

Prevention and management of postharvest diseases with physical means

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Thessaloniki, 23/05/2025

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ARISTOTLE
UNIVERSITY OF
THESSALONIKI

cost
COOPERATION
SCIENCE & TECHNOLOGY



The new European plant health regulation (EU 2016/2031) jointly with the

“European Green Deal”



The 2021-2030 Agenda of 17 Sustainable Development Goals (SDGs)

aims to



- reduction of 50% of pesticides
- increment by 25% of the land used for organic farming

Aspire™



In this regard, microorganisms and Physical means (Heat treatments) can represent an ecological and safe strategy to adopt in agriculture

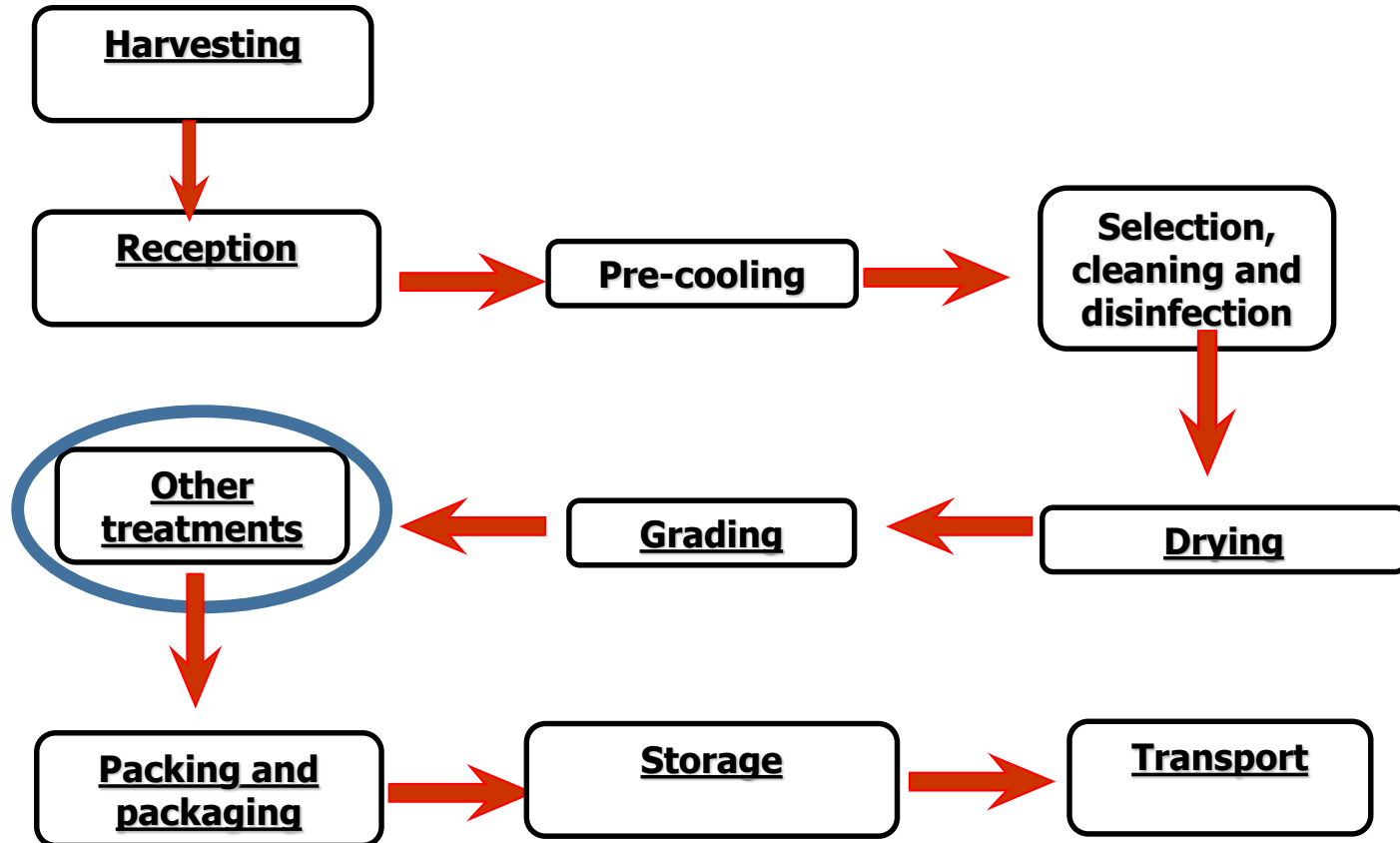
Botector™



Biosave™



Fundamental Steps of Postharvest



Other Treatments



- DA-meter
- **Heat treatments**
- Botanicals
- Biological control agents

Postharvest control strategies

Fungicide treatment	NO!!!!
Sanitation practice	YES!!!
<u>Alternative approach ?</u>	YES!!!

In postharvest phases, **physical means** and in particular **heat treatments** (HTs) may represent a valid approach due to the versatility with which they can be applied



Hot Water dips, rinses or brushing



Vapour and Hot air

HTs can have a twofold effectiveness against fungal diseases: preventative and curative

Type of **fruit**, **temperature**, **time** of exposure to the heat source and **disease** to control

Heat Treatments



HOT WATER:

- immersion in HW or spraying on fruits
- T° between 45 and 60 °C for up to 10 min
- Spraying consists of a pressured spray of HW, often part of a working line where fruits are moved by brush rollers



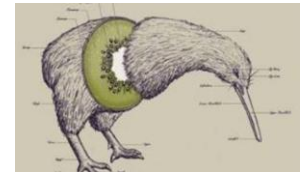
HOT AIR:

- 12 to 96 h and 38 to 46 °C
- changing in function of the **heat transfer, fruit size, and sensitivity**
- Slow heat transfer (small size fruits)
- HTs if not uniform can cause fruit quality damage in firmness and colour

CURING: very simple to apply leaving bins-containing fruit for the required time under a shelter, is adopted in the global kiwi industry to control gray mold (Mari et al. 2015)

OR

2-3 days in an air atmosphere heated to temperatures higher than 30 °C and a RH higher than 90% (Palou, 2013)



HTs: Mechanisms of Action

1) Fungal pathogen inhibition

On fungal spore germination and mycelial growth on fruit surface, so reducing the microbial epiphytic population:

- Direct action
- Accumulating ROS, damage of proteins and lipids

Pathogen	T°	Time
Diversi batteri	60-70	10
Batteri resistenti al calore	90	30
<i>Didymella lycopersici</i>	50	30
<i>F. oxysporum</i> f.sp. <i>dianthi</i>	60	30
<i>F. oxysporum</i> f.sp. <i>gladioli</i>	57	30
<i>Pythium</i> sp.	53	30
<i>P. irregulare</i>	46	20-40
<i>P. ultimum</i>	53	30
<i>R. solani</i>	50	5
<i>Sclerotinia sclerotiorum</i>	53	30
<i>Verticillium albo-atrum</i>	58	30
<i>V. dahliae</i>	90	30
Molti attinomiceti	100	15
Diversi virus	70-80	15
Diverse infestanti	60-70	30

