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Biological Control of Fungal and Bacterial Plant Pathogens
IOBC wprs Bulletin Vol. 25 (10) 2002
pp.29 - 32

Pre- and post-harvest practical application of *Pichia anomala* strain K, β-1,3-glucans and calcium chloride on apples: Two years of monitoring and efficacy against post-harvest diseases

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Abstract: Biological treatment based on a powder of *Pichia anomala* strain K (10^7 cfu/ml), β-1,3-glucans (YGT 2 g/l) and $CaCl_2.2H_20$ (20 g/l) was pre- or post-harvest applied against *Botrytis cinerea* and *Penicillium expansum* on apples under practical conditions. During the first year, the two highest protective levels were obtained with the pre-harvest standard chemical program consisting in 4 successive fungicidal treatments (99.6 %) and with the pre-harvest high volume spraying of the triple composition applied 12 days before harvest (82.6%). During the second year, post-harvest Sumico treatment and post-harvest biological treatment, both by dipping the apples, offered the highest protective levels (84.9 and 69.0 % respectively). A density threshold of 10^4 cfu of strain K/cm^2 of apple surface seemed to be required just after harvest in order to obtain a high protective activity whatever the mode and the time of application. Variations of meteorological conditions between both years were in accordance with strain K population density and efficacy differences observed in case of pre-harvest biological treatments.

Key words: Biological control, *Pichia anomala*, *Botrytis cinerea*, *Penicillium expansum*, apples, practical application, formulation

Introduction

Pichia anomala strain K was previously selected for its high antagonistic activity (even after its mass production and drying) on Golden Delicious apples against *Botrytis cinerea* and *Penicillium expansum*, two wound pathogens provoking economically important losses on storage rooms (Jijakli et al., 1999). Nevertheless, the use of a single antagonist has been sometimes criticized for not providing a reliable protective activity when used under commercial conditions. A proper formulation should be able to stabilize this activity by increasing the survival and/or the antagonistic properties of the biocontrol agent. YGT (containing 71 % of glucans) applied in combination with *P. anomala* strain K offered a higher and longer efficacy than this antagonistic strain used alone (Dicburt et al., 2001). Furthermore, triple composition based on *P. anomala* strain K (10^5 cfu/ml), β-,3-glucans (YGT, 2 g/l) and $CaCl_2.2H_20$ (20 g/l) lead to a higher protective percentage (up to 100%) than the percentage obtained by the sole strain K (10^7 cfu/ml) against both pathogens on apples under controlled conditions.

In this context, our objectives consisted in (1) evaluating the efficacy of the composition including strain K in practical conditions during two years and (2) assessing the population densities of the biocontrol agent in relation to its mode of application and meteorological conditions.

Material and methods

Apple treatments

Apple treatments were carried out in collaboration with the Royal station of fruit research of Gorsem (Belgium). During two successive years (2000 and 2001), the experimental Golden Delicious orchard (planted in 1995) was located in Melveren (Belgium). Temperature, rain and R.H. were determined with a meteorological station located in the orchard. Temperature mean (°C) and total rain (l/m^2) were calculated for each day. Golden Delicious apples were treated with a powder of strain K (10^7 CFU/ml) produced by CWBI (Université de Liège, Belgium) and supplemented with β-1,3-glucans (YGT, 2 g/l) and $CaCl_2.2H_20$ (20 g/l). This triple composition was applied 12, 5 or 2 days before harvest during the first year (15, 7 or 3 days during the second year) by spraying at low (300 l/ha) or high (1000 l/ha) volume. The different batches were artificially wounded just after harvest (4 wounds/apple). The triple composition including strain K was applied on untreated apples (with no chemical nor biological treatments) by dipping or drenching one day after harvesting. The following day, all the batches were artificially inoculated with *B. cinerea* (10^6 spores/ml) and *P. expansum* (10^5 spores/ml). Three controls were carried out as follows: 1) a standard program based on pre-harvest chemical treatments by spraying Bavistin, Phytocap, Sumico and Euparen, respectively 4, 3, 2 and 1 week before harvest, 2) a post-harvest standard treatment with Sumico (1 g/l) by apple dipping, 3) an artificial inoculation of pathogens on untreated apples. Four repetitions of 12.5 kg of fruits were used per treatment. Apples were successively stored at 1°C (15 days), 15°C (1 month) and 20°C (15 days) during the first year of trial (or two days at 20°C, 15 days at 1°C and 65 days at 20°C during the second year) before assessing the infection severity. During the first year, an index of severity (IS) was calculated (IS = number of class 1 lesions + 2 x number of class 2 lesions + n x number of class n lesions /total number of fruits) while lesion diameters were directly measured during the second year of trial. IS and lesions diameters were subjected to analysis of variance (SYSTAT) after log (first year) or square root (second year) transformation. The Duncan test was applied to compare the means at $P = 0.001$. A percentage of protection was also calculated based on the comparison of infection severity of treated batches and untreated but inoculated batch.

