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Biocontrol of postharvest decay using a new strain of *Pseudomonas syringae* CPA-5 in different cultivars of pome fruits

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Epiphytic micro-organisms isolated from fruits and leaves surfaces of apples from different orchards were screened for antagonistic activity against *Penicillium expansum.* From all micro-organisms tested the new strain CPA-5 of *Pseudomonas syringae,* isolated from organic orchard, was selected. This strain was very effective against *Botrytis cinerea*, *P. expansum* and *Rhizopus stolonifer* at various antagonist and pathogen concentrations on 'Golden Delicious' apple, and 'Blanquilla', 'Rocha' and 'Conference' pear. Under cold storage conditions and in semi-commercial trials *P. syringae* (CPA-5) significantly reduced development of *P. expansum* and *B. cinerea* on 'Golden Delicious' apple, and 'Blanquilla' and 'Rocha' pears. Control of *P. expansum* equal to the fungicide imazalil was obtained with CPA-5 at 10^8cfu ml^{-1} on 'Gold Delicious' apple and 'Rocha' pear. The populations of *P. syringae* CPA-5 increased more than 100-fold during the first 50 days, and then remained stable on apple, and slightly decreased on pears. This indicates the high capacity of this antagonist to colonize wound surfaces of pome fruits under cold storage conditions.

Key-words: *Botrytis cinerea, Penicillium expansum, Pseudomonas syringae, Rhizopus stolonifer,* apples, pears, cold storage

Introduction

Worldwide postharvest losses of fruits and vegetables have been estimated at 25% (El-Ghaouth et al. 2002). Postharvest decay of pome fruits are mainly caused by the fungal pathogens *Penicillium expansum* Link, *Botrytis cinerea* Pers. and *Rhizopus stolonifer* (Ehrenb. Ex Link) Lind in Spain and Portugal. Control of these pathogens has been conducted mainly through the use of fungi-

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cides. However, postharvest use of fungicides has been increasingly reduced due to the development of pathogen resistance (Rosenberger and Meyer 1981, Viñas et al. 1991), the public concern about presence of fungicide residues in food and in the environment and the lack of replacement fungicides (Janisiewicz and Korsten 2002). Currently, substantial progress has been made in finding alternatives to synthetic postharvest fungicides (Guinebretiere et al. 2000, Zahavi et al. 2000, Teixidó et al. 2001, Spotts et al. 2002) and several microbial biocontrol agents have been reported to control postharvest decay of pome fruits (Droby et al. 1998, Viñas et al. 1998, Leverentz et al. 2000, Janisiewicz et al. 2001, Usall et al. 2001, Nunes et al. 2002).

For isolation antagonistic micro-organisms most researches prefer natural microflora in fruit and vegetables surface as a source (Barkai-Golan 2001). The isolation of antagonists could be improved by using fruit from unmanaged orchards, where natural microflora have not been disturbed by chemical treatments (Janisiewicz 1996).

To be an effective biocontrol agent the antagonist should meet certain criteria, such as the ability to rapidly colonize wounds and the capacity to be effective against a wide range of pathogens and on different fruits (Wilson and Wisniewski 1989). *Pseudomonas* spp. can grow rapidly, dominate and colonize the new niche where resources are temporarily abundant (Janisiewicz and Korsten 2002). Strains of *Pseudomonas syringae* van Hall have been commercialised as BioSave (EcoScience), for control of blue and grey mould of pome fruits and cherries, and various diseases on citrus, potatoes and sweet potatoes (Janisiewicz and Marchi 1992, Janisiewicz and Jeffers 1997).

A program in organic orchards was initiated to isolate and identify naturally occurring micro-organisms on leaves and fruit surfaces and to screen them for potential biocontrol activity against *P. expansum*, *B. cinerea* and *R. stolonifer* on pome fruits. A new and very effective strain of *Pseudomonas syringae* was selected. The objective of this study therefore was to evaluate the ability of this new strain of *P. syringae* to control the major postharvest diseases in different cultivars of pome fruits.