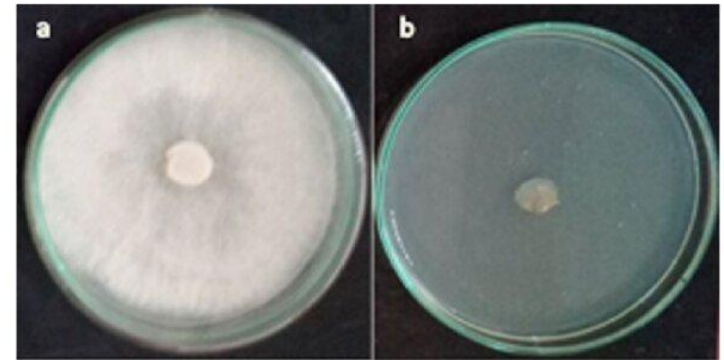
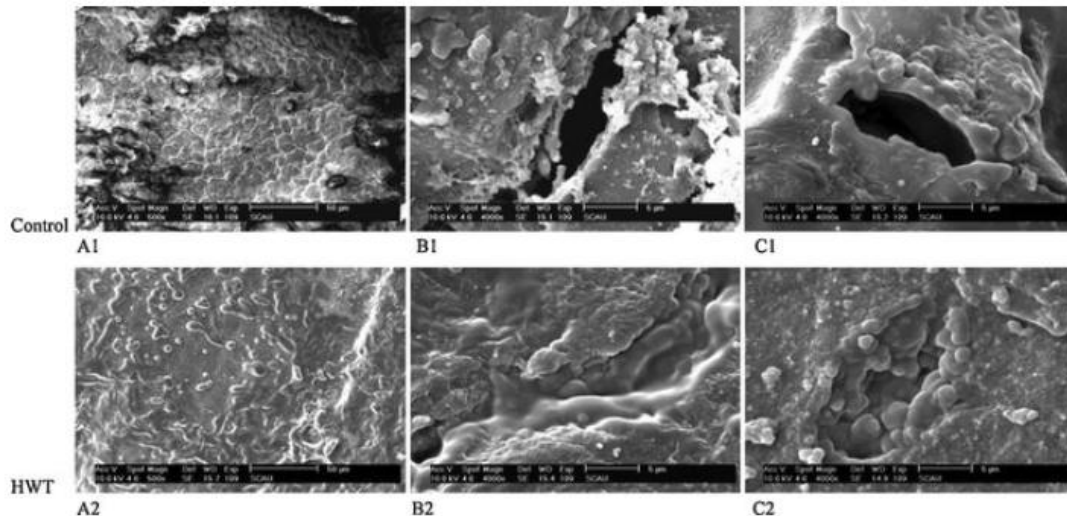


Fig. 3 The *in vitro* sensitivity of *Botrytis cinerea* to hot water treatment (HWT) using agar disk assay. a Untreated agar disk of the pathogen seeded on PDA medium and b hot water-treated (56 °C for 10 s) (Elshahawi et al., 2023)

Wet spores are more sensitive than dry ones to HTs (as well as the germinated than non-germinated ones)

2) Host structural and physiological responses

- sealing fruit entry points with epicuticular waxes (stomata and microcracks)
- host tissues lignification (Bhuiyan et al., 2009)
- improve level of phenolic compounds and increase resistance to fungal pathogen and mycotoxin buildup (Sanzani et al., 2009)

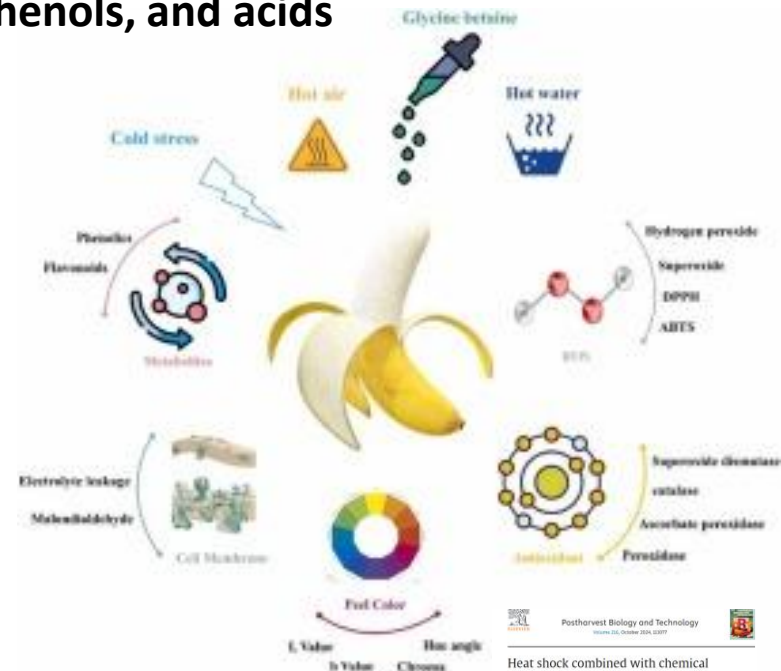


The cracks and most stomata appeared to be partially or completely plugged by the melted wax, thereby providing a mechanical barrier against wound pathogens
(54°C × 4 min)



3) Host genes regulation

- **Heat Shock Proteins (HSPs)** a family of proteins regulated by the heat shock transcription factors (HSTFs) that **perceive abiotic stresses by activating a protection**
- **Pathogenesis Related (PR) proteins**, significantly detectable in response to important stress factors and in particular in response to fungal pathogen infection
- Activating host antioxidant systems such as **catalase (CAT)**, **peroxidase (POD)**, and **superoxide dismutase (SOD)**
- **Genes involved in fruit metabolism of sugars, polyphenols, and acids**



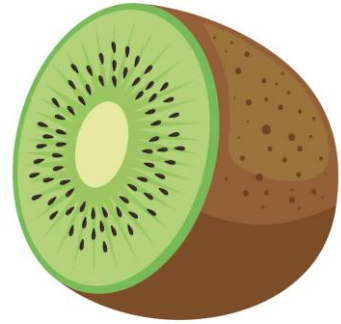
Case studies



1



2



3

Postharvest losses



Brown rot



Bull's eye rot

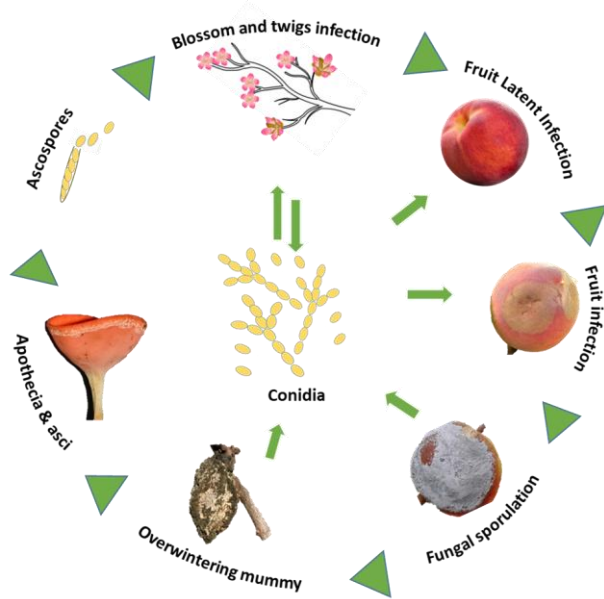


Gray mold

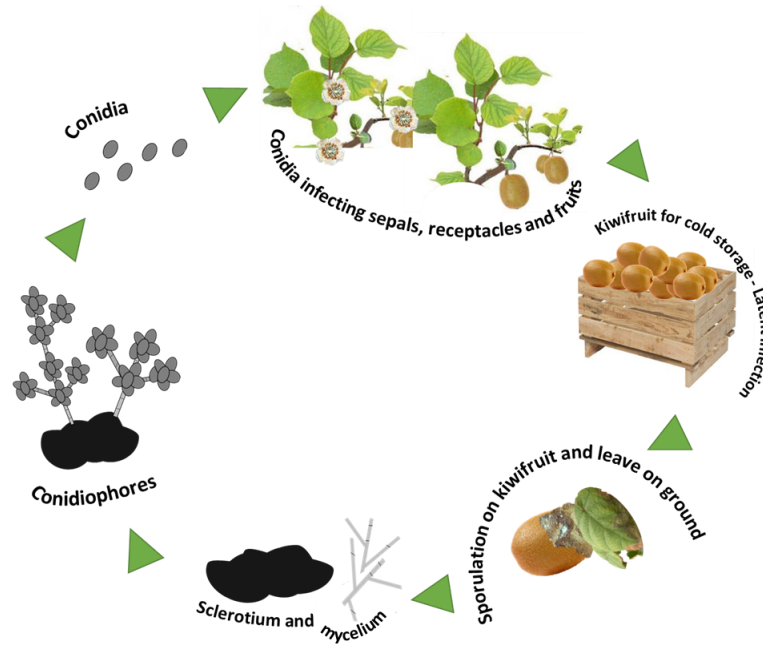


What is the main common characteristic of the three pathogens?

