Alternatives methods to control postharvest diseases of mango

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Geographical situation

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Réunion Island (several projects on mango (agronomy, organic practices, post harvest, entomology)

Little production compared to big producing countries

Difficulties to export mangos

Alternatives Postharvest Treatments

Objective: Find alternatives postharvest treatments using local biodiversity and soft technology

- Post harvest treatment against fruit flies with hypoxia
- Post harvest treatment with Malagasy essential oils against mango anthracnose

Proposed approach

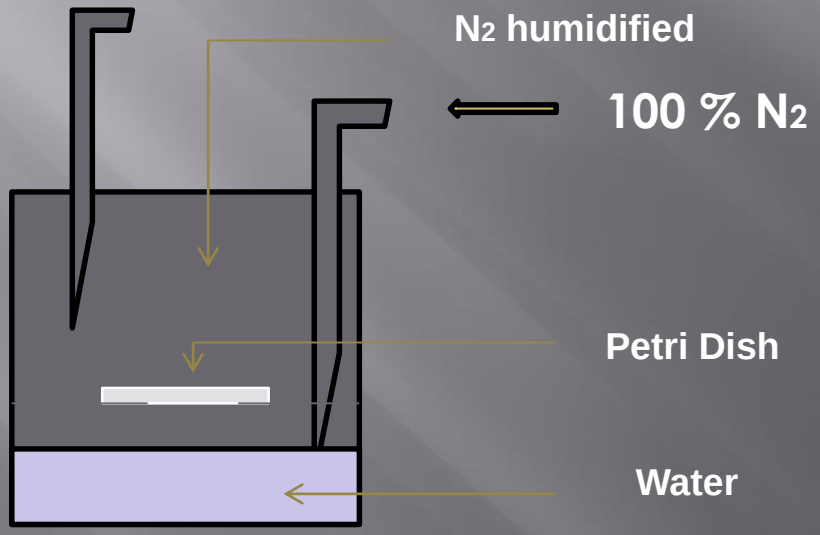
- Test in vitro on the pathogen and the pest
- Fruit physiology
  - Fruit quality
  - Studies on elicitation of resistance mechanism in fruit peel

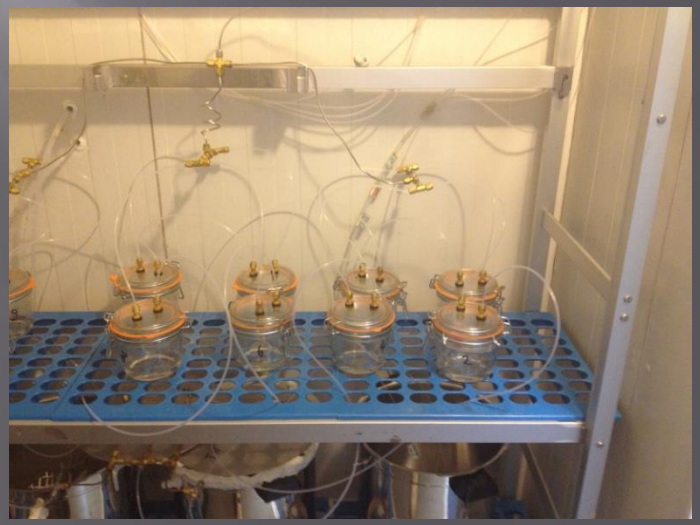
Experiments in vitro

- Experiments on hatching of *Bactrocera* eggs *in vitro*
- Experiments on *Colletrotrichum* growth from conidias, mycelium and appressoria *in vitro*

HYPOXIA on eggs in vitro

- Eggs on Petri dishes (100 eggs /d)
- Gas treatment: Nitrogen flux (to maintain 0 % of O2) to kill the eggs