Material and methods

Antagonist isolation and screening

Potential antagonists were isolated from surfaces of 'Golden Delicious' apple fruits grown on organic and conventional orchards, located in Lleida (Catalonia). Fruits were harvested and washed in sterile water, on a rotary shaker (Gallenkamp, UK) for 10 min at 150 rpm. The fruits were further washed in a 200 ml sterile 0.05 M phosphate buffer [0.2 M KH_2PO_4, 70 ml (Rectapur, France); 0.2 M K_2HPO_4, 30 ml (Rectapur) and deionized water, 300 ml] pH 6.5 for 10 min in an ultrasound bath (Selecta, Abrera, Spain). Washings from sonicated samples were plated on nutrient yeast dextrose agar medium [NYDA: nutrient broth, 8 g (Biokar Diagnostics); yeast extract, 5 g (Biokar Diagnostics); dextrose, 10 g (Rectapur); agar, 15 g (Prolabo) per liter of deionized water] and incubated for 24–48 h at 25ºC. Colonies were isolated on the basis of their different visual characteristics. To screen for antagonists against *Penicillium expansum* the method described by Janisiewicz (1987) with modifications (Nunes et al. 2001) was used. Surface sterilized pears were wounded at the stem and calyx end, by removing blocks of $3 \times 3 \times 3$ mm^3. Then 25µl of water suspension of each isolate micro-organism were pipetted into the wound, followed by inoculation of an aqueous suspension of *P. expansum* conidia (10^4 conidia ml^{-1}). Each fruit constituted a single replicate and each treatment was repeated three times. Lesion diameter was measured after 7 days of incubation at 20±1ºC and 85±5% relative humidity (RH). The isolates were regarded as potential antagonists when the incidence of disease was reduced to less than 50% and inhibition of lesion diameter by more than 75%. The most effective isolates were selected for further studies.

Fruits

For laboratory and semi-commercial trials 'Blanquilla' and 'Conference' pears and 'Golden Delicious' apples were obtained from commercial

orchards in Lleida (Catalonia), and 'Rocha' pears from commercial orchards in Alcobaça (Portugal). Fruits were used just after harvest or after a short time (no longer than 3 months) of storage at 1°C.

Production of antagonist and pathogens

Antagonist suspensions were prepared by growing cultures in nutrient yeast dextrose broth (NYDB) (the same ingredients as NYDA but without the agar) for 24 h at 25±1ºC with shaking at 150 rpm. The culture was harvested by centrifugation (Avanti J-25. Beckman, USA) at 8,315 × g for 10 min, the cells were resuspended in deionized water and adjusted to desired concentrations with a spectrophotometer (CECIL CE 1020. UK) at 420 nm according to a standard curve.

Penicillium expansum, *Botrytis cinerea* and *Rhizopus stolonifer* were isolated from decayed apples after several months in storage and grown on potato dextrose agar medium (PDA: extract of boiled potatoes, 200 ml; dextrose, 20 g; agar, 20 g and deionized water, 800 ml) at 25±1ºC. The concentrations of the conidial suspensions were adjusted using a haemacytometer. The conidial suspensions were prepared from 10, 14 and 7 days old cultures of *P. expansum*, *B. cinerea* and *R. stolonifer*, respectively.

Laboratory trials under room and cold storage conditions

From 131 isolates tested *Pseudomonas syringae* strain CPA-5 was the most effective in controlling decays and was selected for further studies.

To determine the minimum effective concentration of P. syringae CPA-5 against *P. expansum*, *B. cinerea* and *R. stolonifer*, surface sterilized fruit ('Golden Delicious' apple, 'Rocha' and 'Blanquilla' pear) were wounded has described above. Then 25 µl of water suspension of *P. syringae* CPA-5 (10^7, 5×10^7 and 10^8 cfu ml^{-1}) was applied to each wound. After 1 h the wounds were inoculated with 20 µl of an aqueous suspension of pathogens (10^3, 10^4 and 10^5 conidia ml^{-1}). Three fruits constituted a single replicate and each treatment was repeated three times. The fruits were stored at 20±1ºC and 85±5% RH for 7 days and the lesion diameter was measured. The test was repeated twice.

To determine the effectiveness of the strain CPA-5 against *P. expansum* and *B. cinerea* on 'Golden Delicious' apple and 'Blanquilla' and 'Rocha' pear under cold storage, fruits were treated as above with 10^7 and 10^8 cfu ml^{-1} of *P. syringae* and the pathogen concentration of 10^4 conidia ml^{-1}. Ten fruits constituted a single replicate, and each treatment was repeated three times. Fruits were stored in a cold room at 1°C and 90±5% RH for 90 days and the lesion diameter was measured. The test was repeated twice.

Semi-commercial trials under cold storage conditions

This study was conducted on 'Golden Delicious' apple and 'Blanquilla' and 'Rocha' pear. Each fruit was wounded in two locations (midway between the calyx and stem end), to simulate injuries under commercial conditions. The wounds were approximately 1 mm wide and 2 mm deep and were made with a steel rod. Fruits were dipped in a *P. syringae* aqueous suspension at 10^7 and 10^8 cfu ml^{-1} for 30 s. After 1 h the fruits were dipped again for 30 s in a conidial suspension of *P. expansum* or *B. cinerea* (10^4 conidia ml^{-1}). Twenty or forty fruits constituted a single replicate and each treatment was repeated four times. Lesion diameters were measured 4 months after cold storage at 1°C and 90±5% RH. The control treatments were water and the fungicide imazalil (Decozil-S-7.5®. Elf Atochem Agri España, Spain) at a recommended concentration for standard postharvest treatments (0.5%) applied to fruit inoculated with the pathogens. This study was conducted during two storage seasons.

