Combined treatments with BoniProtect *(Aureobasidium pullulans)* and hot water to control storage diseases.

M. Trapman[1], K. De Coninck, K. Konijn

Abstract

Crop losses caused by storage diseases have great impact on the economics of organic fruit production in North Western Europe. Losses vary from less than 5 % in the first months, to occasionally more than 50% after eight months of cold storage. Infection by Gloeosporium spp*. is the predominant cause of fruit rot, but also wound infections by Botrytis cinerea, Nectria galligena and Monilia fructigena can be important. Combinations of pre- and post-harvest applications of BoniProtect aimed at preventing wound infections by storage rots, and hot-water treatments aimed to control* Gloeosporium *fruit rot, where evaluated under practical conditions.*

Pre+post-harvest application of BoniProtect provided a significant reduction by 32.7 % of the total fruit rot that was mainly caused by Gloeosporium*. This is remarkable while BoniProtect is thought not to be effective on* Gloeosporium *fruit rot.*

Hot-water treatment with 2 minutes 51ºC, 2 minutes 52ºC and 3 minutes 52ºC reduced storage rots by 65.0, 75.8, and 78.2 %. However on Pinova 3 minutes 52ºC caused heat damage to up to 10% of the fruits, and when the treatment was prolonged to 4.5 minutes fruit damage increased to 25% of the fruits. The prolonged heat treatment also caused a significant increase in the number of fruits damaged by other rots than Gloeosporium*.*

In all trials, and in all combinations, combinations of hot-water treatment and BoniProtect treatments where more effective than hot-water treatment alone, however these reductions where never statistically significant due to the marginal presence of other rots than Gloeosporium*.*

Keywords: Storage diseases, hot water treatment, *Gloeosporium*, *Aureobasidium pullulans,* apple, organic

Introduction

Crop losses caused by storage diseases have great economical impact on organic fruit production in North Western Europe. These fruits cost money, as they have been grown, picked, stored, graded, and disposed afterwards. Diseases as *Gloeosporium spp.* that continue to develop further during the marketing chain, undermine the confidence of resellers and consumers in the product and can cause a dramatic fall in sales. Crop losses due to storage diseases depend on apple variety, orchard situation, picking time, year, storage conditions and the duration of cold storage. In main apple varieties as Elstar and Topaz losses vary from less then 5 % in the first four months of storage, till occasionally more than 50% after eight months of storage. *Gloeosporium spp*, *Botrytis cinerea* and *Nectria galligena* are the main fungi causing storage rot in organic apple production in The Netherlands and Belgium.Routine treatments during summer to control apple scab with low rates of copper, sulphur or lime sulphur seem to have little or no practical effect on the occurrence of storage rots. The efficacy of post harvest hot-water treatments to prevent the development of *Gloeosporium* fruit rot as discovered by Burchill in 1964 (4) has been confirmed by many others, and possible modes of action have been described. (2,3,8,9,11,12,13,14,15,17) Hot-water treatment is used by a growing number of organic

[1]Marc Trapman, Bio Fruit Advies, Netherlands 4111KT Zoelmond, marc.trapman@biofruitadvies.nl

producers to prevent development of *Gloesporium* fruit rot in high-risk situations. These treatments have however little or no effect on the occurrence of *B. cinerea* and *N. galligena* fruit rot, or even increase losses by *B. cinerea* (P.Creemers, personal communication). Pre- or post-harvest treatments with *Aureobasidum pullans* offer protection against infection of wounds by *B. cinerea* and other rots (10). Positive effects of combinations of heat-treatment and biological control agents have been reported before (5,6,7). The commercial product BoniProtect® contains $5x10^{10}$ cfu of *Aureobasidium pullulans* per gram. A combination of post-harvest treatment with hot-water against *Gloeosporium* combined with a pre- or post-harvest treatment with BoniProtect® against wound infecting fungi seems an interesting broad spectrum strategy for the control of storage diseases in organic apple production. The Council Regulation (EC) No 834/2007 on organic production in Europe does not exclude postharvest treatments. In European countries where post harvest application of BoniProtect® is approved, this strategy could be used in organic production.

