Development of a new tool for pest management in fruit arboriculture using essential oils applied by trunk injection

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Plan

Context : Current pest management strategies of *Dysaphis plantaginea* in orchards

- Sap sucking insects
- Leave deformation /fruits loss
- Honeydew (fungi development)
- Main pest (20% yield loss)

- Chemicals control (synthetic pesticides)
- Environmental risks
- Pesticide-resistant populations
- Public demand for environmentally friendly products

Bangels, E.; Alhmedi, A.; Akkermans, W.; Bylemans, D.; Belien, T. Towards a Knowledge-Based Decision Support System for Integrated Control of Woolly Apple Aphid, *Eriosoma lanigerum*, with Maximal Biological Suppression by the Parasitoid *Aphelinus mali*. *Insects* **2021**, *12*, 479. https://doi.org/10.3390/insects12060479

Context : The case for essential oils in insect pest management

- Neurotoxic mode of action (GABA, AChE)
- Low toxicity on mammalian species
- Compatible with integrated pest management (IPM)
- Rapid degradation and volatilization
- Low specificity
- Cost

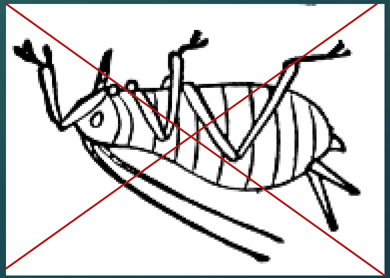
- Alternative plant delivering system- **Trunk injection**
- Target sap sucking pest
- Lower application rate
- Reduced environment load

Tripathi, A. K., & Mishra, S. (2016). Plant Monoterpenoids (Prospective Pesticides). In Ecofriendly Pest Management for Food Security. Elsevier Inc. https://doi.org/10.1016/B978-0-12-803265-7.00016-6
Wise, J., VanWoerkom, A., Aćimović, S., Sundin, G., Cregg, B., & Vandervoort, C. (2014). Trunk Injection: A Discriminating Delivering System for Horticulture Crop IPM. *Entomology, Ornithology & Herpetology: Current Research*, *03*(02), 3–9. https://doi.org/10.4172/2161-0983.1000126

Research objectives

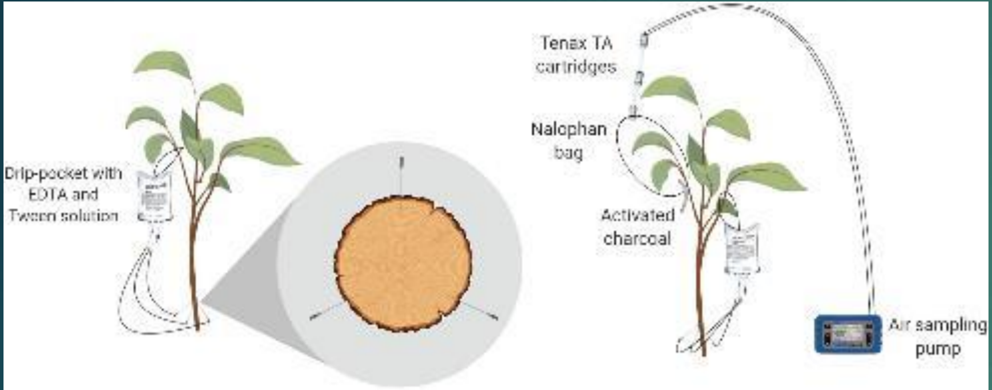
- Demonstrate the systemicity of EO injection