Population dynamics

To study the population dynamics of the antagonist in the semi-commercial trials, fruits were rinsed in fresh water and wounded as above. All fruits were

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dipped into the antagonist suspension (10^8 cfu ml^{-1}) for 30 s. Once dried, fruits were placed on tray packs in plastic boxes and incubated at 1°C and 90±5% RH. Bacterial population was monitored at just prior to storage and after 1, 8, 15, 30, 60, 90 and 126 days.

To recover the antagonist, the weight of fruits was determined, fruits were peeled and the peel was shaken in 150 ml sterile phosphate buffer (pH 6.5) on a rotatory shaker for 20 min at 150 rpm and then sonicated for 10 min in an ultrasound bath. Serial dilutions of the washings were made and plated on NYDA supplemented with imazalil (imazalil sulphate 99%) (0.5 g l^{-1}) to inhibit the growth of fungi and yeasts. Colonies were counted after incubation at 25°C in the dark for 48 h. Population sizes were expressed as cfu g^{-1} of fruit. Four fruits constituted a single replicate and each treatment was replicated four times. The experiment was carried out twice.

Statistical analysis

The incidence and severity of decay were analyzed by an analysis of variance with SAS Software (SAS Institute, version 6.08, Cary N.C.). Statistical significance was judged at the level $P < 0.05$. When the analysis was statistically significant LSD Test was used for separation of means. Data of antagonist populations (cfu g^{-1}) were log transformed to improve homogeneity of variances.

Results

Antagonist isolation and screening

Biocontrol activity of 131 isolates was evaluated. Only 3 reduced severity and incidence by more than 75% and 50%, respectively. Among these, the strain CPA-5 a bacterium identified as *Pseudomonas syringae* by the Centraalbureau voor Schimmelcultures (Delft, Netherlands) was the most effective one. This strain was isolated from the organic orchard and was selected for secondary screenings.

Laboratory trials under room and cold storage conditions

In all the assays on fruit stored at 20ºC for 7 days, *P. syringae* was effective in reducing incidence (% of infected wounds) and severity (lesion diameter) of decay caused by the three pathogens on all cultivars, except against *R. stolonifer* at 10^5 conidia ml^{-1} on 'Blanquilla' pear (Tables 1, 2 and 3).

The severity and incidence of decays differed among the cultivars and depend on type and concentration of the pathogen as well as on the concentration of antagonist (Severity: $F = 7.6$ and $P = 0.0001$; Incidence: $F = 6.2$ and $P = 0.0001$). At the concentration of 10^3 conidia ml^{-1} of the pathogens the incidence and severity of decay was very low. In all cultivars the effectiveness of *P. syringae* in reducing decay ranged between 80 and 100% for *P. expansum*, 71 and 100% *B. cinerea*, and 85 and 100% for *R. stolonifer* decay (except for 'Blanquilla', Table 3), when the pathogens were inoculated at 10^4 conidia ml^{-1}. Increased concentration of the pathogens to 10^5 conidia ml^{-1} slightly reduced the effectiveness of the antagonist, however in several assays total or almost total control was achieved. In general, the most effective results were obtained at 10^8 cfu ml^{-1} of the antagonist (Tables 1, 2 and 3).

On fruits stored at 1ºC for 3 months, all treatments with *P. syringae* (10^7 and 10^8 cfu ml^{-1}) significantly inhibited development of *P. expansum* and *B. cinerea* in 'Golden Delicious' apple, 'Blanquilla' and 'Rocha' pear (Fig. 1). The decreased of the decay was correlated with the increasing concentration of the antagonist. *P. syringae* at 10^8 cfu ml^{-1} reduced *P. expansum* incidence by 91, 95 and 100% and severity by 94, 85 and 100% on 'Golden Delicious', 'Blanquilla' and 'Rocha' pear, respectively (Fig. 1a). Although the control of *B. cinerea* was less effective than *P. expansum*, the reduction of lesion diameter of *B. cinerea* on 'Golden Delicious' apple and 'Rocha' pear was higher than 90% and in 'Blanquilla' was by 75% (Fig. 1b). No differences were observed in the control of *P. expansum* between cultivars. On fruits inoculated with *B. cinerea* the incidence of decay was highest on 'Blanquilla' pear followed

Table 1. Incidence and severity of decay on different apple and pear cultivars protected with *Pseudomonas syringae* CPA-5 and inoculated with *Penicillium expansum*.