Material and Methods

All trial orchards where organic apple orchards in Belgium and The Netherlands where scab control during summer was based on treatments with copper, sulfur, lime sulfur and potassium bicarbonate. For the pre-harvest treatments with BoniProtect the orchards where split in two comparable blocks of 0.5 to 1 hectare each. One block only received the usual scab treatments, the other block received additional treatments with BoniProtect. All pre-harvest BoniProtect treatments where made at the label rate of 1 kg/ha per treatment using standard tractor pulled orchard spraying equipment. For Elstar only fruit from 2nd or 3rd picking round was used as in these fruits most storage rots can be expected to develop. The fruits where randomly picked and stored in EPS boxes containing 11 kg of fruit, on average between 60 and 80 fruits per box. Each box was treated separately in the trial and considered as a replication. The post-harvest hot-water treatments where made pallet wise with a commercial hot-water dipping machine. The post-harvest applications with BoniProtect where made after the hot-water treatment. For the post-harvest application of BoniBrotect a concentration of 33 gr. BoniProtect in 100 liter of water was used. The boxes with fruits where dipped individually in this solution for 2 minutes.

Fruits where stored in normal could storage under atmospheric conditions as CA or ULO storage would suppress the development of fruit rots and symptom expression. Fruits where stored until after their normal commercial storage period to allow for maximum symptom expression. Based on macroscopic symptoms fruit rots where noted as *Gloeosporium*, *Botrytis*, *Monillia*, *Penicillium* or “other fruit rot”.

1- Post-harvest treatments 2006

Fruits from trees of the variety Pinova in an orchard with a history of storage rots where randomly picked. Four treatments where compared in four replications:

1- Untreated
2- Post-harvest dipping in 1% H_2O_2 (2 minutes)
3- Hot water treatment (2 minutes at 51ºC)
4- Hot water treatment + dipping in BoniProtect (2 minutes)

The hot water treatment was made one day after harvest. The dipping in BoniProtect or H_2O_2 two days after harvest. Assessments where made immediately after the fruits left the cold storage on 27-2-2007. Fruits where scored as “healthy” or “rotten”. The types of fruit rot where not assessed.

2- Pre-harvest treatments in 2007

Split plot trials where run in three organic orchards on Elstar, and in the same Pinova orchard as in 2006. BoniProtect was applied three times on Elstar and four times on Pinova at label rate (1 kg./ha) during the last month before harvest. Ten EPS boxes per plot where stored under normal cold storage conditions. In two orchards additional combinations with post-harvest treatments where tested (see below).

On Elstar, observations on storage rots where made immediately after the fruits left the cold storage on 14-2-2008. On Pinova assessments where made directly after storage on 26-2-2008 and again after 14 days storage at 6-8 ºC on 13-3-2008.

3- Pre- and Post-harvest treatment combinations on Elstar in 2007

The following four treatments where compared in ten replications:

1- Untreated
2- 3x BoniProtect pre-harvest
3- Hot-water treatment (2 minutes 52ºC)
4- Hot-water treatment (2 minutes 52ºC) + dipping in BoniProtect

Ten EPS boxes per plot where stored under normal cold storage conditions. Observations on storage rot where made on 14-2-2008 immediately after the fruits left the cold storage.

