Influence of additives on the efficiency of biological control organisms against storage diseases

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Abstract

Storage diseases of pome fruits are caused by different fungal species. Disease management to control storage diseases includes several treatments with different fungicides in the weeks prior to harvest. However, residues on fruits becomes more and more a public and governmental concern. In order to reduce the chemical residue on fruits to a minimum, more research is done on alternative disease management. In this respect, in 2013, a project on 'Nebulisation of biological control organisms in cold storage rooms to control storage diseases', which is funded by the Agency for Innovation by Science and Technology, has started at the pcfruit institute in collaboration with ILVO and the University of Leuven. Here the efficacy of several biological control organisms (BCOs), applied through specific atomization in the cold room, against storage diseases was examined. Besides that, also the influence of additives on the efficiency of BCOs in their control of storage diseases was investigated. Two groups of fungal pathogens causing storage diseases were monitored. The first group comprises the latent fruit rot pathogens like Neofabraea *spp., which infect the fruits already in the orchards through natural openings like lenticels. For this type of pathogens symptom expression is delayed till after a long storage period. The second group are the wound pathogens (*Botrytis cinerea*,* Monilinia *spp.,* Penicillium *spp.) that penetrate the fruits through accidental wounds, for example during picking. However, not only the efficacy of the BCOs is important but also the homogeneous distribution of the compounds in the cold storage room. The first results of this project will be presented.*

Keywords: BCO, Storage diseases, Additives, Cold Storage, Nebulisation

Introduction

Different methods for the control of storage diseases on apple and pear are available for the fruit growers. In general, specific fungicides are applied in the orchard during the last weeks pre-harvest, with the last treatment as close as possible to harvest, depending on the pre-harvest interval of the product. In addition or besides that, postharvest treatments can be executed. The three registered methods in Belgium are: dipping/showering of pears with Philabuster (a.i. imazalil and pyrimethanil), thermonebulisation of apples with Xedathane-A (a.i. pyrimethanil) or the recently registered dipping of pears/apples in Penbotec (a.i. pyrimethanil). Such postharvest treatments are considered as an alternative or supplement for the fungicide treatments during the last weeks before harvest. They are aimed to insure the desirable fungicide residue on the fruits during cold storage, in order to limit storage diseases. However, in practice longer pre-harvest intervals are applied to meet the extra residue requirements (as compared to legal ones; max 4 residues and lower MRL's depending on the product) imposed by retailers.

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Postharvest control of storage diseases with BCOs can offer an alternative and opens perspectives for a further integrated production. In the past several yeasts/fungi were selected for the control of storage diseases by pre harvest applications: Boniprotect (*Aureobasidium pullulans*), Shemer (*Metshnikowia fructicola*), Nexy (*Candida oleophila*) and Serenade (*Bacillus subtilis*) (Kurtzman et al., 2001; Marrone, 2002; Stockwell et al., 2007). These BCOs are active against the major storage pathogens *Botrytis cinerea*, *Penicillium expansum* and *Neofabraea spp.* which cause respectively grey mold, blue mold and Bull's eye rot on apple. Pcfruit started up a BCO-research project together with ILVO and KULeuven. The general objective of this research-project is to develop a suitable technique for post-harvest treatments with BCOs during cold storage by cold fogging or vaporization of the product. BCOs will be selected for vaporization in cold storage rooms based on their biological efficacy and their sensitivity to fungicides which are used in the period before harvest.

Material and Methods

Artificial inoculation tests with *Neofabraea spp*:

For these tests apple fruits (50 untreated fruits/object) of the cultivar Pinova were used. Before wounding the fruits they were disinfected with a 1/20 solution of sodium hypochlorite for 2 minutes, thereafter they were washed with clear water (2 minutes). Wounding of the fruits was done by using a pricker (small nail pushed inside a cork) that causes wounds who are 1 mm diameter and 3 mm deep. Each apple was wounded on 4 sides. The spore solution was made by scraping spores of petri dishes and dissolve them in a 0,05 % Tween 20 solution. The number of spores was counted by using a Bürker counting chamber. Wounded fruits were inoculated with a 1,5 x 10^5 spores/ml solution of *Neofabraea spp*. by using a moving table, above which a construction with nozzles is build. For this trail 3 Teejet 650050 nozzles (1 above and 2 at the sides) were used (spray angle 60 °C), the speed of the moving table was set on 0.19 m/s. The fruits pass underneath the nozzles, the fruits are turned and thereafter they pass a second time under the nozzles, so each side of the fruits is covered with the spore solution. After the artificial inoculations the fruits were incubated at 20 °C and high relative humidity. A curative treatment was performed 24 hours after the artificial inoculation. This treatment was performed with 1) BCOs, 2) BCOs combined with additives or 3) additives alone. As a control disinfected, uninoculated and untreated apples were included in the test. Several BCOs were tested (X2 until X10) and 4 different additives (1 % concentration) were used for these trials: calcium-D-gluconate, calcium nitrate, calcium chlorid and potassium bicarbonate (Teixido *et al*., 2001; Karabulut *et al*., 2003; Spadora *et al*., 2004; Torres *et al*., 2007; Janisiewicz *et al*., 2008; Lui *et al*., 2011). After the curative treatment the fruits were incubated at 20 °C and high relative humidity. Disease symptoms were evaluated 3 weeks after the artificial inoculation, by counting the number of infected lesions and measuring the diameter of the infected lesions. The efficacy of each treatment was determined by using the formula:

