Alteration Of Survival And Oxidative Balance Induced By Subchronic Exposure Of Overwintered Honeybees To Insecticide, Fungicide And Herbicide Combinations

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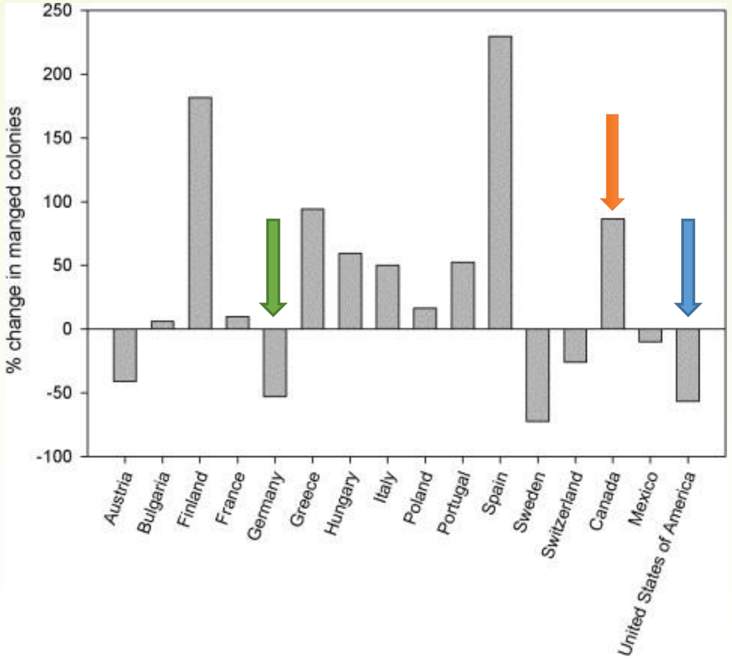
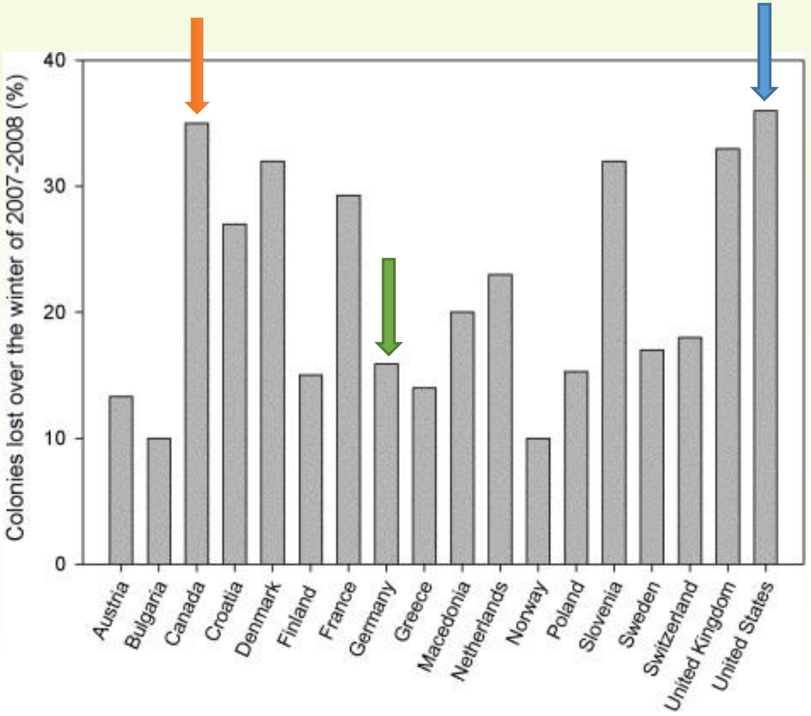
Introduction

The global stocks of honey bees have increased over the last five decades, but not all regions have experienced gains (FAO, 2009)



Percent change in number of managed bee colonies between 1961 and 2006 in selected countries in Europe and North America (FAO, 2009)



Percentage of colony winter losses in 2007/2008 in several countries (vanEngelsdorp, 2010)

United states: High winter losses, ↓ in the number of colonies

Germany : moderate winter losses, ↓ in the number of colonies

Canada : high winter losses, ↑ in the number of colonies

How pesticides affect honey bees?

