Primary report 2015

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

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 *Monilinia* (*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 *P. expansum* or *N. perennans* (Tahir et al., 2014). 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 as well as the relationship between the concentration of AR emulsion and its antifungal activity *in vitro* and *in vivo*.

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. Four emulsions with different ARs concentrations (Ref. 0.0%, AR1. 0.025%, AR2.

0.1% and AR3. 0.2%) were prepared, containing 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

Ninety 'Amorosa' trees were chosen in an organic apple orchard in Kivik, divided into three blocks in a complete randomized design. A total of 42 trees were sprayed with 0.5 l of AR 1 emulsion per tree according to the following schedule:

1. Once at the first week of June.
2. Once at the first week of July.
3. Once at the first week of August.
4. Once at the first week of September.
5. Twice, at the first week of June and again at the first week of August.
6. Twice, at the first week of June and again at the first week of September.
7. Twice, at the first week of August and again at the first week of September.

In addition, another set of 42 trees were sprayed with 0.5 l of Ref emulsion (0.0%) per tree according to the same schedule (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).

Fruit, harvesting and storage

Fallen fruit were removed from the tree rows during June and July to avoid their effect as sources of fungal infection. At harvest date, fallen fruit which dropped during August and September were collected and the fungal decay in these fruit was identified.

Using the Streif index (involving soluble solids concentration, firmness and starch conversion degree), commercial harvesting date was determined and fruit were picked, counted and weighed. The harvested fruits from each treatment were divided into three groups, one group was used to evaluated fruit quality and the others were stored either in cold storage (2 °C and 85% RH) or in CA storage (2.0 kPa O_2 and 2.0 kPa CO_2 and 2 °C). Fruit quality [firmness, soluble solids content (SSC), and color] was estimated on 5 fruits per tree, using penetrometer, refractometer and colorimeter respectively. Stored fruits were evaluated after five months in storage chambers and one week in 20 °C as shelf life. Natural fungal decay was determined as percentage damaged fruit and fruit quality was estimated on 5 fruit per treatment and block as was mentioned above.

Preparation of Inoculums

The pathogens *P. expansum, M. fructigena, N. perennans* and *C. acutatum* were isolated from naturally infected apples, showing typical symptoms of blue mold, brown rot, bull's eye rot and bitter rot respectively. Fruit surface was sterilized for 30 s with 70% ethanol and rinsed with sterile distilled water. Small sections from the growing margin of the fruit rot lesion were plated on PDA at 25 °C for the first two and on MEA at 22 °C for the other two pathogens. The plates were maintained under two weeks and pure cultures were obtained by transferring the hyphal tips to new PDA and MEA plates respectively and incubating at the same temperatures for 3 weeks. Thereafter, the pathogens were maintained separately as pure cultures at 4 ºC. Conidial suspensions of the four pathogens were prepared by removing spores from the surface of the cultures on PDA and MEA, respectively, and suspending them in 5 mL sterile distilled water containing 0.05% (v/v) Tween 80. The suspensions were filtered through four layers of sterile cheesecloth to remove any adhering mycelia, and spore concentrations were determined with a hemacytometer and adjusted to $1x10^5$ conidia mL^{-1}. These suspensions were used to test the antifungal effect of ARs *in vivo*.

Fruit inoculation

'Amorosa' fruits, at the similar maturity level (Streif index) were picked from non-sprayed trees in the same organic orchard, washed with sterilized water, wounded twice on both sides to a depth of 3 mm, and inoculated by pipetting 20 µL of a conidial pathogen suspension into each of the wound sites as following:

- Group 1. a lot of 180 fruits were inoculated with *P. expansum*.
- Group 2. a lot of 180 fruits were inoculated with *M. fructigena.*
- Group 3. a lot of 180 fruits were inoculated with *N. perennans*.

Each group was divided into 5 subgroups. After 3 h of inoculation, four of these subgroups were sprayed with 10 ml of Ref, AR1, AR2, and AR3 respectively, while the fifth subgroup was left without any treatment as control. All groups were stored in cold storage (2 °C and 85% RH) for 8 weeks. At the end of the storage period, decay on fruit inoculated with *P. expansum* and *M. fructigena* was evaluated directly while fruit inoculated with *N. perennans* were transferred to a plastic chamber (18 ± 2 ºC and 80% RH) for one week before evaluations. Decay severity was estimated as surface decay lesion diameter. The inhibition level was calculated as percent related to lesion diameter on non-treated fruit.

In vitro antifungal activity of ARs

Mycelial plugs (10 mm in diameter) of *P. expansum* were transferred from 10-days pure cultures onto 75 plates PDA. After 3 hours, the plates were divided into five groups, 15 plates each; four were sprayed with 1 ml AR1, AR2, AR3 and Ref (0.0%, 0.025%, 0.1% and 0.2 %) respectively while the fifth group was left without any treatment as control. Two other groups of plates, 75 each, were treated by the same method replacing *P. expansum* with *C. acutatum* in the first group and with *N. perennans* in the second group. All plates were incubated at 24 ºC for 10 days and the radial mycelia growth of each pathogen was measured with a calliper in mm and expressed as percentage inhibition of radial mycelial growth. The assays were repeated three times with fife replicates and three plates each.

To evaluate possible effects of ARs at different concentrations (0.0, 0.025, 0.1 and 0.2 %) on the viability of the pathogens' conidia of *P. expansum*, *C. acutatum* and *N. perennans*, 0.5 ml of conidial suspension (10^3 conidia/ml) per pathogen were transferred to five Eppendorf tubes (1.5 ml), four of them contained 0.5 ml of one of the emulsions Ref, AR1, AR2 and AR3 respectively while the fifth was empty (left without any treatment as a control tube). The tubes were incubated at 22 ºC for one day and 100 µl of their content were transferred and uniformly distributed in PDA plates amended with streptomycin sulphate (250 mg/l each). Plates were incubated at 25 ºC and the number of colony forming units (CFU) was recorded after 7 days. This evaluation was repeated three times with five replicates/tubes each.

Statistical analysis

A complete randomized design was adopted in this study, either in the orchard, in the storage house and in the laboratory (*In vitro* or *in vivo*). Data were analysed with a GLM- analysis of variance with emulsions, spraying frequency and time and their interactions as fixed effects for yield, fruit weight, fruit quality at harvest and fruit storage potential (decay occurrence and quality decline). Multiple comparisons were made with Tukey's post hoc test ($\alpha = 0.05$).

The data from the *in vitro* experiments and the *in vivo* trials were subjected to ANOVA (one-way analysis of variance). Means were separated using the LSD test, at $P = 0.05$. All statistics were performed by using Minitab 17.2.4.0 (Minitab Ltd., State College, PA, USA).

Results

Effect on yield and fruit weight

Spraying trees with ARs emulsion decreased yield in comparison to non-sprayed trees (Table 1). Trees which were sprayed with ARs emulsion had 25% fewer yields than trees sprayed only with Ref emulsion (Fig.1). However, Ref emulsion also decreased tree yield but the results were incompatible (Table 1).

Table 1. Tree yield and fruit weight during 2015 season.

Spraying time	Trees sprayed with ARs emulsion		Trees sprayed with Ref emulsion	
	Yield (kg/tree)	Fruit weight (g)	Yield (kg/tree)	Fruit weight (g)
Once, June	2.684 bc	114.6 ab	5.783 ab	128.5 a
Once, July	1.089 c	91.4 bc	2.887 bc	110.4 abc
Once, August	3.953 bc	123.5 a	1.978 c	88.3 bc
Once, September	4.753 b	101.1 abc	5.589 ab	113.8 ab
Twice, June and August	3.900 bc	83.9 bc	3.218 bc	81.2 c
Twice, June and September	1.860 bc	84.1 bc	4.352 bc	114.7 ab
Twice, August and September	4.008 bc	126.5 a	6.057 ab	113.5 ab
Non-sprayed trees	8.535 a	121.8 a	8.535 a	121.8 a

Spraying frequency (once or twice) had the same negative effect on tree yield (Fig. 2). Earlier treatments (June and July) showed higher negative effects on tree yield than the late treatment (September) (Fig.3).

