Application of ARs in an organic apple orchard for protection against storage diseases

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Introduction

Apples show relatively high susceptibility to fungal rots, with *Neofabraea* (*N. alba* and *N. perennans*), *Colletotrichum* (*C. acutatum* and *C. gloeosporioides*), *Penicillium expansum*, *Botrytis cinerea*, and *Monilia* (*M. laxa* and *M. fructigena*) being the most common in Northern Europe (Børve and Stensvand, 2007; Weber and Palm, 2010). Many of these fungi appear to have increased over the world during the latest decades, most likely due to the global temperature increase.

Various strategies against postharvest decay in organically grown fruit have been explored, like the use of certain bioactive compounds that can inhibit fungal growth on apples. Phenolic lipids constitute a highly diversified group of compounds derived from mono- and dihydroxyphenols, i.e., phenol, catechol, resorcinol, and hydroquinone. Two resorcinols characterized in mango, 5-(12-cis-heptadecenyl) resorcinol and 5-n-pentadecylresorcinol, have been shown to inhibit the growth of *Alternaria alternaria* (Droby et al., 1987) and *C. gloeosporioides* (Hassan et al., 2007) in immature mango fruit. In a previous Ekoforsk-funded study by our research group on a very similar topic, we isolated alkylresorcinols (ARs) from commercial grade rye bran, and then produced 19 different emulsions containing ARs (0.25–0.5 mg mL^{-1}) together with different combinations and concentrations of solvents, emulsifiers and stabilizers (Dey et al., 2013). We sprayed these emulsions onto apples that a few hours earlier had been inoculated with 20 µl conidia-spore suspension of *P. expansum*. Size of damaged area on the fruit skin was measured after several weeks of cold storage: on (1) control fruits, (2) fruit sprayed with water and (3) fruit sprayed with AR emulsions, respectively. Most of the AR emulsions produced a significant inhibition of disease symptoms. In a second step two selected AR emulsions were shown to inhibit mycelial growth of bull's eye rot (*N. perennans*) and blue mold (*P. expansum*) *in vitro*, and to significantly reduce postharvest decay in Swedish-grown fruit of four apple cultivars ('Aroma', 'Ingrid Marie', 'Frida' and 'Gloster') that had been inoculated immediately after harvest with either blue mold or bull's eye rot. All of these cultivars are commonly grown in the Nordic countries, and exhibit variable levels of storage disease susceptibility.

The purpose of this work is to investigate the antifungal effect of AR emulsions on storage rots, when they sprayed on trees in organic orchards.

Project achievements in 2014

Materials and methods

Isolation of ARs

ARs were isolated from commercial grade rye bran at the laboratory of Estera Dey, Lund University, using super-critical carbon dioxide ($scCO_2$) extraction (Dey and Mikhailapula 2009, Dey et al. 2013). Attempts were made to remove phenolic residues from the rye bran using

enzymatic pre-treatment and/or a wet oxidation process, and thus made it possible to reduce the isolation process to a one-step $scCO_2$ extraction. The 'AR 1' emulsion, which had previously showed the highest inhibiting effect (Tahir et al., 2014) was prepared, containing 0.025% ARs, 0.1% xanthan gum, 0.5% Synperonic 91/6, 0.2% Tween 20, 1% trioleate, 2% oleylalcohol, 2% PEG 400 and 5% $CaCl_2$.

Treatments in the orchard

Sixty-six 'Amorosa' trees were chosen in an organic apple orchard in Kivik. A total of 30 trees were sprayed with 0.5 l of AR 1 emulsion per tree according to the following schedule:

1. Once at full bloom
2. Once at 4 weeks prior to harvest
3. Twice, at full bloom and at 4 weeks prior to harvest
4. Twice, at 4 weeks prior to harvest and at harvest
5. Twice, at full bloom and at harvest

In addition, another set of 30 trees were sprayed according to the same schedule with an emulsion that contained all the ingredients except ARs (as a control of the effect of ARs). Finally, one set of 6 trees was not sprayed at all (as a control of the effect of spraying).