Neofabraea vagabunda



Monilinia spp.



Botrytis cinerea



Heat treatments for the control of postharvest decay of fresh fruit: case studies of peach brown rot, kiwifruit gray mold and citrus green and blue mold

Alessandra Di Francesco^{1*}, Antonio Ippolito², Gianfranco Romanazzi³

Heat damages (skin browning) can occur when too high or too long dips are used

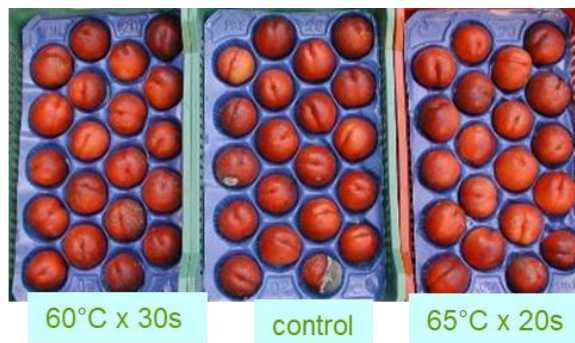


65°C x 40s



70°C x 40s

'Luckily' peach and nectarine are tolerant of HW dips, deadly to *Monilinia* rot



60°C x 30s

control

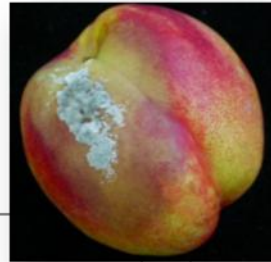
65°C x 20s



Heat treatment trials carried out by immersion in water at 60 °C for 20 and for 60 sec (~70% inhibition) (Spadoni et al., 2015) as curative treatment

Preventative application

Effect of hot water treatment applied **BEFORE** inoculation

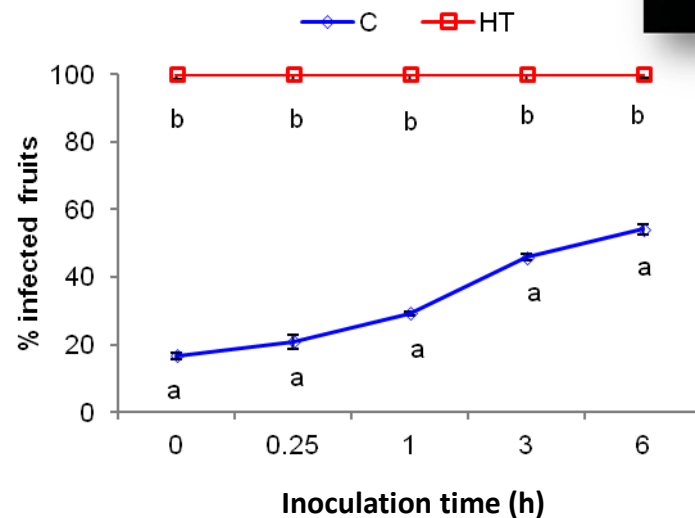


Plant Pathology (2015) 64, 1120–1129

Doi: 10.1111/ppa.12353

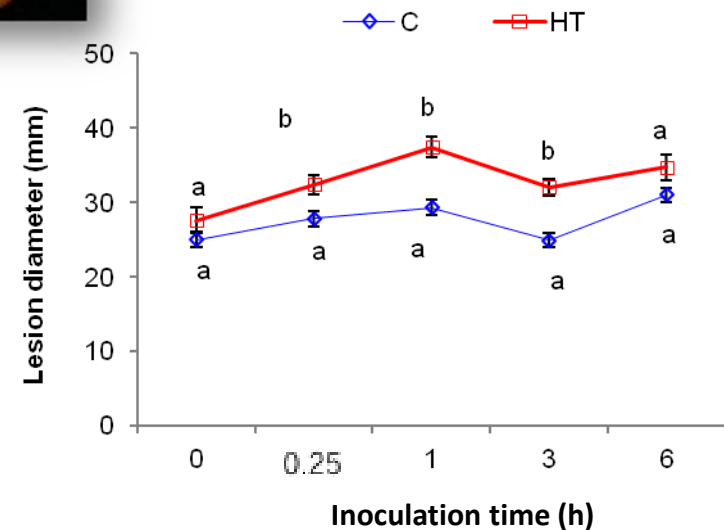
Effect of hot water treatment on peach volatile emission and *Monilinia fructicola* development

A. Spadoni^a, L. Cappellin^b, F. Neri^a, A. Algarra Alarcon^b, A. Romano^b, M. Guidarelli^b, F. Gasperi^b, F. Biasioli^b and M. Mani^{a*}



HWT 60°C×60 sec

Dipping inoculation (10^3 conidia/mL)



HWT 60°C×60 sec

Wound inoculation (1×10^3 conidia/mL)

STIMULATORY EFFECT – after 5 days at 20°C



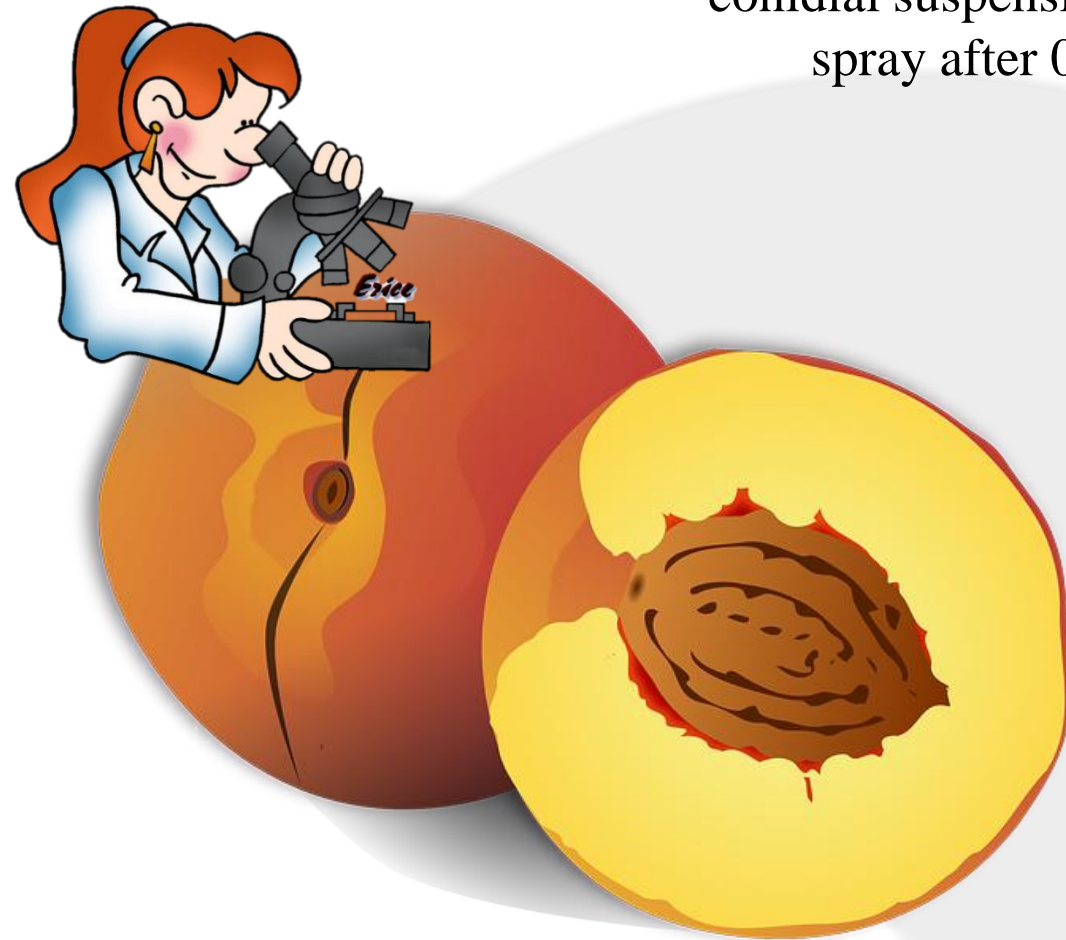
Why heat treatment has not a preventive action?