Fruits	Concentration of *P. expansum* (conidia ml^{-1})	Concentration of *P. syringae* (CFU ml^{-1})							
		0[a]		10^{7}[a]		5×10^{7}[a]		10^{8}[a]	
		Severity (mm)	Incidenc (%)	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)
'Golden Delicious' apple	1×10^{3}	9.2 a	66 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^{4}	16.6 a	88 a	3.1 b	33 b	0.0 b	0 c	0.0 b	0 c
	1×10^{5}	24.0 a	100 a	14.6 b	66 b	10.5 c	55 b	4.7 d	28 c
'Blanquilla' pear	1×10^{3}	27.2 a	89 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^{4}	33.8 a	100 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^{5}	36.8 a	100 a	18.3 b	55 b	5.4 c	22 c	4.4 c	22 c
'Rocha' pear	1×10^{3}	11.6 a	72 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^{4}	23.7 a	93 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^{5}	29.3 a	100 a	1.1 b	11 b	0.0 b	0 c	0.0 b	0 c
'Conference' pear	1×10^{3}	12.8 a	78 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^{4}	21.1 a	94 a	1.1 b	5 b	0.0 b	0 b	0.0 b	0 b
	1×10^{5}	23.2 a	95 a	1.9 b	11 b	1.0 b	10 b	0.0 b	0 c

[a] Means of 18 lesions fruits (two lesion per fruit) measured after 7 days at 20ºC. Within severity or incidence different letters in the same row, indicate significance between means using LSD test ($P < 0.05$).

Table 2. Incidence and severity of decay on different apple and pear cultivars protected with *Pseudomonas syringae* CPA-5 and inoculated with *Botrytis cinerea*.

Fruits	Concentration of *B. cynerea* (conidia ml^{-1})	Concentration of *P. syringae* (CFU ml^{-1})							
		0[a]		10^{7}[a]		5×10^{7}[a]		10^{8}[a]	
		Severity (mm)	Incidenc (%)	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)
'Golden Delicious' apple	1×10^{3}	23.1 a	83 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^{4}	25.0 a	78 a	1.4 b	17 b	0.0 b	0 b	0.0 b	0 b
	1×10^{5}	39.1 a	94 a	29.1 b	66 b	21.5 bc	56 bc	13.1 c	44 c
'Blanquilla' pear	1×10^{3}	16.0 a	50 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^{4}	37.1 a	100 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^{5}	58.4 a	100 a	8.5 b	33 b	8.1 b	27 b	1.4 b	6 c
'Rocha' pear	1×10^{3}	43.9 a	94 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^{4}	24.1 a	100 a	6.7 b	28 b	0.0 c	0 c	0.0 c	0 c
	1×10^{5}	29.5 a	94 a	4.8 b	33 b	3.3 b	22 b	2.1 b	17 b
'Conference' pear	1×10^{3}	48.9 a	100 a	1.1 b	6 b	0.8 b	6 b	0.0 b	0 b
	1×10^{4}	69.0 a	100 a	13.3 b	44 b	2.1 c	22 c	2.0 c	22 c
	1×10^{5}	74.8 a	100 a	28.1 b	66 b	19.5 bc	61 bc	17.2 c	44 c

[a] Means of 18 lesions fruits (two lesion per fruit) measured after 7 days at 20ºC. Within severity or incidence different letters in the same row, indicate significance between means using LSD test ($P < 0.05$).

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Table 3. Incidence and severity of decay on different apple and pear cultivars protected with *Pseudomonas syringae* CPA-5 and inoculated with *Rhizopus stolonifer*.

Fruits	Concentration of *R. stolonifer* (conidia ml^{-1})	Concentration of *P. syringae* (CFU ml^{-1})							
		0[a]		10^{7a}		5×10^{7a}		10^{8a}	
		Severity (mm)	Incidenc (%)	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)
'Golden Delicious' apple	1×10^3	19.2 a	44 a	2.2 b	17 b	0.0 b	0 c	0.0 b	0 c
	1×10^4	53.5 a	100 a	7.5 b	22 b	0.0 c	0 c	0.0 c	0 c
	1×10^5	81.8 a	100 a	41.6 b	83 b	19.2 c	45 c	16.4 d	45 c
'Blanquilla' pear	1×10^3	33.1 a	100 a	4.8 b	22 b	0.6 b	6 bc	0.0 c	0 c
	1×10^4	78.8 a	100 a	52.6 b	66 b	46.8 b	61 b	24.4 c	44 c
	1×10^5	81.4 a	100 a	79.4 a	100 a	76.4 b	94 a	37.4 c	49 a
'Rocha' pear	1×10^3	27.7 a	28 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^4	28.3 a	28 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^5	46.6 a	44 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
'Conference' pear	1×10^3	12.0 a	100 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^4	11.0 a	94 a	0.0 b	0 b	0.0 b	0 b	0.0 b	0 b
	1×10^5	12.0 a	100 a	28.8 b	28 b	0.0 c	0 c	0.0 c	0 c

[a] Means of 18 lesions fruits (two lesion per fruit) measured after 7 days at 20ºC. Within severity or incidence different letters in the same row, indicate significance between means using LSD test ($P < 0.05$).

by 'Rocha' pear and less on 'Golden Delicious' apple. No differences were observed in the severity of the disease (data not shown).