Evaluation of flowering and fruiting

Tree flowering was estimated in May, using a scale of 1–9 where 1 represented very poor flowering and 9 represented vigorous flowering. All trees were harvested at commercial harvesting date as determined using the Streif index (involving soluble solids concentration, firmness and starch conversion degree). Fruits were counted and weighed. Fruit quality [firmness, soluble solids content (SSC), acidity, and color] was estimated on 5 fruits per tree, using penetrometer, refractometer, titration with NaOH and colorimeter respectively).

Fruit inoculation

Penicillium expansum and *Neofabraea* (mostly *N. perennans*) were isolated from naturally infected apples showing typical symptoms of blue mold and bull's eye rot respectively, maintained on Petri dishes with potato dextrose agar (PDA) or (MEA) and stored separately as pure cultures at 4 ºC. Pathogen virulence was confirmed by periodic transfers over time through apples. Conidia were removed from the surface of 10-day-old cultures and suspended in 5 mL sterile distilled water containing 0.05% (v/v) Tween 80. The suspension was filtered through four layers of sterile cheesecloth to remove any adhering mycelia, and spore concentration was adjusted to 1×10^5 conidia per mL by hemacytometer. Fruits were wounded twice on both sides to a depth of 3 mm, and inoculated with *P. expansum* or *N. perennans* by pipetting 20 µL of a conidial pathogen suspension into each of the wound sites.

Postharvest treatments

The harvested fruits were divided into two groups and put in either cold storage (2 °C and 85% RH) or in CA stoarge (2.0 kPa O_2 and 2.0 kPa CO_2 and 2 °C). Each group was divided into four subgroups (15 fruits each) and treated with one of the following alternatives before storage:

1. Left untreated.
2. Sprayed with AR 1.
3. Inoculated with *P. expansum* spores.
4. Inoculated with *N. perennans* spores.

Fruits were evaluated after five months. Natural fungal decay was determined as percentage damaged fruit and fruit quality was estimated as above, in the subgroups 1 and 2. Damage contracted by the artificial inoculation was estimated as mean lesion diameter in the subgroups 3 and 4.

Results

Effect on yield and fruit quality

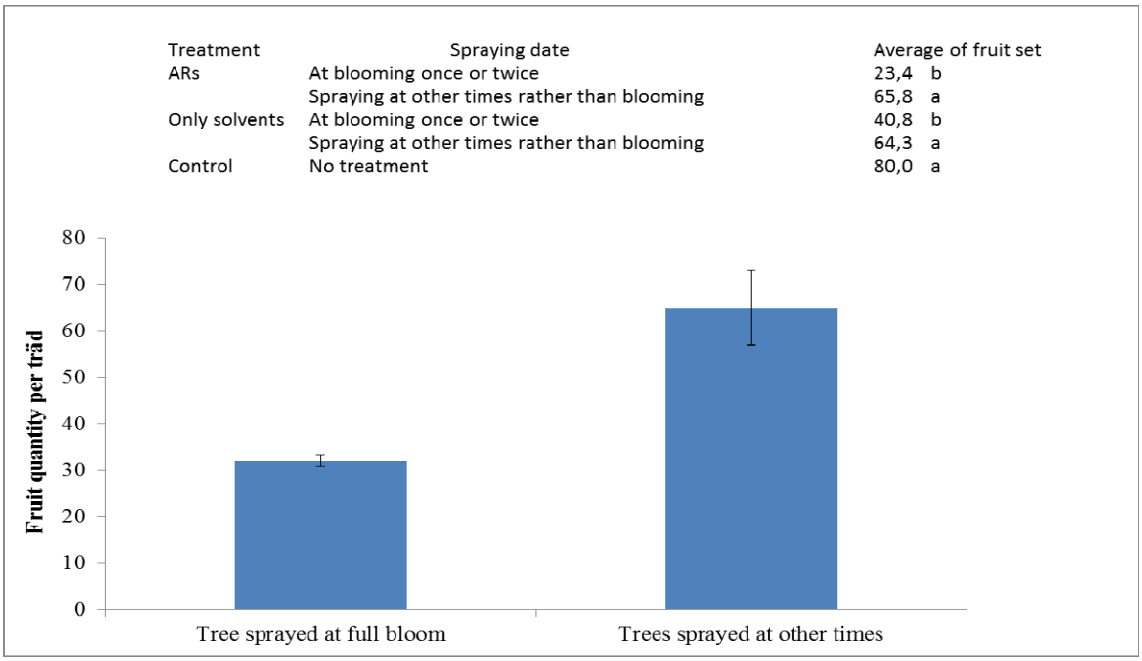
Amount of flowering did not vary significantly between trees used in the different treatments (Table 1). Trees that were sprayed at bloom with AR or with solvents only, had a lower fruit set (Fig. 1). However, no correlation between flowering level and fruit set (r = 0,079, *P = 0,528*) was noted. Spraying at other times, rather than at full bloom, had no significant effect on fruit set and thus no negative effect on tree yield (Table 1).

