

Improving control of storage diseases on apple by combining biological and physical post-harvest methods

B. Vorstermans¹, S. Van Laer¹, P. Creemers¹, H. Jijakli², P. Pujos³

Abstract

Post-harvest non-chemical treatments consists of a large range of different approaches, including strengthening of the commodity's natural defence mechanisms, thermotherapy, application of antagonistic microorganisms and natural antimicrobial substances. NEX0101 is a promising antagonistic biocontrol agent containing the yeast *Candida oleophila* as the active ingredient. NEX0101 was developed by Bionext, a spin-off from the laboratory of Dr. H. Jijakli, and is currently evaluated for commercial use. The product contains a yeast strain isolated from apple fruit and was originally developed for the control of post-harvest diseases on apple. The antifungal effectiveness of this antagonist can be increased by addition of calcium salts. As the mode of action of this yeast is based on the colonisation of wounds, the main targets of NEX0101 are blue mould, caused by *Penicillium expansum*, and grey mould, caused by *Botrytis cinerea*. Facing possible latent infections, thermotherapy by using hot water treatments could provide an advanced control towards lenticelrot (*Gloeosporium* spp.). A combination of both physical and biological treatment techniques could broaden the spectrum to all key pathogens on apple and pear. The use of NEX0101 for the control of *P. expansum* was examined in combination with calcium gluconate. According to the results the combination NEX0101 with calcium gluconate provides an advanced mould control towards *P. expansum*. The best results were achieved using NEX0101 in combination with post-harvest dipping by thermotherapy. The hot water treatment alone was clearly inefficient towards wound parasite *P. expansum*, on the contrary thermotherapy stimulates the decay caused by this postharvest pathogen. For the future a combination of biological and physical treatments could offer a worthy non-chemical alternative for organic and integrated fruit growers towards fruit rot decay, although more research is necessary to implement these methods in practice.

Keywords: *Botrytis*, *Penicillium*, storage

Introduction

The purpose of this trial was to compare biological and physical post-harvest methods of storage disease control on apple, and potentially to improve it by combining them. Concerning the biological control strategy, NEX0101 is included in the trial setup. This promising antagonistic biocontrol agent contains the yeast *Candida oleophila* strain O as the active ingredient and is currently evaluated for commercial use. The antifungal effectiveness of this antagonist can be increased by addition of a specific additive. As the mode of action of this yeast is based on the colonisation of wounds, the main targets of NEX0101 are blue mould, caused by *Penicillium expansum*, and grey mould, caused by *Botrytis cinerea*.

¹ Proefcentrum Fruitteelt* Mycology, De Brede Akker 13, B-3800 Sint-Truiden, Belgium, E-mail: ben.vorstermans@pcffruit.be, stijn.vanlaer@pcffruit.be, piet.creemers@pcffruit.be;

² Unité de Phytopathologie, Faculté Universitaire des Sciences Agronomiques, Passage des déportés, 2, 5030 Gembloux, Belgium, E-mail : jijakli.h@fsagx.ac.be

³ Bionext, Passage des déportés, 2, 5030 Gembloux, Belgium pps@bionext.be

As for the physical control strategy, thermotherapy using hot water treatment was included in this trial. Facing potent latent infections, thermotherapy could provide an advanced control towards lenticelrot (*Gloeosporium* spp.). A combination of both physical and biological treatment techniques could broaden the spectrum to all key pathogens on apple and pear.

Material and Methods

The trial was executed on apple variety Golden delicious. The fruits originated from one orchard and were stored till the start of the trial, under standard storage conditions (1°C) at the Research Station of Gorsem. The fruits were sorted on fruit size and injured apples were removed. Apples with a fruit size of 75-85 mm were intended to be used in the trial.

Artificial wounding

The fruits were infected with the storage disease *Penicillium expansum*. This fungus is a wound parasite, for infection the fruits need to be wounded in advance. In concrete the apples were punctured at 4 places, divided equally over the fruit, using a sterilized nail, mounted in a cork plug, creating a 1-mm wide by 2-mm deep cylindrical wound. As the fruits were dipped before the artificial infection, the trial was set up to evaluate the preventive efficiency of the test objects.