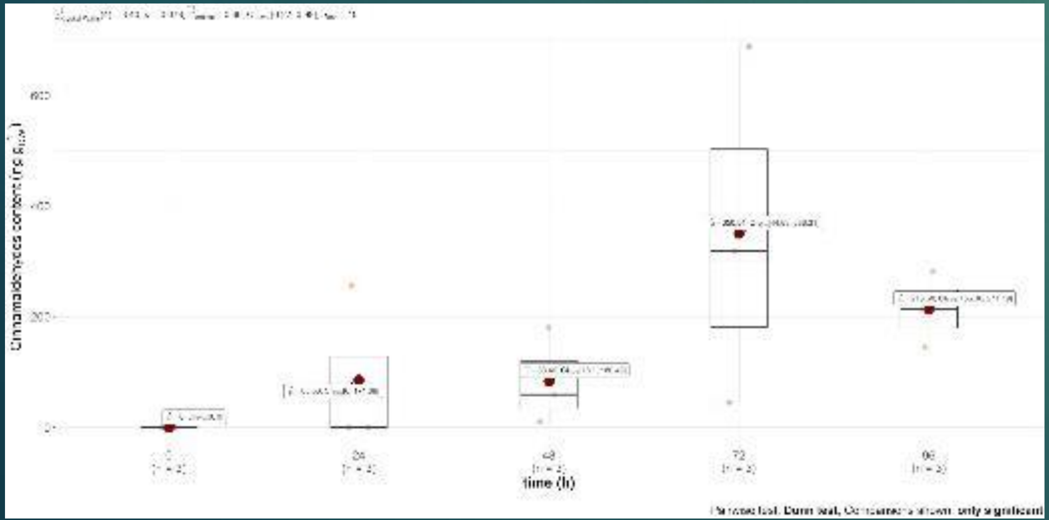
- Characterize the biochemical and physiological impact of EO applications on apple tree (phytotoxicity)

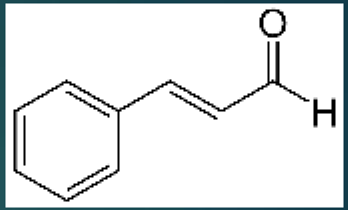


- Investigate the insecticidal properties on *D. plantaginea*

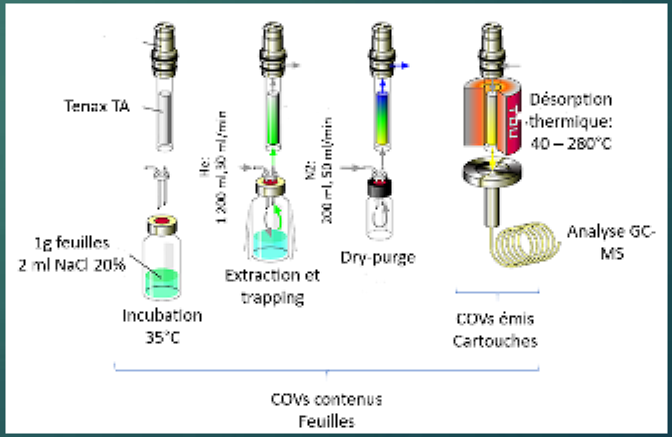
Systemicity of *Cinnamomum cassia* EO trunk injection







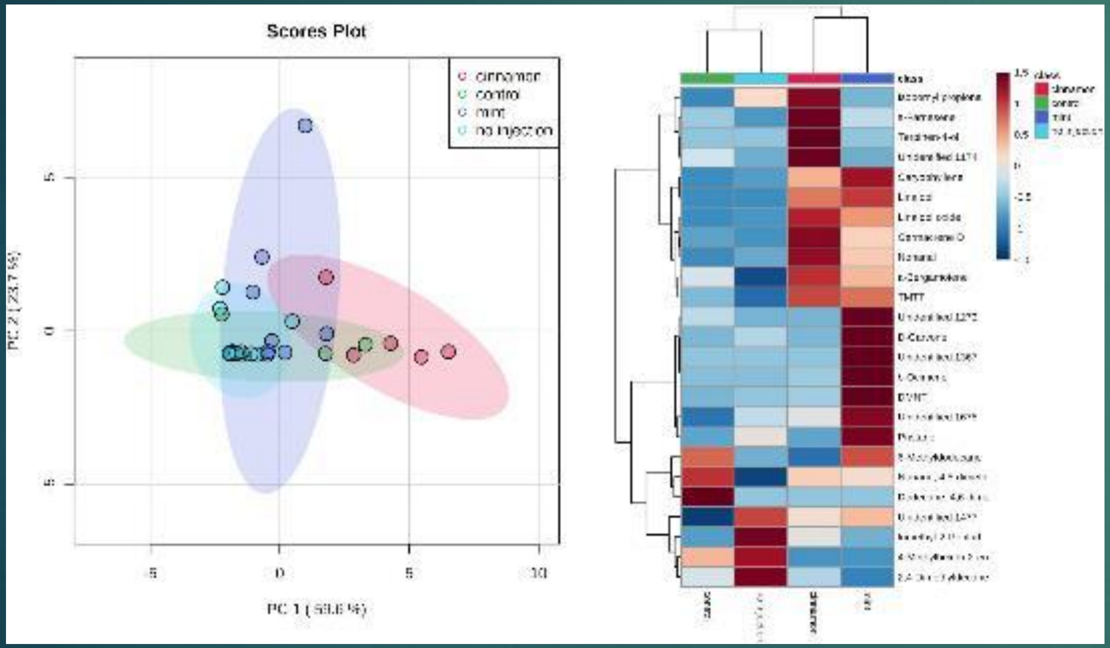
- EO systemicity assayed by:
  - **targeted** Trans-cinnamaldehyde (EO main compound)
  - **Untargeted** Volatile organic compounds (VOCs) analysis
  - **leaf-emitted** VOCs: Thermal desorption TDU-GC-MS
  - **leaf-contained** Dynamic headspace sampling DHS-GC-MS