Table 1. Evaluation of flowering at full bloom and of yield at harvest date.

Treatments	Flowering (1-9)*	Fruit per tree
AR, once at bloom	4.8 a[z]	20.2 b
AR, once at 4 weeks prior to harvest	5.7 a	65.5 a
AR, twice, at bloom and at 4 weeks prior to harvest	5.3 a	25.2 b
AR, twice, at bloom and at harvest	5.2 a	24.8 b
AR, twice, at 4 weeks prior to harvest and at harvest	3.5 a	66.0 a
Solvents only, once at bloom	3.5 a	27.7 b
Solvents only, once at 4 weeks prior to harvest	3.8 a	61.7 a
Solvents only, twice, at bloom and at 4 weeks prior to harvest	3.8 a	30.3 b
Solvents only, twice, at bloom and at harvest	5.3 a	64.3 a
Solvents only, twice, at 4 weeks prior to harvest and at harvest	3.7 a	66.8 a
No spraying, control	5.0 a	80.0 a

**: 1 = very few and 9 = very high. z. Values followed by a common letter in a row for each cultivar are not significantly different at P<0.05.*

Fig. 1. Effect of spraying time on tree yield, 'Amorosa' 2014.



Trees, which were sprayed at bloom with AR or only with solvents, showed lower yield (with 44% and 35% respectively) and larger fruit (with 57% and 22% respectively) compared to non-sprayed trees (Table 2). However, non-sprayed trees had the smallest fruit followed by trees that were sprayed with AR at 4 weeks prior to harvest (Table 2).

Table 2. Tree yield and fruit weight at harvest 2014.

Treatments	Yield (kg per tree)	Fruit weight (g)
AR, once at bloom	4.459 f	197.7 abc
AR, once at 4 weeks prior to harvest	6.155 de	95.0 f
AR, twice, at bloom and at 4 weeks prior to harvest	5.135 ef	214.0 ab
AR, twice, at bloom and at harvest	4.727 f	181.4 bcd
AR, twice, at 4 weeks prior to harvest and at harvest	11.29 a	156.7 d
Solvents only, once at bloom	3.543 f	207.7 abc
Solvents only, once, at 4 weeks prior to harvest	6.525 de	176.9 bcd
Solvents only, twice, at bloom and at 4 weeks prior to harvest	3.567 f	223.8 a
Solvents only, twice, at bloom and at harvest	8.819 bc	169.2 cd
Solvents only, twice, at 4 weeks prior to harvest and at harvest	9.843 ab	150.1 de
No spraying, control	7.525 cd	111.5 ef

Fruits from trees that had been sprayed with AR were 10% firmer at harvest, compared to fruits from trees sprayed with the solvents only. Among AR-treatments, trees sprayed with AR at four weeks prior to harvest or/and at harvest had firmer fruit compared with trees sprayed at other times. No clear effect of treatments on fruit color was found (Table 3).