P. anomala *strain K monitoring*

The different treatments were applied on apples as above described but fruits were not wounded nor inoculated with pathogens and kept at 1°C during one month. After increasing periods, 8 apples per treatment were shaken separately during 20 minutes in 250 ml of KPBT 'washing' buffer (KH_2PO_4 0,034M, K_2HPO_4 0,016M, 0,05% tween 80, pH 6,5). The washing suspensions of the 8 fruits were mixed, serially diluted and plated on a semi-selective medium consisting in PDA supplied with 12.5 mg/l hygromycin B, 0.25 mg/l TMTD (containing thirame as active ingredient) and 5 mg/l Sumico (including 1.25 mg/l carbendazim and 1.25 mg/l diethofencarb). Petri dishes were incubated at 25°C during 4 to 5 days and yeast colonies of white colour were counted.

Results and discussion

During the first year, the best protection (99.6 %) against *B. cinerea* and *P. expansum* was obtained with the pre-harvest chemical treatments consisting in four successive applications of different fungicides. The highest percentages of protection (ranging from 40.3 to 82.6) by using biological treatment were observed in case of pre-harvest treatments with strain K (including YGT and $CaCl_2.2H_20$) whatever the application time. In opposite, the protective level of post-harvest apples treated with this triple composition was not significantly different

to the level observed for untreated apples. The best protective level (82.6 %) against *B. cinerea* and *P. expansum* was offered by the high volume spraying of strain K (12 days before harvest). This protective level was slightly higher (but not significantly) than the one recorded in case of post-harvest standard chemical treatment (74.7 %). More than 10^4 cfu of white yeasts/cm^2 of apple surface were observed just before pathogen inoculation in case of strain K pre-harvest high volume spray, 12 (1.7 10^4 cfu/cm^2) or 2 days (3.1 10^4 cfu/cm^2) before harvest (Figure 1A). This density level was never reached just before pathogen inoculation when apples were treated by dipping the post-harvest apples.

Suspecting a lack of stability of strain K suspension for post-harvest treatments, this suspension was mixed after each treatment during the second year of trial. A percentage of protection of 69.0 or 67.9 % against both pathogens was obtained with the post-harvest application of strain K respectively by dipping or drenching during this second year. These protective levels were lower (but not significantly) than the level reached with the post-harvest standard chemical treatment (84.9). In opposite, the highest percentages of protection obtained for pre-harvest chemical or biological (high volume spray 3 days before harvest) treatments were 59.4 and 38.9 % respectively. A density level of 1.6 10^4 cfu of white yeasts/cm^2 of apple surface was recorded just before pathogen inoculation with post-harvest dipping of apples (Figure 1B) while lower density levels were observed at the same period for pre-harvest treatments (high volume spray 12 or 2 days before harvest).

Table 1: Efficacy of biological and chemical treatments in relation with their mode and time of application

	Treatments	First year		Second year	
Pre-harvest treatments	Low volume spray (12[a] or 15[b] days)	71.8[e]	bcdef[f]	26.9[e]	efghi[f]
	High volume spray (12 or 15 days)	82.6	b	36.8	efgh
	Low volume spray (5 or 7 days)	58.4	cdefgh	10.5	fghi
	High volume spray (5 or 7 days)	40.3	cdefgh	-11.0	hi
	Low volume spray (2 or 3 days)	56.3	cdefgh	-11.5	hi
	High volume spray (2 or 3 days)	65.7	cdefgh	38.9	defgh
	Chemical standard treatment[c]	99.6	a	59.4	bcdef
Post-harvest treatments	Dipping	-4.0	fgh	69.0	bcd
	Drenching	-8.1	fgh	67.9	bcde
	Chemical standard treatment[d]	74.7	bcde	84.9	ab
Control	Untreated apples	0.0	h	0.0	ghi