- Honey bees are exposed to pesticides through acute and chronic exposure
- Lethal and sublethal effects may appear during or after plant protection treatments or by consumption of food contaminated by pesticide residues (Belzunces et al, 2012)
- More than 121 different pesticides and metabolites can be present in beehive matrices with an average of 6 pesticides per sample, including varroacides, fungicides, herbicides and insecticides (Mullin et al, 2010)

Belzunces, L.P., S. Tchamitchian, and J.L. Brunet, Neural effects of insecticides in the honey bee. Apidologie, 2012. 43(3): p. 348-370.

Mullin, C.A., et al., High levels of miticides and agrochemicals in north american apiaries: implications for honey bee health. Plos One, 2010. 5(3): p. 19.

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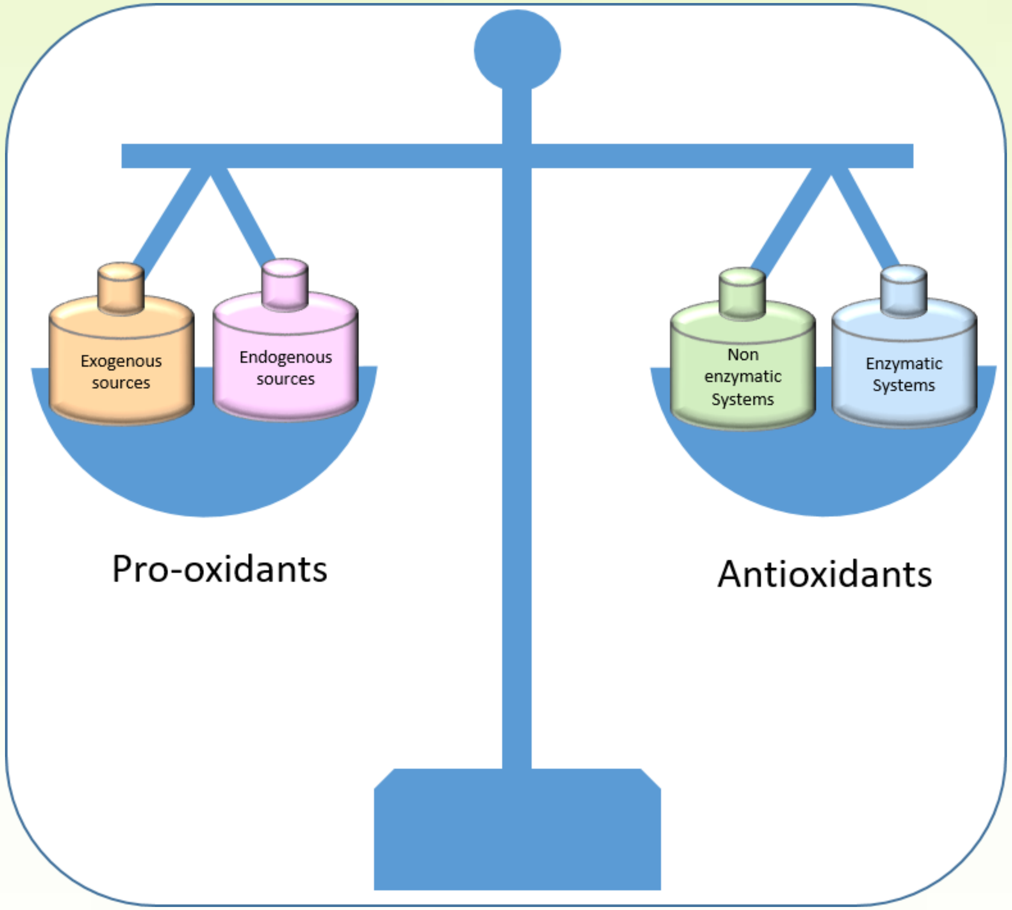
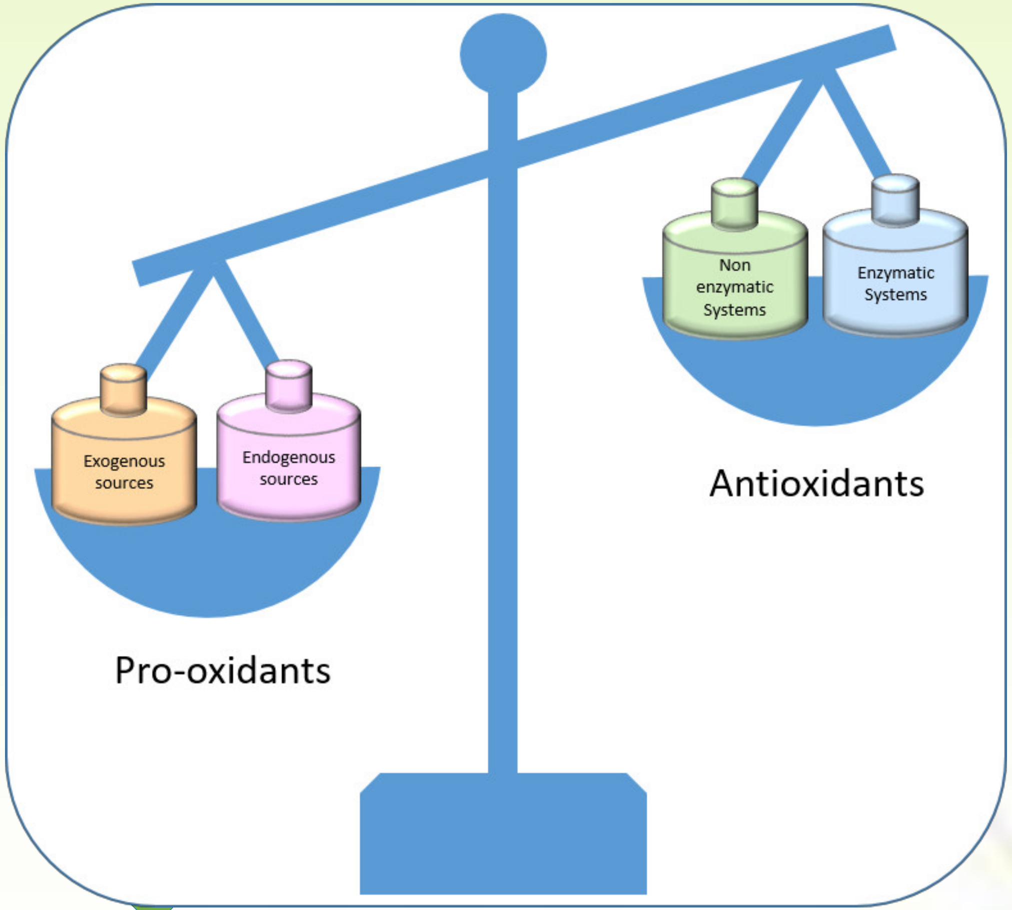
Objective