1° - HW treatment of peach fruit (60°C *60 sec) or tap water (control)

2° - Inoculation with *Monilinia fructicola* conidial suspension (10^6 conidia/mL) by spray after 0 and 24h from TRT

3° - HW and C peach fruit stored at 25°C for 18 h in a humid box

4° - Microscope analysis lactophenol blue (4×4 mm epidermal layer)



Why heat treatment has not a preventive action?

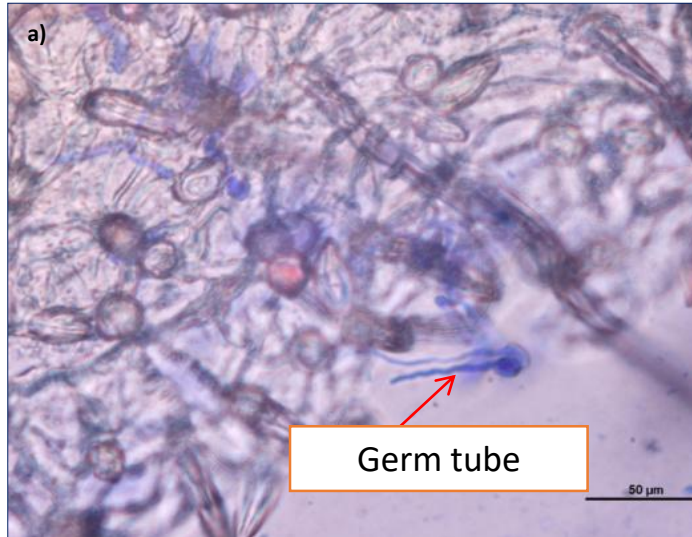


Inoculation time AFTER the HWT

0 h

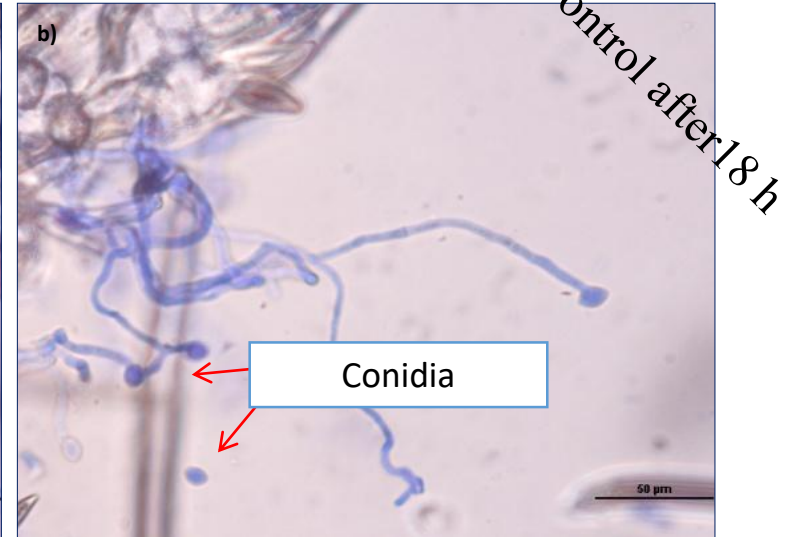
24 h

Control



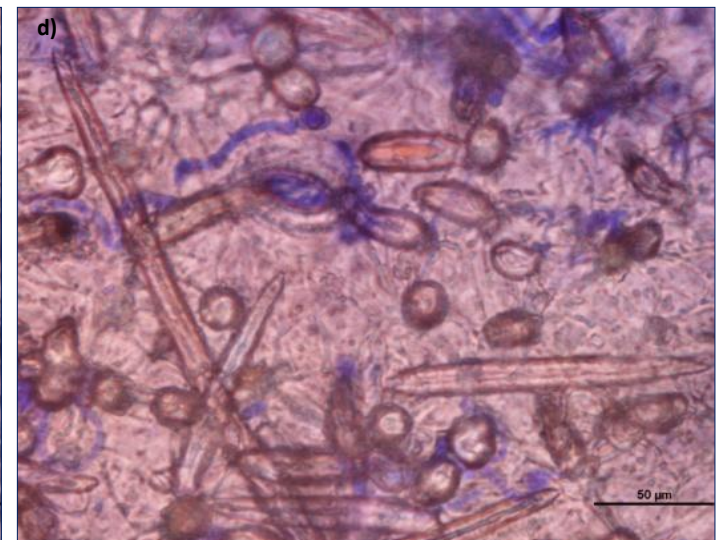
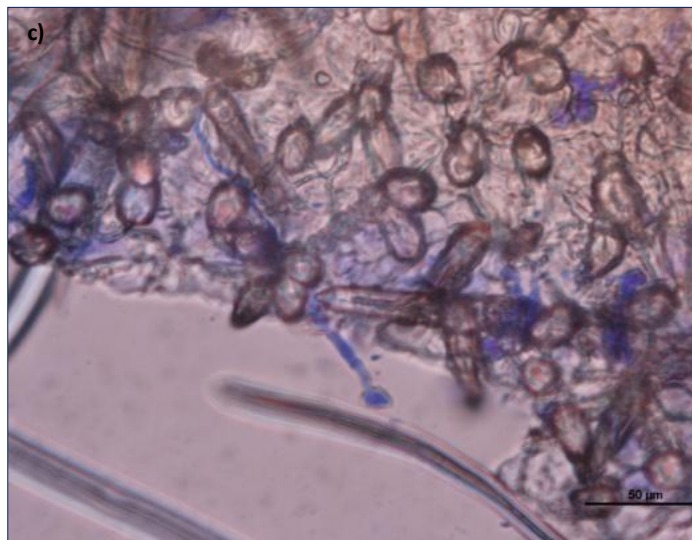
Germ tube

Heat Treated



Conidia

Control after 18 h



Why heat treatment has not a preventive action?