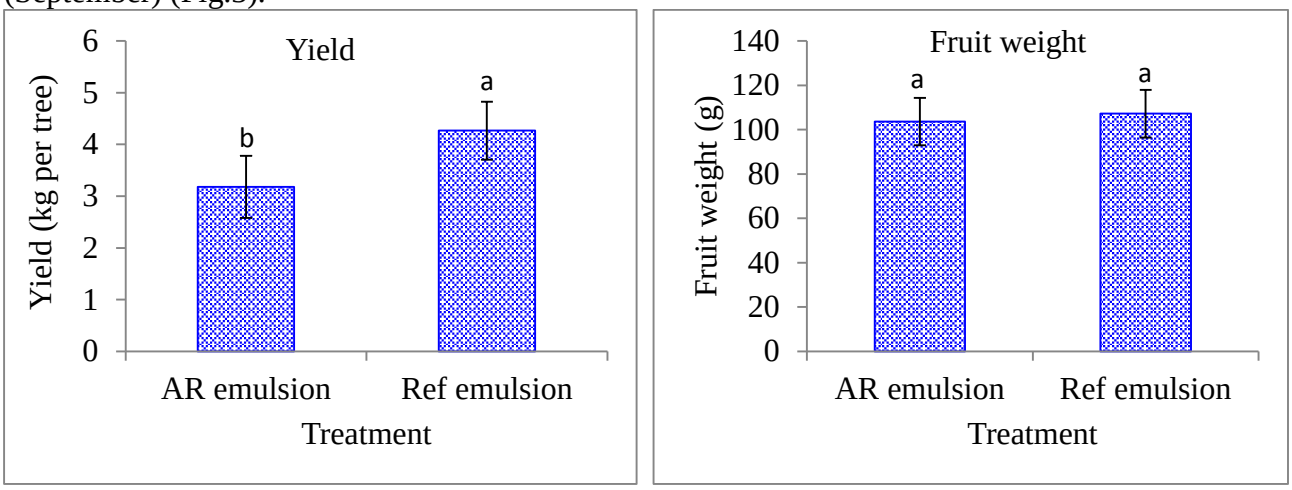


Fig. 1. Comparison between the effects of emulsions on tree yield and fruit weight (2015). Interactions between emulsion and each of spraying frequency and spraying time were non-significant at p=0.05.

AR respective Ref treatments did not affect fruit weight (Fig.1). Spraying trees with AR, twice a season decreased fruit weight by 15% compared to spraying trees once a season. Such negative effect did not observe when trees were sprayed only with Ref emulsion (Fig. 2). Spraying time did not affect fruit weight neither when AR emulsion was sprayed nor when Ref emulsion was sprayed (Fig.3).

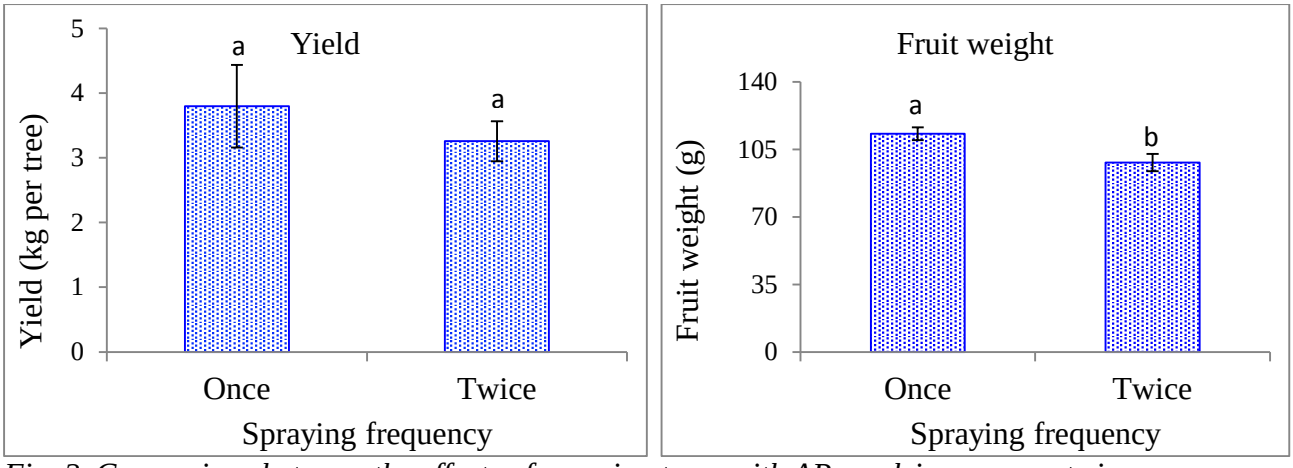


Fig. 2. Comparison between the effects of spraying trees with AR emulsion once or twice a season on tree yield and fruit weight, 2015. Interactions between emulsion and each of spraying frequency and spraying time were non-significant at p=0.05.

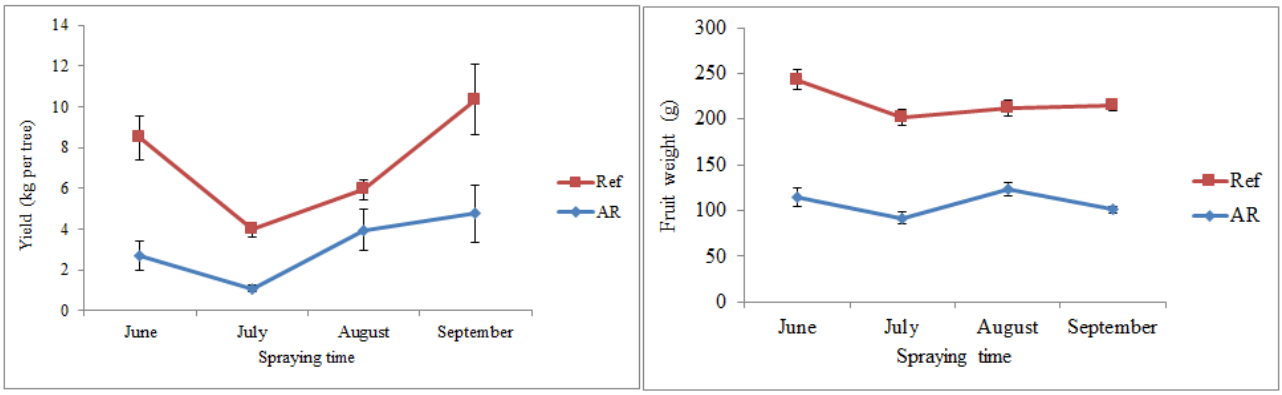


Fig. 3. Relationships between spraying time with different emulsions and tree yield respective fruit weight, 2015. Interaction between spraying frequency and spraying time was non-significant at p=0.05.

Effect on fallen fruit

A lot of 127 fallen fruit were collected from the experiment rows immediately before harvesting. All of them showed fungal decay (mostly due to *Monilinia fructigena*). Treatment with AR emulsion may be decreased fallen fruit quantity and fungal infection compared to non-sprayed trees (Table 2). Spraying time and frequency did not show any effect of ARs on fallen fruit. Spraying trees with ARs once or twice a season decreased fallen and infected fruit by 59% and 76% respectively compared to spraying trees with Ref emulsion once or twice a season (Fig.4). Ref emulsion had not clear effect on fallen fruit because no clear significant differences were found between sprayed and non-sprayed trees (Table 2).

Table 2. Effect of tree treatments on infection of fallen fruit by different fungi, 2015.

Spraying time	Trees sprayed with	
	ARs emulsion	Ref emulsion
Once, June	1.30 b	1.70 b
Once, July	1.20 b	2.50 ab
Once, August	0.73 b	2.00 ab
Once, September	0.50 b	2.00 ab
Twice, June and August	0.83 b	1.80 ab
Twice, June and September	0.17 b	1.50 b
Twice, August and September	0.17 b	1.50 b
Non-sprayed trees	3.30 a	3.30 a

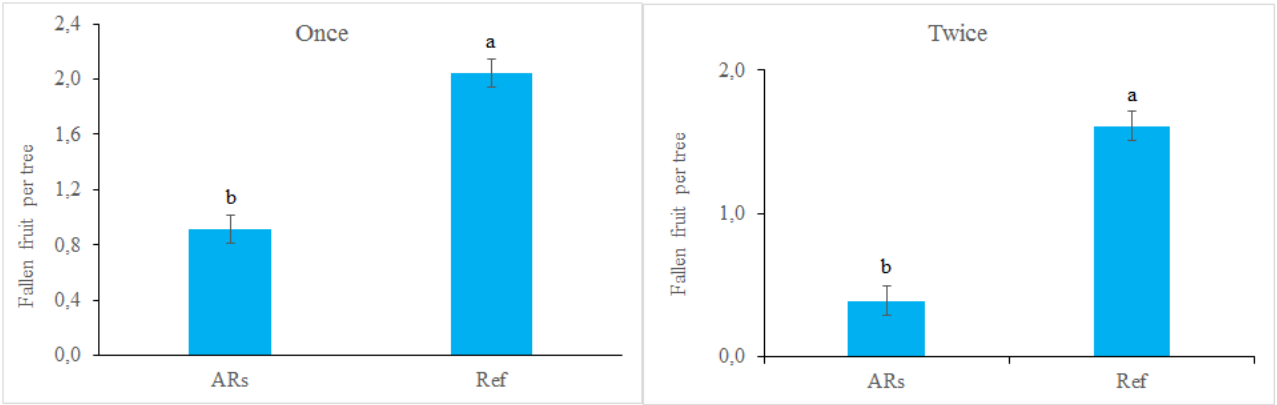


Fig. 4. Relationships between spraying with different emulsions and fallen fruit per tree at harvesting, 2015. Interaction between spraying frequency and spraying time was non-significant at p=0.05.