Impact of pesticide combinations on the survival and oxidative balance of winter honeybees

What is the Oxidative stress ?





Superoxide dismutase (SOD)
Catalase (CAT)
Glutatione reductase (GR)
Glutathione-S-transferase (GST)
Glutatione peroxidase (GPox)
Glucose-6-phosphate dehydrogenase (G6PDH)

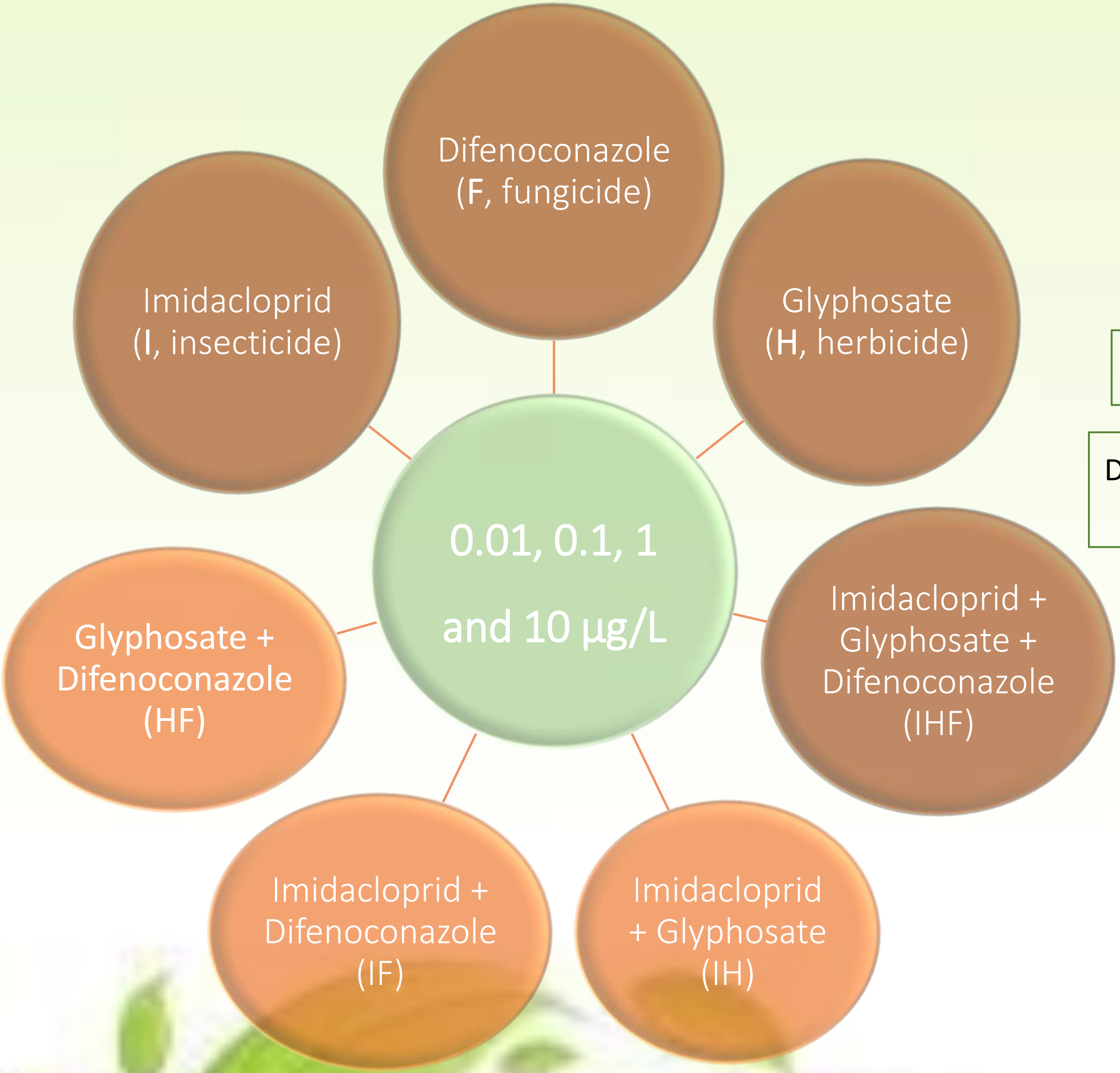
DNA, lipid and protein oxidation

Pesticides of interest

Pesticide	Imidacloprid	Difenoconazole	Glyphosate
Family	Neonicotinoid	Triazole	Amino-phosphonate (glycine)
Mode of action	Agonist of cholinergic neurones	Biosynthesis ergosterol inhibitor	Blocks the activity of the enzyme enolpyruvylshikimate-3-phosphate synthase (EPSPS) of the plant
Examples of formulation	Confidor® (200SL) Gaucho® (350FS)	Score®(250 EC)	Roundup® (720 g/kg)
Residues in the colony	0.14 to 0.7 µg/kg in honey (Chauzat. 2009, Lambert. 2013)	0.6 µg/Kg in honey 1 µg/kg in wax (Kubik. 2000) (Herrera lopez. 2016)	64 µg/kg in honey (Rubio. 2014)
Acute LD_{50} ***	Contact LD50 : 0.081 µg / bee Oral LD50 : 0.0037 µg / bee	Contact LD50 >100 µg/ bee Oral LD50 >177 µg/ bee	Contact LD50 >100 µg/ bee Oral LD50 : 100 µg/ bee