Semi-commercial trials under cold storage conditions

In semi-commercial trials, on fruit stored at 1ºC during 4 months, all treatments significantly reduced decays and as the concentration of the antagonist increased decays decreased (Fig. 2). *P. syringae* at 10^8 cfu ml^{-1} reduced *P. expansum* incidence by 97% in 'Golden Delicious' apple and Rocha pear and no differences were observed between this treatment and the postharvest fungicide imazalil (Fig. 2a). On 'Blanquilla' pear, *P. syringae* 10^8 cfu ml^{-1} reduced *P. expansum* incidence by 70% (Fig. 2a).

Control of *B. cinerea* by *P. syringae* 10^8 cfu ml^{-1} was slightly inferior to that of *P. expansum*, however reduction in incidence of the decay on 'Golden Delicious' apple, 'Blanquilla' and 'Rocha' pear was 65, 80 and 71%, respectively, and lesion diameter was reduced more than 75% (Fig. 2b).

Population dynamics

Population of the antagonist was affected by the type of fruit and higher populations were recovered from 'Golden Delicious' apples than from 'Blanquilla' pear (Fig. 3). The initial applied concentration of *P. syringae* was the same in pears and apples, however the population recovered at time 0 in pears was about one log unit lower than on apples. On pears, *P. syringae* population reached the maximum population at day 30, increasing during this time more than 150-fold (8.3×10^3 cfu g^{-1}). Then the population started to decline and at the end of the storage was only 3×10^3 cfu g^{-1}. On apples, *P. syringae* population increased during the first month more than 50 fold (3.1×10^4 cfu g^{-1}). Then the growth slowed, and the highest population (6.3×10^4 cfu g^{-1}) was reached at day 90. At the end of the experiment (day 126) the population on 'Golden Delicious' apple was 5.4×10^4 cfu g^{-1}.

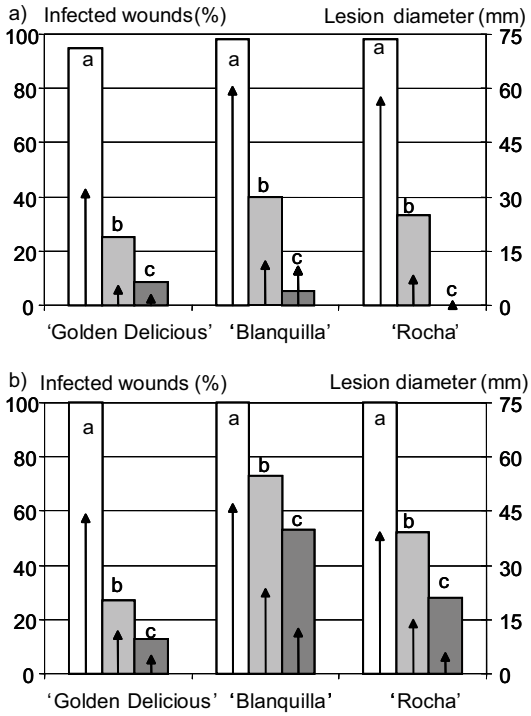

Fig. 1. Suppression of a) blue mould and b) grey mould of 'Golden Delicious' apple, 'Blanquilla' and 'Rocha' pear by *Pseudomonas syringae* (CPA-5). Fruits were wounded and inoculated with antagonist at 0, 10^7 and 10^8 cfu·ml^{-1} and *Penicillium expansum* and *Botrytis cinerea* at 10^4 conidia ml^{-1}. Fruits were stored at 1ºC for 3 months. ▲ Represents lesion diameter (mm) and columns represent the incidence of decay. Columns with the same letters for each cultivar are not significantly different according to LSD Test (P = 0.005).