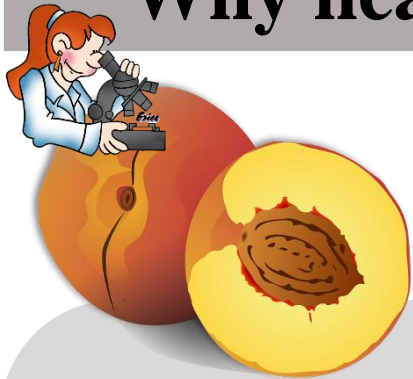
Monilinia fructicola germ tube length (μm)
after 18 h of incubation at 25°C

Time of inoculation from the treatment (h)	Control	Heat treated
0	58 \pm 4.5 a*	135 \pm 7.8 b
24	73 \pm 6.3 a	72 \pm 4.8 a

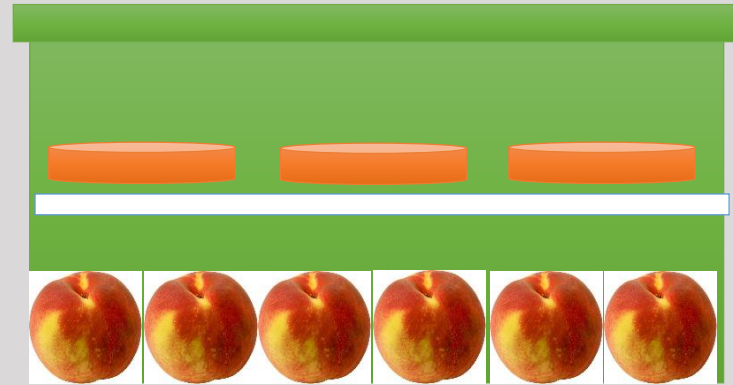
> 2 times longer



Why heat treatment has not a preventive action?



Inoculated Petri dishes overturned



Heat treated peaches

PDA Petri dishes were spread with *M. fructicola* 10^3 conidia/ mL

Cv used: Springbelle, Redhaven and Lucie Tardibelle



Why heat treatment has not a preventive action?

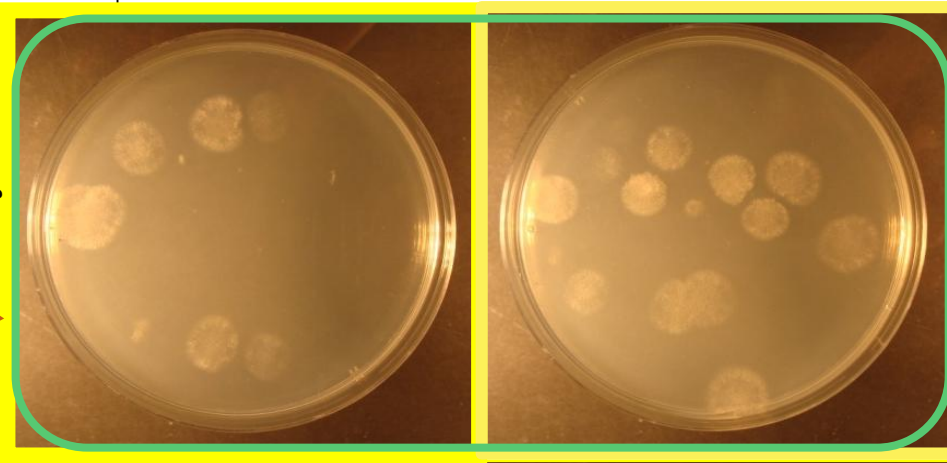
Cultivar	Control	HT	Stimulation (%)
'Springbelle'	14.1 ± 1.3 b	39.0 ± 2.8 a*	64.1
'Redhaven'	12.4 ± 1.1 b	19.8 ± 1.8 a	40.0
'Lucie'	8.3 ± 0.8 b	12.0 ± 1.3 a	33.3

Control

Heat Treated

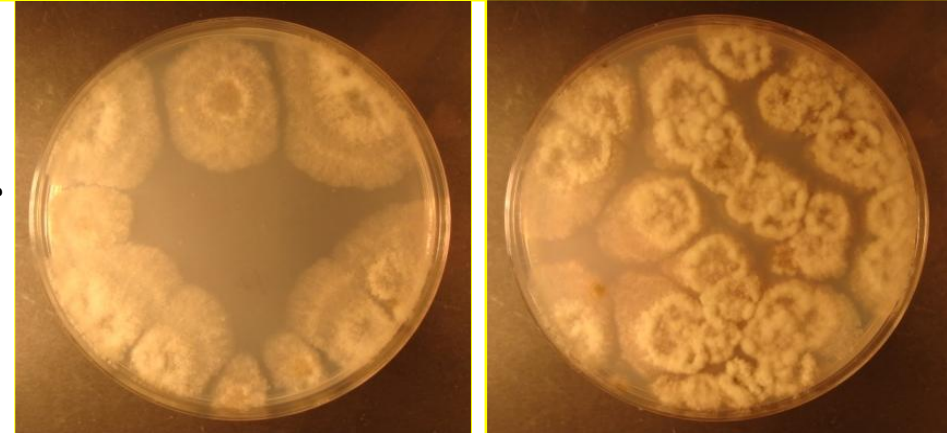
Incubation time inside the box

4 days

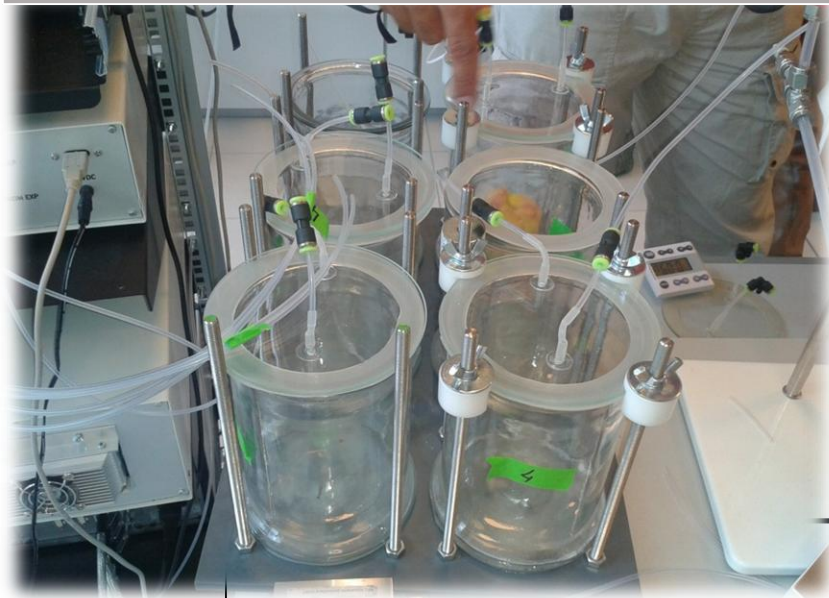


The role of peach VOCs

7 days



Why heat treatment has not a preventive action?



The role of peach VOCs

PTR-ToF-MS analysis

		CONTROL		HEAT TREATED			
Ion sum formula	Annotation	MEAN (ppbv)	SD (ppbv)	MEAN (ppbv)	SD (ppbv)	P-value	<i>P</i> <0.05
CH5O+	Methanol	95.7	76.5	84.4	54.6	0.755	
C2H5O+	Acetaldehyde	27.5	22.4	445.5	294.7	0.003	*
C2H7O+	Ethanol	5.1	3.5	143.0	96.3	0.003	*
C2H5O2+	Acetate fragment	6.3	1.1	16.5	9.8	0.018	*
C10H17+	Monoterpenes	3.6	2.0	4.4	1.7	0.887	

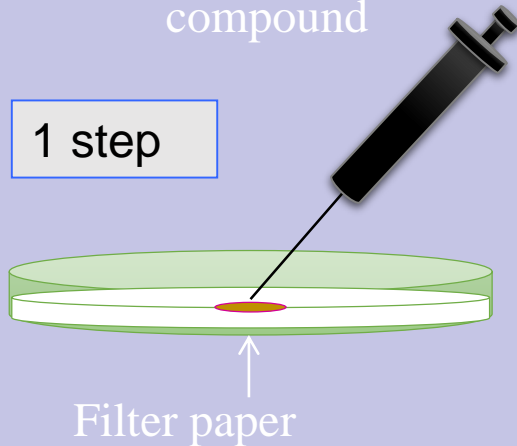


Why heat treatment has not a preventive action?