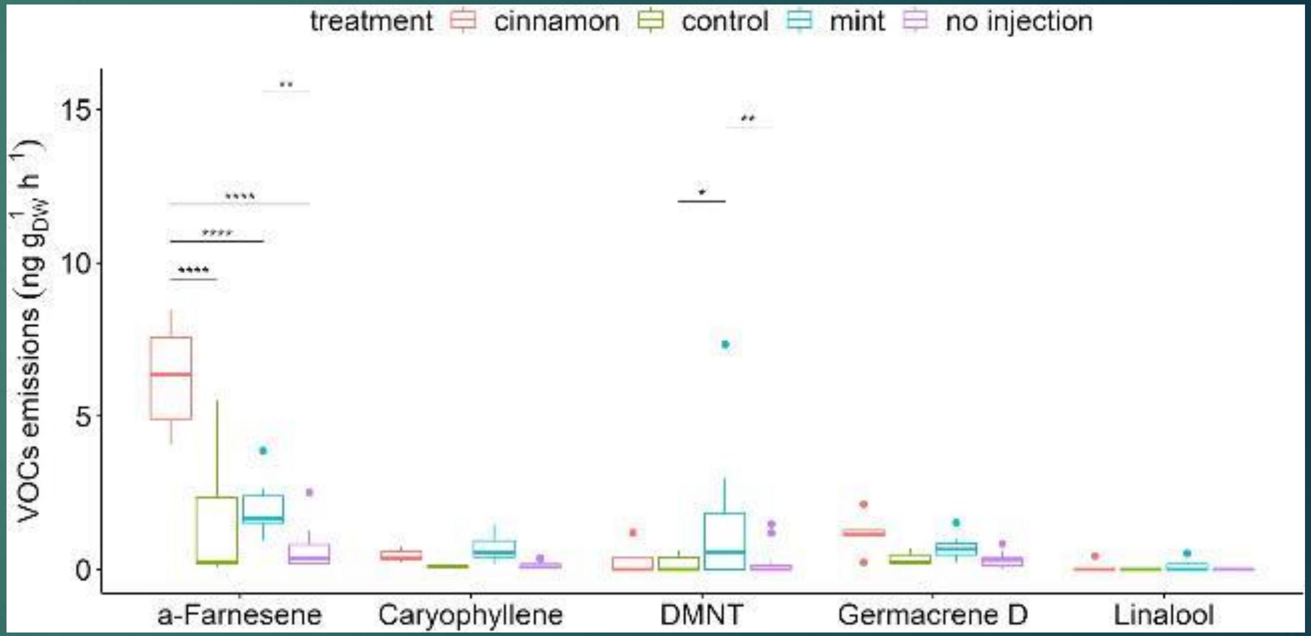


Werrie P-Y, Burgeon C, Le Goff GJ, Hance T and Fauconnier M-L (2021) Biopesticide Trunk Injection Into Apple Trees: A Proof of Concept for the Systemic Movement of Mint and Cinnamon Essential Oils. *Front. Plant Sci.* 12:650132

