladoporium* rots. Myc was not effective in controlling the natural incidence of *Alternaria–Cladosporium;* however, it showed a protective effect on fruit wounds. This active principle is mainly used for the control of foliar diseases. Preharvest applications with this active ingredient, may be ineffective by the composition of the cuticle of the fruit, which would not allow entry and movement of fungicides in them (Solel and Edgington, 1973). Moreover, adding sprays of this fungicide of effective control on powdery mildew and scab, with the aim of controlling postharvest diseases, could be risky for the development of resistant strains to these diseases (Braun, 1994; Hetherington and Gunning, 2003; Chapman et al., 2011).

The activity of fungicides applied before harvest in the protection of after harvest wounds and surrounding fruit tissue may be due to its direct action on fungal inoculation on the fruit surface, to the fact that the fungicide is mechanically driven into the wound as is created, or to the general penetration of the fruit cuticle and sub-cuticular tissues. Xiao and Boal (2009) suggested that effects of pre-harvest treatment with Pristine fungicide (Pyr + Bosc) were evident in non- wounded pears that had been washed and brushed at packing 4 months after harvest.

The results of our study indicate the importance of treatments in orchard before harvest to storage pears for medium − long term. Applications with preharvest fungicide of low risk, in addition to the good practices of crops and biocontrol in postharvest (Droby et al., 2009; Robiglio et al., 2011; Lutz et al., 2012), could be an important tool for reducing post-harvest diseases of pear and improving the quality of the exportable fruits.

The preharvest fungicide application is not a management practice used in the main region producing pome fruits, Rio Negro and Neuquen. In this study, one preharvest application of fungicides to control postharvest diseases is being proposed for the first time for this region. However, epidemiological studies of disease should be performed to establish which would be the optimal time to control all detected pathogens in our environmental conditions and pear cultivars.

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