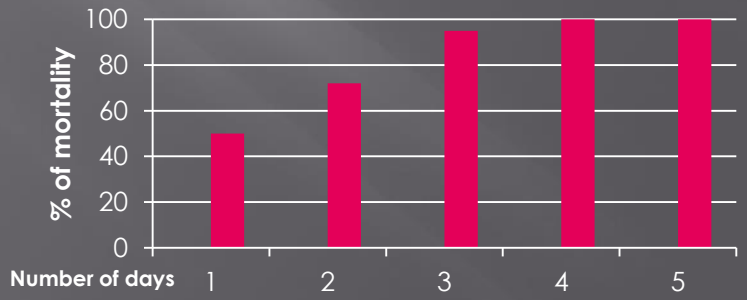


Different treatment time (1 day, 2 days, etc …) + 3 days at normal atmosphere

hatched egg counting

After hypoxia treatment, 0 % of hatched eggs

3 days after back to air



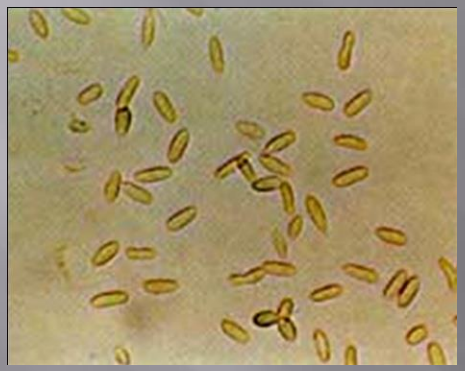
- 96 h at 21°C and 100 % HR to kill 100 % of eggs flies in vitro

Anthracnose due to *Colletotrichum gloeosporioides*

- Fungus
- Fruit pathogen (postharvest disease) but anthracnose affects leaves and flowers too
- Contamination at the field
- Necrosis during storage and ripening