Systemicity of *Cinnamomum cassia* EO trunk injection

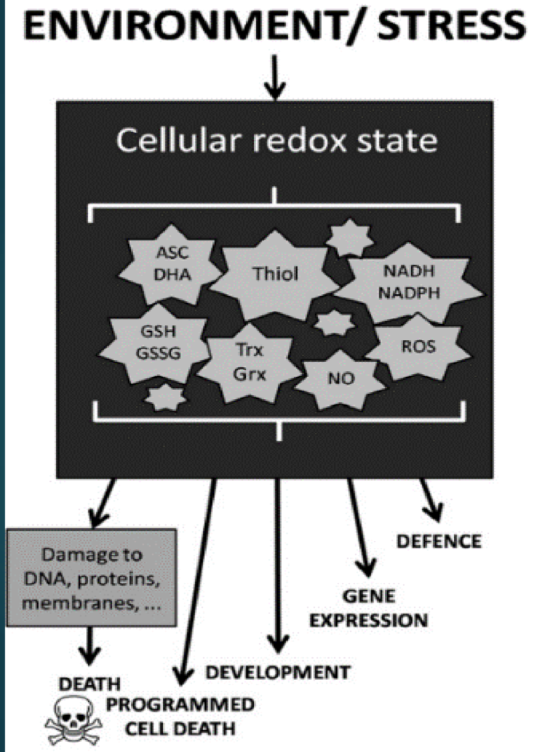
- Untargeted volatile organic compounds (VOCs) analysis



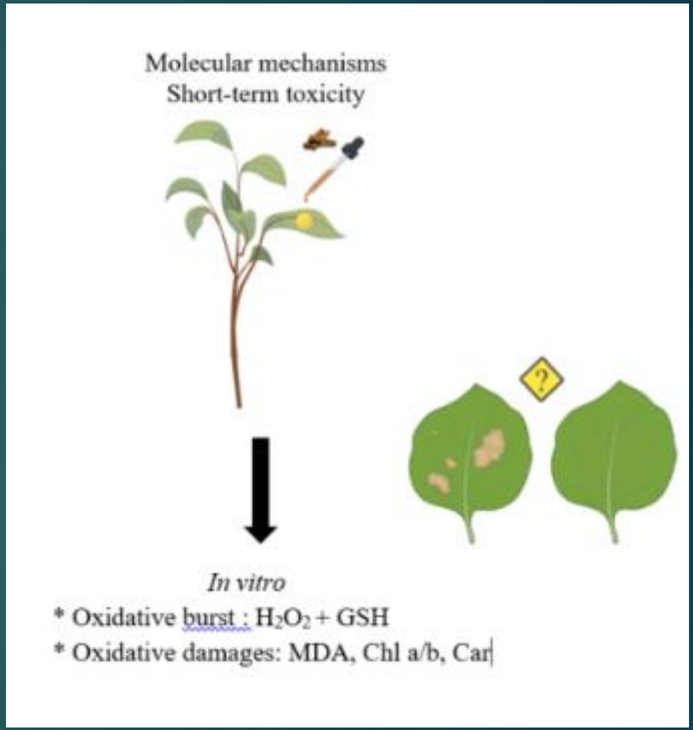


- VOC profile emissions alteration
- Increase in **a-farnescene production**
  - Aphid repellent
  - Produced during systematic acquired resistance (SAR) elicitation

Biochemical and physiological impact of EO application

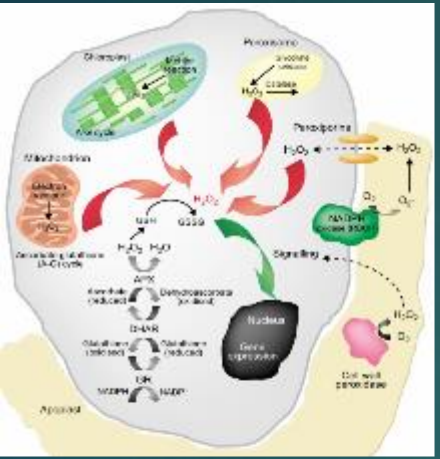


- Plant responses to abiotic stress
- **Reactive oxygen species** (ROS)
- toxic by-products **AND** key regulators of growth/defense pathways
- Molecular mechanism investigated by:
  - Redox state alteration (GSH)
  - Oxidative damage (MDA)
  - Defense gene induction (RT-PCR)