The role of peach VOCs

Liquid solution of a specific volatile compound

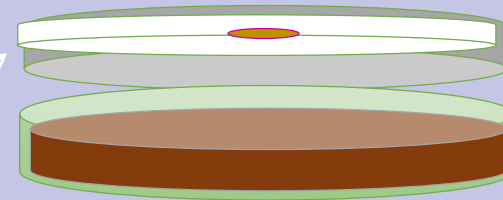
1 step



2 step

Filter paper

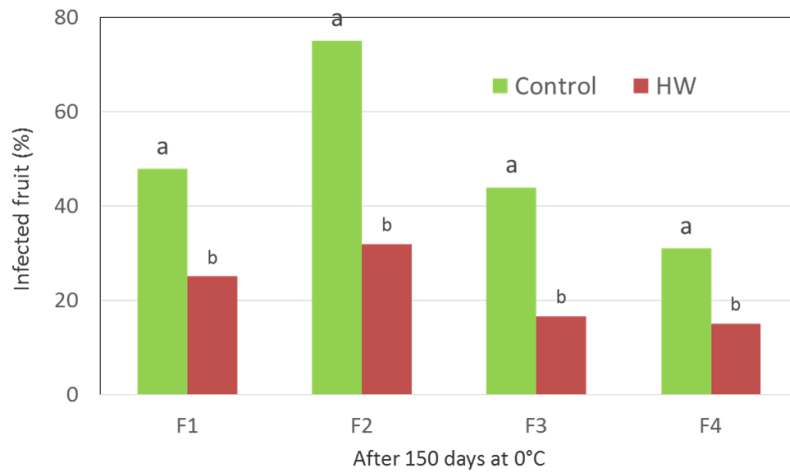
Petri dishes with *M. fructicola* conidia or mycelia



	Control	Acetaldehyde 0.6 ppm	Ethanol 0.2 ppm
CFUs	48.2 ± 6.2 a	<u>77.3 ± 2.4 b*</u>	57.5 ± 6.4 a
Ø colony (mm)	33.6 ± 1.1 a	32.0 ± 3.2 a	<u>39.0 ± 0.4 b</u>



Hot water treatment



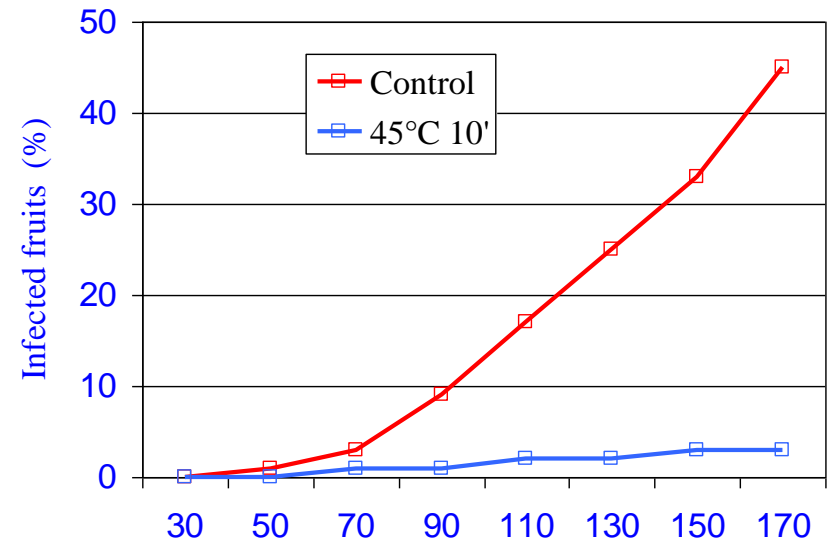
EI %

47,7%

57,4%

62,3%

51,4%



45°C × 10 min

Hot Water Enzyme Effect in Apple



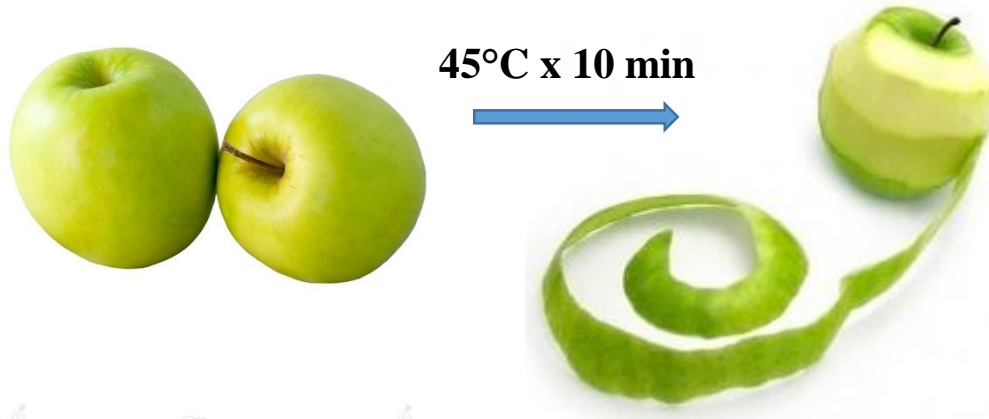
Scientia Horticulturae
Volume 227, 3 January 2018, Pages 181-186