One main problem for exportation

Anthracnose / *Colletotrichum gloeosporioides*

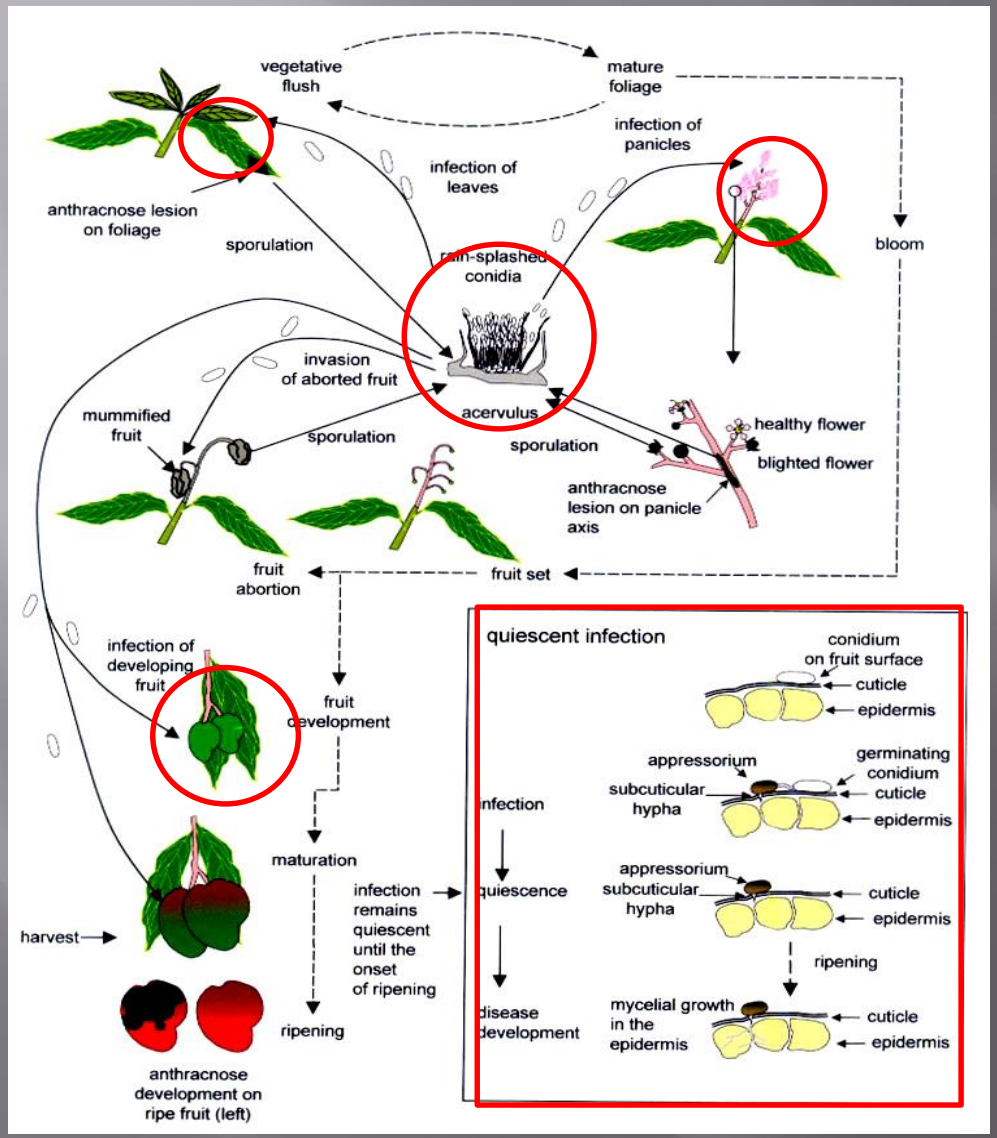


Conidias

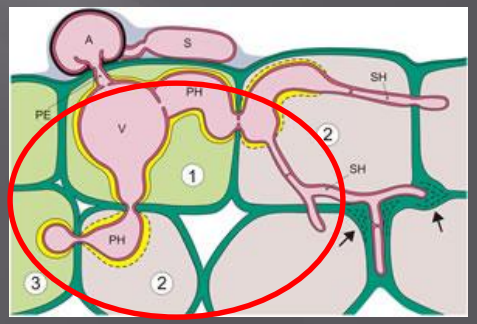
Culture on PDA

Symptoms

Anthracnose due to *Colletotrichum gloeosporioides*



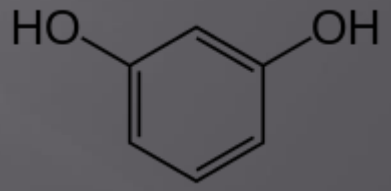
- Disease cycle
- Quiescent infection
- Appressorium formation
- Subcuticular hypha



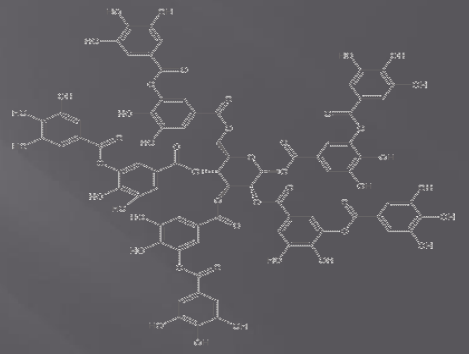
(In Araux, 2000)

Biochemical resistance of the fruit

. Resorcinols (diphenol)



. Gallotanins (Polyphenolic compounds)



. Chitinase activity

Objective: Find alternatives postharvest treatments that can be used in organic production systems

We works with : biological control, modified atmosphere, controlled atmosphere, temperature, CO2 Shock

Post harvest treatment with 2 **essential oils** against mango anthracnose

Proposed approach

- EO characterization with CG-MS
- Pathogens collection - characterization
- Test in vitro on pathogens
- Test in vivo with inoculated fruits
- Fruit quality
- Study the elicitation of resistance mechanism in fruit peel

EO characterization by CG-MS

	X2	X5
4-carène	0,18%	
carvone	60,17%	
caryophyllene	6,92%	
D-limonène	0,69%	
eugenol	30,67%	
humulène	1,26%	
propylène glycol (thymol adjuvant)		25,02%
thymol		74,98%
Y-terpinène	0,12%	

•**Collection of strains of *C. gloeosporioides* from Réunion island**

- **isolation of pathogens from necrosis on mango**
- **Molecular characterization (PCR / ITS1 and ITS4)**
- **Test of pathogenicity**

