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Postharvest Biological Control of Citrus Fruit

Carla A. Nunes* • **Teresa Manso** • **Mª Emília Lima-Costa**

Centro de Desenvolvimento de Ciências e Técnicas de Produção Vegetal (CDCTPV), University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

Corresponding author: * **canunes@ualg.pt**

ABSTRACT

Economical losses due to postharvest decays are very important worldwide, and fungicides are the primary means to control these losses. Public concern in food safety and the increase of pathogen resistant population has enhanced the interest in developing alternatives fungicides to control postharvest fruit diseases. The research in biological control using antagonistic microorganisms has been developed as an important food safety alternative. Biocontrol of postharvest products has the advantage to be in a controlled environment which can be manipulated to favor the biocontrol agent. Actually there are already in the market three biofungides to control postharvest diseases of fruits, including citrus fruit. It is likely that several more products will enter the market in the near future, as the result of the biological control research programs worldwide. The development of a biocontrol system requires several steps in order to isolate, test and select a potential biocontrol agent. Bioassays at a pilot and commercial scale must be addressed; the antagonistic mechanism of the microorganism has to be understood. For commercial application, biocontrol agent has to be produced and formulated at an industrial scale, maintaining its biocontrol activity. This paper presents an overview of postharvest biological control approaches especially of citrus fruit and explores new possibilities of research to improve biocontrol activity.

Keywords: antagonists, blue mould, green mould, *Penicillium digitatum*, *Penicillium italicum*

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INTRODUCTION

Postharvest losses of fruits and vegetables reach very high values depending on species, harvest methods, storage, transportation, etc., representing more than 25% of the total production in industrialized countries (Harvey 1978) and more than 50% in developing countries where postharvest handling and storage conditions are not optimal (Eckert and Ogawa 1985). Much of these losses are due to the attack of several fungi and bacteria pathogens because of the high amount of nutrients and water content and as after harvest fruits and vegetables have lost most of the intrinsic resistance that protects them while they are attach to the plant (Droby *et al*. 1992). Because of the low pH of citrus fruits the majority of the decays are caused by pathogenic fungi. In contrast to pathogens that attack in the field, most of the postharvest pathogens are incapable of penetration directly through the cuticle, requiring a wound to their penetration. Generally these wounds are made during harvest, transport, packinghouses operations and storage process (Barkai-Goland 2001).

Penicillium digitatum Sacc. causing green mould and *Penicillium italicum* Wehmer causing blue mould are wound pathogens, and the most common and devastating postharvest pathogens of citrus growing countries. They can infect the fruit in the field, in the packinghouse, in transportation and in the market. Both pathogens are specific of all citrus varieties, however *P. digitatum* is more widespread and the most economically important in Mediterranean countries, California and all production areas with low summer rainfall (Eckert and Eaks 1989). In other areas such as China, the largest world producer of citrus fruit, *P. italicum* can

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Invited Review

Table 1 Biological control agents of postharvest citrus fruit.

Biocontrol agent	Pathogen	References
Bacteria		
Bacillus pumilus	*Penicillium digitatum*	Huang *et al*. 2004
Bacillus subtilis	*Penicillium digitatum*	Gutter and Littauer 1953; Shing and Deverall 1984; Obagwu and Korsten 2003a; Leelasuphakul *et al*. 2008
Pantoea agglomerans	*Penicillium digitatum* *Penicillium italicum*	Viñas *et al*. 1999; Teixidó *et al*. 2001; Manso *et al*. 2004, 2006; Torres *et al*. 2007; Nunes *et al*. 2008
Pseudomonas cepacia	*Penicillium digitatum*	Smilanick and Denis-Arrue 1992; Huang 2000; El-Ghaouth *et al*. 2002
Pseudomonas glathei	*Penicillium digitatum*	Huang *et al*. 1995
Pseudomonas syringae Bio-save®10 LP	*Penicillium digitatum* *Penicillium italicum* *Geotrichum candidum*	Bull *et al*. 1998
Serratia plymuthica	*Penicillium digitatum* *Penicillium italicum*	Meziane *et al*. 2006
Fungi		
Candida famata	*Penicillium digitatum*	Arras 1996
Candida oleophila Aspire™	*Penicillium digitatum* *Penicillium italicum*	Droby *et al*. 1998; Bar-Shimon *et al*. 2004
Candida saitoana	*Penicillium digitatum*	El-Ghaouth *et al.* 2000a, 2000b
Cryptococcus laurentii	*Penicillium italicum*	Zhang *et al*. 2005
Kloeckera apiculata	*Penicillium italicum*	Long *et al*. 2005
Metschnikowia fructicola Shemer®	*Penicillium digitatum* *Penicillium italicum*	Droby 2006
Metschnikowia pulcherrima	*Penicillium digitatum*	Kinay and Yildz 2008
Muscodor albus	*Penicillium digitatum* *Geotrichum candidum*	Mercier and Smilanick 2005
Pichia anomala	*Penicillium digitatum* *Penicillium italicum*	Lahlali *et al*. 2004
Pichia guillermondii (ex *Debaryomyces hansenii*)	*Penicillium digitatum* *Penicillium italicum* *Geotrichum candidum*	Droby *et al*. 1989, 1993; Chalutz and Wilson 1990; Arras *et al*. 1998; Kinay and Yildz 2008
Rhodotorula glutinis	*Penicillium digitatum*	Zheng *et al.* 2005

cause losses of 30-50% (Long *et al*. 2005).

Conidia of both fungi are present during the season in the atmosphere of citrus growing areas, particularly in packinghouses, in their equipment and in their surroundings (Barkai-Goland 2001). The fungus reproduces very rapidly and if there are inappropriate packinghouses sanitation measures, the inocula level in packinghouses may gradually increase during the season (Palou *et al*. 2001a).

Another important postharvest disease of citrus fruit is the sour rot caused by *Geotrichum candidum* Link. This disease is less important than the others, but it should not be underestimated because initial infections are easily overgrown by other moulds (Smoot *et al*. 1983). The incidence of fruit decay caused by *G. candidum* increases after prolonged wet seasons (Smoot *et al*. 1983) and when harvesting occurs after abundant rainfall (Tuset 1987). Sour rot is primarily disease in storage and in transit and it was reported most often on lemons (*Citrus limon* (L.) Burm.f), limes (*Citrus aurantifolia* (Christm.) Swing) and grapefruits (*Citrus paradise* Macf.), which are often stored for long periods (Barkai-Goland 2001). Other important postharvest diseases that can occur in citrus fruit are Stem-end rots caused by *Phomopsis citri* Fawcet or *Alternaria citri* Ell & Pierce, brown rot caused by *Phytophthora* spp. and anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Sacc. They are, in general, infections of immature fruit in preharvest but are manifested only after harvest.

Postharvest losses can be reduced by preventing the development of the infections. Careful handling throughout the process of harvest, transportation and packinghouse management can minimize mechanical injuries; sanitation procedures in field, including the removal of fallen fruits have also a significant impact on fruit decay. Packinghouse sanitation is one of the most important control practices associated with citrus fruit handling, providing the reduction of inocula of *P. digitatum*, *P. italicum*, *G. candidum* and *Phytophthora* spp. (Brown and Miller 1999). Maintaining natural fruit resistance by harvesting with adequate maturity, using hormones, cold storage to delay senescence also helps in preventing fruit decays (Shweleft 1986).

Nevertheless, the use of these beneficial practices are not sufficient to avoid the infection on fruits, so the primary means to control all these diseases are the use of postharvest synthetic fungicides (Eckert 1990). However the repeated and continue use of fungicides has led to the development of resistant strains of *P. digitatum* and *P. italicum* (Holmes and Eckert 1999; Kinay *et al*. 2007). *Penicillium* spp. has the ability to produce large numbers of airborne spores, so can rapidly produce some resistant spores. Normally these resistance spores constitute a minor component of the population without any ecological advantage, however once population is subjected to selection pressure by continuous application of a selective fungicide, such imazalil or tiabendazol, the fungicide resistant mutate are able

to proliferate free of competition (Brown and Miller 1999). In addition the growing concern for human safety and protection of the environment, the increase interested in sustainable agriculture, integrated crop management and organic production have resulted in the need to developing other methods to control postharvest decays. Biological control using microbial antagonist has been considered a desirable alternative to synthetic fungicides for the protection of fruits.

During the last 25 years several research programs have been developed all over the world, and numerous biological control agents have been investigated against different postharvest diseases of fruits (**Table 1**) (Janisiewicz and Roitman 1988; Chalutz and Wilson 1990; McLaughlin *et al*. 1992; Janisiewicz and Jeffers 1997; Viñas *et al*. 1998; Fan *et al*. 2000; Zahavi *et al*. 2000; Nunes *et al*. 2001a; Teixidó *et al*. 2001; Adikaram *et al*. 2002; Zheng *et al*. 2005; Bleve *et al*. 2006; Govender and Korsten 2006; Nunes *et al*. 2007a). Some of them have been patented and tested on large scale commercial conditions (Janisiewicz and Jeffers 1997; Droby *et al*. 1998; Viñas *et al*. 1999; El-Ghaouth *et al*. 2000a; Usall *et al*. 2001; Arras *et al*. 2002; Torres *et al*. 2007). Despite of this only a few commercial products are available.

The purpose of this article is to review significant research work in biological control of postharvest diseases of citrus fruit.

BIOLOGICAL CONTROL IN POSTHARVEST

Postharvest environment represents a particular advantage to develop biological control when compared with field environment. Injuries made during harvest and transport to packinghouse can be protected from wound pathogens with only a single application of the biocontrol product directly to infection site (harvested fruit), using the existing facilities (drenchers, on-line sprayers, on-line dips) (Janisiewicz and Korsten 2002). During storage period fruits are kept in a constant physical environment, which can be controlled to favor the antagonist growth. In addition, the fact that almost every fruits, including citrus are washed before postharvest treatments, make that the antagonists encounter minimal competition from other microorganisms and from other interfering factors such as dust particles. The high value of the commodities in postharvest makes the application of a biocontrol fungicide more justified than in the field. And, for some commodities, such as citrus fruit, protection from postharvest diseases is needed for a short period time (Wisniewski and Wilson 1992). Although the postharvest environment presents an uniqueness biocontrol system, Chalutz and Droby (1997) suggest that specific difficulties should be taken in account: the required high level of disease control (95-98%) in postharvest, food safety requirements with the application of live microorganisms in food and the relatively small potential market for the use of postharvest biofungicides.