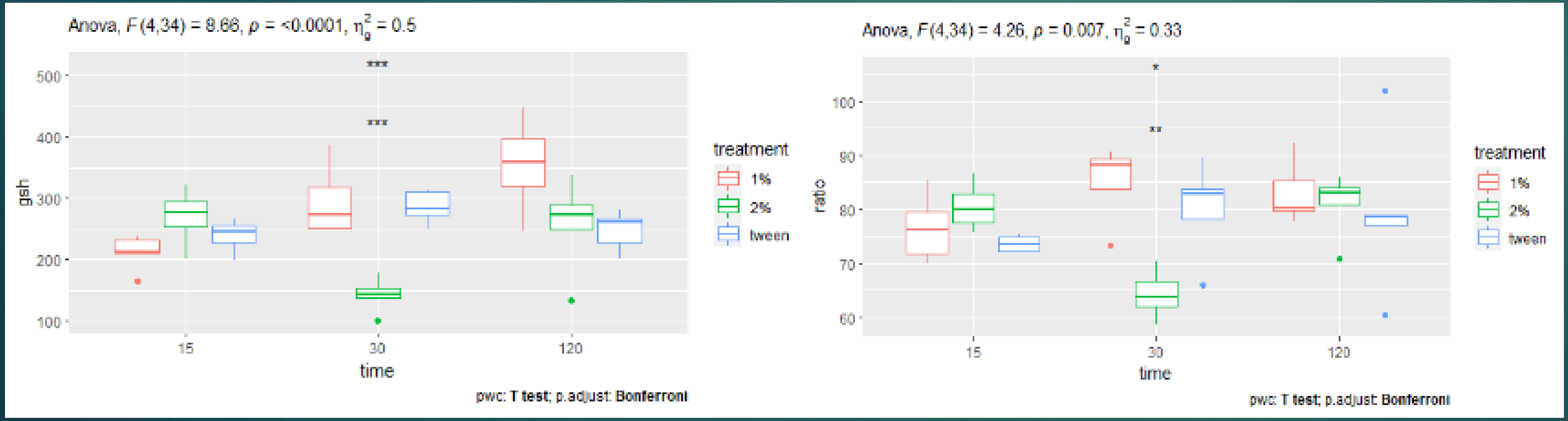


Xia, X. J., Zhou, Y. H., Shi, K., Zhou, J., Foyer, C. H., & Yu, J. Q. (2015). Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. In *Journal of Experimental Botany* (Vol. 66, Issue 10, pp. 2839–2856). https://doi.org/10.1093/jxb/erv089

Biochemical and physiological impact of EO application



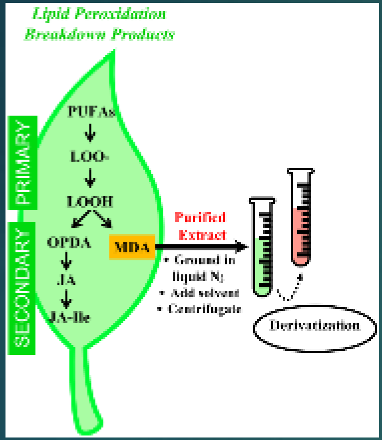
- Glutathione = tripeptide, non-enzymatic antioxidant in plant cell (GSH/GSSG)
- Role in:
  - ROS scavenging
  - Stress perception/signalling
  - Defence reactions



- Glutathione redox status rapid alteration at high EO concentration !