Effect on fruit quality at harvest

Fruit firmness increased when trees were sprayed with ARs emulsion once in August or in September as well as twice in June and August and in June and September in comparison with non-sprayed trees (Table 3a and 3b). ARs treatments (once or twice a season) improved the soluble solid concentration (SSC) regardless the spraying time in comparison with non-sprayed trees (Table 3a and 3b). ARs spraying had no significant effect on fruit ground or superficial color (Table 3.a and 3b).

Spraying trees with Ref emulsion showed incompatible effects on fruit firmness and SSC at harvest while Ref emulsion did not affect the fruit coloration (Table 3a and 3b).

Spraying time and frequency with either ARs or Ref emulsions did not show any significant relationships with fruit firmness and SSC. ARs emulsion which was sprayed once or twice a season increased fruit firmness and SSC in comparison with Ref emulsion (Fig.5).

Table 3. Effect of spraying trees with ARs and Ref emulsions on fruit quality at harvesting (2015).

Table 3a. Once a season

Treatment	Tree were sprayed with ARs				Tree were sprayed with Ref.			
	Firmness	SSC	Ground color	Red color	Firmness	SSC	Ground color	Red color
In June	8.3 ab	13.3 a	6.7 a	57 a	8.1 b	12.6 b	6.8 a	70 a
In July	8.2 ab	13.7 a	7.2 a	66 a	7.3 c	13.4 ab	6.8 a	53 a
In August	8.8 a	13.5 a	6.8 a	68 a	8.8 a	13.6 a	7.2 a	70 a
In September	8.6 a	13.4 a	7.1 a	63 a	7.8 bc	13.0 ab	6.8 a	63 a
Non-sprayed trees	7.8 b	12.4 b	6.5 a	66 a	7.7 bc	12.4 b	6.5 a	66 a

Table 3b. Twice a season

Treatment	Tree were sprayed with ARs				Tree were sprayed with Ref.			
	Firmness	SSC	Ground color	Red color	Firmness	SSC	Ground color	Red color
In June and Aug.	8.2 ab	13.3 b	6.0 a	64 a	8.1 a	14.0 a	6.9 a	67 a
In June and Sep.	8.8 a	14.4 a	6.3 a	65 a	7.7 a	13.5 a	6.5 a	65 a
In Aug. and Sep.	7.9 bc	13.3 b	6.5 a	61 a	7.2 b	13.3 ab	6.7 a	51 a
Non-sprayed trees	7.7 c	12.4 c	6.5 a	66 a	7.7 a	12.4 b	6.5 a	66 a

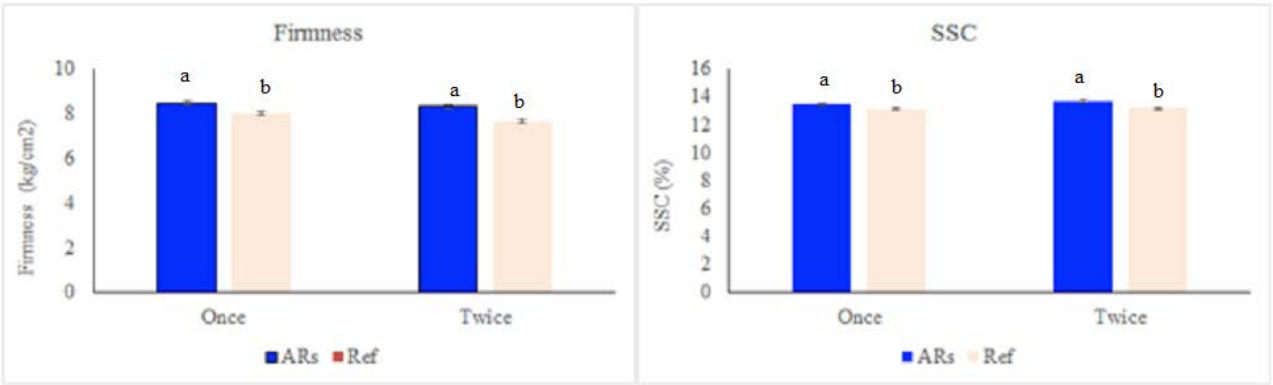


Fig 5. ARs spraying caused higher firmness and sweater fruit at harvest in comparison with spraying with Ref emulsion (2015).

Effect of tree treatments on fruit storability

Weight loss during storage

Spraying trees with ARs or Ref emulsions did not affect weight loss during storage in regular air or ULO in comparison with non-sprayed trees (Table 4a and 4b). No significant differences were found

between the effects of ARs and Ref emulsions on weight loss in fruit, stored in regular air or ULO (Fig 6). Interactions between the effects of emulsions, spraying frequency and time were not significant (Table 5). However, ULO storage in general caused lower weight loss by 50% than storage in regular air (Fig 7).

Fungal decay

ARs treatment decreased fungal decay occurrence during storage in comparison with non-sprayed trees regardless the storage method and spraying frequency or time (Table 4a and 4b). Spraying trees with Ref emulsion showed incompatible effect on fungal decay occurrence in fruit stored in regular air while Ref emulsion did not affect fungal decay occurrence in fruit stored in ULO (Table 4a and 4b). Interactions between the effects of emulsion, storage method and spraying times were significant (Table 5).

Table 4. Effects of tree treatments on fruit storability (weight loss and fungal decay), 2015.

Table 4a. Trees were treated once a season

Tree were sprayed	Trees were sprayed with ARs emulsion				Trees were sprayed with Ref emulsion			
	Regular air storage		ULO storage		Regular air storage		ULO storage	
	Weight loss %	Decay %	Weight loss %	Decay %	Weight loss %	Decay %	Weight loss %	Decay %
In June	7.5 a	14.2 b	2.1 ab	6.7 b	3.7 a	15.8 bc	3.0 a	13.3 a
In July	5.2 ab	11.7 bc	3.3 ab	6.7 b	5.7 a	20.8 ab	1.9 a	15.6 a
In August	6.7 ab	8.3 cd	1.7 b	5.6 b	6.9 a	21.7 ab	3.5 a	10.0 a
In September	6.3 ab	5.8 d	3.7 a	4.4 b	6.9 a	14.2 c	2.4 a	8.9 a
Non-sprayed	4.6 b	24.1 a	2.8 ab	15.6 a	4.6 a	24.1 a	2.8 a	15.6 a

Table 4b. Trees were treated twice a season

Tree were sprayed	Trees were sprayed with ARs emulsion				Trees were sprayed with Ref emulsion			
	Regular air storage		ULO storage		Regular air storage		ULO storage	
	Weight loss %	Decay %	Weight loss %	Decay %	Weight loss %	Decay %	Weight loss %	Decay %
In June & Aug.	5.3 ab	13.3 b	2.9 a	3.3 b	4.9 a	20.8 ab	3.1 a	8.9 a
In June & Sep.	5.3 ab	11.7 b	2.9 a	2.2 b	5.9 a	20.6 ab	2.8 a	13.3 a
In Aug. & Sep.	6.6 a	8.4 b	2.6 a	2.2 b	6.0 a	17.5 b	2.3 a	10.0 a
Non-sprayed	4.6 b	24.1 a	2.8 a	15.6a	4.6 a	24.1 a	2.8 a	15.6 a

ARs spraying decreased fungal decay in comparison with Ref spraying, by 46% and 44% in regular air when trees were sprayed once and twice a season respectively and by 47% and 75% in ULO, when trees were sprayed once and twice a season respectively (Table 4a and 4b and Fig 6). Spraying frequency showed positive effect only when fruit were stored in ULO (Fig 6). In general, ULO storage decreased fungal decay by 50% in comparison with storage in regular air (Fig.7). Spraying trees with ARs, later in the season (August and September), once or twice, showed higher protective effect than spraying with ARs earlier in June (Table 4a and 4b) (***see attached 1***).

Physiological disorders and total losses

No relationships between sparying with ARs respective Ref emulsion and occurrance of physiological disorders could be noted (Fig 6). ULO storage decreased physiological disorders in comparison with storage in regular air (Fig 7). However, trees sprayed with ARs earlier in the season showed a slight higher soft scald than fruit which were sprayed later (data not shown).