Before becoming an economically feasible a biocontrol agent of postharvest disease has to satisfy different requirements. The first one is that any potential antagonist must have the ability to rapid colonize and be persistent in the wound site and still be metabolic active at storage temperatures (Janisiewicz and Korsten 2002). Wisniewski and Wilson (1992) indicated that the ideal antagonist for the postharvest environment should be: genetically stable, effective at low concentrations against a wide range of pathogens and commodities, able to survive under adverse environmental conditions, simple and inexpensive nutritional requirements, inexpensive to produce and formulate with long shelf life, easy to dispense, compatible with handling and storage practices and resistant to pesticides, non pathogenic for the human health and host commodity. Concerning with these characteristics several interests have been driven to yeasts. Yeast can colonize a surface for long periods under dry conditions, produce extracellular polysaccharides that enhance their survival and restrict wound colonization and flow germination of fungi, rapidly use the available nutrients and be minimally impact by pesticides (Janisiewicz 1988). Despite this, several bacteria have been shown to have also a great potential as biocontrol agents. Bacteria have the ability to grow in substrates with very low amino acid and carbohydrates contents, but also produce potent antibiotics. Special attention should be given to their use in postharvest, in a way that their antagonistic activity will not be due to an antibiotic production.

SELECTION OF A POSTHARVEST BIOCONTROL AGENT

The development of a biological control agent requires several steps. The first one is the isolation, test efficacy, identification and selection. Screening for antagonists has been practiced in almost all research laboratories dealing with biocontrol. Different strategies have been used; however most of the research has been focused in isolating naturally occurring microorganisms from fruit just before harvest or during storage (Janisiewicz 1988, 1997; Viñas *et al*. 1998; Jijakli *et al*. 1999; Nunes *et al*. 2001a, 2007a). In fact the fructoplane has provided the most abundant and desirable source of antagonists against postharvest fruit diseases (Janisiewicz and Korsten 2002). However microorganisms with antagonist capacity of fruit pathogens have been isolated from soil and leaves (Jijakli *et al*. 1999). *Bacillus subtilis* strains isolated from soil showed high antagonistic activity against *P. digitatum* (Leelasuphakul *et al*. 2008).

As we reported several strategies have been used, but since the antagonists are applied to consumable products, they need to have strict requirements including non production of toxic metabolites. For that reason researchers often evaluate the efficacy of microorganisms on wounded fruits instead of on *in vitro* studies. Although the direct screening on fruit is very laborious it was the most efficient methodology to obtain antagonistic microorganisms of wound postharvest pathogens (Janisiewicz 1997). *In vitro* assays normally select microorganism with the ability to produce antifungal metabolites and discard potential non-antibiotic-producing antagonists (Andrews 1985). After selection a potential antagonist the next step is the secondary screening to determine the minimum effective concentration. In general only 2-5% of all the isolates are selected in secondary screening. Palau *et al.* (2002) test in mandarins (*Citrus reticulate* Blanco) and oranges (*Citrus sinensis* (L.) Osb.) the activity of 212 bacteria and yeasts against *P. digitatum* and only 3 (1.4%) inhibited the decay by 50% or more. In other study conducted by our research group, from 800 microorganisms isolated from the surface of leaves and fruits of oranges only 4 (0.5%) show potential as a biocontrol agent against green mould in oranges (Manso *et al*. 2004).

To develop a promising biological control system the screening program should simulate natural wounding and inoculation, and the inoculum should be applied in the proper time. We already state that in citrus, wounds are inflected and inoculated during harvest and handling in packinghouse, so in contrast to other crops, like pome fruits, in experimental assays the application of the antagonist should be done after pathogen inoculation. This fact implicate that the biocontrol agent has to be able to eradicate infection and protect wounds. Different times of effectiveness were observed with different antagonists. The biocontrol agent *Pseudomonas cepacia* was effective in controlling green mould in lemons if applied within 12 h after inoculation (Smilanick and Denis-Arrue 1992), while the yeast *Pichia guillermondii* (originally described as *Debaryomyces hansenii*, McLaughlin *et al*. 1990) was effective against green and blue mould and sour rot in lemon and grapefruits if applied after 3 h of pathogen inoculation (Chalutz and Wilson 1990). This particularity of citrus makes the biological control less attractive and effectiveness in this crop than the others, where wounds are infected in packinghouse after postharvest treatment. In addition the density of the inocu-

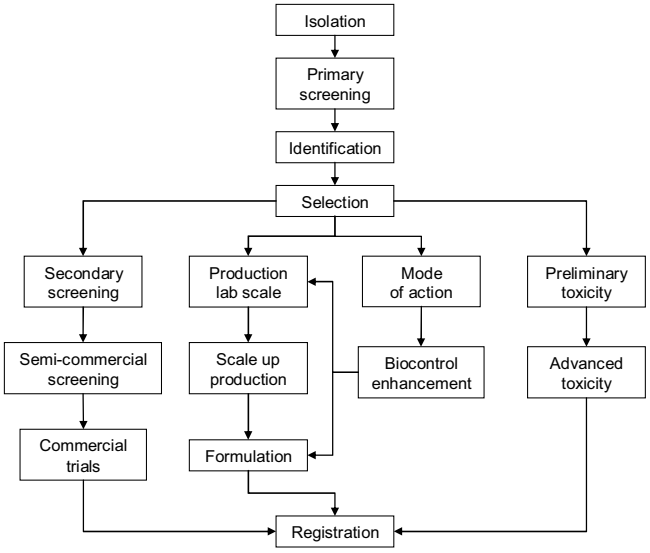

Fig. 1 Flow diagram of development of a postharvest biocontrol agent.

lums recommended for evaluation of postharvest treatments in citrus to control green and blue mould is 10^6 spores/mL (Eckert and Brown 1986), 100-fold more than in pome fruits.

Another important factor is that the antagonists selected to develop at a commercial scale must be effective at reasonable concentrations for commercial development (Janisiewicz 1997). Reported concentrations to control postharvest citrus diseases varied in bacteria from 2×10^8 cfu/mL of *Pantoea agglomerans* CPA-2 (Nunes *et al*. 2008) to 1.9×10^9 cfu/mL of *Pseudomonas glathei* (Huang *et al*. 1995) and in yeasts from 3×10^8 cfu/mL of *Kloeckera apiculata* (Long *et al*. 2005) to 2×10^9 cfu/mL of *Pichia guillermondii* (Droby *et al*. 1997).

The development of a biocontrol agent for postharvest diseases is an interactive process and is difficult to model all the steps involved in a flow diagram (Janisiewicz 1997), however we make one to better understand the process (**Fig. 1**). As we already see this steps include tests reflecting conditions encountered in commercial packinghouses, such different cultivars, compatibility with postharvest practices. For citrus fruit this involves studies with degreening process, application methods such drencher, spay and dip, storage temperatures, wax, etc. These tests are conducted at a large or pilot scale.

At the same time scale-up the production and the development of a formulated product of the biocontrol agent as well the enhancement of biocontrol activity should be accessed, as we will see in this review.

MODE OF ACTION OF POSTHARVEST BIOCONTROL AGENTS

The antagonistic activity of bacteria and yeast biocontrol agents has been demonstrated on citrus fruits, as well as on other fruits, at different level, including commercial application. However the mechanisms of action of most biocontrol agents of postharvest diseases are poorly understood. It is generally assumed that involves a complex interaction between host, pathogen, antagonists and environment, comprising process of antibiosis, nutrient and space competition, induced resistance, parasitism and lytic enzymes production. Often more than one mechanism is implicated.

Antibiosis

In the case of bacteria it has been suggested that activity may be in part due to antibiotic production. In fact one of the first observations of a potential microbial control of postharvest diseases of citrus fruit was reported in 1953 by Gutter and Littauer, with the bacteria *Bacillus subtilis*. This microorganism has been reported as antagonistic of postharvest diseases of citrus fruit (Obagwu and Korsten 2003a) and other fruits (Sholberg *et al*. 1995; Fan *et al*. 2000; Korsten *et al*. 2007). However almost all the strains inhibit the pathogens by producing antibiotics (Gutter and Littauer 1953; Singh and Deverall 1984; Leelasuphakul *et al*. 2008). Other bacterial biocontrol agent *Pseudomonas syringae* effective against *Penicillium* moulds in citrus fruit produces the antibiotic syringomycin E (Bull *et al*. 1998) and *Pseudomomas cepacia* and *Serratia plymuthica* produce pyrrolnitrin (Smilanick and Denis-Arrue 1992; Meziane *et al*. 2006). This leads us to the debate if an antibiotic-producing microorganism should be used in postharvest phase, due to the concern of introducing an antibiotic into food and the possible development of a pathogen resistance. On the other hand, and especially in citrus were wound infection occurred prior to biological control agent application the antibiotic-producing microorganisms may be particularly effective (Chalutz and Droby 1997). However for the same microorganisms the role of antibiotics in antagonistic activity was not full elucidated, since some pyrrolnitrin non production mutant maintain their antagonistic activity (El-Ghaouth *et al*. 2002) and *P. digitatum* isolates resistant to pyrrolnitrin were still controlled in lemons by *P. cepacia* (Smilanick and Denis-Arrue 1992). This suggests that the antibiotic substances produced by these microorganisms are not the only means to control the pathogen, and other factors may be involved. Besides antibiotic producing these bacterial antagonist rapid growth and colonize the wounds, meaning that competition may play a major role.