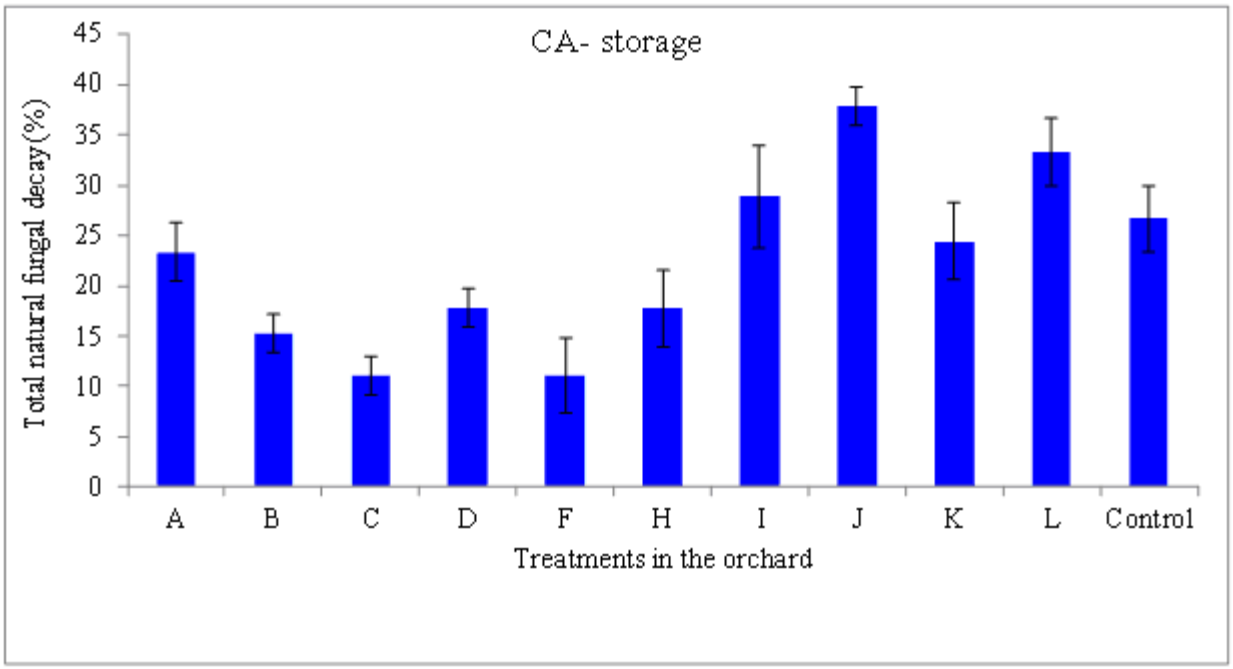
Table 3. Fruit quality at harvest date, cv. Amorosa, Kivik, 2014.