4- Pre- and post-harvest treatment combinations on Pinova in 2007

The following treatments where compared in eight replications:

1- Untreated
2- 4x BoniProtect (BP) pre-harvest
3- BP post-harvest
4- 4x BP pre-harvest + BP post-harvest
5- 4x BP pre-harvest + Hot Water treatment (3 minutes 52ºC) post-harvest
6- 4x BP pre-harvest + Hot Water treatment (3 minutes 52ºC) post-harvest + BP post-harvest
7- Hot-water treatment post-harvest + BP post-harvest
8- Hot-water treatment (3 minutes 52ºC)
9- Hot-water treatment extra long (4.5 minutes 52ºC)

The pre-harvest treatments with BoniProtect where made 30, 19, 12 and 5 days before harvest. Hot-water treatment was made the first day after harvest. The post-harvest treatments with BoniProtect where made within 8 hours after the hot-water treatment. Eight EPS boxes per plot where stored under normal cold storage conditions. Observations on storage rots where made immediately after the fruits left the cold storage on 26-2-2008 and on 13-03-2008 after 14 days of storage at 6-8ºC.

For statistical analyses of the results ANOVA followed by Tukey multiple range test was performed on the Log+1 transformed percentages rotten fruits per box.

Results and Discussion

The results of the trial series 1 to 4 are summarized in Table 1 to 4 and in Figure 1.

Table 1: Results of post-harvest treatments on Pinova in 2006
(Diseased fruits after storage as percentage of total number of stored fruits)

	Post-harvest treatments	Total fruit rots	Effect (Abbot)
1	Untreated	52.9 a	
2	1 % H_2O_2	57,8 a	- 9,1 %
3	Hot-water 2 min 51 ºC	18,6 b	65,0 %
4	Hot-water 2 min 51 ºC + BoniProtect	9,5 b	82,1 %

(ANOVA and Tukey-HSD after log+1 transformation: values followed by a different character differ sign.at alpha =0.05)

Table 2: Results of pre-harvest treatments with BoniProtect on Elstar in two orchards in 2007
(Diseased fruits after storage as percentage of total number of stored fruits)

Pre-harvest treatments		*Gloeosporium*	*B. cinerea*	Other rots	Total rots	Effect (Abbot)
Orchard A	Untreated	27,9 a	0,9 a	0,2 a	29,0 a	
	3x BoniProtect	19,9 a	0,3 a	0,2 a	20,4 a	29.8%
Orchard V	Untreated	4,7 a	1,6 a	2,9 a	9,2 a	
	3x BoniProtect	3,8 a	4,0 a	1,5 a	9,2 a	0 %

(ANOVA and Tukey-HSD after log+1 transformation: values followed by a different character differ sign. at alpha =0.05)

Table 3: Results of combinations of pre- and post-harvest treatments on Elstar in 2007
(Diseased fruits after storage as percentage of total number of stored fruits)

Treatments		*Gloeosporium*	*B. cinerea*	Other rots	Total rots	Effect (Abbot)
1	Untreated	19,4 a	3,0 a	1,0 a	23,5 a	
2	3x BoniProtect pre-harvest	22,7 a	0,7 a	1,0 a	24,5 a	- 4,3 %
3	Hot-water 2 minutes 52 ºC	0,8 b	3,8 a	1,1 a	5,7 b	75,8 %
4	Hot-Water 2 minutes 52 ºC + BoniProtect post-harvest	0,8 b	0,9 a	0,3 a	2,0 b	91,6 %

(ANOVA and Tukey-HSD after log+1 transformation: values followed by a different character differ sign. at alpha =0.05)

Table 4: Results of combinations of pre- and post-harvest treatments on Pinova in 2007
(Diseased fruits after storage as percentage of total number of stored fruits)