Post-harvest dipping/drenching treatment

On day one, the wounded fruits were dipped in the treatment solution containing the test product NEX0101. To prepare the test solution the biomass granules of NEX0101 have been reactivated in a small volume of water (13.33g in 0.5l). The suspension was smoothly agitated for one hour to homogenize. As the antifungal effectiveness of this biocontrol agent can be increased by addition of a specific additive, the trial was set up using the biomass in combination with the additive, at a dose rate of 2g/l with the target concentration of the biomass (0.033%). In this trial each test object contains 4 replicates of 50 fruits. Next to the untreated dry check, one water dipped check object was included and dipped for 2 minutes, just as was done for NEX0101. Also one object was included facing a submersion time of 30 seconds. Dipping was done in plastic baskets with a content of 60 l and filled up with 40 l of the test solution. Perforated baskets containing 50 fruits were dipped in the test solution. Four dips were needed for the four replicates of 50 fruits per test object. In the trial setup also one object was included using post-harvest drenching. In concrete a spray equipment was used. The construction consists of a spray tower and a moving table. In the spray tower, 3 different nozzles are available, one above the fruits (spray tip: Twin Jet TJ-60 8004 VS) and two sideways (spray tip: Teejet UB8502 SS) guaranteeing a high flow rate (drenching). The fruits were placed on the moving table (0.045 m/s) and treated with the test solution (spray pressure 3 bar). Next to the biological control of *Penicillium expansum* also a physical control strategy was evaluated towards blue mould. In the trial setup, two objects were included facing thermotherapy (a hot water treatment for 2 minutes at 50°C) respectively alone or in combination with a biological dip treatment with NEX0101. An incubation time of about one hour was respected between both post-harvest dip treatments. After all objects were treated, the fruits were placed in small wooden boxes (50 fruits/box = 1 replicate), marked and wrapped in plastic foil leaving holes to insure a high level of humidity. The boxes were placed at random on pallets in a 100% humidified room (with a mist defensor) and incubated for 24 hours at about 20°C.

Artificial infection

On day two, four replicates of 50 fruits per object were inoculated with a conidial suspension of *Penicillium expansum*. The conidial suspension was made from a culture plated on PDA-agar (Potato dextrose agar 39g/l, Merck, Germany). The spores of 10 days old *Penicillium* cultures, sporulated under normal light, were used to inoculate the fruits. The sporulating plates were rinsed using sterilized demineralised water, containing 0.05% of Tween 20 (Fluka Chemie, Switzerland). After counting in a Burkler cel, the conidial suspension was assessed, by controlling the germination of the conidia on a PDA-agar. In this trial the germination level was more than 90%. To infect the fruits, a spray equipment was used. The construction (spray tower and a moving table) was also used for the object using post-harvest drenching. Only some preset parameters have been adjusted: in the spray tower 3 flat spray tips (TeeJet 650050, spraying angle 65°) are installed and the speed of the mobile table amounts to 0.19 m/s. The fruits were placed on the moving table and infected with the conidial suspension (spray pressure: 2 bar). When they were passed the nozzles, the fruits were turned and infected in the same way on the other side. Afterwards the fruits were collected again in wooden boxes and wrapped in plastic foil leaving holes to insure a high level of humidity. The boxes were stored on pallets in a 100% humidified room (with a mist defensor) and incubated for 48 hours at room temperature (20°C). The wooden boxes were arranged at random on the pallets.

On day four, the pallets were stored in a cold storage room at 1°C for one week. Afterwards the pallets were placed at 20°C and regularly moistened till wet to accelerate the development of the disease.

Assessment on fruit rot decay

About 18 days later the degree of decay of the apple fruits was evaluated. A second assessment of *Penicillium* took place one week later. To assess the wounded fruits on decay, per fruit the diameter of the lesion area is measured for each of the 4 wounds (mm). The total diameter of the lesions is expressed per fruit and when the whole fruit was infected with the disease, a total maximum diameter of 200mm was noted. The efficacy (Abbott value) of the test substances is calculated on the total diameter of lesions on 50 fruits. The number of infected fruits and the percentage of infected fruit were also calculated.