Competition for nutrients and/or space

Competition for nutrients and space is reported has the major mode of action of postharvest biocontrol agents (Droby *et al*. 1989; Viñas *et al*. 1998; Janisiewicz *et al*. 2000; Nunes *et al*. 2001b; Bencheqroun *et al*. 2007). This hypothesis is supported by the fact that biocontrol activity of an antagonist depends on their concentration in the wound (Janisiewicz 1997). As it was mentioned before in addition to other characteristics the ideal biocontrol agent should be grown rapidly, use low concentrations of nutrients and be better adapted to the environment. So it can be considered if an antagonist rapidly grows by depleting the available nutrients in the wound, will prevent the possibility of the pathogen to use these nutrients to germinate and initiate the infection process. Thus, it was observed that oranges treated with the bacteria *Pantoea agglomerans* (CPA-2) and maintained during 24 h at 20°C before cold storage, the population of *P. agglomerans* (CPA-2) increased more than 10-fold (Nunes *et al*. 2008), and achieved better decay control when compared with fruits immediately stored at cold temperature (unpublished data), suggesting that this time is necessary to this biocontrol agent colonize the fruit surface. In fact, other work showed that if fruits were stored at cold temperature immediately after *P. agglomerans* treatment, during the first 3 days the population decreased, and the increased of 10-fold was only observed after 7 days of cold storage (Teixidó *et al.* 2001). It was also observed a rapid growth of the yeast *Pichia guillermondii* on grapefruit what allow it to colonize the wound in 24 h, while *P. digitatum* spores are still in their initial stages of germination (Droby *et al*. 1992). In most reports on biological control of postharvest diseases a quantitative relationship has been demonstrate between the antagonist concentration in the wound and the efficacy of the biocontrol agent (Teixidó *et al*. 2001; Nunes *et al*. 2002a). On oranges the antagonistic activity of several biocontrol agents increases by increasing their concentration and decreasing the pathogen inocula (El-Ghaouth *et al*. 2002).

Parasitism and lytic enzyms production

In postharvest very little information is available on biocontrol agents that directly parasitize pathogens. However Arras *et al.* (1998) observed the attachment of *P. guillermondii* to the mycelium of *P. digitatum* and subsequent changes in hyphae indicating that hyperparasitism is also involved in antagonistic activity of this yeast. It is possible that the attachment facilitates a more efficient depletion of nutrients from the area subjacent to the mycelium or serves as a mechanical barrier to nutrient uptake by the fungi (Droby *et al*. 1992). Moreover, *P. guillermondii* shows a high activity of the enzyme β-1,3-glucanase that could result in the degradation of the fungal cell walls (Jijakli and Lepoivre 1998). Scanning electron microscope observations of *P. digitatum* and *Candida famata* (F35), a biocontrol agent of green mould, in wounds sites revealed numerous yeast cells strongly attached to the hyphae, exhibiting lyctic activity and rapid alterations of hyphal tissue (Arras 1996).

Candida oleophila, is the base of the commercial product Aspire™ for control postharvest disease of citrus and pome fruits. Competition for nutrients and space is believed to be the major mode of action. However, the involvement of fungal cell wall-degrading enzymes, such exo-β-1,3-glucanase, chitinase and proteases is also suggested to play a role in it mechanism of action (Bar-Shimon *et al*. 2004). In this study it was observed that the production of these enzymes was stimulated by the presence of cell wall fragments of *P. digitatum* in the growth medium, in addition to glucose. It was also provided evidence for the role of exo-β-1,3-glucanase (*CoEXG1*) in biocontrol activity of *C. oleophila* by testing *CoEXG1*-knockouts and double-*CoEXG1* over-production transformants.

Induce resistance in fruits

The resistance of fruits to pathogens is mainly due to physical and chemical barriers as a response to biotic or abiotic stress and the microflora present on the fruit (Arras 1996). Induced defense reactions can be restricted to tissue close to the site of the stimulus or can be expressed systematically throughout the tissue. Fruits use a wide range of physical and biochemical strategies to defend themselves from attack by pathogenic microorganisms. Defense response of the fruits include production of inhibitors of cell wall-degrading enzymes of the pathogen, the activity of antifungal compounds, such phenolic compounds and phytolaexins, active oxygen species, and reinforcement of the cell wall of the host.

Several antagonists have been reported to induce specific host response in fruits. *P. guillermondii* has been shown to stimulate the production of ethylene in grapefruit (Wisniewski *et al*. 1991). Ethylene actives the phenylalaninammonium-lyase (PAL) an enzyme involved in the synthesis of phenols, phytoalexins and lignins. *C. famata* (F35) was also found in oranges to stimulate the production of the phytoalexins, scoparone and scopoletin in the wound site (Arras 1996). These phytoalexins are known to inhibit spore germination of *P. digitatum*, however are dependent of concentration. The biosynthesis of scoparone and scopoletin is related with factors such as citrus fruit species, size of the wound, antagonists species, concentration and time of inoculation (Arras 1996). Scoparone production was 890 µg/g fresh peel in Oroblanco grapefruit and only 260 µg/g fresh peel in Valencia oranges, five days after inoculation of *P. guillermondii* (Rodov *et al*. 1994). *C. oleophila* was found to induce resistance to *P. digitatum* when applied in the surface of both wounded and unwounded grapefruit (Droby *et al*. 2002). However, the responses were higher in wounded fruit and decrease when the distance to wound site increase. The induced responses reported in that work was the increase of ethylene production, PAL activity, phytoalexin biosynthesis and accumulation of chitinase and β-1,3-glucanase.

Volatile compounds

Biofumigation refers the use of antimicrobial volatiles produced by biocontrol microorganisms (Strobel *et al.* 2001; Mercier and Jiménez 2004), and has been introduced as a better alternative because there is no contact with the food (Schotsmans *et al*. 2008). Moreover the application of postharvest biofumigation will be advantageous since less manipulation of the commodities would be involved.

A good candidate for biocontrol by biofumigation *Muscodor albus*, an endophytic fungus isolated from a cinnamon tree in Honduras (Strobel *et al.* 2001). This fungus inhibit or kill a broad range of bacteria and fungi through the production of a mixture of volatile organic compounds (Strobel *et al*. 2001; Ezra *et al.* 2004; Mercier and Jiménez 2004; Schotsmans *et al*. 2008).

Several reports have shown the effect of biofumigation with *M. albus* in controlling postharvest diseases of fruit (Mercier and Jiménez 2004; Mercier and Smilanick 2005; Mercier *et al*. 2005; Gabler *et al*. 2006; Schnabel and Mercier 2006). Mercier and Smilanick (2005) related the capability of *M. albus* control green and sour rot of lemon by biofumigation. The control of green mould was also higher when combined with the degreening process. In this study, *in vitro* growth of *P. digitatum* and *Geotrichum citri-aurantii* was completely inhibited by exposure of volatiles of *M. albus* during 3 days. And when the spores were transferred to fresh medium all spores exposed to volatiles were confirmed dead. The most abundant volatiles produced by *M. albus* are 2-methyl-1-butanol, isobutyric acid, ethyl propionate and phenethyl alcohol (Mercier and Jiménez 2004). However other studies indicate that the composition of the medium used to support the growth of *M. albus* greatly influences the quality and effectiveness of the volatiles emitted by this organism (Ezra and Strobel 2003). Schotsmans *et al*. (2008) also demonstrate that the efficacy of this antagonist was temperature dependent, reducing effectiveness at low temperature. The mode of action of *M. albus* is little know, but the antibiotic effect of the volatile organic compounds is strictly related to the synergistic activity of the compounds in the gas phase (Strobel 2006).

A commercial formulation developed by AgraQuest (Davis, California, USA) has received US Environmental Protection Agency and the California Department of Pesticide Regulation approval and has been registered as a natural biofumigant and an alternative to methyl bromide for agricultural applications. Research now is focused on determining optimum production methods and formulation for best efficacy and cost–benefit (Anonymous 2008).

Volatiles generated by *Bacillus subtilis* JA was also reported to significantly inhibited both spore germination and elongation of germ tubes in *Botrytis cinerea in vitro*. The volatiles caused protoplasm retraction from the hyphal tips to the spores (Chen *et al.* 2008). Leelasuphakul *et al.* (2008) also reported the *in vitro* inhibition of *P. digitatum* by volatiles of *Bacillus* spp. However, in this study the volatile organic compounds produced by *Bacillus* spp. growing with PDA medium were less inhibitory and only fungistatic while their water soluble compounds present in 1:32 dilutions of bacterial culture fluids were more effective and fungicidal.

The biofumigation could be important to be used as in-package fumigant, or combining with degreening of citrus fruit to provide protection during this process (Mercier and Smilanick 2005). An emerging advantage of *M. albus* for postharvest citrus protection is that it has the flexibility to be integrated into the various phases of degreening, cold storage, packing, etc. (Anonymous 2008).

Thus, the biocontrol activity of an antagonist not only may be dependent on its ability to rapidly colonize the wound site and compete for nutrients, but may also depend on its ability to attach firmly to hyphae of the pathogen, produce cell wall degrading enzymes or volatile compounds. The understanding of the mode of action of the antagonists in relation with the etiology of the disease is essential for

the success of biocontrol process, this knowledge will increase with further research. It is necessary to fully understand the complex reactions of induced resistance and chemical and physical elicitors, as well the wound competence at the molecular level using the new tools of molecular biology. As we see, more recently, the development of molecular techniques are innovative alternative tools for understanding and demonstrating the mechanisms of biocontrol systems. The identification of genes involved in biological property allows understanding the genetic basis of mechanism of action. Gene inactivation and overexpression studies can provide information on the transcription and regulation of these genes (Massart and Jijakli 2007). Understanding the mode of action of postharvest biocontrol agents will contribute to improve the selection procedures of more active antagonists, for optimization their application in fruits, developed appropriate formulation to enhance effectiveness, and facilitate the registration for commercial use (Droby and Chalutz 1994).

ENHANCEMENT OF BIOLOGICAL CONTROL

The development occurred during the first decade studying postharvest biocontrol system is called the "First Generation of Biocontrol Products", since the definition of biocontrol was adopted by the entomology, which involves the control of one organism by another. However a plant disease is not an organism, it is a process. Actually the definition adopted by plant pathologist is that the biological control of a disease is the "control of a plant disease by a biological process or the product of a biological process" (Wisniewski *et al*. 2007). From this definition new approaches in developing biocontrol systems in order to overcome the existing limitations and a new concept have been used to develop the second generation of postharvest biocontrol agents.

The main approaches to improve and developed new biocontrol systems are: (i) antagonistic mixture; (ii) manipulation of nutritional environment; (iii) pre-harvest application; (iv) manipulation of antagonists; (v) production and formulation, and (vi) integration with other methods. These approaches are expected to overcome the problems of the first generation biocontrol agents such as a narrow range of activity either of fruits or/and diseases, under particular environmental conditions, to promote high and constant efficacy and make possible the control of previously established and latent infection.