Experiments in vitro

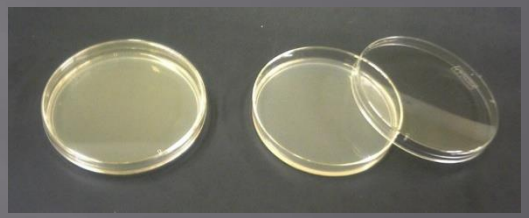
- Experiments on *Colletotrichum gloeosporioides* with the more pathogenic strain of our collection (Nurc MG01)

  - Mycelium growth on PDA medium

  - Germination of conidias (on PDA)

  - Germination of appressoria : difficulties to produce and to stimulate germination

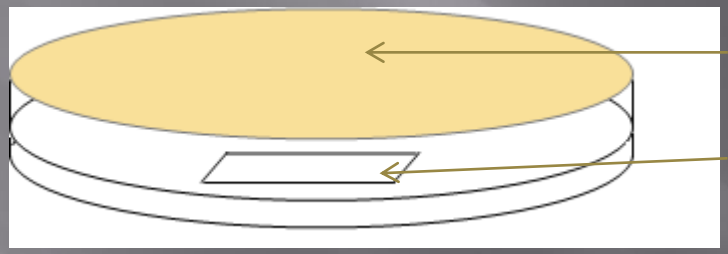
Experiment (volatiles oils)



Test in Petri dishes

(7 days with oil / 7 days without)

Photo by Mrc Chillet



PDA

Glass slide with 5-10 µl of essential oil

Test with spores, on Dutcher slide to count spore germination by microscopy (10 µl of EO)

Photo by Cesar Calderon

Photo by Maria do Ceu Silva

Mycelium growth of *Colletotrichum gloeosporioides*

Treat.	Vol	Mycelial area after 7 days under treatment			MGI	Mycelial area after 7 days of treatments and 7 days without treatment			MGI
	µL	mm²			%	mm²			%
C	10	54,27	±	3,84		33,8	±	2,26	
	5	49,54	±	2,21		49,38	±	7,88	
X2	10	0,32	±	0,52	99,41	15,24	±	13,16	54,91
	5	0,04	±	0,1	99,92	36,29	±	7,96	26,51
X5	10	0	±	0	100,00	0	±	0	100,00
	5	0	±	0	100,00	0	±	0	100,00

Germination of *Colletotrichum gloeosporioides*

	% of germination after 6 h with EO	% of germination after 6 h with EO and 48 h without EO
Control	100	100
X2 (Eugenol, Carvone)	0	80
X5 (Thymol)	0	0

Effect of the treatment on *C. gloeosporioides in vivo*

Evaluation by comparison of necrosis development on inoculated control fruits and treated ones.

Inoculation steps (10 µl at 10^6 sp/ml)
Inoculation time : 48 hours
4 days of treatment in a box (20 liter) :

- **Control : 300 µl of water**
- **X2 : 300 µl of EO X2**
- **X5 : 300 µl of EO X5**

Inoculated mangoes during maturation

Test *in vivo* with artificial inoculation of *Colletotrichum gloeosporioides*

Treatment	Surface necrosis (mm2)
Control	125.3 ± 67.1 (ns)
X2 (4 days)	113.8 ± 57.8 (ns)
X5 (4 days)	122.7 ± 66.3 (ns)
F	0.0801
P	0.923

No effect of the two EO treatments / 4 days of treatment is not sufficient because the appressoria seems to be resistant to both EO

Effect of the treatments on fruit physiology

- Physico-chemical characteristics of quality (at commercial maturity)

- Elicitation of metabolic pathway implicated in host/pathogen interactions

EO on quality parameters (pH)

Treatment	Pulp pH
Control	8.05 ± 0.4(ns)
X2	8.22 ± 0.1(ns)
X5	8.37 ± 0.2(ns)
F	0.07
P	0.95