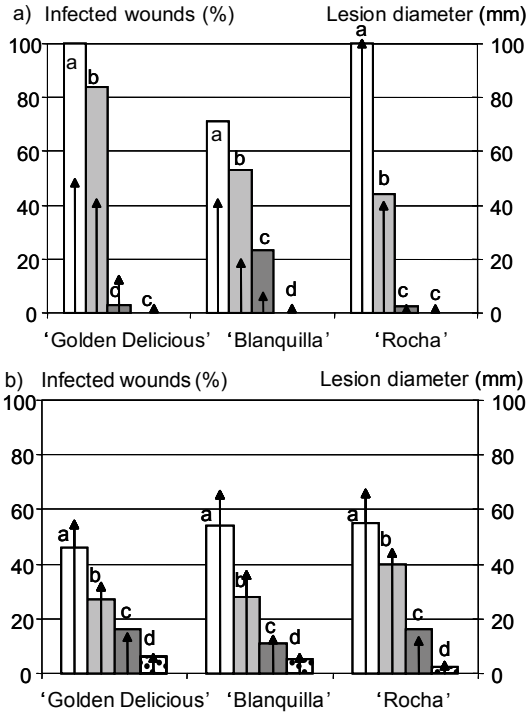

Fig. 2. Suppression of a) blue mould and b) grey mould of 'Golden Delicious' apple, 'Blanquilla' and 'Rocha' pear by *Pseudomonas syringae* (CPA-5) in semi-commercial trial. Fruits were wounded and submerged for 30 s in a CPA-5 aqueous suspension at 0, 10^7 and 10^8 cfu ml^{-1} or an aqueous suspension of Imazalil then submerged again for 30 s in a conidial suspension of *Penicillium expansum* (10^4 conidia ml^{-1}). Fruits were stored at 1ºC for 4 months. ▲ Represents lesion diameter (mm) and columns represent the incidence of decay. Columns with the same letters for each cultivar are not significantly different according to LSD Test (P = 0.005).

Discussion

From all micro-organisms isolated in fruit surface the strain CPA-5 of *Pseudomonas syringae* was the most effective against postharvest decays of apple and pear fruits caused by *P. expansum*¸ *B. cinerea* and *R. stolonifer*. The fact that this strain showed potential as a biocontrol agent supports earlier findings that unmanaged orchards, with natural microflora undisturbed by chemical treatments is a good site for isolating antagonists (Janisiewicz 1996). Biocontrol agents with a large potential market on a variety of crops and for different diseases would have the most change for commercial success (Froyd 1997).

Pseudomonas syringae has been described as providing biological control of postharvest fruit diseases and is commercialized under the name of Biosave 11 and Biosave 110 (EcoScience Crop, USA) to control decay on apples and pears caused by *P. expansum*, *B. cinerea* and *Mucor piriformes* Fischer (Janisiewicz and Marchi 1992, Janisiewicz and Jeffers 1997) and various decays on cherries, citrus, potatoes and sweet potatoes (US EPA

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registration). Our studies confirmed the potential of this species to control *P. expansum*, *B. cinerea* and also *R. stolonifer* in different cultivars of apple and pears.

Many biotic and abiotic factors, such locations, disease and fruit variety could influence the magnitude of control performed by the antagonists (Bull et al. 1997). In fact Biosave 10 and Biosave 110 applied in the same conditions do not control diseases to the same extent (Janisiewicz and Marchi 1992, Jeffers and Wright 1994). Although these two products are both *P. syringae* they represent different strains that differ in the spectrum of biocontrol activity (Smilanick et al. 1996).

In this work, disease control studies were conducted with fruits from different locations, on different cultivars and at different concentrations of the three pathogens. Results of our experiments indicated that *P. syringae* CPA-5 provides excellent control against the all tested pathogens on all fruits variety, however differences in level of control were observed. Concentrations of 5×10^7 or 10^8 cfu ml^{-1} of *P. syringae*, in general, provided total or almost total control of the pathogens at all pathogens concentrations. At room temperature, in the experiments carried out on 'Golden Delicious' apple, the effectiveness of the antagonist in controlling incidence of the decays did not differ between the pathogens. Among pear cultivars for all pathogens the best decay reduction was observed on 'Rocha' pears treated with CPA-5.

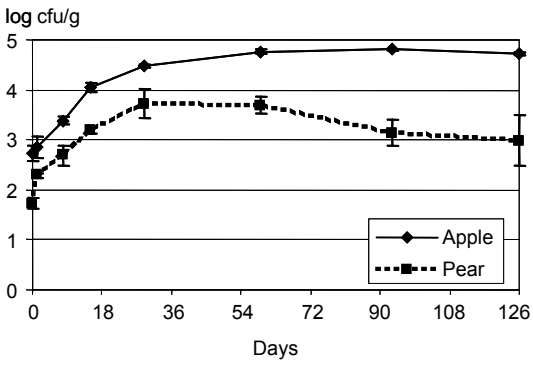

Fig. 3. Population dynamics of *Pseudomonas syringae* CPA-5 on surface of wounded 'Golden Delicious' apple and 'Blanquilla' pear, incubated at 1°C for up to 126 days. Wounded fruits were dipped in the antagonist suspension (10^8 cfu ml^{-1}) for 30 s. The vertical bars represent standard errors of the means of four replicates.