ARs treatment decreased total losses by 35% and 18% when trees sprayed once and twice respectively and fruit stored in regular air in comparison with Ref treatment. In ULO storage, ARs decreased total losses by 36% and 60% when trees were sprayed once and twice respectively in comparison with

Ref treatment (Fig. 6). In general, ULO storage decreased total losses by 56% in comparison with storage in regular air (Fig 7). Interactions between the effects of emulsion, storage method and spraying times and frequency were significant (Table 5). Spraying trees with ARs, later in the season (August and September), once or twice, showed lower total losses than spraying with ARs earlier in June (Table 4a and 4b). Spraying frequency had positive effect on total losses only when fruit were stored in ULO (Fig 6).

Table 5. Interactions between effects of various factors (emulsion, spraying frequency and time, storage method) on fruit storability, 2015.

Factors	Weight loss %	Fungal decay %	Disorders %	Total loss %
Storage method	0.000	0.000	0.000	0.000
Emulsions	ns	0.000	ns	0.000
Spraying frequency	ns	ns	ns	ns
Spraying month	ns	0.000	0.006	ns
Storage x emulsion	ns	ns	ns	ns
Storage x frequency	ns	0.007	ns	0.028
Storage x month	ns	ns	0.001	ns
Emulsion x Frequency	ns	ns	0.000	ns
Emulsion x month	ns	0.034	ns	0.001
Frequency x month	ns	ns	0.047	ns
Storage x emulsion x frequency	ns	ns	0.000	0.001
Storage x emulsion x month	0.011	ns	ns	ns
Storage x frequency x month	ns	ns	0.013	ns
Emulsion x frequency x month	ns	ns	0.013	0.000
Storage x emulsion x frequency x month	ns	0.022	0.047	ns

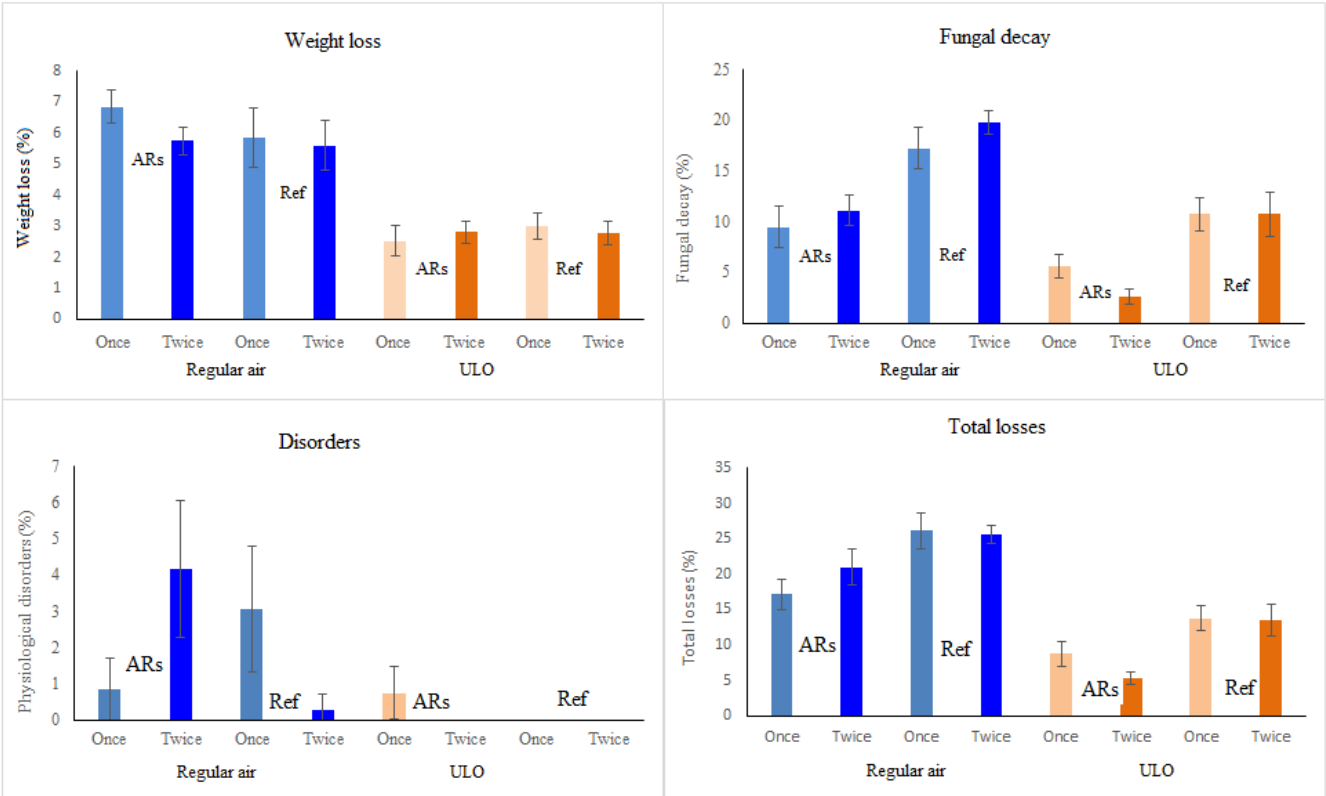


Fig. 6. Effects of various treatments on the fruit storability (2015), Interactions between spraying frequency and treatment were non-significant at $p<0.05$.

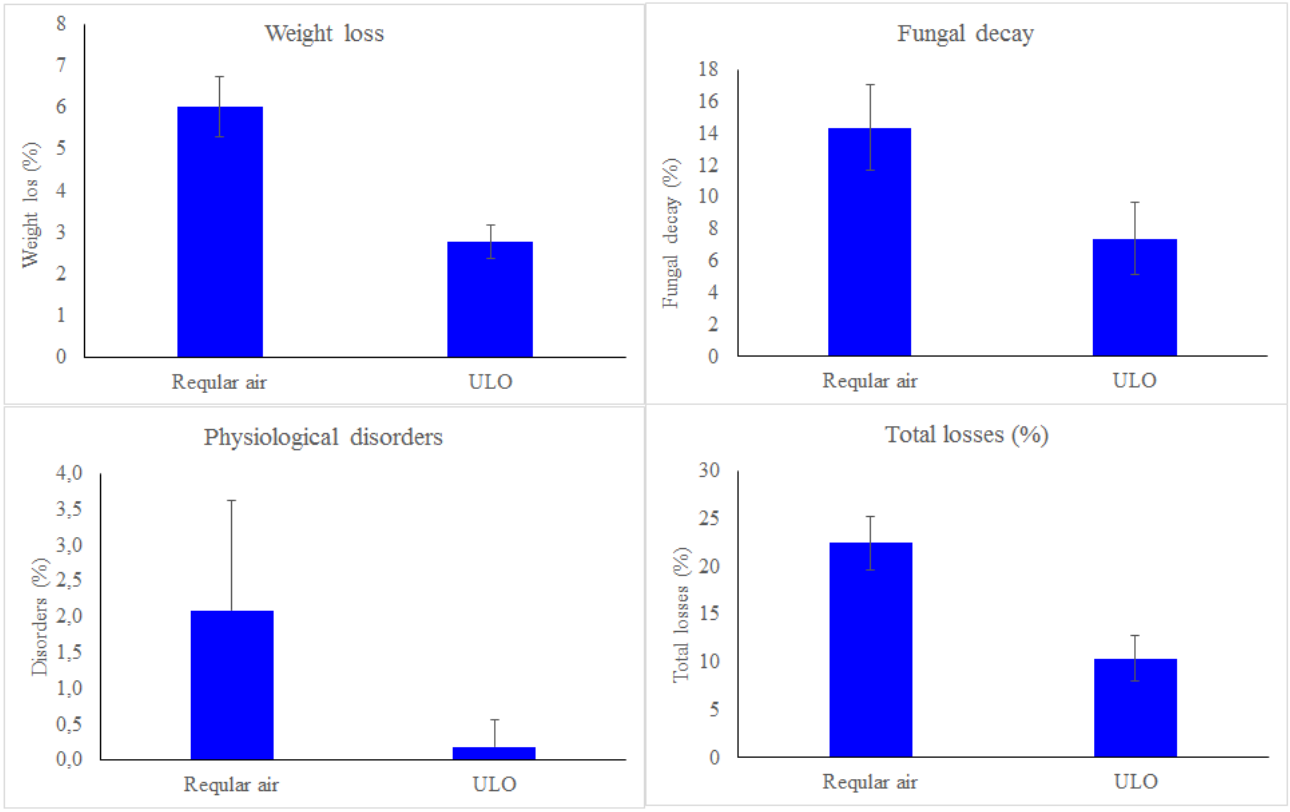


Fig.7. ULO storage decreased losses in comparison with storage in regular air (2015). Interactions between storage methods, spraying frequency and treatemnts were non-significant at $p<0.05$.