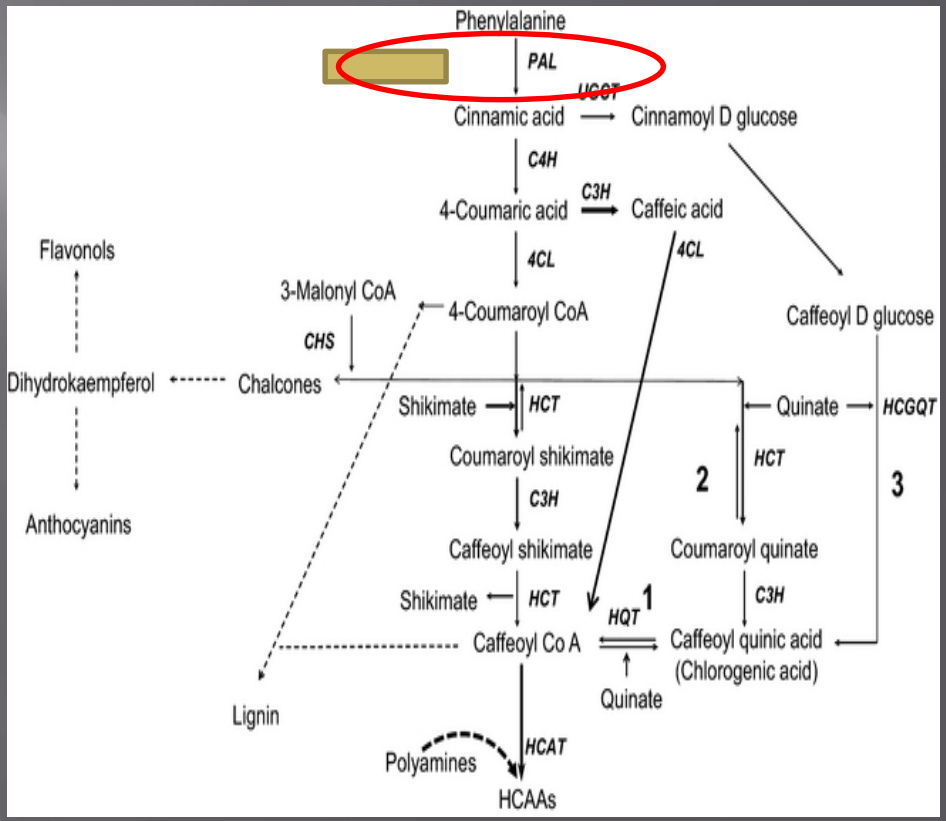
EO on quality parameters (°Brix)

Treatment	Pulp °Brix
Control	9.40 ± 2.5 (ns)
X2	10.24 ± 2.0(ns)
X5	9.16 ± 2.9(ns)
F	0.064
P	0.943