	Treatments	*Gloeosporium*			Other rots	Total rots	Effect (Abbot)	Heat damage
		26-2*)	13-3**)	Total				
1	Untreated	57,4	22,7	80,1 a	0 c	80,1 a	-	0 c
2	BoniProtect (=BP) pre-harvest	22,9	36,8	59,7 a	0,7 bc	60,4 ab	24,6 %	0 c
3	BP post-harvest	33,6	29,5	63,1 a	0 c	63,1 ab	21,3 %	0 c
4	BP pre + BP post-harvest	20,9	32,7	53,7 a	0,2 bc	53,9 b	32,7 %	0 c
5	BP pre + Hot-water 3 min. 52ºC post-harvest	1,6	8,1	9,7 bc	2,3 ab	11,9 c	85,1 %	9,9 b
6	BP pre + Hot-water 3 min. 52ºC + BP post-harvest	4,5	10,8	15,3 b	0,4 bc	15,7 c	80,4 %	3,0 b
7	Hot-water 3 minutes 52ºC + BP post-harvest	5,3	9,4	14,8 b	0,8 bc	15,6 c	80,6 %	3,3 b
8	Hot-water 3 minutes 52ºC	8,4	7,8	16,2 b	1,3 bc	17,5 c	78,2 %	7,9 b
9	Hot-water 4.5 minutes 52ºC	2,1	2,8	4,9 c	5,9 a	10,8 c	86,5 %	25,9 a

**) Gloeosporium fruit rot visible immediately after storage*
***) Additional Gloeosporium fruit rot that became visible 14 days after the fruits left the could storage*
(ANOVA and Tukey-HSD after log+1 transformation: values followed by a different character differ sign. at alpha =0.05)

The trials were set up to find a strategy to control the spectrum of storage diseases occurring in organic apple production with a combination of methods acting on different types of storage diseases. The assessments in the untreated trial objects however show that *Gloeosporium* was the predominating disease in all trials, and in most cases less than 1% of the stored apples was infected by other diseases. The absence of “other fruit rots” in the observations of some plots with a high percentage of fruits infected by *Gloeosporium* could be due to the artefact that rotten fruits where noted only for their primary cause of decay. When infected by *Gloeosporium*, secondary fruit rot on the same fruit will not have been noted.

Pre-harvest orchard treatments with BoniProtect in four orchards in 2007 reduced the total loss by fruit rots during storage by 29.8%, 0%, -4.3% and 24,6%. (Table 2, 3 and 4). This is less than the 47% and 45% efficacy achieved in practical applications in Germany and Austria (10,1), and is comparable to the results published by Zimmer. (17). Post-harvest application of BoniProtect alone was not more effective than pre-harvest application. Only pre+ post-harvest application of BoniProtect provided a significant 32.7% reduction of the total fruit rot that was mainly caused by *Gloeosporium* (Table 4).
The reduction of *Gloeoesporium* by BoniProtect is remarkable. BoniProtect was developed and tested to reduce wound-invading pathogens (16), and most fruits are infected by G*loeosporium* trough lenticels and not though wounds. However also Römer found that three pre-harvest applications of BoniProtect where as effective on *Gloeosporium* as two treatments with Captan. (11)

Post-harvest dipping of apples in hot water is known to be very effective against *Gloeosporium* fruit rot. But although this effectiveness was already shown by Burchill in 1964, it was only recent that this method was developed to a practical level. In our trials hot water treatments with a commercial machine in *2* minutes 51ºC, 2 minutes 52ºC and 3 minutes 52ºC reduced storage rots by 65.0, 75.8 and 78.2 %(Table 1, 3, and 4) . However on Pinova 3 minutes 52ºC caused heat damage on up to 10% of the fruits, and with the heat treatment prolonged to 4.5 minutes the fruit damage increased to 25% of the fruits. The prolonged heat treatment also caused a significant increase in the number of fruits damaged by other rots than *Gloeosporium*.

In all trials and in all combinations, successive treatments with hot-water and Boni-Protect reduced the loss by fruit rots compared to hot-water treatment alone, however these reductions where never statistically significant. (Figure 1)

Post-harvest dipping of fruits in a 1% H_2O_2 solution did not reduce fruit decay by *Gloeosporium* or other fruit rots at all. In two other trials that are not presented here we had the same result. Our findings in this are consistent with these presented by Weibel in 2004 (15).

Conclusion

The theoretical advantage of the combination of hot water treatment with pre- or post-harvest application of BoniProtect to control a wider spectrum of storage diseases on apples could be demonstrated, but could not been scientifically proven as in the trials *Gloeosporium* was by fare the most abundant fruit rot. BoniProtect was primarily developed to control other wound infecting fungi on fruits.