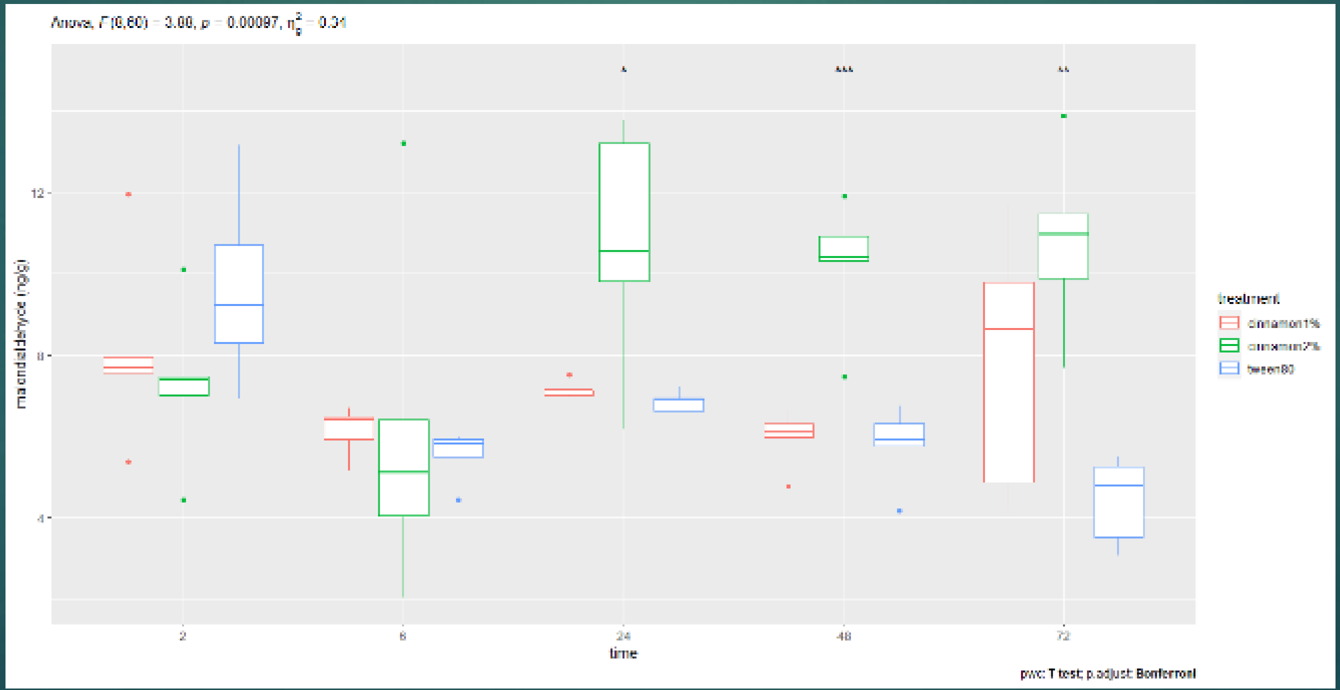
Noctor, G. (2006). Metabolic signalling in defence and stress: The central roles of soluble redox couples. *Plant, Cell and Environment*, 29(3), 409–425. https://doi.org/10.1111/j.1365-3040.2005.01476.x

Biochemical and physiological impact of EO application



(Morales & Munné-Bosch, 2019)

- Malondialdehyde = secondary product of lipid peroxidation
- Marker of oxidative damages



- Oxidative damages occur at high concentration
- Dose-dependent phytotoxicity of C. *cassia*

Weber, H., Chételat, A., Reymond, P., & Farmer, E. E. (2004). Selective and powerful stress gene expression in Arabidopsis in response to malondialdehyde. *Plant Journal*, *37*(6), 877–888. https://doi.org/10.1111/j.1365-313X.2003.02013.x