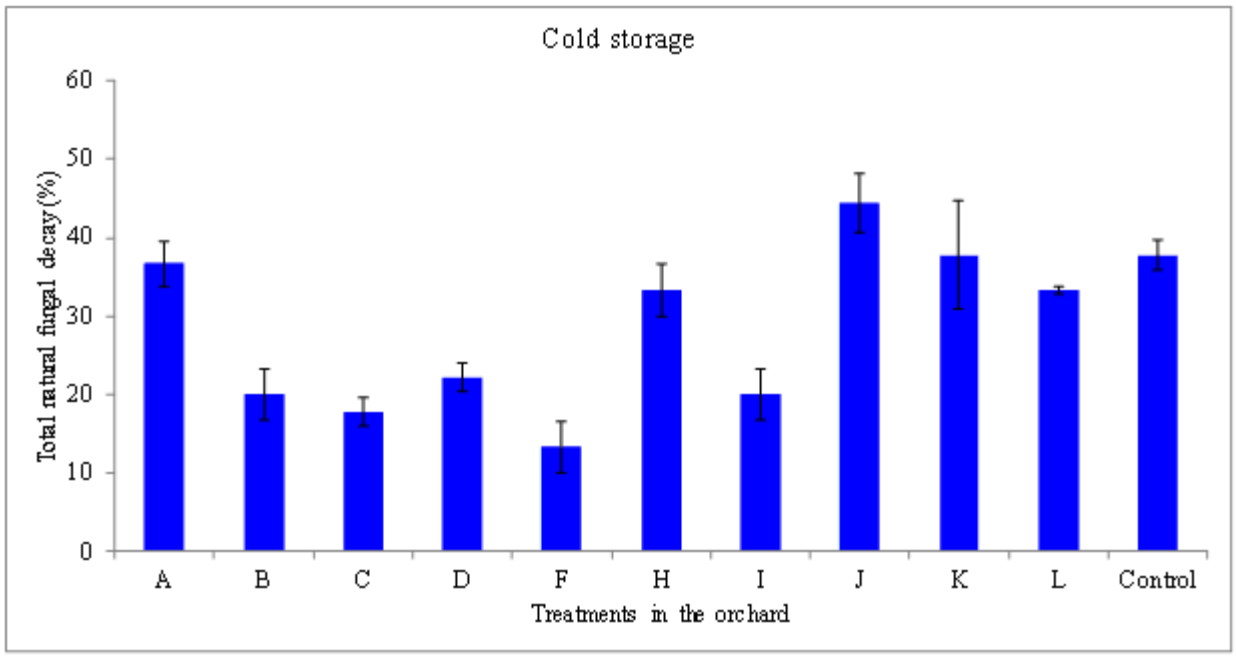
Treatments	Firmness, Kg/cm^2	Color index
AR, once at bloom	5.9 cd	15.4 b
AR, once at 4 weeks prior to harvest	6.9 ab	17.8 b
AR, twice, at bloom and at 4 weeks prior to harvest	5.3 d	26.3 ab
AR, twice, at bloom and at harvest	6.2 bc	16.6 b
AR, twice, at 4 weeks prior to harvest and at harvest	6.6 ab	25.1 ab
Solvents only, once at bloom	5.4 cd	29.1 ab
Solvents only, once at 4 weeks prior to harvest	5.9 cd	25.6 ab
Solvents only, twice, at bloom and at 4 weeks prior to harvest	5.6 cd	13.9 b
Solvents only, twice, at bloom and at harvest	5.4 cd	17.6 b
Solvents only, twice, at 4 weeks prior to harvest and at harvest	5.3 d	18.5 b
No spraying, control	7.5 a	38.1 a

AR and natural fungal decay during storage

In general, fruits from trees that had been sprayed with AR showed less natural decay when compared to fruits from non-sprayed trees or to fruits from trees that had been sprayed with the solvents only (Fig. 2). Spraying with solvents showed in general no significant effects on the occurrence of natural fungal decay during storage (Fig. 2).

Fig. 2. Effects of different orchard treatments on the occurrence of natural fungal decay during storage (A, B, C, D and F trees were sprayed with AR while H, I, J, and K trees were sprayed with the solvents only; A and H were sprayed at full bloom, B and I were sprayed at 4 weeks prior to harvest, C and J were sprayed twice (at full bloom and at four weeks prior to harvest), D and K were sprayed twice (at full bloom and prior to harvest) and F and L were sprayed twice (at four weeks prior to harvest and at harvest)).





Significant effects of orchard treatments, storage methods as well as the impacts of postharvest treatments were noted on the occurrence of natural fungal decay during storage (Table 4). No significant interactions between these various treatments were found.

Table 4. ANOVA – GLM, natural fungal decay 2014
Six treatments in the orchard, 2 storage methods, 2 postharvest handlings, 3 replications.

Factors			*DF*	*P*
Spraying time (6 variables)			5	*0.000* ***
Storage method (2 variables)			1	*0.000* ***
Postharvest treatments (2 variables)			1	*0.000* ***
Spraying time	Storage method		5	*0.374* *ns*
Spraying time	Postharvest treatment		5	*0.703* *ns*
Storage method	Postharvest treatment		1	*0.634* *ns*
Spraying time	Storage method	Postharvest treatment	5	*0.852* *ns*
Error			48	
Total			71	

**** = <0.001, **=>0.01 *= <0.05 ns. Not significant*

Table 5. Effect of field treatment, postharvest treatment with AR and storage method on the occurrence of natural fungal decay in 'Amorosa' apple.