It was therefore remarkable that in both pre- and post-harvest treatments BoniProtect also reduced losses by *Gloeosporium* fruit rot. All tested combinations of hot-water treatment and applications of BoniProtect where more effective than hot-water treatment alone, but due to the marginal presence of other rots than *Gloeosporium* this gain in effectiveness could not be proven statistically.

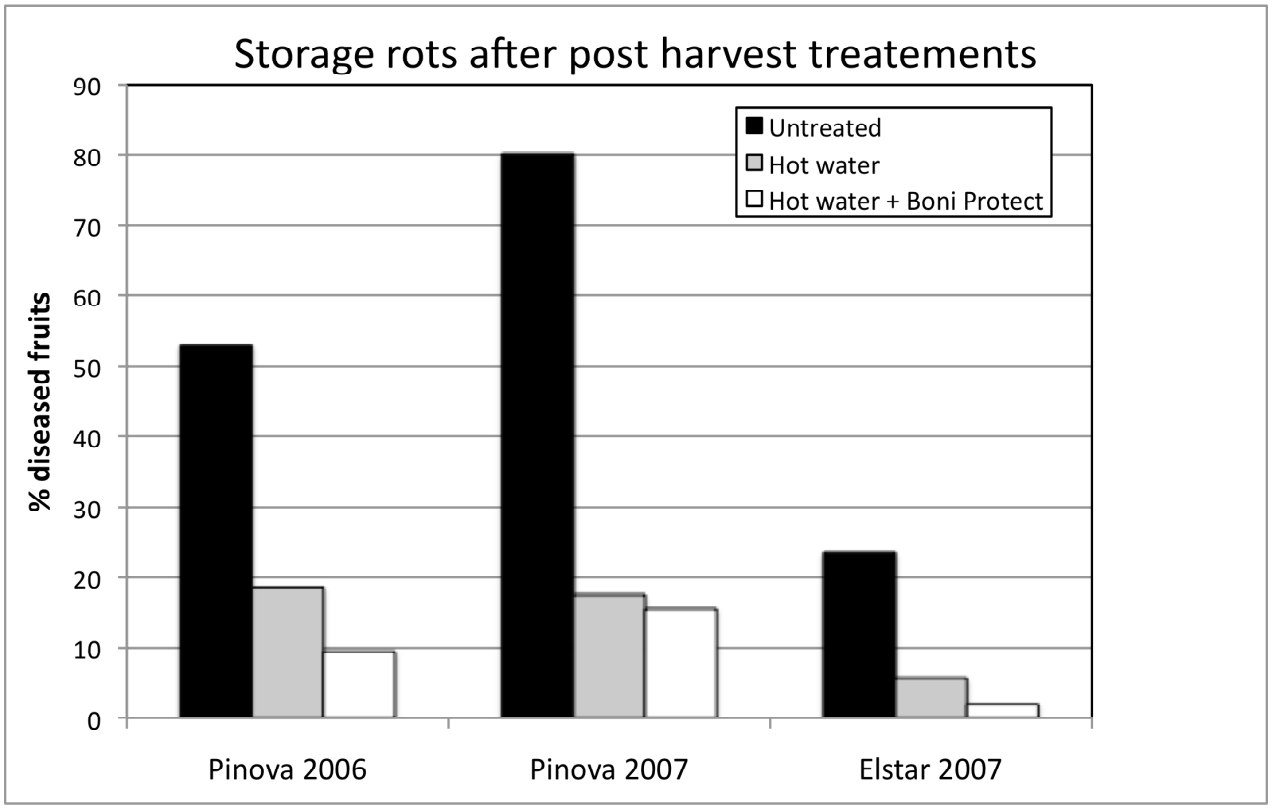

Figure 1: Losses by storage rots in three trials after hot-water treatment alone, and hot-water treatment followed by post harvest treatment with BoniProtect.

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