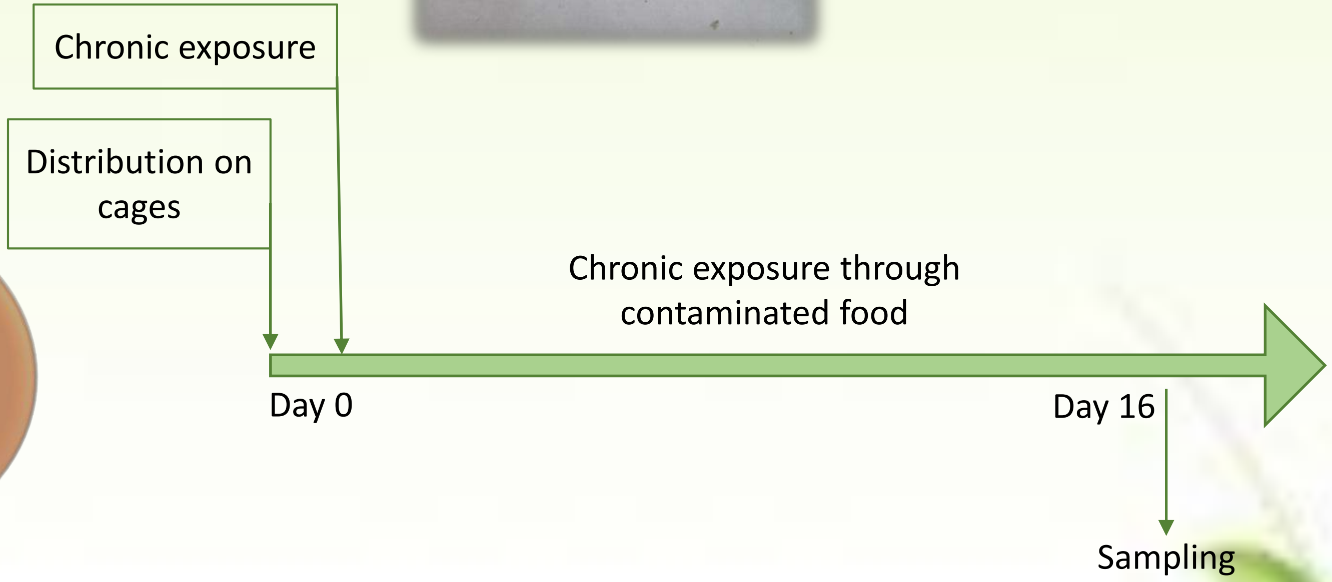
***AGRITOX : http://www.agritox.anses.fr/php/fiches.php

Experimental design



14 replicates/treatment

30 honey bees/replicate



The studied parameters

- Daily mortality
- Daily food consumption
- Physiological life history traits of oxidative stress

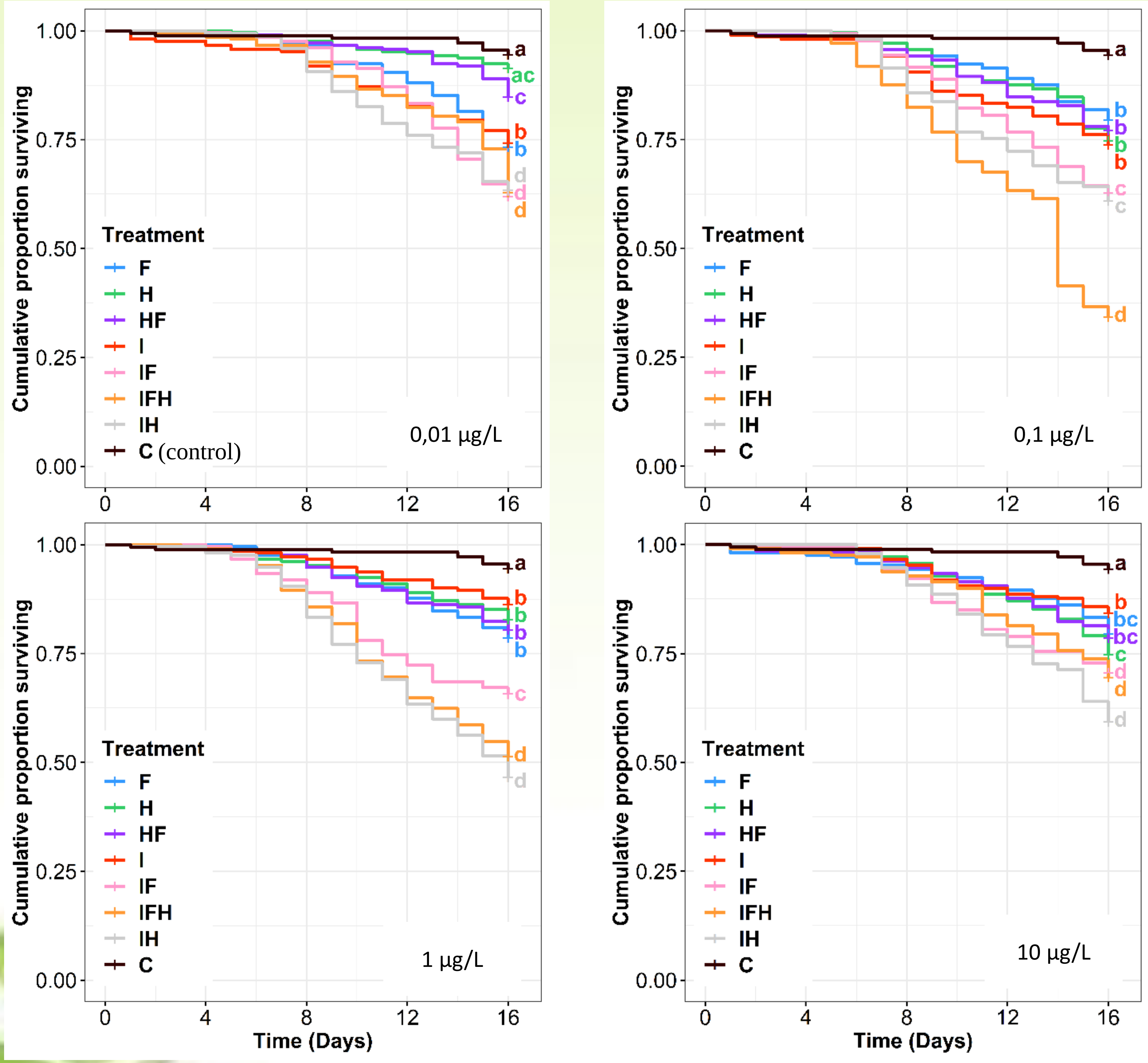
Head	Abdomen	Midgut
Catalase (CAT)	Glucose-6-phosphate dehydrogenase (G6PDH)	Catalase (CAT)
Superoxide dismutase (SOD)		Superoxide dismutase (SOD)
Glutathione-S-transferase (GST)		Glutathione-S-transferase (GST)
Glutatione reductase (GR)		Glucose-6-phosphate dehydrogenase (G6PDH)
Glutatione peroxidase (GPox)		