Efficacy = (100 – ((100 / % infected wounds control) * % infected wounds object))

Results

Biological efficacy of different BCOs (and additives) towards *Neofabraea spp.* fruit rot:

The BCO (+ additive) treated objects were compared with the untreated control (64,50 % infected wounds). The columns in Table 1 shows the results of the treatments for each BCO separately and in combinations with the additives, with efficacies between 50,00 % and 100,00 % (green), efficacies between 25,00 % and 49,99 % (yellow) and efficacies below 25,00 % (red). For example BCO X4 which had the best efficacy in combination with calcium chloride. But for this BCO all the combination with additives (calcium nitrate, calcium-D-gluconate or potassium bicarbonate) obtained good efficacies. BCO X4 applied alone had an efficacy between 25,00 % and 49,99 %. For BCO X5 the best results were obtained in combination with calcium-D-gluconate, calcium chloride and calcium nitrate. All of treatments had an efficacy between 50,00 % and 100,00 %. Only the combination with potassium bicarbonate had a much lower efficacy, even below 25,00 %. BCO X5 applied alone had an efficacy between 25,00 % and 49,99 %. For BCO X10 the bests results were obtained in combination with calcium chloride and calcium-D-gluconate. The other combinations with potassium bicarbonate or calcium nitrate gave a lower efficacy between 25,00 % and 49,99 %. BCO X10 applied alone had the same efficacy (25,00 % - 49,99 %) towards *Neofabraea spp*. For the BCO Boni Protect the best results were obtained in combination with calcium chloride and calcium-D-gluconate, with an efficacy in het highest category between 50,00 % and 100,00 %. The combination Boni Protect and potassium bicarbonate gave a lower efficacy, but still an efficacy between 25,00 % and 49,99 % was obtained. The combination Boni Protect and calcium nitrate gave an efficacy below 25,00 %. BCO Boni Protect applied alone had an efficacy between 25,00 % and 49,99 %. Also the additives applied alone gave sometimes reasonable results for calcium chloride and calcium-D-gluconate with an efficacy between 25,00 % and 49,99 %. Their efficacy was relatively higher than calcium nitrate and potassium bicarbonate, which had an efficacy below 25,00 %. From all these results some combinations of BCOs and additives (Table 1, marked with an *) performed significantly better as the solo treatments of the different compounds, this was the case for BCO X4 combined with calcium chloride, BCO X10 combined with calcium chloride and BCO X10 combined with calcium-D-gluconate. In these cases a synergistic effect of the BCO and the additive was observed.

Table 1: Biological efficacy of different BCOs (and additives) towards *Neofabraea spp.* fruit rot.

Efficacy	(green)	100,00 % - 50,00 %	(yellow)	49,99 % - 25,00 %	(red)	< 25,00 %

(yellow)	X2
(red)	X2 + calcium-D-gluconate
(yellow)	X2 + calcium chlorid
(red)	X2 + calciumnitrate
(yellow)	X2 + potassium bicarbonate

(red)	X3
(yellow)	X3 + calcium-D-gluconate
(yellow)	X3 + calcium chlorid
(green)	X3 + calcium nitrate
(red)	X3 + potassium bicarbonate

(yellow)	X4
(green)	X4 + calcium-D-gluconate
(green) *	X4 + calcium chlorid
(green)	X4 + calcium nitrate
(green)	X4 + potassium bicarbonate

(yellow)	X5
(green)	X5 + calcium-D-gluconate
(green)	X5 + calcium chlorid
(green)	X5 + calcium nitrate
(red)	X5 + potassium bicarbonate

(yellow)	X6
(red)	X6 + calcium-D-gluconate
(yellow)	X6 + calcium chlorid
(red)	X6 + calcium nitrate
(yellow)	X6 + potassium bicarbonate

(yellow)	Boni Protect
(green)	Boni Protect + calcium-D-gluconate
(green)	Boni Protect + calcium chlorid
(red)	Boni Protect + calcium nitrate
(yellow)	Boni Protect + potassium bicarbonate

(red)	X9
(red)	X9 + calcium-D-gluconate
(red)	X9 + calcium chlorid
(red)	X9 + calcium nitrate
(red)	X9 + potassium bicarbonate

(yellow)	X10
(green) *	X10 + calcium-D-gluconate
(green) *	X10 + calcium chlorid
(yellow)	X10 + calcium nitrate
(yellow)	X10 + potassium bicarbonate

(yellow)	Calcium-D-Gluconaat
(yellow)	Calciumchloride
(red)	Calciumnitraat
(red)	Kaliumbicarbonaat

Discussion

The combination of BCOs with additives give promising results concerning enhanced efficacies against fruit rot (*Neofabraea spp.*). The best results were achieved by using BCO X4 combined with the additive calcium chloride and BCO X5 combined with the additive calcium-D-gluconate. In general 2 combinations of a BCO with this additives leads to a higher efficacy towards *Neofabraea spp.* fruit rot. The same trend was seen in similar trials with artificial *Botrytis cinerea*-infections (not shown in this contribution).

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