Antagonistic mixture

The use of antagonists in mixtures could improve the spectrum of activity and reduced the cost of treatments by allowing the concentration of antagonists to be reduced (Nunes *et al*. 2002b). The antagonist action will result not from an activity of one species but from the action of a community of microorganisms that suppress disease through different mechanisms of action (Janisiewicz and Korsten 2002). There are no reports in using a combination of antagonists in citrus fruit, however in other biocontrol systems successful has been achieved (Janisiewicz 1988; Janisiewicz and Bors 1995; Janisiewicz 1996; Guetsky *et al*. 2001, 2002; Nunes *et al*. 2002b, 2005). One strategy to select the antagonist for mixtures proposed by Janisiewicz (1997) is based in a microbial succession at the wound site. It was proposed that after depletion of the nutrients by one microorganism, another originally less competitive may take over colonization of the wound, further depleting the remaining nutrients for the pathogen. However, better understanding of microbial ecology in wound site is necessary to take advantage of this microbial competitive approach.

Manipulation of nutritional environment

Besides effectiveness the cost is one of the most important factors that will determine the feasibility of any biocontrol system. As it was reported higher concentration of the antagonist must be applied to achieve more effective control, but increasing the microorganism population makes biocontrol less economical. Nutritional amendments may result in a stimulation of antagonist growth and better colonization of wound site. The nutrients should be chosen preferably by being metabolized by the antagonist and not by the pathogen (Janisiewicz 1997). The application of sugar analog, 2-deoxy-D-glucose at 0.2%, showed to improve *Candida saitona* biocontrol activity of green mould in lemon and oranges to levels similar to imazalil (El-Ghaouth *et al*. 2000b). This sugar analog, molecule is known to have a fungicidal effect however the enhancement appears to be due a synergy between *C. saitona* and 2-deoxy-D-glucose. Similar protective effects were reported with other biocontrol agents on pome fruits (Janisiewicz 1994; El-Ghaouth *et al*. 2000b; Nunes *et al*. 2001b). The addition of nitrogenous compounds was also reported to enhanced biocontrol activity of the *Pantoea agglomerans* CPA-2 in controlling green mould in oranges and mandarins (Nunes *et al*. unpublished).

Nutritional composition can also influence the production of metabolites, such cell wall-degrading enzymes (Wisniewski *et al*. 1991). More studies of the effects of nutrients in the antagonist and in the pathogens are necessary to improve biocontrol system by manipulating the nutritional environment.

Pre-harvest application

Pre-harvest application can enhance the biocontrol system because will allow the antagonist to have longer interact with the pathogen, to colonise tissues before the arrival of pathogen, such as latent infection and incipient infections occurring through wounds resulting from harvesting period (Ippolito and Nigro 2000). In citrus this is import since often infections occur prior to harvest. Droby *et al*. (1993) suggested the possibility of reducing postharvest decay in citrus by pre-harvest application of *Pichia guillermondii.*

However, successful of pre-harvest application are dependent of the tolerance to environmental stress such as, dry conditions, direct UV irradiation, high temperatures, low nutrient availability, rapid climatic changes, etc. Very few reports indicate the possibility of a microorganism accomplished these characteristics without cells manipulation or adaptation. In the sub-heading of formulations we will discuss the improvement tolerance of the antagonists to field conditions.

Manipulation of antagonists

Genetic manipulation of a postharvest biocontrol agent is a very new field. Current efforts are focused on developing efficient and rapid procedures for tracking antagonists than for enhancing biocontrol (Schena *et al*. 2000; Janisiewicz and Korsten 2002; Schena *et al*. 2002; Massart *et al*. 2005; Nunes *et al*. 2008). However genes responsible for biocontrol activity or for increasing the ecological competence could be introduced in antagonists microbial. For example insertion of genes or over-expression of endogenous genes responsible for antifungal activity, such as cell wall degrading enzymes or, insertion of genes for better utilization of available nutrients could be effective in enhance biocontrol activity. More recently few attempts were made in transformed antagonists to enhance biocontrol activity. However *in vivo* assays showed no differences between wild-type and transformer antagonists (Nigro *et al*. 1999; Bar-Shimon *et al*. 2004).

Formulation

Production and formulation processes can affect many aspects of biocontrol activity, shelf-life, and safety and may enhance or diminish control. The efficacy of most postharvest biocontrol agents is directly related to the number of viable cells, so if the formulate product allows the application of a high number of viable and effectiveness cells of the antagonists we are already improving biocontrol. There are several reports on enhancing viability, efficacy and shelf-life of the formulated cells when compared with fresh cells of the biocontrol agent. Postharvest biocontrol agents has been formulated into a refrigerated liquid (Abadias *et al*. 2003), a solid formulation using freeze-drying (Costa *et al*. 2000; Abadias *et al*. 2001), spray drying (Costa *et al*. 2002; Abadias *et al*. 2005), or fluidized bed drying (Mounir *et al*. 2007), wettable refrigerated powder (Janisiewicz and Jeffers 1997), frozen pellets (Janisiewicz and Korsten 2002) and granulates (Kinay and Yildiz 2008). All these techniques of formulated cells had particularly effects on cells viability. For example for the citrus postharvest biocontrol agent *Pantoea agglomerans* CPA-2 formulated product obtained by spray-drying drastically reduce cell viability (Costa *et al*. 2002). Another factor of dry formulation that enhanced survival is the growth, protective and rehydration media. Important and intensive research in this filed has been done with the biocontrol agent *P. agglomerans* CPA-2. Teixidó *et al*. (2005) significantly improve its tolerance to dehydration and to high temperatures using modified growth media with NaCl. This tolerance was attributing to the intracellular accumulation of compatible solutes glycine-betaine and ectoine. The osmotic-adapted cells also demonstrate better survival during spray drying and maintaining its biocontrol activity to control green mould of citrus (Teixidó *et al*. 2006). Regarding to the effect of protective and rehydration media in freeze-drying of *P. agglomerans* CPA-2, the use of 5% trehalose as a protective agent achieved the viability of 83% whereas with 5% fructose the viability was only 35%; and 100% of viability was obtained using 10% of non-fat skim milk as a rehydrated media (Costa *et al*. 2000). Kinay and Yildiz (2008) observed that the viability and effectiveness of several granular formulations of the citrus postharvest biocontrol agents *Metschnikowia pulcherrima* and *Pichia guillermondii* were dependent of the carriers and adjuvants used in formulation. For both antagonists they observed that the formulation containing talc as a carrier and sodium alginate (1.5%) as adjuvant had high viability, and if sucrose (1%) and yeast extract (1%) were added shelf life and efficacy were enhanced. Previously we explained that pre-harvest application of the antagonist could lead to an improvement of effectiveness. However applying and keeping an active microorganism in such stress environment is a limiting factor. It is known that is possible to adapt a microorganism to unfavorable environment by induction of stress responses. Cañamas *et al*. (2008a, 2008b) maintained the survival, stability and effectiveness of *P. agglomerans* CPA-2 under field conditions by integrating certain formulation strategies: adding additives, ecophysiological osmotic adaptation and lyophilization.

Improvements in production and formulation with systematic and integrated approach can result in important progress of biocontrol systems. However more studies to better understand the mechanisms of induced tolerance to stress conditions are needed.

Integration with other methods

Several non-fungicidal methods to control postharvest diseases in fruits have been developed for various commodities. Successful control of postharvest citrus fruit were obtained using physical methods: thermotherapy treatments with hot air (Ben-Yehoshua *et al*. 1987; Plaza *et al*. 2003; Pérez *et al*. 2005; Nunes *et al*. 2007b) and hot water (Ben-Yehoshua *et al*. 2000; Porat *et al*. 2000; Nafussi *et al*. 2001; Smilanick *et al*. 2003) and UV-C illumination (Wilson *et al*. 1997; Arcas *et al*. 2000; Kinay and Yildiz 2006). Chemical methods such food additives or compounds classified by FDA (USA) as GRAS (Generally Regarded as Safe) (Lesar 2008) like ozone (Palou *et al*. 2001b, 2003), sodium carbonate and bicarbonate (Smilanick *et al*. 1997, 1999; Palou *et al*. 2001a), potassium sorbate (Salazar *et al*. 2008; Smilanick *et al*. 2008), ethanol (Smilanick *et al*. 1995), natural products (Arras and Usai 2001; Obagwu and Korsten 2003b) and chitosan (Chien and Chou 2006). However none of these methods, including biological control, when used alone provided the control of diseases needed at postharvest phase, more than 95%. Therefore one approach to use these methods as an alternative to synthetic fungicides is the integration of different treatments, taking advantage of the additive or synergistic effects in order to overcome the performance and improve the efficacy of each method.

The biocontrol activity of *Bacillus subtillis* against postharvest diseases of 'Valencia' and 'Shamouti' oranges was significantly improved when was combined with sodium bicarbonate or hot water (Obagwu and Korsten 2003a). Combining *Pantoea agglomerans* with sodium bicarbonate fully control green mould after 2 months storage at 3ºC (Teixidó *et al*. 2001). When solutions of 3% of sodium bicarbonate were heated at 50ºC followed by the application of fresh cells or formulated cells of *P. agglomerans*, the control of decay was similar or superior to imazalil (Nunes *et al*. 2004; Manso *et al*. 2007a; Torres *et al*. 2007). This strategy combined complementary modes of action with curative and preventive activities, and, is effective and reliable enough to be implemented at a commercial scale because are compatible with existing facilities in several packinghouses (Usall *et al*. 2008).

Combining chitosan (0.2% of glycolchitosan) with *Candida saitoana* improved control of green mould in different varieties of oranges and in 'Eureka' lemons, with equivalent control to that imazalil (El-Ghaouth *et al*. 2000a).

Biocontrol agents has been also combined with low doses of synthetic fungicides, providing similar controlled to that of the same fungicide at commercial doses (Droby *et al*. 1998; Arras *et al*. 2002)

Curing treatments have also been successful when were combined with biocontrol agents. Positive synergistic effect occurred with the combination of *Candida famata* with curing at 37°C during 72 h in controlling green mould in stored grapefruit (D'Hallewin *et al*. 1999). Plaza *et al*. (2004) reported the application of *P. agglomerans* followed by curing treatment at 33°C for 6 h to control established infections of *P. digitatum* on lemons either at room or cold storage.

Although at less extent other physical methods have been combined with biocontrol. 'Dancy' tangerines inoculated with *P. italicum* and exposure to UV-C dose of 1.3 kJ/m^2 for 1.75 min and treated with *Pichia guillermondii* after 48 h show completely control of decay (Stevens *et al*. 1997). Similar results were obtained in 'Navel' oranges (D'Hallewin *et al*. 2005).