a and b = moment of treatment application before harvest during the first ([a]) or the second year ([b]). c = spraying of Bavistin, Phytocap, Sumico and Euparen at the authorised Belgian dose, respectively 4, 3, 2 and 1 week before harvest, d = dipping in Sumico (1 g/l), e = Percentage of protection based on the infection severity of untreated but inoculated batch, f = Duncan test was carried out on infection severity means (not shown). Treatments with a common letter do not differ significantly ($P \leq 0.001$).

The contradictory results of efficacy between years 2000 and 2001 for biological treatments could be partially explained by strain K population dynamic differences since a density threshold of 10^4 cfu of strain K/cm^2 of apple surface seems to be required just after harvest in order to obtain a high antagonistic activity against *P. expansum* and *B. cinerea* whatever the mode and the time of application. Variations of meteorological conditions between both years of trials are in accordance with population density and efficacy differences observed in case of pre-harvest treatments based on strain K. During the first year

of trial, just one peak of rain was recorded in the orchard during 10 hours with a maximal intensity of 1.4 l/m².h (results not shown). In opposite, 4 peaks of more intense rain (up to 5 l/m².h) were detected during the second year and could cause washing off of biological but also chemical treatments. Pre-harvest temperatures were ranged between 18 and 25°C during year 2000 while lowest temperature (from 12 to 19°C) were observed during year 2001 and could be less favourable for strain K development. The overall results highlight the necessity to develop a novel formulation, which will take into account strain K adherence, suspension stability and protection against climatic detrimental factors.

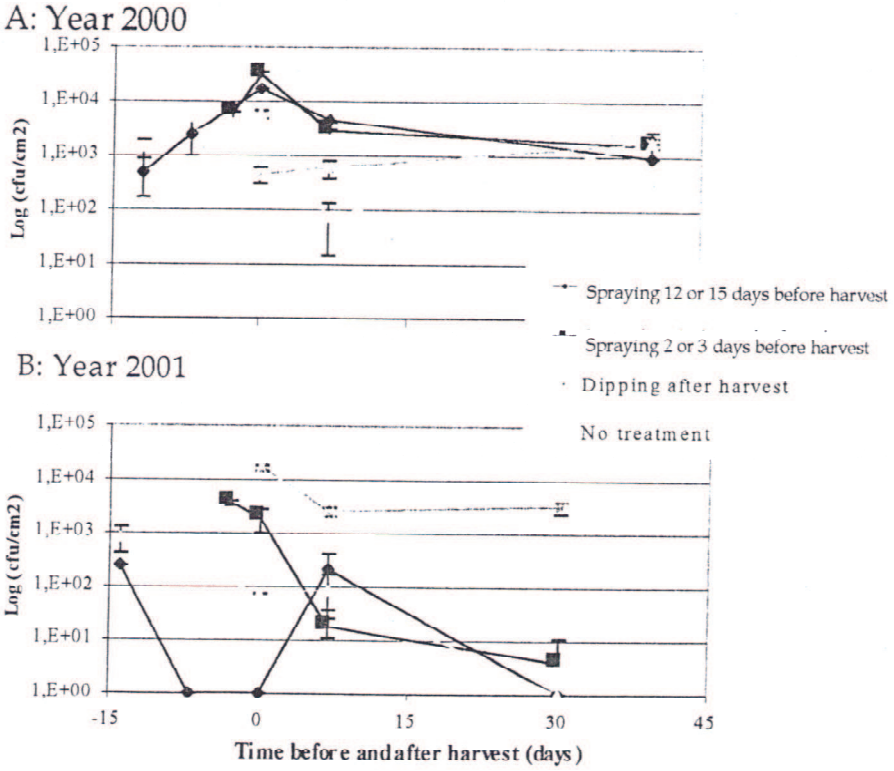

Figure 1: Monitoring of strain K applied in pre-harvest high volume spray 12 or 2 days before harvest during year 2000 (15 or 3 days during year 2001) or by post-harvest dipping. Vertical bars are standard errors of their respective means expressed as log (cfu/cm² of apple surface).

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