Fruit quality after storage

Color

ARs and Ref treatments in the orchard did not show any effect on fruit color (ground or superficial) (data not shown).

Firmness and soluble solid concentration

Fruit from trees, sprayed once with ARs had better firmness after storage in regular as well as in ULO storage in comparison with fruit from non-sprayed trees (Table 6a). This positive effect was also found when trees were sprayed twice and fruit were stored in regular air (Table 6b). Spraying trees with Ref emulsion, once or twice a season showed incompatible effect on fruit firmness after storage in regular air or ULO (Fig. 6a and 6b). No interaction was found between the effect of storage, emulsion and spraying frequency on fruit firmness after storage. However, spraying trees with ARs later in the season (August and September) showed better effect than spraying trees earlier in the season (June) (Table 6a and 6b). Firmness showed lower decline when fruit stored in ULO in comparison with storage in regular air.

Fruit from trees sprayed with ARs had in general higher soluble solid concentration after storage than fruit from non-sprayed trees (Table 6a and 6b). Spraying frequency and time did not affect this relationship. Spraying with Ref emulsion twice a season also increased fruit soluble solid concentration after storage (Table 6a and 6b). ULO stored fruit had higher soluble solid concentration than regular air stored fruit (Table 6a and 6b).

Table 6. Effects of tree treatments on fruit storability (weight loss and fungal decay), 2015.

Table 6 a. Trees were treated once a season

Tree were sprayed	Trees were sprayed with ARs emulsion				Trees were sprayed with Ref emulsion			
	Regular air storage		ULO storage		Regular air storage		ULO storage	
	Firm.	SSC	Firm.	SSC	Firm.	SSC	Firm.	SSC
In June	4,4 c	15,5 a	4,9 ab	14,8 a	4,3 b	13,2 a	4,8 b	14,1 bc
In July	4,5 bc	14,0 bc	5,5 a	14,8 a	4,5 b	13,5 a	5,1 ab	14,9 ab
In August	4,9 ab	14,7 ab	5,5 a	15,2 a	5,5 a	14,5 a	5,5 a	15,1 a
In September	5,0 a	13,6 cd	5,8 a	14,4 ab	4,4 b	13,3 a	5,3 ab	14,5 abc
Non-sprayed	4,4 c	12,8 d	4,7 b	13,8 b	4,5 b	12,8 a	5,0 ab	13,8 c

Table 6 b. Trees were treated twice a season

Tree were sprayed	Trees were sprayed with ARs emulsion				Trees were sprayed with Ref emulsion			
	Regular air storage		ULO storage		Regular air storage		ULO storage	
	Firm.	SSC	Firm.	SSC	Firm.	SSC	Firm.	SSC
In June & Aug.	4,2 b	12,8 b	5,1 a	14,2 b	4,8 a	13,1 ab	5,6 a	14,3 ab
In June & Sep.	5,7 a	14,4 a	5,7 a	14,5 ab	4,6 a	14,4 a	4,9 b	15,0 a
In Aug. & Sep.	5,3 a	14,9 a	5,2 a	14,9 a	4,2 a	13,6 ab	5,1 ab	14,2 ab
Non-sprayed	4,4 b	12,8 b	5,0 a	13,8 b	4,5 a	12,8 b	5,0 b	13,8 b

Fir. Firmness kg/cm^2; SSC. Soluble solid concentration (%)

Preventive effect of ARs on artificially inoculated fruit

P. expansum

Treatments with AR 1, AR 2 and AR 3 (0.025%, 0.1% and 0.2%) decreased lesion area diameter caused due to inoculation with *P. expansum* by 27%, 44% and 61% respectively in comparison with non treated fruit (Fig 8). A very strong correlation was found between ARs concentration and leasion area diameter (Table 7). However, treatment with Ref emulsion also decreased lesion area by 12% in comparison with non-treated fruit (***see attached 2***).

M. fructigena

Treatments with AR 1, AR 2 and AR 3 decreased lesion area diameter caused due to inoculation with *M. fructigena* by 16%, 24% and 29% respectively in comparison with non treated fruit (Fig 8). No significant differenc was found between AR 1 and AR2 as well as bwteen AR 2 and AR 3, mentioned a weak correlation between ARs concentration and leasion area diameter (Table 7). Treatment with Ref emulsion had not effect on lesion area diameter.

N. perennans

Treatments with AR 1, AR 2 and AR 3 decreased lesion area diameter caused due to inoculation with *N. perennans* by 19%, 30% and 40% respectively in comparison with non treated fruit (Fig 8). A clear correlation was found between ARs concentration and leasion area diameter (Table 7). AR2 and AR 3 showed smaller lesion area. Treatment with Ref emulsion did not affect the decay occurrance due to inoculation with *N. Perennans* (***see attached 3***)

In the whole experiment, organic Amorosa showed clear correlation between the susceptibility to the three pathogens (Table 7).

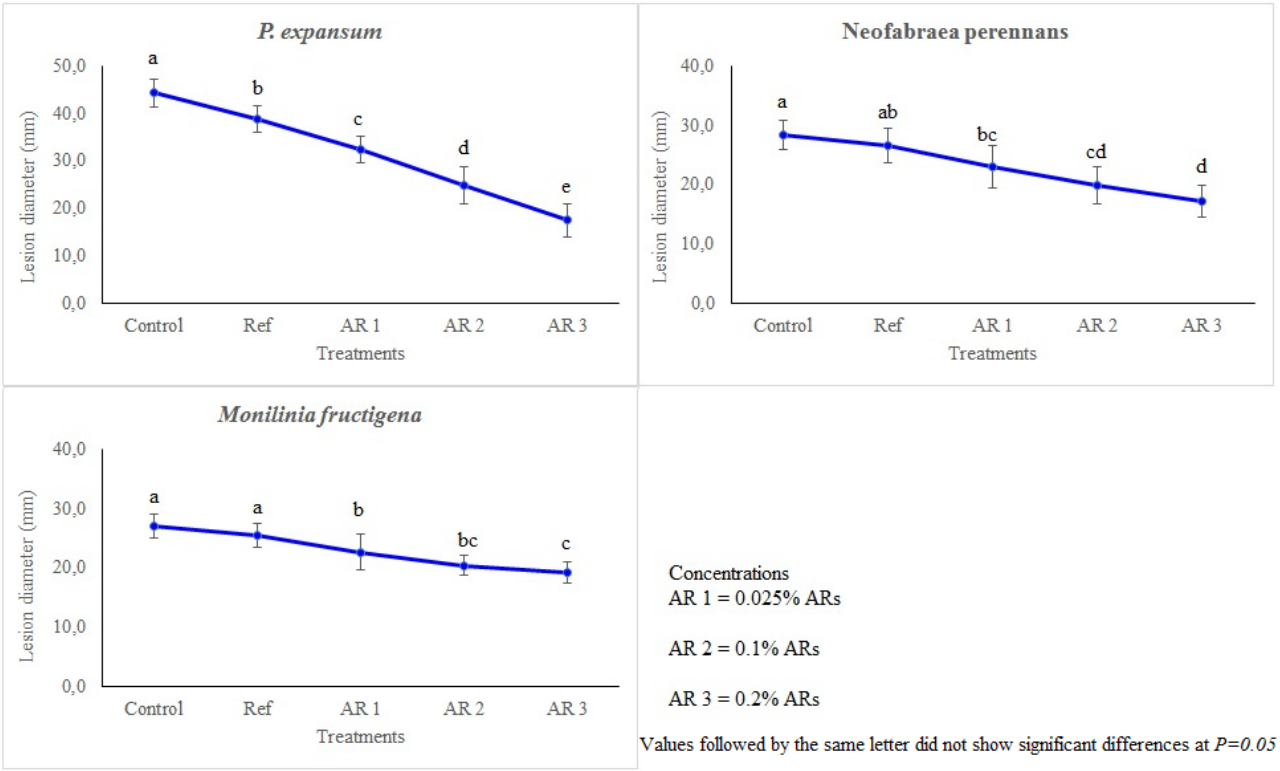


Fig.8. Effect of postharvest treatment on lesion area diameter caused by inoculation with three fungi (2015).

Table 7. Correlations between ARs treatments and fungal decay caused by inoculation with different fungi (2015).

Factors	Correlations between pathogen and decay occurrance			Correlation between ARs concentrations and decay occurrance		
	P.ex & M.f	P.ex & N.p	M.f & N.p	Diameter & P.ex	Diameter & P.ex	Diameter & P.ex
r	0,56	0,557	0,388	-0,778	-0,50	-0,528
P	0,000	0,000	0,000	0,000	0,000	0,000
Equetion	5,2+1,15x	11,0+0,89x	16,6+0,27x	39,4-119x	25,0-33,2x	26,1-49,1x
R^2	25,6%	31,1%	15,1%	61%	25%	28%

P.ex= Penicillium expansum; M.f.= Monilinia fructigena; N.p.= Neofabraea perennans; Diameter= Lesion area diameter.