DEVELOPMENT AND COMMERCIAL APPLICATION

From an industry point of view, a biocontrol agent should meet certain criteria, involving studies to test the antagonist in different fruits and cultivars, with postharvest practices, such application and storage conditions. These tests have to be conducted at a large scale simulating or/and under commercial conditions using fresh and formulated product. Pilot or semi-commercial test involves a large amount of fruits, should be carried out in packinghouses and in different locations with natural infections.

At the same time scale-up the production and the development of a formulated product of the biocontrol agent maintaining the antagonist efficacy should be assessed. We will focus in mass production since in this review we already see the formulation (**Fig. 1**).

The purpose of production is to produce the greatest

quantity of efficacious cells in short period of time. Mass production of biocontrol agents must be a cost-effective process, using as a growth medium by-products from food industries, will balance to supply an optimal ratio between nitrogen and carbon, and fermentation should be completed within 24 to 39 h (Hofstein and Chapple 1998).

Costa *et al*. (2001) have demonstrated that *Pantoea agglomerans* CPA-2 can be produced in different media, using various organic nitrogen sources such as yeast extract, dry beer yeast or soy powder and inexpensive carbohydrates such as sucrose and molasses, whilst maintaining the efficacy of the biocontrol agent, reducing decay by more than 66% and 77% for *P. digitatum* and *P. italicum*, respectively. Other factors must be studied in laboratory scale to provide relevant information for further scale-up production. Operating conditions such aeration, agitation, pH, temperature may also affect the quality and quantity of the microorganisms (Churchill 1982). Inoculum is another important factor to be studied; it must be healthy, active to minimize length of lag phase, free of contaminants and must retain its product-forming capabilities.

Manso *et al*. (2006) reported a maximum biomass production of *P. agglomerans* PBC-1 using as carbon source 5 g/L of sucrose and the fermenter broth evidenced a behavior as a non-Newtonian fluid regime. High biomass productivity of this biocontrol agent was also obtained using as carbon source by-products from carob industry (Manso *et al*. 2007b), and from citrus industry (Manso *et al*. unpublished data). In these studies sugar consumption, pH, dissolved oxygen, K_La, respiration rate were monitored using different aeration and agitation, as well different spargers and impellers geometry for optimal bioreactor design for high-density and active biocontrol agent cultures.

As indicated the formulation of the product is a most critical aspect of the entire development program. The formulation has to allow the microorganism to retain its biocontrol activity, providing a significant extension of shelf-life of at least 6 months, preferably 18, at ambient conditions, to be stored over two seasons, and allowing to be applied with the existing application equipment (Hofstein and Chapple 1998). In general in citrus packinghouses fungicides are applied on-line spray, by drench applications and more recently by dipping fruits in tanks, alone or as in mixture with coating waxes. The first two ones are the most suitable for the application of microbial agents (Droby *et al*. 2001). The yeast product, Aspire™, was successfully applied onto citrus fruit using an on-line drench system (Droby *et al*. 1998).

Registration of a biocontrol product is required before any commercial use as an usual safety food procedure. However, this step has been faced formal restrictions in Europe. In USA the registration processes are easier and are not as expensive or time consuming. In Europe, the placing of plant protection products on the market is regulated by Council Directive 91/414/EEC, which is a longer registration and more difficult process, are subject to extremely high registrations fees and costs for providing data (Alabouvette *et al*. 2006). This is the reason why in Europe there are several postharvest biocontrol agents but none of them has already been registered. It must be noticed that these microbial agents used as biocontrol agents are not risky for human health and on contrary, are a safe alternative postharvest agent which should be implemented a short–term in all world markets.

In addition the global market of biocontrol agents is different from pesticides. Biocontrol agents are produced by small companies compared to chemical where 90% of market is in about seven multinational companies (Pertot and Gessler 2007). For example exploitation rights of the biocontrol agent *Pantoea agglomerans* CPA-2 patented in Spain with extension to Europe (Viñas *et al*. 1999) were transferred to DOMCA, SA., a small Spanish company (Immaculada Viñas, pers. comm.).

Through in this review was reported several microorganisms that have been identified as a biocontrol agents against citrus postharvest diseases, however there are only three biological products available in the market for citrus. Aspire™ based in *Candida oleophila*, produced by Ecogen and limited to USA and Israel, Bio-save™ constituted by *Pseudomonas syringae*, produced initially by EcoScience and know by Jet Harvest Solutions and limited to USA, and Shemer™ based in *Metschnikowia fructicola*, produced by Agrogreen and limited to Israel. New products are in process of registration. Pantovital based in *Pantoea agglomerans* are expected to be commercialized in Spain during 2009 (Immaculada Viñas, pers. comm.). Citrigreen® constituted with *Bacilus subtilis* and *B. licheniformis* in South Africa (Obagwo and Korsten 2003), and a second generation products as been developed and patented such as Biocoat whose main components are *Candida saitoana* with chitosan or Biocure also with *C. saitoana* and lysozyme. Both products also contains other additives such sodium bicarbonate (Wisniewski *et al*. 2007).

CONCLUDING REMARKS

This review reports the extensive research and significant progress made worldwide in the last two decades in postharvest diseases of fruits. There are already some products in the market with ability to control several diseases in different crops, including citrus fruit.

The search for new antagonists should be permanent as in chemical industry the search for new molecules are constant, as well the objective to broaden the use of biocontrol agents to different diseases and commodities. The postharvest environment is a unique opportunity to use microorganisms, but pre-harvest application may become more common in order to control latent and quiescent infections and improve the wound colonization. Detailed ecophysiological studies to understand and improve the behavior of the antagonists under stress conditions, in fruit surface and with microbial community are necessary. Development of microbial strains more adapted to field conditions is a challenging for the researchers.

Studies for improving the knowledge of the mode of action need to be more investigated, and molecular approaches may prove useful in understanding and enhanced the biocontrol activity. It is equally important to develop formulation that improve efficacy and could be delivery with the minimal modification of current practices.

At the present besides registration, the use of postharvest biocontrol agents are constrained by the lack of consistent efficacy and the high level of control required at postharvest. However, the second generation of biocontrol agents will overcome the limitations of the first generation, and by employing integrated approaches using biological, chemical and physical methods, will probably provide control level similar to those of synthetic fungicides without posing any food safety to consumer.

In the future the commercial use of these products will depend on the market. Worldwide organic producing are growing, representing a huge market, since the use of synthetic fungicide is not allowed. Furthermore, nowadays some supermarkets already demands for fruits and vegetables free of residues of postharvest products, because the chemical residues are more likely to be present when fruits will be consumed.

REFERENCES

Abadias M, Benabarre A, Teixidó N, Usall J, Viñas I (2001) Effect of freeze drying and protectans on viability of the biocontrol yeast *Candida sake*. *International Journal of Food Microbiology* **65**, 173-182

Abadias M, Teixidó N, Usall J, Solsona C, Viñas I (2005) Survival of the postharvest biocontrol yeast *Candida sake* CPA-1 after dehydratation by spray-drying. *Biocontrol Science and Technology* **15**, 835-846

Abadias M, Usall J, Teixidó N, Viñas I (2003) Liquid formulation of the postharvest biocontrol agent *Candida sake* CPA-1 in isotonic solutions. *Phytopathology* **93**, 436-442

Adikaram NKB, Joyce DC, Terry LA (2002) Biocontrol activity and induced resistance as a possible mode of action for *Aureobasidium pullulans* against

grey mould of strawberry fruit. *Australasian Plant Pathology* **31**, 223-229

Alabouvette C, Olivain C, Steinberg C (2006) Biological control of plant diseases: the European situation. *European Journal of Plant Pathology* **114**, 329-341

Andrews JH (1985) Strategies for selecting antagonistic microorganisms for the phylloplane. In: Windels CE, Lindow SE (Eds) *Biological Control of the Phylloplane*, The American Phytopathological Society Press, St Paul, MN, pp 31-44

Anonymous (2008) Endophyte spices up biofumigation. *Biocontrol News and Information* **28**, 69N-74N

Arcas MC, Botía JM, Ortuño AM, Del Río JA (2000) UV irradiation alters the levels of flavonoids involved in the defence mechanism of *Citrus aurantium* fruits against *Penicillium digitatum*. *European Journal of Plant Pathology* **106**, 617-622

Arras G (1996) Mode of action of an isolate of *Candida famata* in biological control of *Penicillium digitatum* in orange fruits. *Postharvest Biology and Technology* **8**, 191-198

Arras G, De Cicco V, Arru S, Lima G (1998) Biocontrol by yeasts of blue mould of citrus fruits and the mode of action of an isolate of *Pichia guilliermondii*. *Journal of Horticultural Science and Biotechnology* **73**, 413-418

Arras G, Scherm B, Migheli Q (2002) Improving biocontrol activity of *Pichia guillermondii* against post-harvest decay oranges in commercial packinghouses by reduced concentrations of fungicides. *Biocontrol Science and Technology* **12**, 547-553

Arras G, Usai M (2001) Fungitoxic activity of 12 essential oils against four postharvest citrus pathogens: Chemical analysis of *Thymus capitatus* oil and its effect in subatmospheric pressure conditions. *Journal of Food Protection* **64**, 1025-1029

Barkai-Goland R (2001) *Postharvest Diseases of Fruits and Vegetables. Development and Control*, Elsevier, Amsterdam, 418 pp

Bar-Shimon M, Yehuda H, Cohen L, Weiss B, Kobeshnikov A, Daus A, Goldway M, Wisniewski M, Droby S (2004) Characterization of extracellular lytic enzymes produced by the yeast biocontrol agent *Candida oleophila*. *Current Genetics* **45**, 140-148

Ben-Yehoshua S, Barak E, Shapiro B (1987) Postharvest curing at high temperatures reduces decay of individually sealed lemons, pomelos, and other citrus fruit. *Journal of American Society for Horticultural Science* **112**, 658-663

Ben-Yehoshua S, Peretz J, Rodov V, Nafussi B, Yekutieli O, Wiseblum A, Regev R (2000) Postharvest application of the hot water treatment in citrus fruits: the road from the laboratory to the packing-house. *Acta Horticulturae* **518**, 19-28