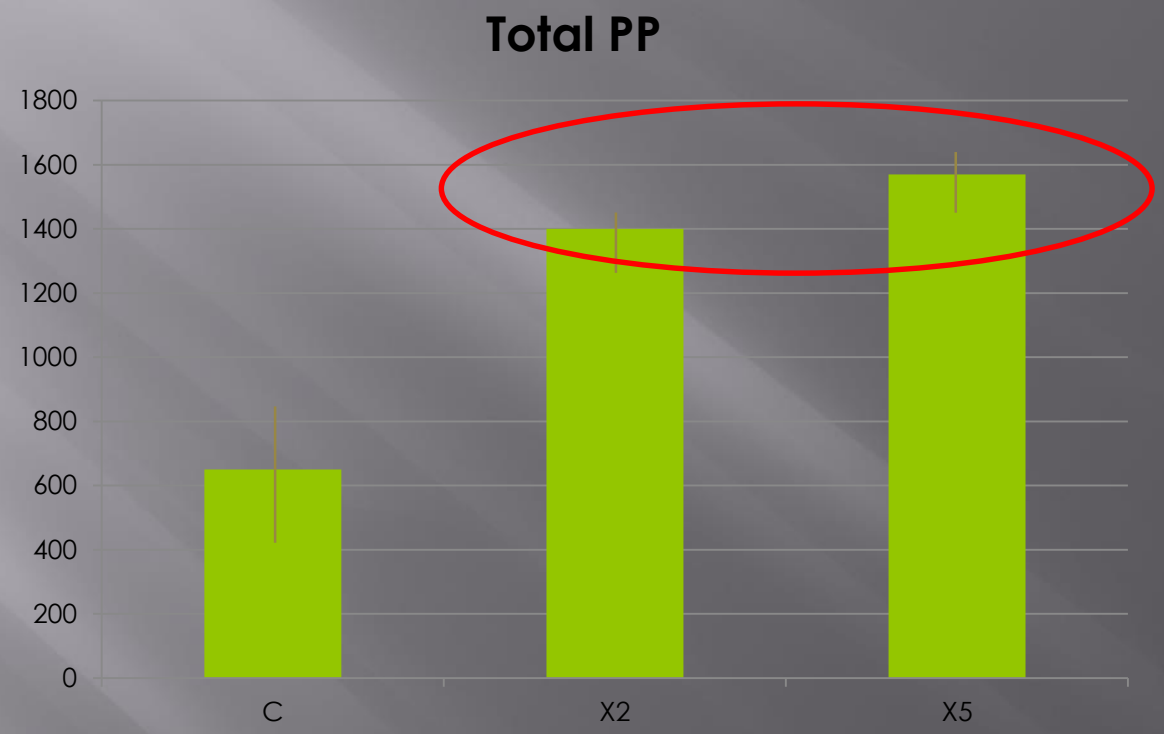
Effect of the essentials oils on resistance mechanisms

- PAL activity
- Polyphenol content
- Gallic acid content (base of the gallotannins)
- 5-pentadecyl resorcinol

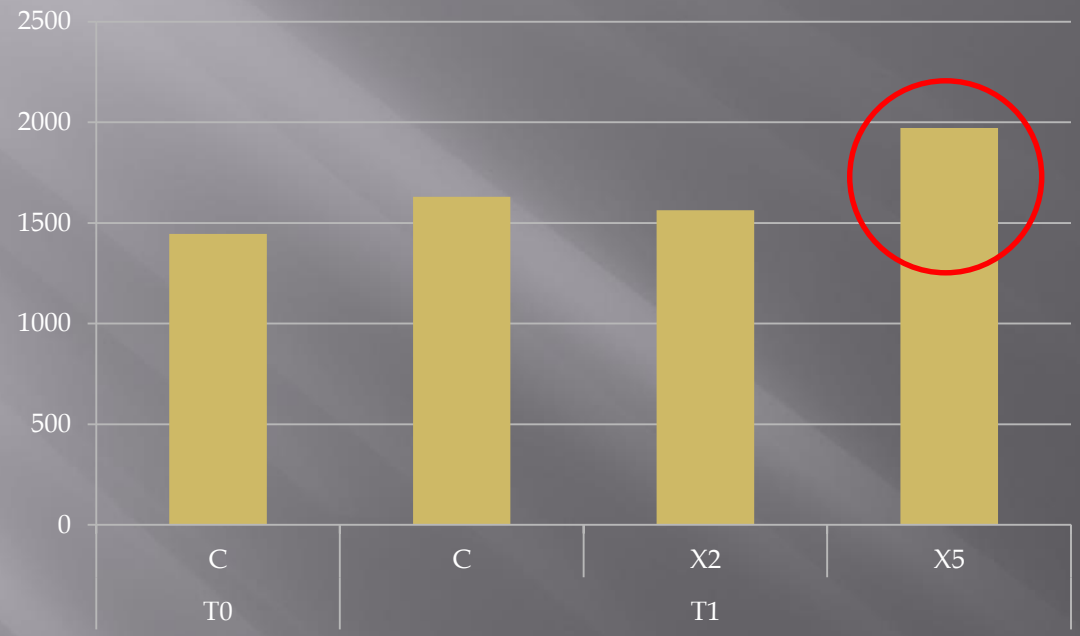
Effect on the polyphenols biosynthesis pathway



Total Polyphenols (after 4 days)