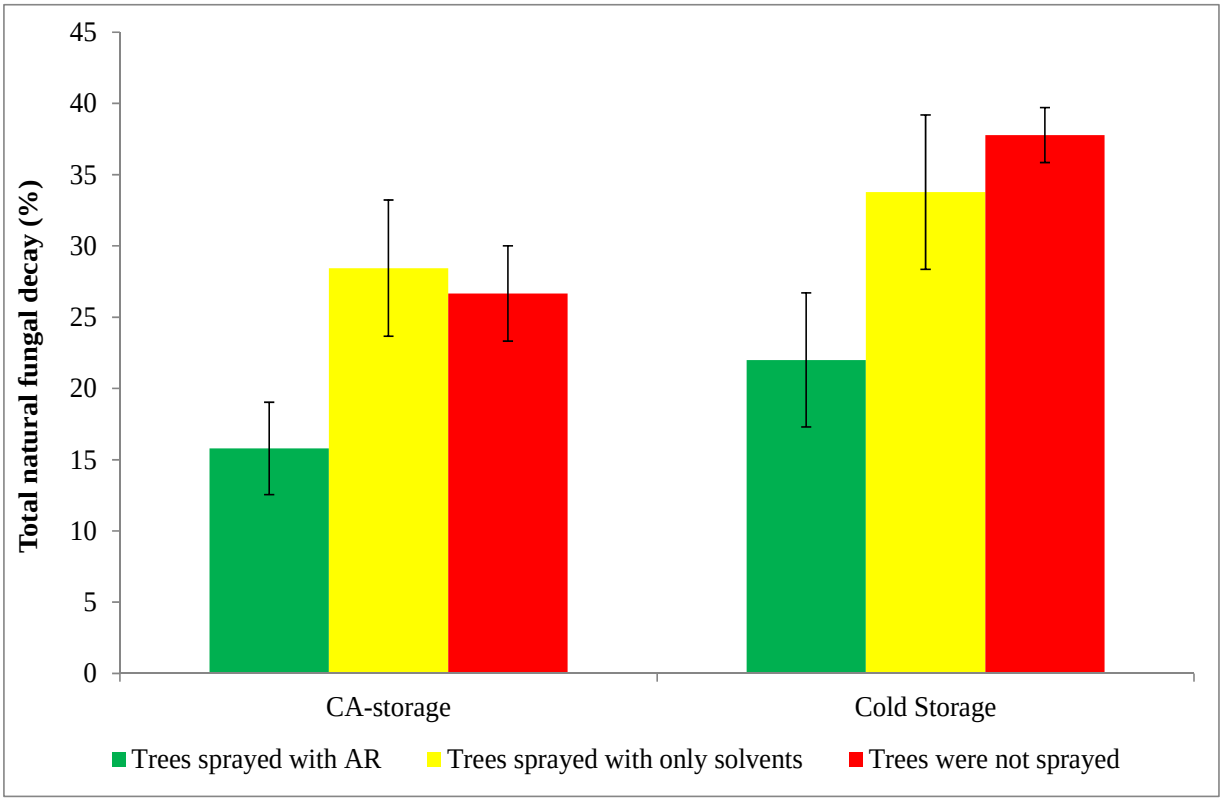
Spraying times	Storage method	Postharvest	*Neofabraea* spp.	*Colleto-trichum* spp.	*P. expansum*	Total natural decay %
At full bloom	CA	Treatment	6.7 abc	10.0 ab	0.0 b	16.7 bcde
		No treatment	6.7 abc	6.7 ab	10.0 a	23.3 abcd
	Cold	Treatment	6.7 abc	6.7 a	6.7 ab	26.7 abc
		No treatment	13.3 ab	21.1 a	0.0 b	36.7 a
Four weeks before harvest	CA	Treatment	2.2 bc	2.2 b	0.0 b	6.7 de
		No treatment	4.4 bc	8.9 ab	2.2 ab	15.6 bcde
	Cold	Treatment	4.4 bc	6.7 ab	0.0 b	11.1 bcde
		No treatment	11.1 abc	8.9 ab	0.0 b	20.0 abcd
twice (at full bloom and 4 weeks before harvest)	CA	Treatment	4.4 bc	4.4 b	0.0 b	8.9 cde
		No treatment	2.2 bc	6.7 ab	2.2 ab	11.1 bcde
	Cold	Treatment	8.9 abc	6.7 ab	0.0 b	15.6 bcde
		No treatment	6.7 abc	8.9 ab	0.0 b	17.8 bcde
Twice (at full bloom and at harvest)	CA	Treatment	4.4 bc	2.2 b	0.0 b	6.7 de
		No treatment	6.7 abc	8.9 ab	0.0 b	17.8 bcde
	Cold	Treatment	6.7 abc	8.9 ab	0.0 b	20.0 abcde
		No treatment	11.1 abc	11.1 ab	0.0 b	22.2 abcd
Two times (4 weeks before harvest and at harvest)	CA	Treatment	0.0 c	2.2 b	0.0 b	2.2 e
		No treatment	4.4 bc	4.4 b	2.2 ab	11.1 bcde
	Cold	Treatment	0.0 c	2.2 b	0.0 b	6.7 de
		No treatment	4.4 bc	6.7 ab	0.0 b	13.3 bcde
Control, no spraying	CA	Treatment	6.7 abc	6.7 ab	2.2 ab	17.8 bcde
		No treatment	11.1 abc	8.9 ab	4.4 ab	26.7 abc
	Cold	Treatment	17.7 a	8.9 ab	2.2 ab	28.9 ab
		No treatment	11.1 abc	13.3 ab	8.9 ab	37.8 a