Bencheqroun SK, Bajji M, Massart S, Labhilili M, Jaafari S, Jijakli H (2007) *In vitro* and *in situ* study of postharvest apple blue mold biocontrol by *Aureobasidium pullulans*: Evidence for the involvement of competition for nutrients. *Postharvest Biology and Technology* **46**, 128-135

Bleve G, Grieco F, Cozzi F, Logrieco A, Visconti A (2006) Isolation of epiphytic yeasts with potential for biocontrol of *Aspergillus carbonarius* and *A. niger* on grape. *International Journal of Food Microbiology* **108**, 204-209

Brown GE, Miller WR (1999) Maintaining fruit health after harvest. In: Timmer LW, Duncan LW (Eds) *Citrus Health Manager*, The American Phytopathological Society Press, St. Paul, MN, pp 175-192

Bull CT, Wadsworth ML, Sorensen KN, Takemoto JY, Austin RK, Smilanick JL (1998) Syringomycin E produced by biological control agents controls green mold on lemons. *Biological Control* **12**, 89-95

Cañamás TP, Vinas I, Usall J, Casals C, Solsona C, Teixidó N (2008a) Control of postharvest disease on citrus fruit by preharvest application of the biocontrol agent *Pantoea agglomerans* CPA-2. Part I. Study of different formulation strategies to improve survival of cells in unfavourable environmental conditions. *Postharvest Biology and Technology* **49**, 86-95

Cañamás TP, Viñas I, Usall J, R T, Anguera M, Teixidó N (2008b) Control of postharvest disease on citrus fruit by preharvest application of biocontrol agent *Pantoea agglomerans* CPA-2. Part II. Effectiveness of different cell formulations. *Postharvest Biology and Technology* **49**, 96-106

Chalutz E, Droby S (1997) Biological control of postharvest diseases. In: Boland GJ, Kuykendall LD (Eds) *Plant-Microbe Interactions and Biological Control*, Marcel Dekker, Inc, New York, pp 157-170

Chalutz E, Wilson CL (1990) Postharvest biocontrol of green and blue mold and sour rot of citrus fruit by *Debaryomyces hansenii*. *Plant Disease* **74**, 134-137

Chen H, Xiang X, Wang J, Wu L, Zheng Z, Yu Z (2008) Antagonistic effects of volatiles generated by *Bacillus subtilis* on spore germination and hyphal growth of the plant pathogen, *Botrytis cinérea*. *Biotechnology Letters* **30**, 919-923

Chien PJ, Chou CC (2006) Antifungal activity of chitosan and its application to control post-harvest quality and fungal rooting of Tankan citrus fruit (*Citrus tankan* Hayata). *Journal of the Science and Food Agriculture* **86**, 1964-1969

Churchill BW (1982) Mass production of microorganisms for biological control. In: Charudattan R, Walker HL (Eds) *Biological Control of Weeds with Plant Pathogens*, John Wiley & Sons, New York, pp 139-156

Costa E, Teixidó N, Usall J, Atarés E, Viñas I (2001) Production of the biocontrol agent *Pantoea agglomerans* strain CPA-2 using commercial products by-products. *Applied Microbiology and Biotechnology* **56**, 367-371

Costa E, Teixidó N, Usall J, Fons E, Gimeno V, Delgado J, Viñas I (2002) Survival of *Pantoea agglomerans* strain CPA-2 in a spray-drying process. *Journal of Food Protection* **65**, 185-191

Costa E, Usall J, Teixidó N, Garcia N, Viñas I (2000) Effect of protective agents, rehydratation media and initial cell concentration on viability of *Pantoea agglomerans* strain CPA-2 subjected to freeze-drying. *Journal of Applied Microbiology* **89**, 793-800

D'Hallewin G, Arras G, Dettori A, Schirra M (1999) Citrus green mould control in stored 'Star Ruby' grapefruit by the use of a bio-control yeast under curing conditions. *Acta Horticulturae* **485**, 111-115

D'Hallewin G, Arras G, Venditti T, Rodov V, Ben-Yehoshua S (2005) Combination of ultraviolet-C irradiation and biocontrol treatments to control decay caused by *Penicillium digitatum* in 'Washington navel' orange fruit. *Acta Horticulturae* **682**, 2007-2012

Droby S (2006) Biological control of postharvest diseases of fruits and vegetables: difficulties and challenges. *Phytopathology Polish* **39**, 105-117

Droby S, Chalutz E (1994) Mode of action of biocontrol agents of postharvest diseases. In: Wilson CL, Wisniewski ME (Eds) *Biological Control of Postharvest Diseases. Theory and Practice*, CRC Press, Boca Raton, pp 63-75

Droby S, Chalutz E, Wilson CL, Wisniewski ME (1989) Characterization of the biocontrol activity of *Debaryomyces hansenii* in the control of *Penicillium digitatum* on grapefruit. *Canadian Journal of Microbiology* **35**, 794-800

Droby S, Chalutz E, Wilson CL, Wisniewski ME (1992) Biological control of postharvest diseases: a promising alternative to the use of synthetic fungicides. *Phytoparasitica* **20**, 149-153

Droby S, Cohen L, Daus A, Weiss B, Horev B, Chalutz E, Katz H, Kere-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

Droby S, Hofstein R, Wilson CL, Wisniewski ME, Fridlender B, Cohen L, Weiss B, Daus A, Timar D, Chalutz E (1993) Pilot test of *Pichia guillermondi*: A biocontrol agent of postharvest diseases of citrus fruit. *Biological Control* **3**, 47-52

Droby S, Vinokur V, Weiss B, Cohen L, Daus A, Goldschmidt EE, Porat R (2002) Induction of resistance to *Penicillium digitatum* in grapefruit by the yeast biocontrol agent *Candida oleophila*. *Phytopathology* **92**, 393-399

Droby S, Wisniewski ME, Cohen L, Weiss B, Eilam Y, Chalutz E (1997) Influence of $CaCl_2$ on *Penicillium digitatum*, grapefruit pell tissue, and biocontrol activity of *Pichia guillermondii*. *Phytopathology* **87**, 310-315

Droby S, Wilson CL, Wisniewski ME, El-Ghaouth A (2001) Biologically based technology for the control of postharvest diseases of fruits and vegetables. In: Wilson CL, Droby S (Eds) *Microbial Food Contamination*, CRC Press, Boca Raton, pp 187-205

Eckert JW (1990) Recent development in the chemical control of postharvest diseases. *Acta Horticulturae* **269**, 477-494

Eckert JW, Brown GE (1986) Evaluation of postharvest treatments for citrus fruit. In: Hickey KD (Ed) *Methods for Evaluating Pesticides for Control of Plant Pathogens*, The American Phytopathological Society Press, St. Paul, MN, pp 92-97

Eckert JW, Eaks IL (1989) Postharvest disorders and disease of citrus fruit. In: Reuther W, Calavan EC, Carman GE (Eds) *The Citrus Industry* (Vol 5), University of California, Oakland, pp 179-260

Eckert JW, Ogawa JM (1985) The chemical control of postharvest diseases: subtropical and tropical fruits. *Annual Review of Phytopathology* **23**, 421-454

El-Ghaouth A, Smilanick JL, Brown GE, Ippolito A, Wisniewski ME, Wilson CL (2000a) Application of *Candida saitoana* and glycolchotosan for the control of postharvest diseases of apple and citrus fruit under semi-commercial conditions. *Plant Disease* **84**, 243-248

El-Ghaouth A, Smilanick JL, Wisniewski ME, Wilson CL (2000b) Improved control of apple and citrus fruit decay with a combination of *Candida saitoana* and 2-deoxy-D-glucose. *Plant Disease* **84**, 249-253

El-Ghaouth A, Wilson CL, Wisniewski M, Droby S, Smilanick JL, Korsten L (2002) Biological control of postharvest diseases of citrus fruit. In: Gnanamanickam S (Ed) *Biological Control of Crop Diseases*, Marcel Dekker, Inc, New York, pp 289-312

Ezra D, Jasper J, Rogers T, Knighton B, Grimsrud E, Strobel G (2004) Proton transfer reaction-mass spectrometry as a technique to measure volatile emissions of *Muscodor albus*. *Plant Science* **166**, 1471-1477

Ezra D, Strobel G (2003) Effect of substrate on the bioactivity of volatile antimicrobials produced by *Muscodor albus*. *Plant Science* **165**, 1229-1238

Fan Q, Tian SP, Li Y-Y, Xu Y, Wang Y (2000) Biological control of postharvest brown rot in peach and nectarine fruits by *Bacillus subtilis* (B-912). *Acta Botanica Sinica* **42**, 1137-1143

Gabler F, Fassel R, Mercier J, Smilanick J (2006) Influence of temperature, inoculation interval, and dosage on biofumigation with *Muscodor albus* to control postharvest gray mold on grapes. *Plant Disease* **90**, 1019-1025

Govender V, Korsten L (2006) Evaluation of different formulations of *Bacillus licheniformis* in mango pack house trials. *Biological Control* **37**, 237-242

Guetsky R, Shtienberg D, Elad Y, Dinoor A (2001) Combining biocontrol agents to reduce the variability of biological control. *Phytopathology* **91**, 621-627

Guetsky R, Shtienberg D, Elad Y, Fischer M, Dinoor A (2002) Improving

biological control by combining biocontrol agents each with several mechanisms of disease suppression. *Phytopathology* **92**, 976-985

Gutter Y, Littauer P (1953) Antagonistis action of *Bacillus subtilis* against citrus fruit pathogens. *Bulletin of the Research Council of Israel* **3**, 192-196

Harvey JM (1978) Reduction of losses in fresh market fruits and vegetables. *Annual Review of Phytopathology* **16**, 321-341

Holmes GJ, Eckert JW (1999) Sensitivity of *Penicillium digitatum* and *P. italicum* to postharvest citrus fungicides in California. *Phytopathology* **89**, 716-721

Hofstein R, Chapple A (1998) Commercial Development of Biofungicides. In: Frhajj Menn (Ed) *Biopesticides: Use and Delivery*, Humana Press Inc, Totowa, NJ, pp 77-102

Huang Y, Deverall BJ, Morris SC (1995) Postharvest control of green mould on oranges by a strain of *Pseudomonas glathei* and enhancement of its biocontrol by heat treatment. *Postharvest Biology and Technology* **5**, 129-137