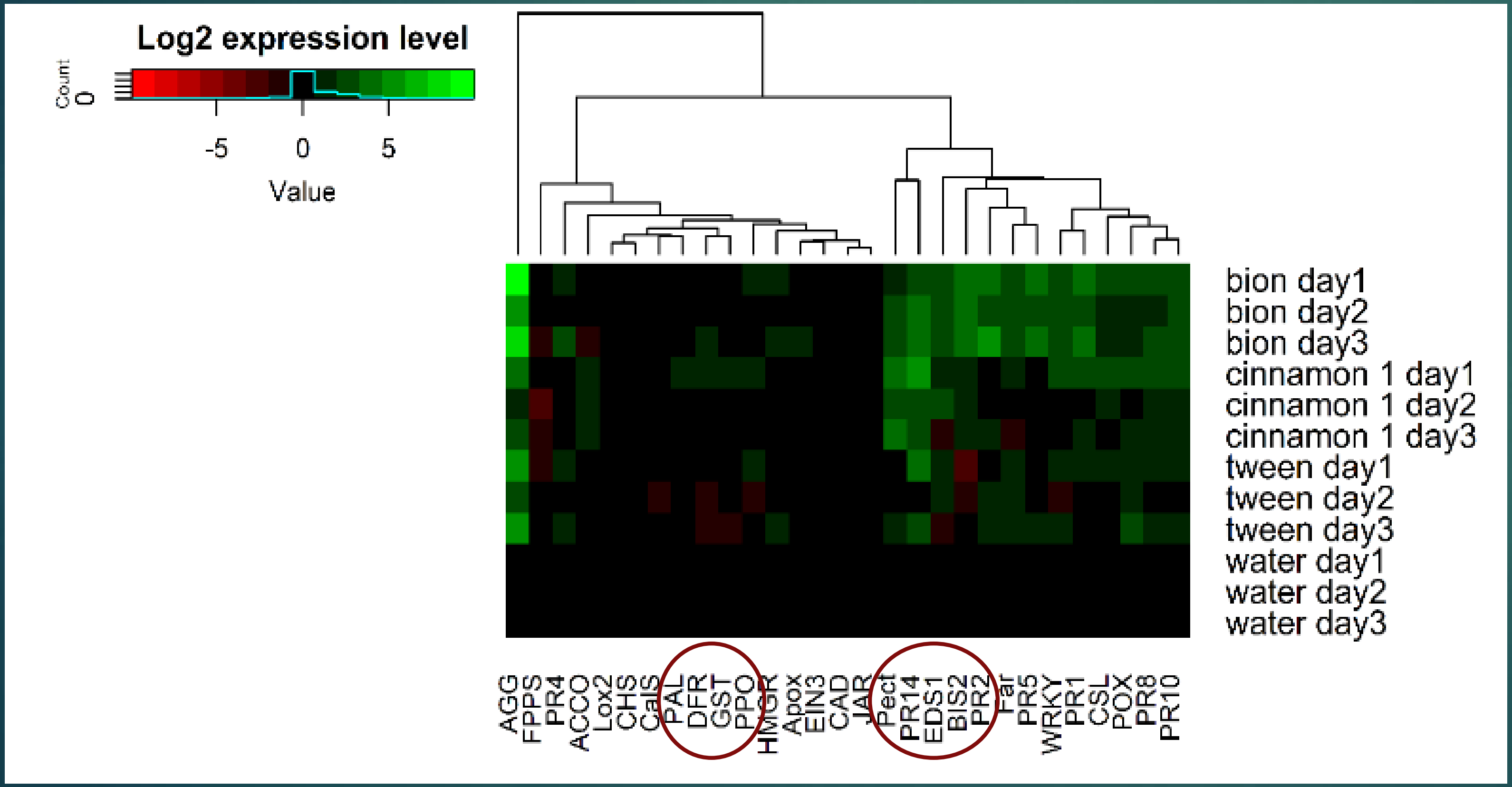
Biochemical and physiological impact of EO application

Defence Classes and Subclasses		Defence Genes	
		Gene Codes	Complete Names
Chemical and/or physical barriers	PR proteins	PR-1	Pathogenesis-related protein 1
		PR-2	Pathogenesis-related protein 2 (glucanases)
		PR-4	Pathogenesis-related protein 4 (hevein-like)
		PR-5	Pathogenesis-related protein 5 (thaumatin-like, osmotin)
		PR-8	Pathogenesis-related protein 8 (class III chitinase)
		PR-14	Pathogenesis-related protein 14 (lipid transfer protein)
		PR-15	Pathogenesis-related protein 15 (oxalate oxidase)
	Phenylpropanoids	PAL	Phenylalanine ammonia-lyase
		CHS	Chalcone synthase
		DFR	Dihydroflavonol reductase
		ANS	Anthocyanidin synthase
		PPO	Polyphenol oxidase
	Isoprenoids	HMGR	Hydroxymethyl glutarate-CoA reductase
		FPPS	Farnesyl pyrophosphate synthase
		Far	(E,E)-alpha-farnesene synthase
	Cysteines	CSL	Alliinase
	Oxidative stress	APOX	Ascorbate peroxidase
		GST	Glutathion S-transferase
		POX	Peroxidase
	Parietal modification	CalS	Callose synthase
		Pect	Pectin methyl esterase
		CAD	Cinnamyl alcohol dehydrogenase
	Agglutinin	AGG	Agglutinin synthetase
Hormonal signalling	Salicylic acid (SA)	EDS1	Disease resistance protein EDS 1
		WRKY	WRKY transcription factor 30
	Jasmonic acid (JA)	LOX2	Lipoxygenase AtLOX2
		JAR	Jasmonate resistant 1
	Ethylene (ET)	ACCO	1-aminocyclopropene-1-carboxylate oxidase
		EIN3	EIN3-BINDING F BOX PROTEIN 1