Fruits harvested from trees that had been sprayed twice with AR (at 4 weeks prior to harvest and at harvest), showed minimum natural decay, especially when treated with AR again after harvesting and stored in CA. The second best results were obtained for trees that had been sprayed with AR, once (at 4 weeks prior to harvest) or twice (at full bloom and at 4 weeks prior to harvest), treated with AR again after harvesting and stored in CA (Table 5). Total natural fungal decay in these fruits was 40% lower than in fruits from non-sprayed trees (Fig. 3). No significant differences between non-sprayed trees and trees sprayed only with solvents were observed (Fig. 3).

The most important pathogens causing natural decay were *Neofabraea* spp., *C. acutatum* and *P. expansum* (Table 5). Very few infections with *Monilinia spp.* and *B. cinerea* were found.

The pre- and postharvest treatments which were most effective in reducing total natural decay, also reduced natural decay caused by *Neofabraea spp.* and *Colletotrichum spp.* Fruits from trees sprayed twice (at 4 weeks prior to harvest and at harvest) did not show any symptoms of *Neofabraea* spp. and very few symptoms of *C. acutatum* after storage (Table 5). Most treatments resulted in no symptoms of *P. expansum* while non-sprayed fruit suffered from this pathogen (Table 5).

Fig. 3. Effect of orchard spraying with AR on the occurrence of total natural decay, 'Amorosa', 2014, bars are SD± P=0.05