Research Paper

Defense response against postharvest pathogens in hot water treated apples

Alessandra Di Francesco ^a, Marta Mari ^a, Roberta Roberti ^b,



0 - 3 - 6 – 24 h from HWT



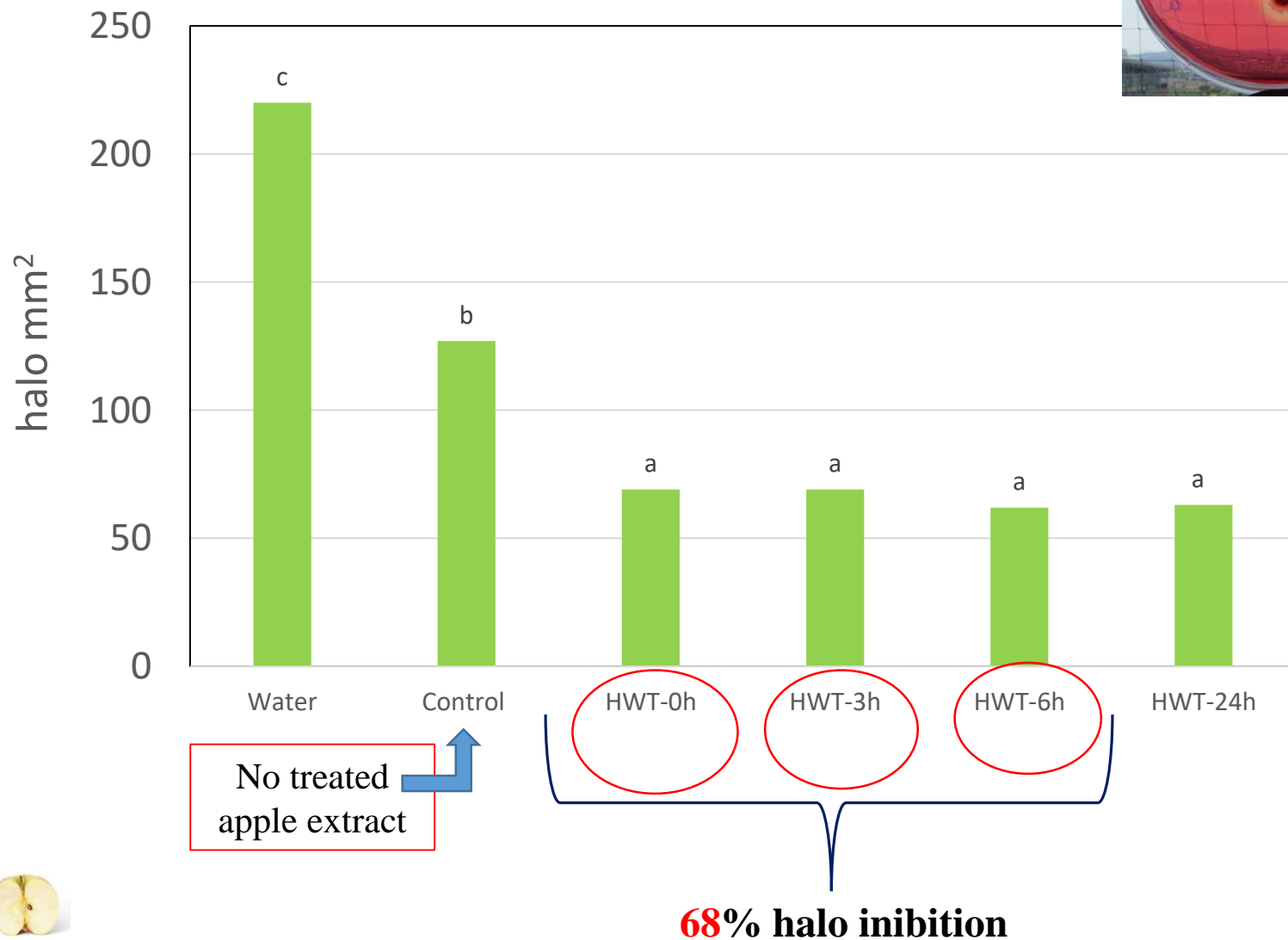
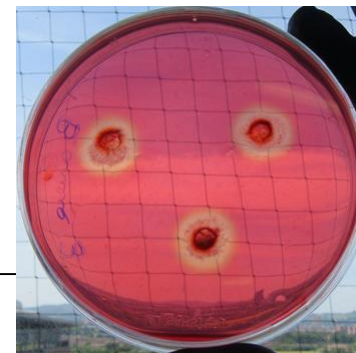
Vs

Neofabraea vagabunda pathogenic enzymes





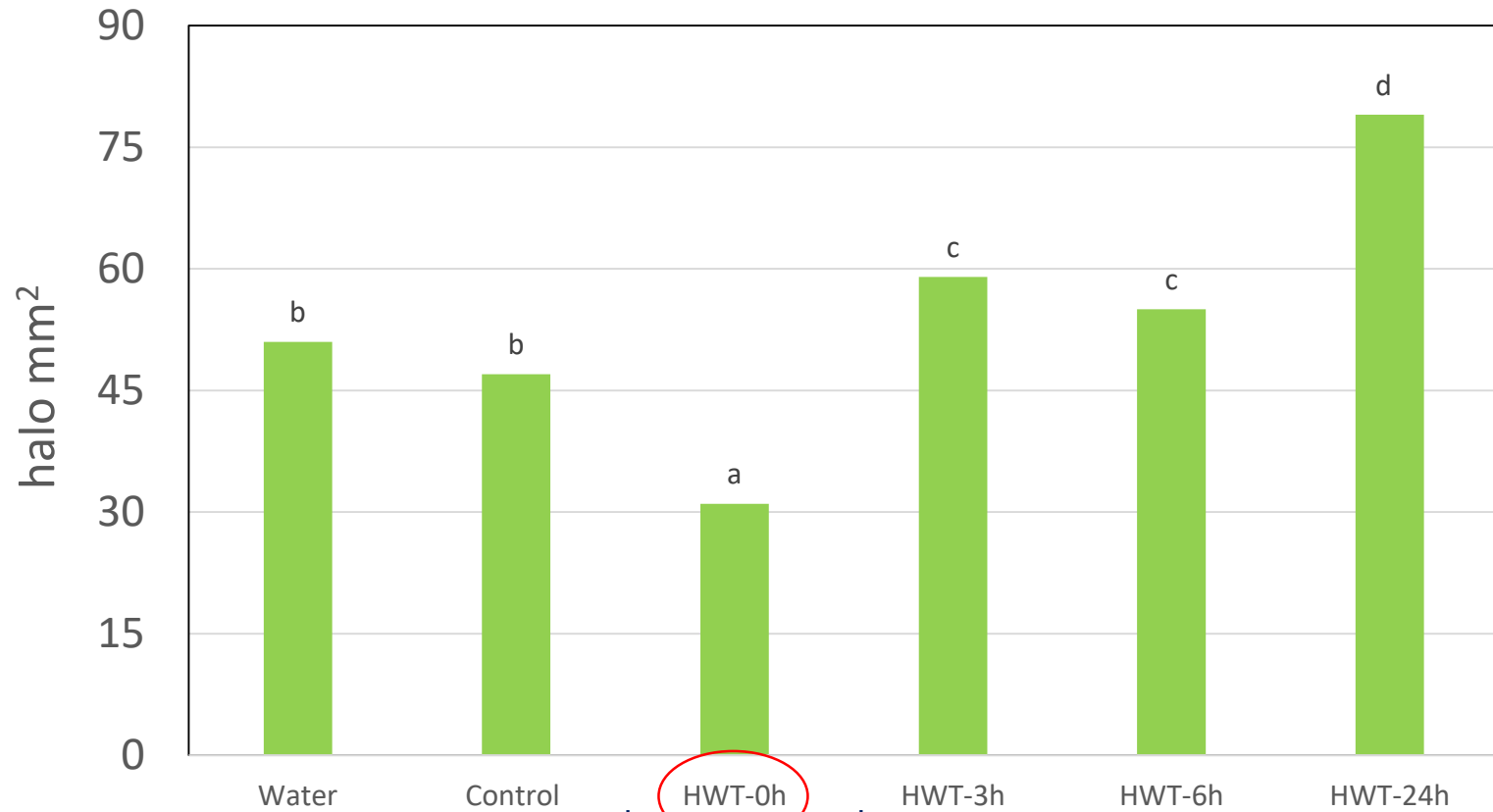
Endo-1,4-β-glucanase activity (cellulase)