Huang Y, Deverall BJ, Morris SC (2000) Effect of *Pseudomonas cepacia* on postharvest biocontrol of infection by *Penicillium digitatum* and on wound responses of citrus fruit. *Australasian Plant Pathology* **22**, 84-93

Huang Y, Wild BI, Morris SC (2004) Postharvest biological control of *Penicillium digitatum* decay on citrus fruit by *Bacillus pumilus*. *Annals of Applied Biology* **120**, 367-372

Ippolito A, Nigro F (2000) Impact of preharvest application of biological control agents on posthavest diseases of fresh fruits and vegetables. *Crop Protection* **19**, 715-723

Janisiewicz WJ (1988) Biocontrol of postharvest diseases of apples with antagonist mixtures. *Phytopathology* **78**, 194-198

Janisiewicz WJ (1994) Enhancement of biocontrol of blue mold with the nutrient analog 2-deoxy-D-glucose on apples and pears. *Applied and Environmental Microbiology* **60**, 2671-2676

Janisiewicz WJ (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 WJ (1997) Biocontrol of postharvest diseases of temperate fruits. In: Boland GJ, Kuykendall LD (Eds) *Plant-Microbe Interactions and Biological Control*, Marcel Dekker, Inc, NY, pp 171-198

Janisiewicz WJ, Bors B (1995) Development of a microbial community of bacterial and yeast antagonists to control wound-invading postharvest pathogens of fruits. *Applied and Environmental Microbiology* **61**, 3261-3267

Janisiewicz WJ, Jeffers S (1997) Efficacy of commercial formulation of two biofungicides for control of blue mold and gray mold of apples in cold storage. *Crop Protection* **16**, 629-633

Janisiewicz WJ, Korsten L (2002) Biological control of postharvest diseases of fruits. *Annual Review Phytopathology* **40**, 411-441

Janisiewicz WJ, Roitman J (1988) Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. *Phytopathology* **78**, 1697-1700

Janisiewicz WJ, Tworkoski TJ, Sharer C (2000) Characterization the mechanism of biological control of postharvest diseases on fruits with a simple method to study competition for nutrients. *Phytopathology* **90**, 1196-1200

Jijakli H, Lepoivre L (1998) Characterization of an exo-β-1,3-glucanase produced by *Pichia anomala* strain K, antagonist of *Botrytis cinerea* on apples. *Phytopathology* **88**, 335-343

Jijakli H, Lepoivre L, Grevsse C (1999) Yeast species for biocontrol of apple postharvest diseases: an encouraging case of study for practical use. In: Mukerji KG, Chamola BP, Upadhyay RK (Eds) *Biotechnological Approach in Biocontrol of Plant Pathogens*, Kluwer Academic/Plenum Publishers, NY, pp 31-49

Kinay P, Mansour MF, Mlikota F, Margosan DA, Smilanick JL (2007) Characterization of fungicide-resistant isolates of *Penicillium digitatum* collected in California. *Crop Protection* **26**, 647-656

Kinay P, Yildiz M (2006) Studies on effect of UV-C light on postharvest *Penicillium* decays of Satsuma mandarins. In: Erkan M, Ben-Yehoshua S (Eds) *The Use of UV as a Postharvest Treatment: Status and Prospects*, Conference Proceedings, Antalya, Turkey, pp 49-53

Kinay P, Yildiz F (2008) The shelf life and effectiveness of granular formulations of *Metschinikowia pulcherrima* and *Pichia guillermondii* yeast isolates that control postharvest decay of citrus fruit. *Biological Control* **45**, 433-440

Korsten L, Havenga W, Zeeman K, Regnier T (2007) Mode of action of *Bacilus subtilis* as a biocontrol agent of fruit diseases. *Bulletin OILB* **30 (6)**, 107-111

Lahlali R, Serrhini MN, Jijakli H (2004) Efficacy assessment of *Candida oleophila* (strain O) and *Pichia anomala* (strain K) against major postharvest diseases of citrus fruits in Morocco. *Communications in Agricultural and Applied Biological Sciences* **69**, 601-609

Leelasuphakul W, Hemmanee P, Chuenchitt S (2008) Growth inhibitory properties of *Bacillus subtilis* strains and their metabolites against the green mold pathogens (*Penicillium digitatum* Sacc.) of citrus fruit. *Postharvest Biology and Technology* **48**, 113-121

Lesar KH (2008) The potential role of GRAS (Generally Regarded as Safe) chemicals, alone and in combination with the post-harvest fungicides, in the control of the major postharvest citrus pathogens, *Penicillium digitatum* (citrus green mold) and *Geotrichum candidum* (sour rot). In: Bertolini P (Ed) *Novel Approaches for the Control of Postharvest Diseases and Disorders*, International Congress Proceedings, Bologna, Italy, pp 122-129

Long C-A, Wu Z, Deng B-X (2005) Biological control of *Penicillium italicum* of citrus and *Botrytis cinerea* of grape by strain 34-9 of *Kloeckera apiculata*. *European Food Research Technology* **221**, 197-201

Manso T, Antunes F, Nunes C, Neto D, Pardão J, Raposo S, Lima-Costa ME (2007b) Production of the biocontrol agent *Pantoea agglomerans* PBC-1 using by-product from carob industry as carbon source. In: *II International Conference on Environmental, II Industrial and Applied Microbiology (BioMicroWold2007)*, Seville, Spain, p 723

Manso T, Isaac M, Duarte A, Torres R, Usall J, Nunes C (2007a) Enhancement of *Pantoea agglomerans* CPA-2 by the combination with curing to control postharvest diseases on oranges. *Bulletin OILB* **30 (6)**, 461

Manso T, Sequeira S, Lima-Costa ME, Nunes C (2004) Isolamento e avaliação da eficácia de microrganismos epifíticos no controlo de *Penicillium digitatum* em citrinos. In: Nolasco G, Nunes C (Eds) *4º Congresso da Sociedade Portuguesa de Fitopatologia*, Faro, Portugal, pp 94-98 (in Portuguese)

Manso T, Raposo S, Nunes C, Lima-Costa ME (2006) Production and rheological characterization of the biocontrol agent *Pantoea agglomerans* strain 125-STC using different carbon sources. In: *12th Congress of the Mediterranean Phytopathological Union*, Congress Proceedings, Rhodes, Greece, pp 588-590

Massart S, De Clercq D, Salmon M, Dickburt C, Jijakli M (2005) Development of real-time PCR using Minor Groove Binding probe monitor the biological control agent *Candida oleophila* (strain O). *Journal of Microbiology Methods* **60**, 73-82

Massart S, Jijakli H (2007) Use of molecular techniques to elucidate the mechanisms of action of fungal biocontrol agents: A Review. *Journal of Microbiology Methods* **69**, 229-234

McLaughlin RJ, Wilson CL, Chalutz E, Kurtzman CP, Fett WF, Osman SF (1990) Characterization and reclassification of yeast used for biological control of postharvest diseases of fruits and vegetables. *Applied Environmental Microbiology* **56**, 3583-3586

McLaughlin RJ, Wilson CL, Droby S, Ben-Arie R, Chalutz E (1992) Biological control of postharvest diseases of grape, peach, and apple with the yeasts *Kloeckera apiculata* and *Candida guilliermondii*. *Plant Disease* **76**, 470-473

Mercier J, Jiménez JI (2004) Control of fungal decay of apples and peaches by the biofumigant fungus *Muscor albus*. *Postharvest Biology and Technology* **31**, 1-8

Mercier J, Smilanick JL (2005) Control green mold and sour rot of stored lemon by biofumigation with *Muscodor albus*. *Biological Control* **32**, 401-407

Mercier J, Walgenbach P, Jiménez J (2005) Biofumigation with *Muscodor albus* pads for controlling decay in commercial table grape cartons. *HortScience* **40**, 1114

Meziane H, Gavriel S, Ismailov Z, Chet I, Chernin L, Hofte M (2006) Control of green mould on orange fruit by *Serratia plymuthica* strains IC14 and IC1270 and putative modes of action. *Postharvest Biology and Technology* **39**, 125-133

Mounir R, Durieux A, Bodo E, Allard C, Simon J-P, Achbani E-H, el-Jaafari S, Douira A, Jijakli MH (2007) Production, formulation and antagonistic activity of the biocontrol like-yeast *Aureobasidium pullulans* against *Penicillium expansum*. *Biotechnology Letters* **29**, 553-559

Nafussi B, Ben-Yehoshua S, Rodov V, Peretz J, Ozer BK, D'Hallewin G (2001) Mode of action of hot-water dip in reducing decay of lemon fruit. *Journal of Agricultural and Food Chemistry* **49**, 107-113

Nigro F, Finetti Sialer MM, Gallitelli D (1999) Transformation of *Metschnikowia pulcherrima* 320, biocontrol agent of storage rot, with the green fluorescent protein gene. *Journal of Plant Pathology* **81**, 205-208

Nunes C, Bajji M, Stepien V, Manso T, Torres R, Usall J, Jijakli H (2008) Development and application of a SCAR marker to monitor and quantify populations of the postharvest biocontrol agent *Pantoea agglomerans* CPA-2. *Postharvest Biology and Technology* **47**, 422-428

Nunes C, Torres R, Manso T, Usall J, Duarte A, Plaza P, Palou L, Viñas I (2004) Combination of *Pantoea agglomerans*, hot water and sodium bicarbonate, to control the major postharvest diseases in citrus from different locations. *International Citrus Congress 10*, Congress Proceedings, Orlando, USA (Vol III), pp 1045-1048

Nunes C, Usall J, Manso T, Torres R, Olmo M, Garcia JM (2007b) Effect of high temperature treatments on growth of *Penicillium* spp. and their development on 'Valencia' oranges. *Food Science and Technology International* **13**, 63-68

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

Nunes C, Usall J, Teixidó N, Miró M, Viñas I (2001b) Nutritional enhancement of biocontrol activity of *Candida sake* (CPA-1) against *Penicillium expansum* on apples and pears. *European Journal of Plant Pathology* **107**, 543-551

Nunes C, Usall J, Teixidó N, Torres R, Viñas I (2002b) Control *of Penicillium expansum* and *Botrytis cinerea* on apples and pears with the combination of *Candida sake* (CPA-1) and *Pantoea agglomerans* (CPA-2). *Journal of Food Protection* **65**, 178-184

Nunes C, Usall J, Teixidó N, Viñas I (2001a) Biological control of postharvest

pear diseases using a bacterium, *Pantoea agglomerans* CPA-2. *International Journal of Food Microbiology* **70**, 53-61