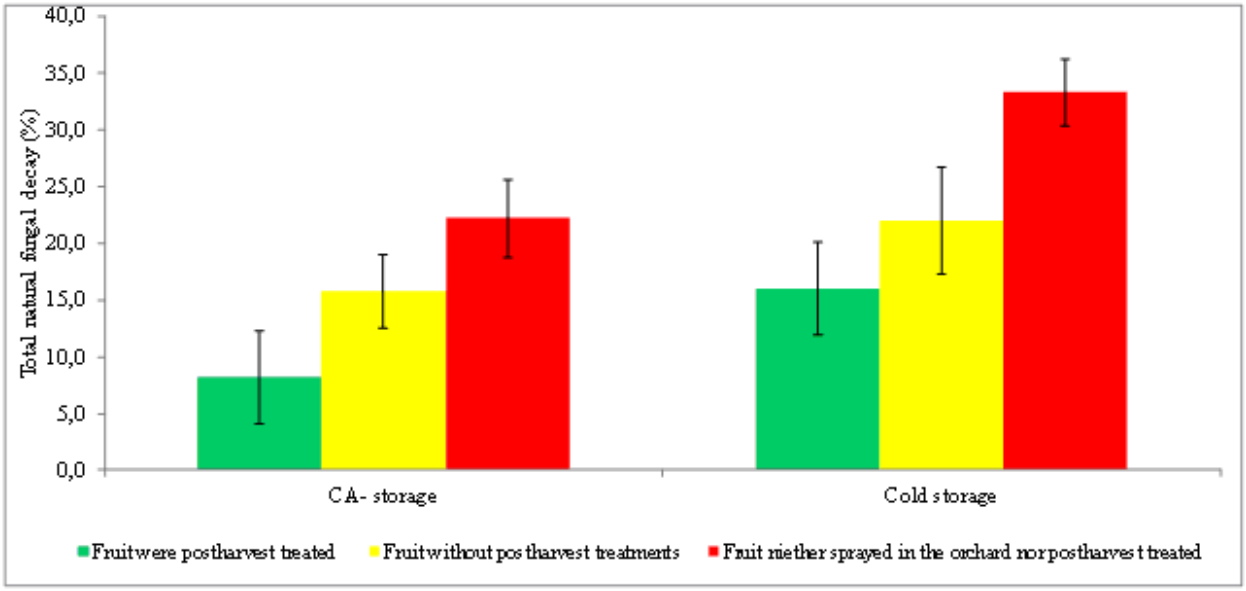


Postharvest treatment with AR improved the efficiency of orchard treatments on the occurrence of natural decay, but only for fruits in CA storage (Fig. 4). This positive effect was not evident for fruits in cold-storage. However, regardless of postharvest treatment, fruits from sprayed trees showed lower natural infection compared with fruits from non-sprayed trees (Fig. 4).

AR-inhibiting effect on damage caused by inoculation with* P. expansum *and* Neofabraea *spp.

Orchard treatments influenced the size of lesions caused by artificial inoculation with *P. expansum* and *Neofabraea* spp. (Table 6). Fruit from trees sprayed twice with AR (at 4 weeks prior to harvest and at harvest) had 30% and 28% smaller lesion area when inoculated with *P. expansum* and *Neofabraea spp.* respectively, compared to fruits harvested from non-sprayed trees and postharvest inoculated with the same pathogens (Table 6). Spraying trees with the solvents only had not clear effect on the lesion area after artificial inoculation (Table 6).

Fig. 4. Effect of postharvest treatment with AR on the occurrence of total natural decay, 'Amorosa', 2014, bars are SD± P=0.05



A significant correlation was found between lesion area due to inoculation with *P. expansum* and with *Neofabraea* spp. (r = 0.531, *P = 0.001*). No significant difference between the effect of one or two sprayings on the lesion area caused by *P. expansum* was noted (61 and 60 mm respectively). Lesion area diameter caused by *Neofabraea* spp. was reduced from 56 mm when trees received one spraying to 48 mm when trees were sprayed twice.

Table 6. Effect of orchard treatments on the the lesion diameter caused by artificial inoculation with two pathogens, 2014, 'Amorosa'.

Material	*Treatments*	Lesion diameter (mm)	
		P. expansum	*Neofabraea* spp.
AR	Once at bloom	69.0 a	63.0 bc
	Once at 4 weeks prior to harvest	52.5 b	48.8 bcd
	Twice, at bloom and at 4 weeks prior to harvest	60.0 ab	45.7 d
	Twice, at bloom and at harvest	68.6 a	51.2 bcd
	Twice, at 4 weeks prior to harvest and at harvest	52.2 b	47.5 cd
Only solvents	Once at bloom	60.0 ab	64.7 b
	Once at 4 weeks prior to harvest	68.7 a	83.6 a
	Twice, at bloom and at 4 weeks prior to harvest	69.2 a	61.7 bcd
	Twice, at bloom and at harvest	69.9 a	81.4 a
	Twice, at 4 weeks prior to harvest and at harvest	59.8 ab	60.9 bcd

No spraying, control	74.4 a	63.8 b

Effect of orchard treatments on fruit quality after storage

Orchard treatments had no significant effect on fruit color, fruit firmness or soluble solids content after storage. However, fruit from CA storage were firmer than fruit from cold storage.

Literature

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