There is a quantitative relation between concentration of an antagonist, a pathogen and resulting control of decay (Janisiewicz 1987, Chand-Goyal and Spots 1997, Viñas et al. 1998, Nunes et al. 2001). The excellent control results at the concentration of 10^8 cfu ml^{-1} of *P. syringae* (CPA-5) on different fruits and cultivars in cold storage indicate that this concentration may be adequate under commercial conditions. This concentration is low enough to be considered viable for commercial purposes as the high limit for bacteria seems to be about 10^9 cfu ml^{-1} (Janisiewicz 1997).

In selecting an antagonist suitable for postharvest application to control diseases in pome cultivars, it is necessary to look for those that should be able to function under cold-storage conditions (Barkai-Golan 2001). *P. syringae* CPA-5 provided excellent control of decay after 4 months of storage at 1ºC. On the three cultivars control of *P. expansum* on fruit treated with *P. syringae* at 10^8 cfu ml^{-1} after 4 months of cold storage was similar to that obtained by strain MA-4 of this bacterium under the same storage conditions on 'Empire' and 'Golden Delicious' apples but at a concentration of *P. expansum* at 10^3 conidia ml^{-1} (Zhou et al. 2001).

It has been suggested that the biocontrol activity of bacterial antagonists may be in part associated with the production of antibiotics (El-Ghaouth et al. 2002), and indeed there are some reports indicating that some species of *Pseudomonas* inhibit germination of *Penicillium* sp. spores and may reduce decay by the production of antibiotic substances such as pyrrolnitrin and syringomycin (Janisiewicz and Roitman 1988, Bull et al. 1998). However the production of these antibiotics has not been conclusively shown to be involved in mechanism of biocontrol on fruits. The mechanism by which CPA-5 of *P. syringae* reduce the development of diseases is not clear, however the studies to determine the mode of action showed that this strain does not produce any antibiotics *in vitro* (data not shown), and thus CPA-5 of *P. syringae* probably does not inhibit diseases by production of antibiotic substances. Competition

for limiting nutrient and space could be the mode of action of CPA-5. This is supported by our results from population studies showing high capacity of this strain to colonize wounds. *P. syringae* CPA-5 increased rapidly on apple or pear surface during the first 30 days at cold storage. Rapid colonization of wounds by an antagonist controlling wound invading pathogens is a prerequisite for successful biocontrol (Janisiewicz 1997).

In conclusion, the new strain CPA-5 of *P. syringae* controlled the most important diseases of pome fruits, on several cultivars and under different storage conditions. Further studies, under commercial conditions, are necessary to determine the commercial potential of this strain. This will require the application of this biological control agent at the growers' packinghouses. Also, laboratory studies are needed to optimize growth and formulation of this antagonist.

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References

Barkai-Golan, R. 2001. *Postharvest diseases of fruits and vegetables. Development and control*. Elsevier, Amsterdam. 432 p.

Bull, C.T., Stack, J.P. & Smilanick, J.L. 1997. *Pseudomonas syringae* strains ESC-10 and ESC-11 survive in wounds on citrus and control green and blue molds of citrus. *Biological Control* 8: 81–88.

Bull, C.T., Wadsworth, M.L., Sorenson, K., Takemoto, J., Austin, R. & Smilanick, J.L. 1998. Syringomycin E produced by biological agents controls green mould on lemons. *Biological Control* 12: 89–95.

Chand-Goyal, T. & Spotts, R.A. 1997. Biological control postharvest diseases of apple and pear under semi-commercial and commercial conditions using three saprophytic yeasts. *Biological Control* 10: 199–206.

Droby, S., Cohen, L., Daus, A., Weiss, B., Horev, B., Chalutz, E., Katz, H., Keren-Tzur, M. & Shachnai, A. 1998. Commercial testing of aspire: A yeast preparation for the biological control of postharvest decay of citrus. *Biological Control* 12: 97–101.

El-Ghaouth, A., Wilson, C.L., Droby, S., Smilanick, J. & Korsten, L. 2002. Biological control of postharvest diseases of citrus fruit. In: Gnanamanickam, S.S. (ed.). *Biological control of crop diseases*. Marcel Dekker, New York. p. 289–312.

Froyd, J.D. 1997. Can synthetic pesticides be replaced with biological-based? An industry perspective. *Journal of Industrial Microbiology & Biotechnology* 19: 192–195.

Guinebretiere, M.H., Nguyen-The, C., Morrison, N., Reich, M. & Nicot, P. 2000. Isolation and characterization of antagonists for the biocontrol of the postharvest wound pathogen *Botrytis cinerea* on strawberry fruits. *Journal of Food Protection* 63: 386–394.

Janisiewicz, W. 1987. Postharvest biological control of blue mold of apples. *Phytopathology* 77: 481–485.

Janisiewicz, W. 1996. Ecological diversity, niche overlap, and coexistence of antagonists used in developing mixtures for biocontrol of postharvest diseases of apples. *Phytopathology* 86: 473–479.