Nunes C, Usall J, Manso T, Teixidó N, Vinas I (2005) Biocontrol of postharvest blue mould on pear and apple fruits with the combination of *Candida sake* (CPA-1) and *Pseudomonas syringae* (CPA-5). *Acta Horticulturae* **682**, 2101-2108

Nunes C, Usall J, Teixidó N, Abadias I, Asensio A, Viñas I (2007a) Biocontrol of postharvest decay using a new strain of *Pseudomonas syringae* CPA-5 in different cultivars of pome fruits. *Agricultural and Food Science* **16**, 56-65

Obagwu J, Korsten L (2003a) Integrated control of citrus green and blue molds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water. *Postharvest Biology and Technology* **28**, 187-194

Obagwu J, Korsten L (2003b) Control of citrus green and blue mold with garlic extracts. *European Journal of Plant Pathology* **109**, 221-225

Palou L, Usall J, Teixidó N, Viñas I (2002) Evaluación de microorganismos antagónicos para el control biológico de las podredumbres verde y azul en post-cosecha de cítricos. In: *Avaluació de sistemes alternatius als fungicidas sintéticos per al control de les podridures verda i blava en postcollita de citrics*. PhD thesis, Universitat de Lleida, Lleida, Spain, pp 183-196 (in Spanish)

Palou L, Smilanick JL, Crisosto CH, Mansour M (2001b) Effect of gaseous ozone exposure on the development of green and blue molds on cold stored citrus fruit. *Plant Disease* **85**, 632-638

Palou L, Smilanick JL, Crisosto CH, Mansour MF, Plaza P (2003) Ozone gas penetration and control of the sporulation of *Penicillium digitatum* and *Penicllium italicum* within commercial packages of oranges during cold storage. *Crop Protection* **22**, 1131-1134

Palou L, Smilanick JL, Usall J, Viñas I (2001a) Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. *Plant Disease* **85**, 371-376

Pérez AG, Luaces P, Olmo M, Sanz C, Garcia JM (2005) Effect of intermittent curing on mandarin quality. *Journal of Food Science* **70**, 64-68

Pertot I, Gessler C (2007) Is it possible to improve biocontrol efficacy in some plant/pathogen systems? *IOBC/WPRS Bulletin* **30**, 3-9

Plaza P, Usall J, Smilanick JL, Lamarca N, Viñas I (2004) Combining *Pantoea agglomerans* (CPA-2) and curing treatments to control established infections of *Penicillium digitatum* on lemons. *Journal of Food Protection* **67**, 781-786

Plaza P, Usall J, Torres R, Lamarca N, Asensio A, Viñas I (2003) Control of green and blue mould by curing on oranges during ambient and cold storage. *Postharvest Biology and Technology* **28**, 195-198

Porat R, Daus A, Weiss B, Cohen L, Fallik E, Droby S (2000) Reduction of postharvest decay in organic citrus fruit by a short hot water brushing treatment. *Postharvest Biology and Technology* **18**, 151-157

Rodov S, Ben-Yehoshua S, Fang DQ, D'Hallewin G, Castia T (1994) Accumulation of phytoalexins scoparone and scopoletin in citrus fruits subjected to various postharvest treatments. *Acta Horticulturae* **381**, 517-523

Salazar M, Manso T, Simões D, Monteiro D, Afonso M, Nunes C (2008) Studies of physicochemical methods to control *Penicillium expansum* and *P. digitatum.* In: Bertolini P (Ed) *Novel Approaches for the Control of Postharvest Diseases and Disorders*, International Congress Proceedings, Bologna, Italy, pp 409-413

Schena L, Ippolito A, Zahavi T, Cohen L, Droby S (2000) Molecular approaches to assist the screening and monitoring of postharvest biocontrol yeasts. *European Journal of Plant Pathology* **106**, 681-691

Schena L, Sialer M, Gallitelli D (2002) Molecular detection of strain L47 of *Aureobasidium pullulans*, a biocontrol agent of postharvest diseases. *Plant Disease* **86**, 54-60

Schnabel G, Mercier J (2006) Use of a *Muscodor albus* pad delivery system for the management of brown rot of peach in shipping cartons. *Postharvest Biology and Technology* **42**, 121-123

Schotsmans W, Braun G, DeLong J, Prange R (2008) Temperature and controlled atmosphere effects on efficacy of *Muscodor albus* as a biofumigant. *Biological Control* **44**, 101-110

Shing V, Deverall BJ (1984) *Bacillus subtilis* as a control agent against fungal pathogens of citrus fruit. *Transactions of the British Mycological Society* **83**, 487-490

Sholberg PL, Marchi A, Bechard J (1995) Biocontrol of postharvest diseases of apple using *Bacillus* spp. isolated from stored apples. *Canadian Microbiology* **41**, 247-252

Shweleft RL (1986) Postharvest treatment for extending the shelf-life of fruits and vegetables. *Food Technology* **40**, 70-80

Smilanick JL, Denis-Arrue R (1992) Control of green mold of lemons with *Pseudomonas* species. *Plant Disease* **76**, 481-485

Smilanick JL, Mackey BE, Reese R, Usall J, Margosan DA (1997) Influence of concentration of soda ash, temperature, and immersion period on the control of postharvest green mold of oranges. *Plant Disease* **81**, 379-382

Smilanick JL, Mansour MF, Gabler FM, Sorenson D (2008) Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides. *Postharvest Biology and Technology* **47**, 226-238

Smilanick JL, Margosan DA, Henson DJ (1995) Evaluation of heated solutions of sulfur dioxide, ethanol, and hydrogen peroxide to control postharvest green mold of lemons. *Plant Disease* **79**, 742-747

Smilanick JL, Margosan DA, Mlikota F, Usall J, Michael IF (1999) Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. *Plant Disease* **83**, 139-145

Smilanick JL, Sorenson D, Mansour M, Aieyabei J, Plaza P (2003) Impact of a brief postharvest hot water drench treatment on decay fruit appearance, and microbe populations of California lemons and oranges. *HorTechnology* **13**, 333-338

Smoot JJ, Houck LG, Johnson HB (1983) *Market Diseases of Citrus and Other Subtropical Fruits*, Agricultural Handbook 398, USDA, ARS, Washington, DC, 115 pp

Stevens C, Khan VA, Lu JY, Wilson CL, Pusey PL, Igwegbe ECK, Kabwe K, Mafolo Y, Liu J, Chalutz E, Droby S (1997) Integration of ultraviolet (UV-C) light with yeast treatment for control of postharvest storage rots of fruits and vegetables. *Biological Control* **10**, 98-103

Strobel G (2006) *Muscodor albus* and its biological promise. *Journal of Industrial and Microbiological Biotechnology* **33**, 514-522

Strobel G, Dirkse E, Sears J, Markworth C (2001) Volatile antimicrobials from *Muscodor albus*, a novel endophytic fungus. *Microbiology* **147**, 2943-2950

Teixidó N, Cañamás TP, Usall J, Torres R, Magan N, Viñas I (2005) Accumulation of the compatible solutes, glycine-betaine and ectoine, in osmotic stress adaptation and heat shock cross-protection in the biocontrol agent *Pantoea agglomerans* CPA-2. *Letters in Applied Microbiology* **41**, 248-252

Teixidó N, Cañamás TP, Abadias M, Usall J, Solsona C, Casals C, Viñas I (2006) Improving low water activity and desiccation tolerance of the biocontrol agent *Pantoea agglomerans* CPA-2 by osmotic treatments. *Journal of Applied Microbiology* **101**, 927-937

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

Torres R, Nunes C, García JM, Abadias M, Viñas I, Manso T, Olmo M, Usall J (2007) Application of *Pantoea agglomerans* CPA-2 in combination with heated sodium bicarbonate solutions to control the major postharvest diseases affecting citrus fruit at several mediterranean locations. *European Journal of Plant Pathology* **118**, 73-83

Tuset JJ (1987) *Podredumbres de los Frutos Citricos*, Conselleria d'Agricultura i Pesca, Generalitat Valenciana, Spain, 206 pp (in Spanish)

Usall J, Smilanick J, Palou L, Denis-Arrue N, Teixidó N, Torres R, Viñas I (2008) Preventive and curative activity of combined treatments of sodium carbonates and *Pantoea agglomerans* CPA-2 to control postharvest green mold of citrus fruit. *Postharvest Biology and Technology* **50**, 1-7

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 mold on apple fruit. *Postharvest Biology and Technology* **21**, 147-156

Viñas I, Usall J, Nunes C, Teixidó N (1999) Nueva cepa de la bacteria *Pantoea agglomerans* (Beijerinck, 1998) Gavini, Mergaert, Beji, Mielcareck, Izard, Kerstersy De Ley y su utilización como agente de control biológico de las enfermedades fúngicas de frutas. Solicitud P9900612. Oficina Española de Patentes y Marcas (in Spanish)

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 CL, El-Ghaouth A, Upchurch B, Stevens C, Khan V, Droby S, Chalutz E (1997) Using an on-line UV-C apparatus to treat harvested fruit for controlling postharvest decay. *HorTechnology* **7**, 278-282

Wisniewski ME, Biles C, Droby S, McLaughlin R, Wilson CL, Chalutz E (1991) Mode of action of the postharvest biocontrol yeast, *Pichia guilliermondii*. I. Characterization of the attachment to *Botrytis cinerea*. *Physiological and Molecular Plant Pathology* **39**, 245-258

Wisniewski ME, Wilson CL (1992) Biological control of postharvest diseases of fruits and vegetables: recent advances. *HortScience* **27**, 94-98

Wisniewski ME, Wilson CL, Droby S, Chalutz E, El-Ghaouth A, Stevens C (2007) Postharvest Biocontrol: new concepts and applications. In: Vincent C, Goettel MS, Lazarovits G (Eds) *Biological Control: a Global Perspective*, CAB International, Oxfordshire, pp 262-273

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

Zhang HY, Zheng XD, Xi YF (2005) Biological control of postharvest blue mold of oranges by *Cryptococcus laurentii* (Kufferath) Skinner. *BioControl* **50**, 331-342

Zheng XD, Zhang HY, Sun P (2005) Biological control of postharvest green mold decay of oranges by *Rhodotorula glutinis*. *European Food Research Technology* **220**, 353-357