In vitro antifungal activity of ARs treatment

Effect on mycelial growth

In vitro assays showed a significant antifungal activity of ARs emulsions. Mycelial growth of *P. expansum* was decreased due to treatments with AR 1, AR 2 and AR 3 by 51%, 83% and 86% respectively in comparison with non-treated plates. Ref emulsion also decreased mycelial growth of this pathogen in lower level (23%) (Fig. 9). The same inhabitory effects on mycelial growth of *C. acutatum* were found whereas treatments with AR 1, AR 2 and AR 3 decreased the growth by 58%, 73% and 80% in comparison with non-treated plates (Fig 9). Ref emulsion also had significant effect on mycelial growth of this pathogen which was decreased by 15%. Mycelial growth of *N. Perennans* decreased by 74%, 85% and 90% when the plates were treated by AR 1, AR 2 and AR 3 respectively (Fig 9). Ref emulsion decreased mycelial growth of this pathogen by 12%. (***See attached 4***)

Strong correlations between ARs concentration and mycelial growth of the three pathogens were found (Table 7). However, no significal differences were found between AR 2 and AR3 whereas both concentrations caused the highest inhabition (Table 7).

Effect on conidia viability

Viability of *P. expansum* conidia was inhibited by 46%, 72% and 79% due to treatments with AR 1, AR 2 and AR 3 respectively in comparison with control (Fig 10). Ref emulsion had no effect on conidia viability of this pathogen. No significant difference was found between AR 2 and AR 3 which were caused the highest inhibition.

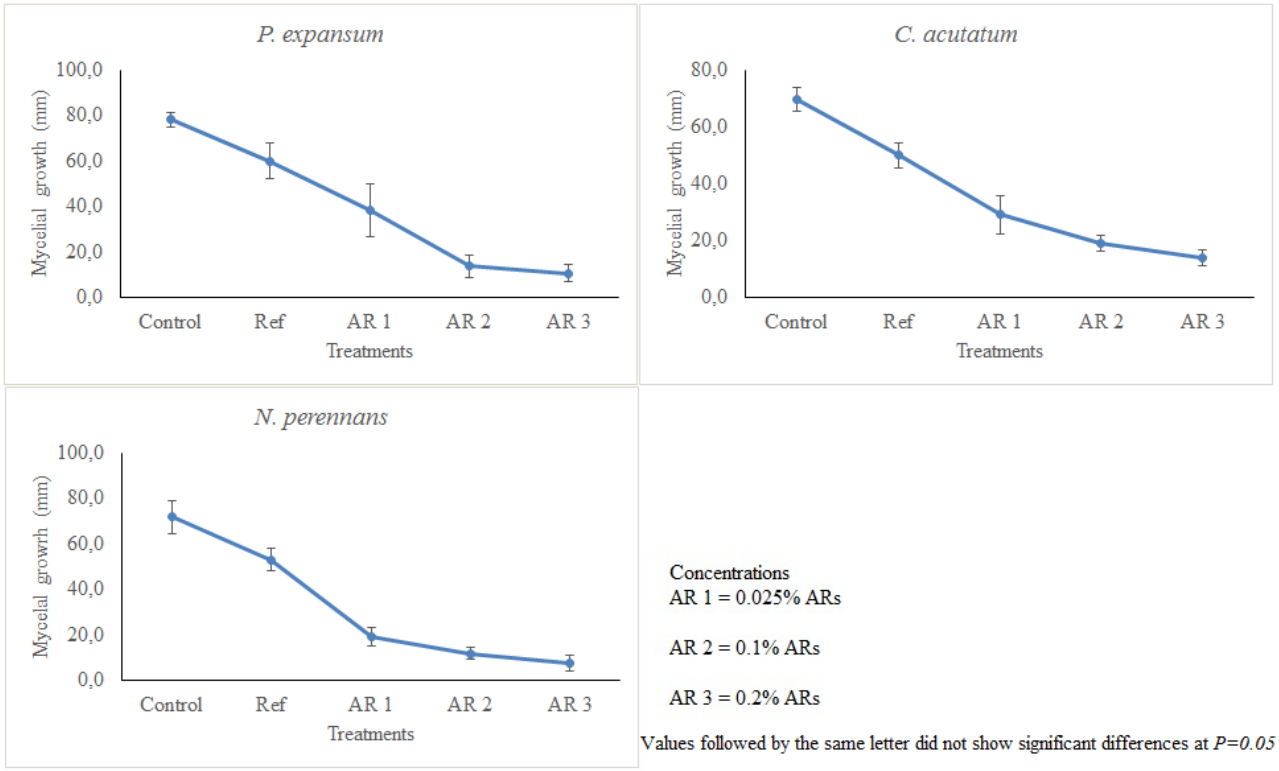


Fig. 9. Effect of treatments with various ARs concentrations on mycelial growth of different pathogens (2015).

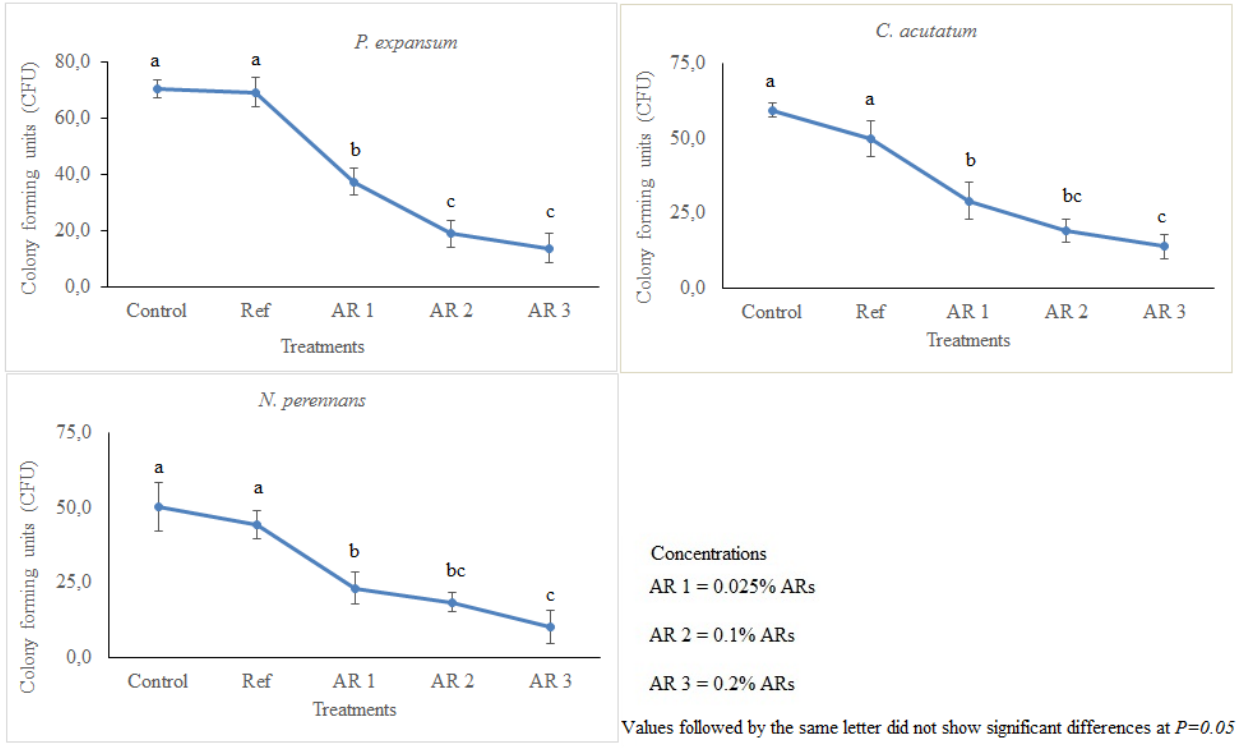


Fig. 10. Effect of treatments with various ARs concentrations on conidia vialbility of different pathogens (2015).

ARs treatwment decreased the viability of *C. acutatum* conidia. AR 1, AR 2 and AR 3 decreased this viability by 38%, 60% and 74% respectively in comparsion with non-treated plates (Fig 10). Again Ref emulsion had no effect and the inhibitory effect of AR 2 and AR 3 was the same (Fig 10).

Conidia of *N. perennans* also lost their viability when they were treated with ARs. Treatment with AR1, AR 2 and AR3 decreased the viability by 54%, 68% and 80% (Fig 10). AR 3 showed the highest inhibitory effect.