- qRT-PCR decipher the potential elicitor properties
- RNA Transcripts from major pathways:
  - PR proteins
  - Phenylpropanoids
  - Isoprenoids
  - Oxidative stress
  - Parietal modifications
  - Hormonal signalization (SA,JA,ET)

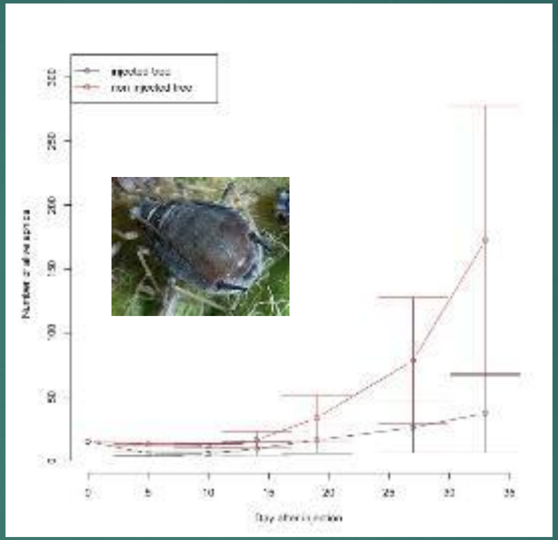
Dugé de Bernonville, T., Marolleau, B., Staub, J., Gaucher, M., & Brisset, M. N. (2014). Using molecular tools to decipher the complex world of plant resistance inducers: An apple case study. *Journal of Agricultural and Food Chemistry*, 62(47), 11403–11411. https://doi.org/10.1021/jf504221x

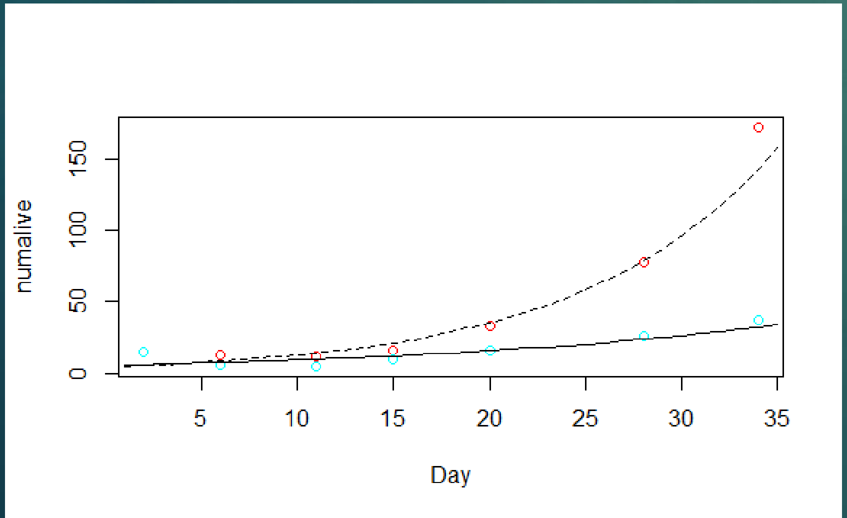
Biochemical and physiological impact of EO application



- Induction of defense gene expression by *C. cassia* during 3 days
- Pathway different of SAR elicitor bion

Impact of C. *cassia* injection on population dynamics





- Injection affects survival and growth
- GLM modelling on aphids population
- Effects of time and injection significant(p-values <0.001)

Discussion

- Generation of oxidative burst- other oxidative damage marker to consider (protein/DNA)
- Hormonal imbalance - Long term phytotoxicity ?
- Population control sufficient to prevent economical impact (yield)

Perspectives

- Field efficiency ?
- Fruit residue ?
- Defence against other pathogens (scab, oidium) ?

Questions

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