Janisiewicz, W. 1997. Biocontrol of postharvest diseases of temperate fruits. Challenges and opportunities. In: Boland, G. & Kuykendall, L. (ed.). *Plant-microbe interactions and biological control*. Marcell Dekker, New York. p. 171–198.

Janisiewicz, W. & Jeffers, S.N. 1997. Efficacy of formulation of two biofungicides for control of blue mould and grey mould of apples in cold storage. *Crop Protection* 16: 629–633.

Janisiewicz, W. & Korsten, L. 2002. Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology* 40: 411–441.

Janisiewicz, W. & Marchi, A. 1992. Control of storage rots on various pear cultivars with a saprophytic strain of *Pseudomonas syringae*. *Plant Disease* 76: 555–560.

Janisiewicz, W. & Roitman, J. 1988. Biological control of blue-mould and gray-mold on apple and pear with *Pseudomonas cepacia*. *Phytopathology* 78: 1697–1700.

Janisiewicz, W., Tworkoski, T.J. & Kurtzman, C.P. 2001. Biocontrol potential of *Metchnikowia pulcherrima* strains against blue mould of apple. *Phytopathology* 91: 1098–1108.

Jeffers, S.N. & Wright, T.S. 1994. Biological control of postharvest diseases of apples: progress and commercial potential. *New England Fruit Meetings* 100: 100–106.

Leverentz, B., Janisiewicz, W.J., Conway, W.S., Saftner, R.A., Fuchs, Y., Sams, C.E. & Camp, M.J. 2000. Combining yeast or a bacterial biocontrol agent and heat treatment to reduce postharvest decay of 'Gala' apples. *Postharvest Biology and Technology* 21: 87–94.

Nunes, C., Usall, J., Teixidó, N. & Viñas, I. 2001. Biological control of postharvest pear diseases using a bacterium *Pantoea agglomerans* CPA-2. *Journal of Food Microbiology* 70: 53–61.

Nunes, C., Usall, J., Teixidó, N., Fons, E. & Viñas, I. 2002. Postharvest biological control by *Pantoea agglomerans* (CPA-2) on Golden Delicious apples. *Journal of Applied Microbiology* 92: 247–255.

Rosenberger, D.A. & Meyer, F.W. 1981. Postharvest fungicides for apples: development of resistance to benomyl, vinoclozoline and iprodione. *Plant Disease* 65: 1010–1013.

Smilanick, J.L., Gouin-Behe, T., Margosan, D.A., Bull, C.T. & Mackey, B.E. 1996. Virulence on citrus of *Pseudomonas syringae* strains that control postharvest green mold of citrus fruit. *Citograph* 80: 1123–1128.

Spotts, R.A., Cervantes, L.A. & Facteau, T.J. 2002. Integrated control of brown rot of sweet cherry with a preharvest fungicide, a postharvest yeasts, modified atmosphere packaging, and cold storage temperature. *Postharvest Biology and Technology* 24: 251–257.

Teixidó, N., Usall, J., Palou, L., Asensio, A., Nunes, C. & Viñas, I. 2001. Improving control of green and blue moulds of oranges by combining *Pantoea agglomerans* (CPA-2) and sodium bicarbonate. *European Journal of Plant Pathology* 107: 685–694.

Usall, J., Teixidó, N., Torres, R., Ochoa De Eribe, X. & Viñas, I. 2001. Pilot tests of *Candida sake* (CPA-1) applications to control postharvest blue mould on apple fruit. *Postharvest Biology and Technology* 21: 147–156.

Viñas, I., Usall, J. & Sanchis, V. 1991. Tolerance of *Penicillium expansum* to postharvest fungicide treatments in apple packinghouses in Lleida (Spain). *Mycopathologia* 113: 15–18.

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Viñas I., Usall, J., Teixidó, N. & Sanchis, V. 1998. Biological control of major postharvest pathogens on apple with *Candida sake*. *International Journal of Food Microbiology* 40: 9–16.

Wilson, C.L. & Wisniewski, M.E. 1989. Biological control of postharvest diseases of fruit and vegetables: an emerging technology. *Annual Review of Phytopathology* 27: 425–441.

Zahavi, T., Cohen, L., Weiss, B., Schena, L., Daus, A., Kaplunov, T., Zutkhi, J., Ben-Arie, R. & Droby, S. 2000. Biological control of *Botrytis*, *Aspergillus* and *Rhizopus* rots on table and wine grapes in Israel. *Postharvest Biology and Technology* 20: 115–124.

Zhou, T., Chu, C.-L., Liu, W.T. & Schaneider, K.E. 2001. Postharvest control of blue mould and grey mould on apples using isolates of *Pseudomonas syringae*. *Canadian Journal of Plant Pathology* 23: 246–252.