Clear correlations were found between the AR concentration and the three pathogen viability (Table 7). The lowest concentration (AR 1, 0.025%) had alwayes the lowest inhibitory effect among the ARs treatments.

In all trials, mycelium colour was changed to be more pale and some time brown. Treated fruit did not show any modification of color or morphology.

Conclusion

ARs treatment in the orchard decreased infected fallen fruit, decay occurrence and total losses during storage as well as improved fruit quality at harvest and caused better maintain of fruit quality during storage. Spraying frequency showed incompatible effects while later spraying in the season (August and September) improved the positive effect of ARs. Postharvest treatment with ARs showed a preventive effect on artificially inoculated fruit with various fungi. *In vitro* treatment of three pathogens (*P. expansum, C. acutatum* and *N. perennans*) with ARs decreased mycelial growth as well as inhibited pathogen viability. Higher ARs concentration (0.1% or 0.2%) improved the activity. However, the negative side effect of using ARs as anti-fungi agent was yield reduction. In conclusion the results of this project provides evidence that ARs has a high potential to be implemented in postharvest control strategies as a natural safe and eco-friendly extract. Evaluation of the anti-fungal effects of ARs in the orchard, in vivo and in vitro highlighted its importance as hopeful natural fungicide. Further- more, the absence of signs of possible phytotoxic effect during in vivo trials and the wide availability of the ray as a waste product of the processing factories, may facilitate the development of ARs as a commercial formulation.

Literature

Ahmadi-Afzadi M, Tahir I, Nybom H. 2013. Impact of harvesting time and fruit firmness on the tolerance to fungal storage diseases in an apple germplasm collection. Postharvest Biol. Technol. 82: 51–58.

Andersson U, Dey SE, Holm C, Degerman E. 2011. Rye bran alkylresorcinols supress adipocytes lipolysis and hormone-sensitive lipase activity. Mol. Nutrition Food Res. 55: S290–S293.

Børve J, Stensvand A. 2007. *Colletotrichum acutatum* found on apple buds in Norway. Plant Health Progress doi, 10.1094/PHP-0522-01-RS.

Dey ES, Mikhailapula K. 2009. A food grade approach for the isolation of major alkylresorcinols from rye bran applying tailored supercritical carbon dixodie ($scCO_2$) extraction combined with HPLC 51: 167–173.

Dey ES, Ahmadi-Afzadi M, Nybom H, Tahir I. 2013. Alkylresorcinols isolated from rye bran by supercritical fluid of carbon dioxide and suspended in a food-grade emulsion show activity against *Penicillium expansum* on apples. Arch. Phytopathol. Plant Protect. 46: 105–119

Droby S, Prusky D, Jacoby B, Goldman A. 1987. Induction of antifungal resorcinols in flesh of unripe mango fruits and its relation to latent infection by *Alternaria alternata*. Physiol. Mol. Plant Pathol. 30: 285–292.

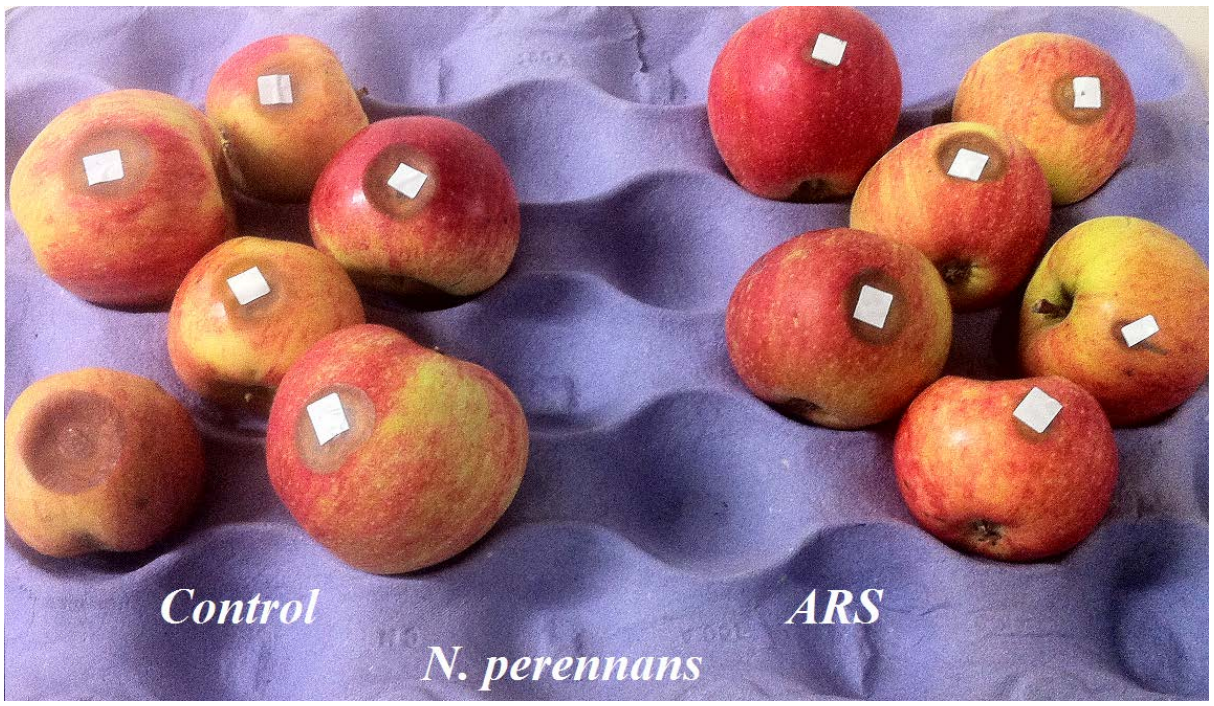
Hassan MK, Dann EK, Irving DE, Coates LM. 2007. Concentrations of constitutive alk(en)ylresorcinol in peel of commercial mango varieties and resistance to postharvest anthracnose. Physiol. Mol. Plant Pathol. 71: 158–165.

Tahir I, Ahmadi-Afzadi M, Nybom H, Dey ES. Alkylresorcinols isolated from rye bran inhibit growth of *Penicillium expansum* and *Neofabraea perennans* in vitro and on fungal-inoculated fruits of four apple cultivars. Europ. J. Hort. Sci. 79: 218–225.

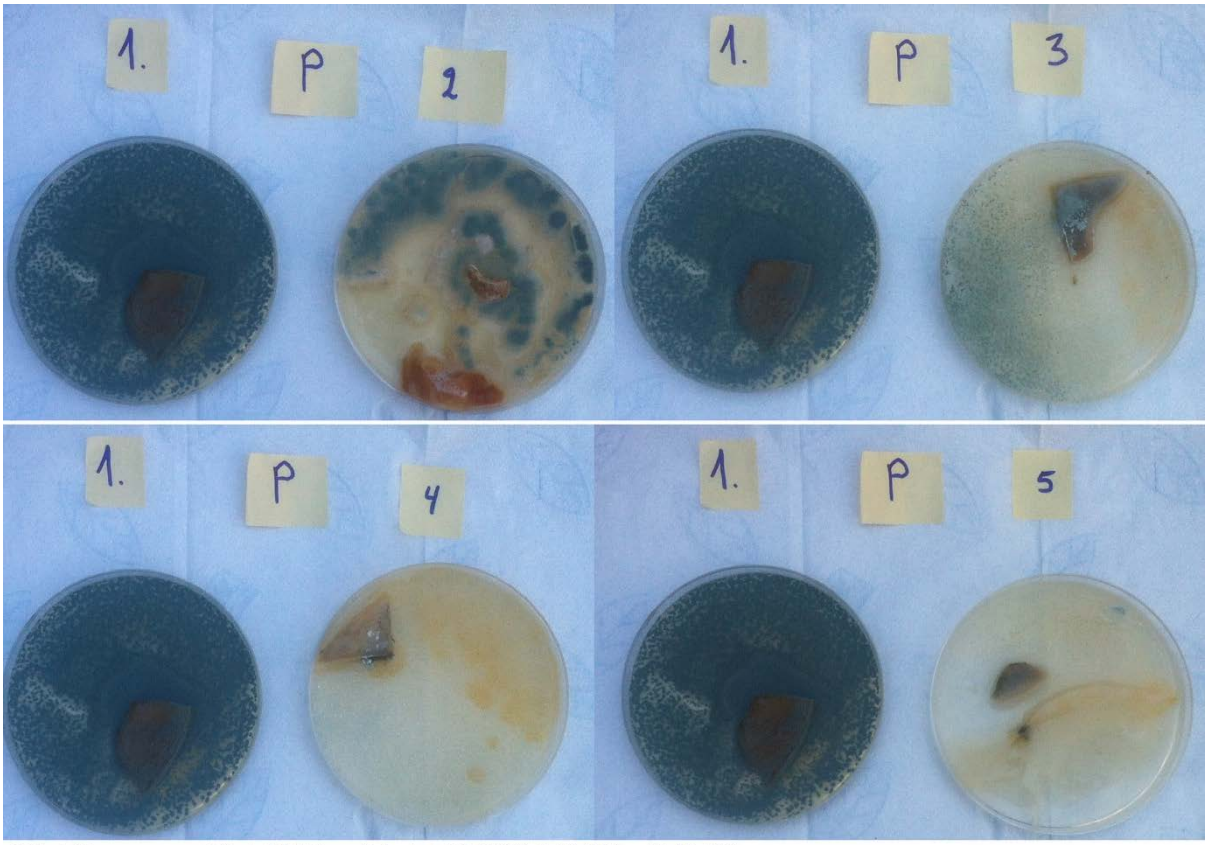
Weber RWS, Palm G. 2010. Resistance of storage rot fungi *Neofabraea perennans*, *N. alba*, *Glomerella acutata* and *Neonectria galligena* against thiophanate-methyl in Northern German apple production. J. Plant Dis. Protect. 117: 185–191.