2

Polygalacturonase activity



40% halo inhibition



Scientia Horticulturae
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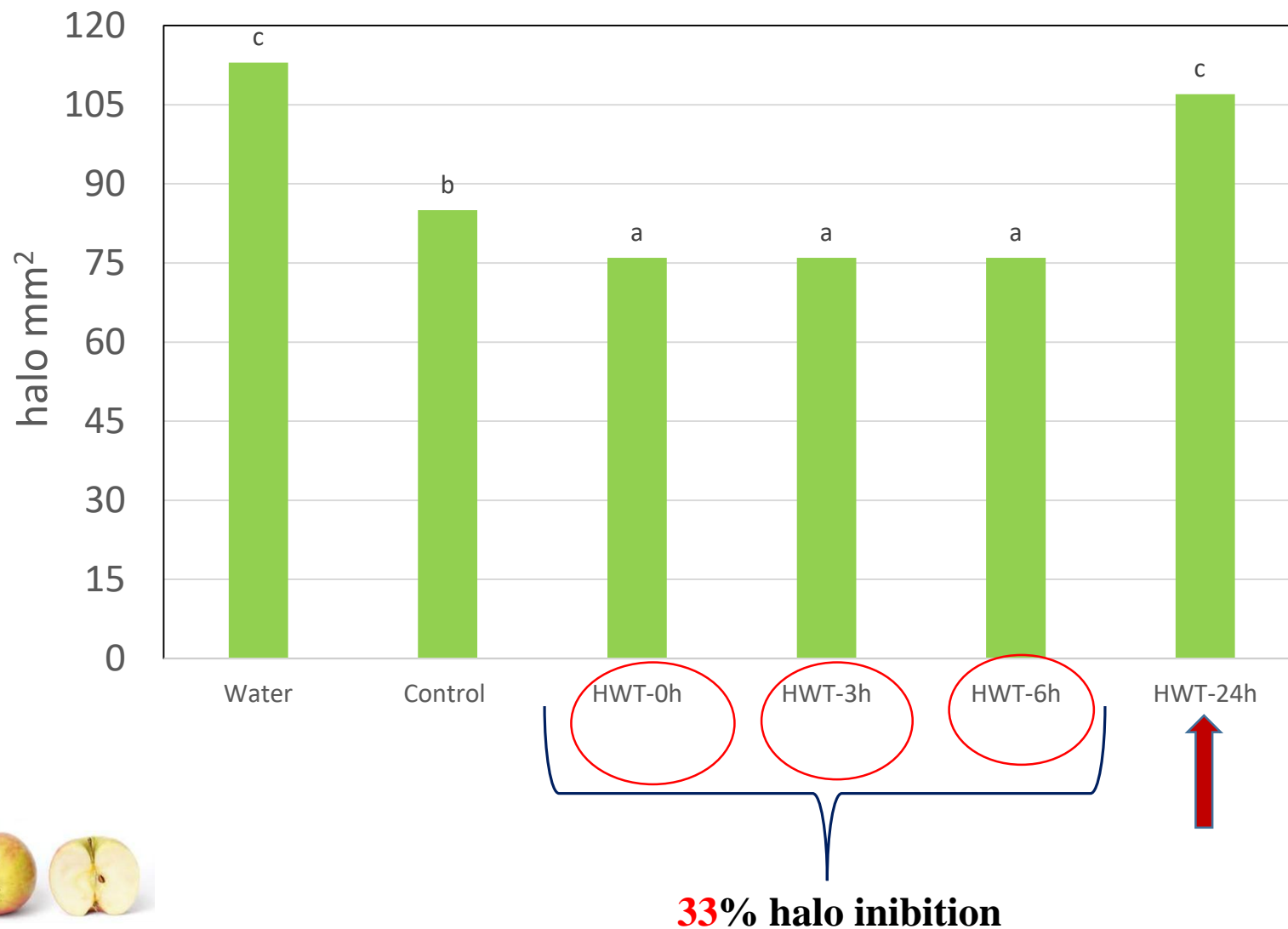
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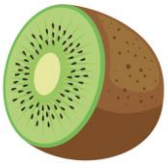
Polymethyl-galacturonase activity (pectinase)



HW dipping



50, 55, and 60 °C for 1.5 and 3 min



3

completely inhibited gray mold at the stem end of kiwifruit
(*Koukounaras et al., 2008*)

Received: 31 August 2021 | Accepted: 18 October 2021
DOI: 10.1111/ppa.13503

Plant Pathology WILEY

ORIGINAL ARTICLE

Heat treatment effect on *Cadophora luteo-olivacea* of kiwifruit

Alessandra Di Francesco¹ | Michele Di Foggia² | Amedeo Fasano¹ | Elena Baraldi¹



FT-IR analysis

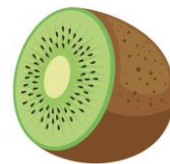
HW (70°C × 3 min) on kiwifruit skin:
waxes and cellulose bands decreased, while
phenolics, flavonoids, and glucose bands
increased



Under review

Postharvest Biology and Technology

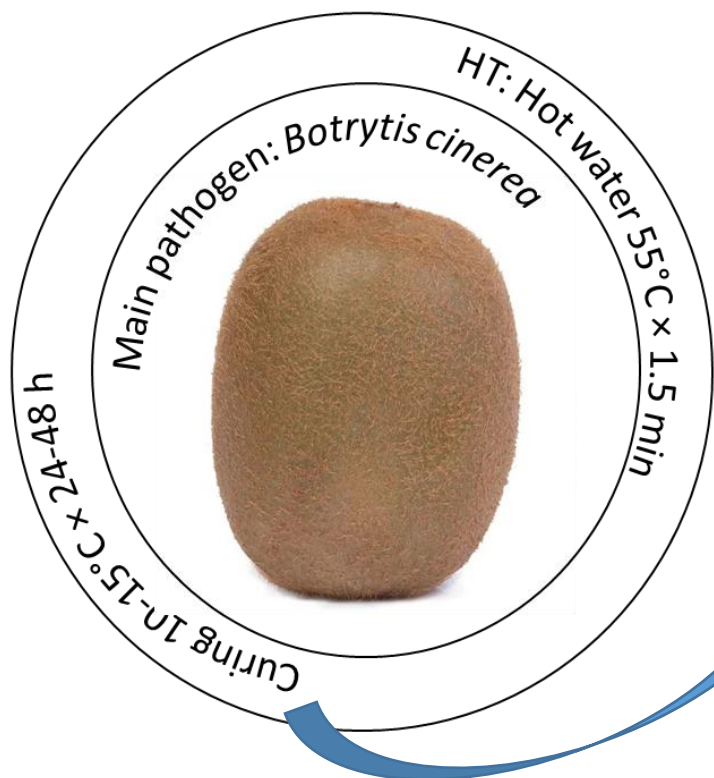
Supports open access



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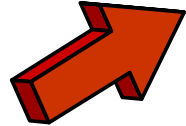
Heat treatments for the control of postharvest decay of fresh fruit: case studies of peach brown rot, kiwifruit gray mold and citrus green and blue mold

Alessandra Di Francesco^{1*}, Antonio Ippolito², Gianfranco Romanazzi³



Beneficial effect of curing:

- **chitinase, phenylalanine ammonia-lyase, polyphenoloxidase**
- **phenolic compounds** and **suberin** in stem plugs and pericarp (Ippolito et al., 1995; Wurms, 2005).
- **longer curing treatments (72-96 h) did not induce any further significant reduction of infection**



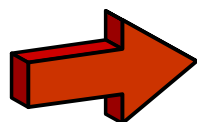
Final considerations

Why Heat Treatments?

- Safe (no fruit residue no waste, water disposal)
- Non selective vs fungal pathogens
- No registration
- Easy to apply
- Economic (recovery of heat for cooling/storage systems)



Take
Note



Final Considerations

- The development of **time × temperature combination** remains the main challenge to keep fruit quality and resistance during postharvest phases
- In some cases, HTs do not have an improving effect on fruits (preventative)

However, the Research must always be kept active to try to find **the best combinations of alternative protection systems**, given the great emergence of new fungal pathogens



Take
Note

Aiming to ensure

Environmental
sustainability

Food
security

Thanks for your attention





What is your opinion now on the strategies for preventing pre- and postharvest losses?