Results

The infection with *Penicillium expansum* was successful, about 43.5% of all apple fruits were infected in the untreated dry check. In the untreated dry check, the total average diameter per object of all infected fruits amounted to 1393.8mm. NEX0101 provided an advanced mould control towards *P. expansum*. All NEX0101 treated objects gave a significant reduction in decay. All objects testing NEX0101 gave a statistically identical result, whatever the dipping time (2' or 30''), the application technique (dipping or drenching), or the addition of thermotherapy to NEX0101. The best results were achieved using NEX0101 in combination with post-harvest dipping by thermotherapy (89.4% efficacy). The hot water treatment alone was clearly inefficient towards wound parasite *P. expansum*, on the contrary thermotherapy stimulates the decay caused by this post-harvest pathogen. In this object 63.0% of all fruits were infected compared to 43.5% in the non-treated dry check object.

Discussion

The results obtained are consistent with other results published by the lab of H. Jijakli on the use of the *Candida oleophila* strain O. The effect of hot water dipping on *P. expansum* is in agreement with what is seen in practice. However this negative effect is not always visible. Probably the physiological condition of the fruit at harvest and the interval between harvest and dipping play a major role in determining the outcome of hot water dipping towards the infection of *P. expansum*. For the future NEX0101 appears to be a flexible biological solution able to be used with different dipping times, application techniques, and alone or after thermotherapy.

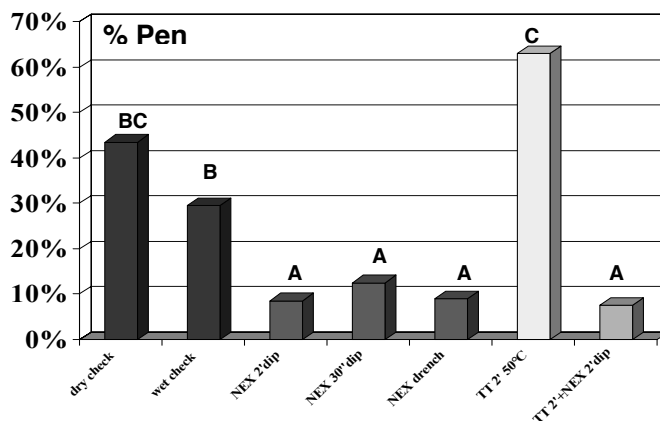


Figure 1. Percentage of *P. expansum* infected fruits per object (Statistics: Anova, Duncan $p < 0.05$)

Table 1. Total evaluation of *Penicillium* fruit rot infestation

	Fungicide	Dose (%)	<i>Penicillium expansum</i>		Efficacy ABB	
			% infection	Diameter total lesions		
			Mean	Mean		
1	Check dry		43.5	bc	1393.8	b
2	Check water treated		29.5	b	891.3	b
3	NEX0101 dipping 2' Biomass + Additive	0.033 + 0.200	8.5	a	201.3	a
4	NEX0101 dipping 30" Biomass + Additive	0.033 + 0.200	12.5	a	312.5	a
5	NEX0101 drenching Biomass + Additive	0.033 + 0.200	9.0	a	213.8	a
6	Thermotherapy 2' in hot water (50°C)		63.0	c	1923.8	c
7	Thermotherapy 2' + dipping NEX0101 (2') (Biomass+Additive)	0.033 + 0.200	7.5	a	147.5	a

(Statistics: Anova, Duncan $p < 0.05$)

Acknowledgement

Research centre subsidized by the Ministry of the Flemish Community

References

- Creemers P., Van Laer S., Dickburt C., Jijakli H. (Mars 2004). Biological control of storage rot on apple by postharvest treatments with NEX0101. International Workshop: Development of Biocontrol Agents of Diseases for Commercial Applications in Food Production Systems, Sevilla, Spain.
- Jijakli H., De Clercq D., Dickburt C., Lepoivre P. (2002). 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. IOBC/WPRS Bulletin: Biological Control of Foliar and Post-Harvest Diseases, Vol 25, 29-32.
- Massart S., Sens-Espel R., Lepoivre P. Jijakli H (2002). Application of RAP-PCR and cDNA-AFLP to isolate genes of *Candida oleophila* (strain O) induced by the presence of galacturonic acid. IOBC/WPRS Bulletin: Biological Control of Foliar and Post-Harvest Diseases, 25, 399-402.
- Unistat 5.5. Statistical Package for Windows. User's Guide, 930 pp. Version 5.5 November 2002. Unistat House, Shirland Mews, 4, London W9 3DY, England.
- Zar J.H. 1999. Biostatistical analysis. Fourth Edition, Prentice-Hall, Upper Saddle River, New Jersey. 663 pp. plus Appendices. ISBN: 0-13-081542-X.