Attached 1. Spraying trees with ARs once in September (C) or twice in June and August (D) decreased fungal decay during storage.

Attached 2. High ARs concentrations decreased lesion area diameter caused by P. expansum

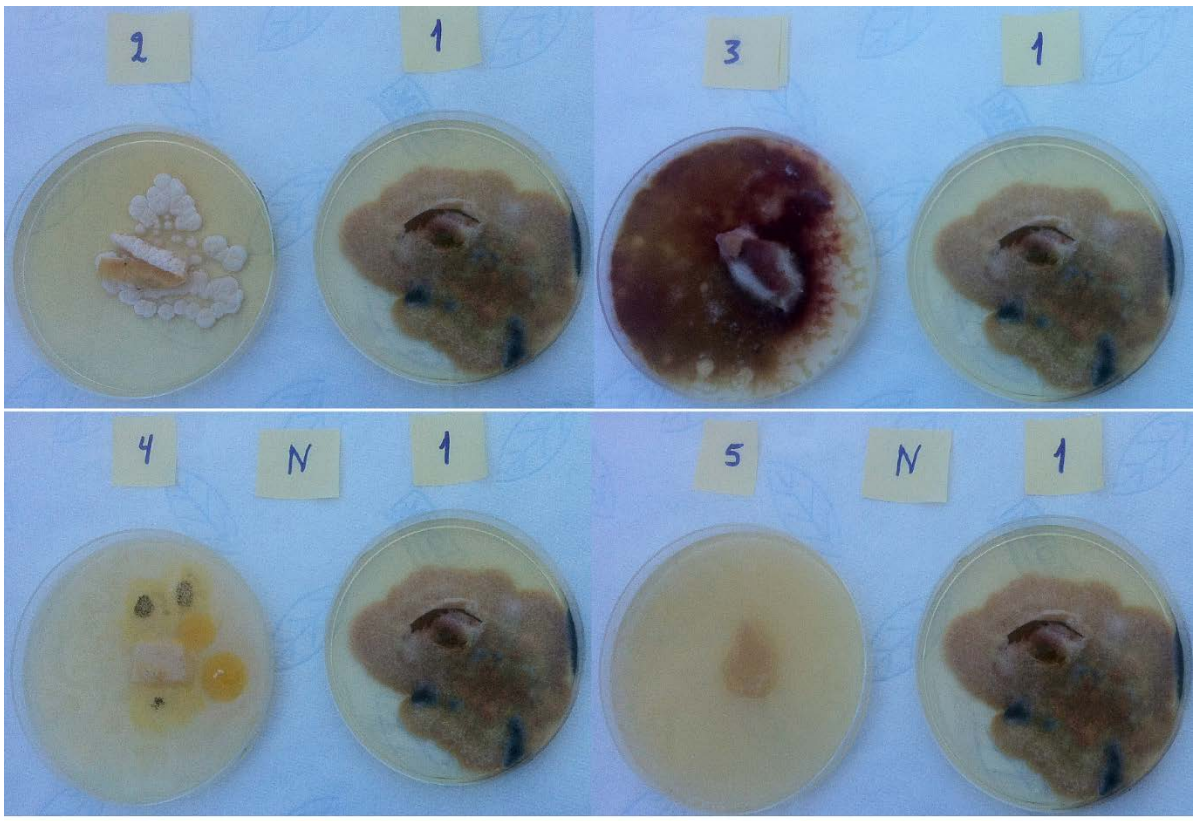


Attached 3. High ARs concentrations decreased lesion area diameter caused by N. perennans



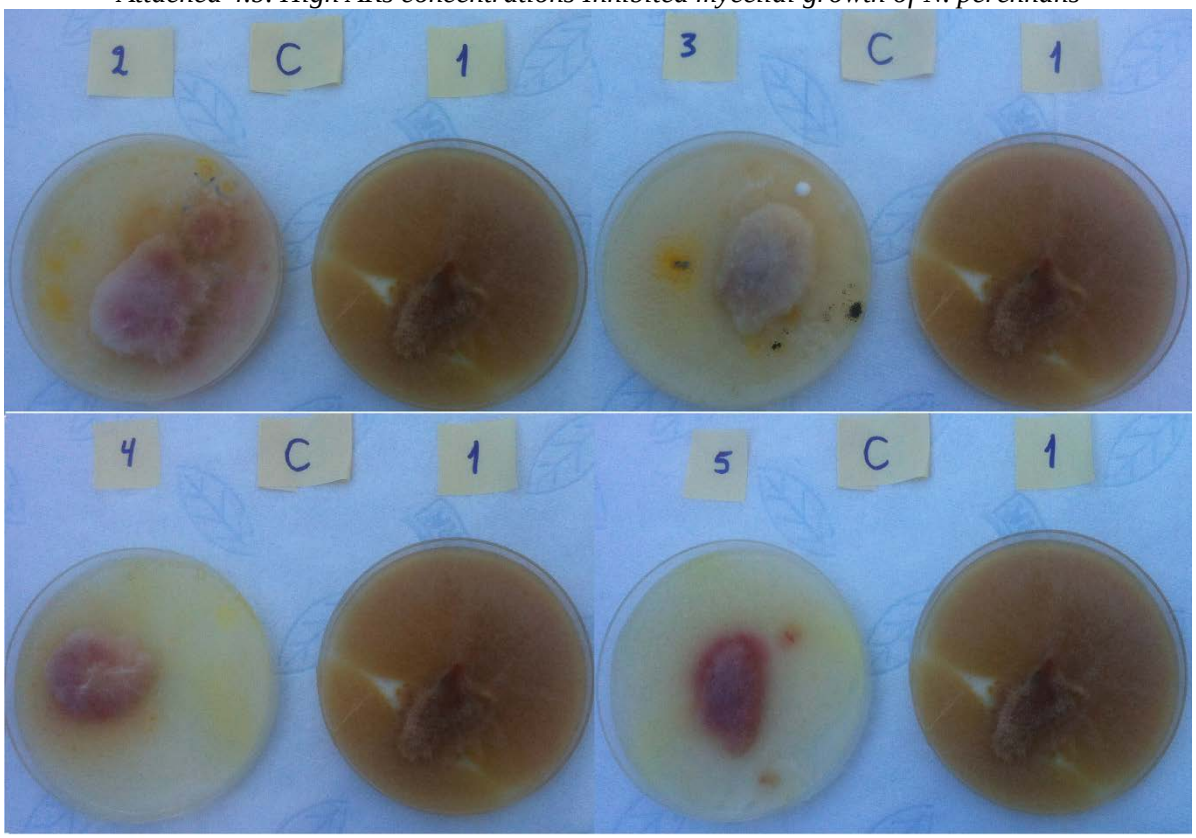
P. *Penicillium expansum* **1. Control, 2. Reference/solvents, 3. ARs 0.025%, 4. ARs 0.1% and 5. ARs 0.2%.**

Attached 4.a. High ARs concentrations inhibited mycelial growth of P. expansum



N. Neofabarea perennans 1. Control, 2. Reference/solvents, 3. ARs 0.025%, 4. ARs 0.1% and 5. ARs 0.2%.

Attached 4.b. High ARs concentrations Inhibited mycelial growth of N. perennans



C. Colletotrichum acutatum 1. Control, 2. Reference/solvents, 3. ARs 0.025%, 4. ARs 0.1% and 5. ARs 0.2%.

Attached 4.c. High ARs concentrations inhibited mycelial growth of C. acutatum