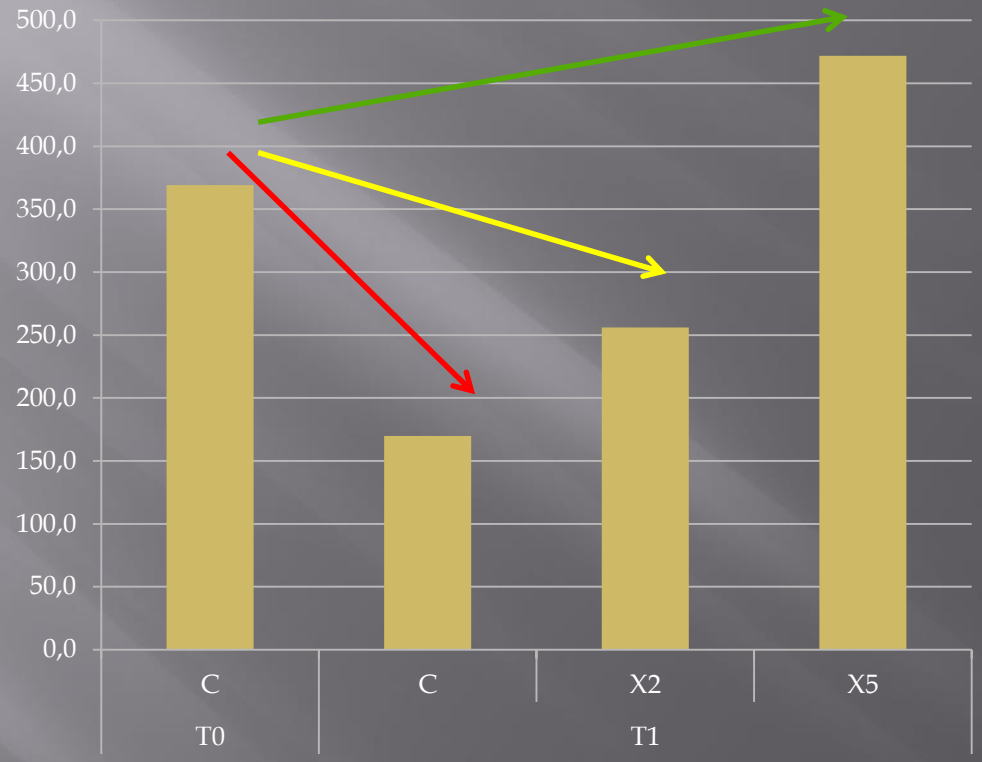


Gallic acid content (in µg/gDM)



Gallic acid content in the fruit peel after 4 days of treatment with X2 and X5 essential oils

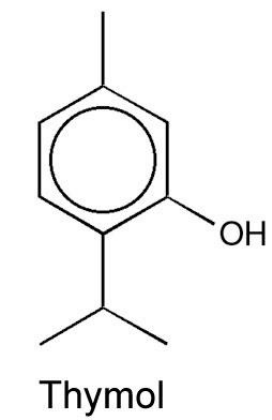
Resorcinols (5-pentadecyl resorcinol) en µg/gDM



Resorcinols content in fruit peel after treatment with X2 or X5 oils, at the harvest stage (T0), after treatment time (T1))

To resume all results :

- Thymol oil has a lot of effect on the pathogen and on fruit physiology
- Thymol oil seems to be better for post harvest treatment than Eugenol-Carvone oil



Conclusions

Thymol oil can block totally the mycelium development

Thymol oil can block totally the spore germination

Thymol oil stimulate PAL activity. X2 oil too

Thymol oil stimulate gallic acid production

Thymol oil permit an increase of resorcinol content

To resume, thymol oil has the properties to be used in postharvest treatment. In our test in vivo, it doesn't work because of the mode of treatment (4 days volatilisation) / and effect on appressorium ?

Future and perspectives

Appressorium ?

Chitinase activity (and other enzymes)

Treatment mode (using thymol impregnated coating, wax, etc …)

Combination of treatment (CO_2, modified atmosphere, …)

Thank you for your attention