- Lipid peroxidation (lipid oxidation)
- Protein carbonylation (protein oxidation)

Results

Effect of chronic exposure on survival

- The highest toxicities were observed at intermediate doses (0,1 and 1 µg/L)
- A Higher number of substances in a mixture does not always produce higher toxicity
- The exposure to pesticides decreases their survival rate
- The highest dose didn't induce the highest mortality
- The highest dose induces a mortality similar to the lowest dose



Effect of chronic exposure on survival

- 81% of mixtures induce a sub-additive, additive and synergistic effects
- **19% induce antagonistic effects**

Antagonism	Sub-additive effect	Additive effect	Synergism
HF 0,01	HF 10	IF 0,1	IF 1
HF 0,1	IF 0,01	IH 0,01	IF 10
HF 1	IH 0,1		IH 1
	IHF 0,01		IH 10
	IHF 10		IHF 0,1
			IHF 1

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Modulation of physiological markers by pesticides

	No modulation	Decrease of enzymatic activity	Increase of enzymatic activity
1 µg/L	✓ head CAT ✓ abdomen G6PDH	✓ head SOD (except HF) ✓ gut CAT (except IH and IHF)	✓ head GST (except HF) ✓ head GR (except HF) ✓ head Gpox (except HF) ✓ gut SOD (except HF) ✓ gut GST (except HF) ✓ gut G6PDH (except I and HF)

- At 1 µg/L : massive modulation of antioxidant defenses
- For each enzyme : similar modulation at the different exposure conditions (except with HF)

Modulation of physiological markers by pesticides

	No modulation	Decrease of enzymatic activity	Increase of enzymatic activity	Mixte cases (Increase or decrease depending on exposure)
0,1 µg/L	✓ head GST ✓ head GR ✓ abdomen G6PDH	✓ gut GST (↓I, ↓F, ↓H)	✓ head SOD (↑F) ✓ head GPox (↑I) ✓ gut SOD (↑IF,↑IH,↑IHF) ✓ gut G6PDH (↑F)	✓ head CAT (↓I, ↓F, ↓H,↑HF) ✓ gut CAT (↓ I, ↑F)

- At 0,1 µg/L : complex pattern of modulation of enzymes involved in antioxidant defenses
- The binary and ternary mixtures induce lower modulations of enzymes compared with the 1 µg/L concentration

Modulation of physiological markers by pesticides

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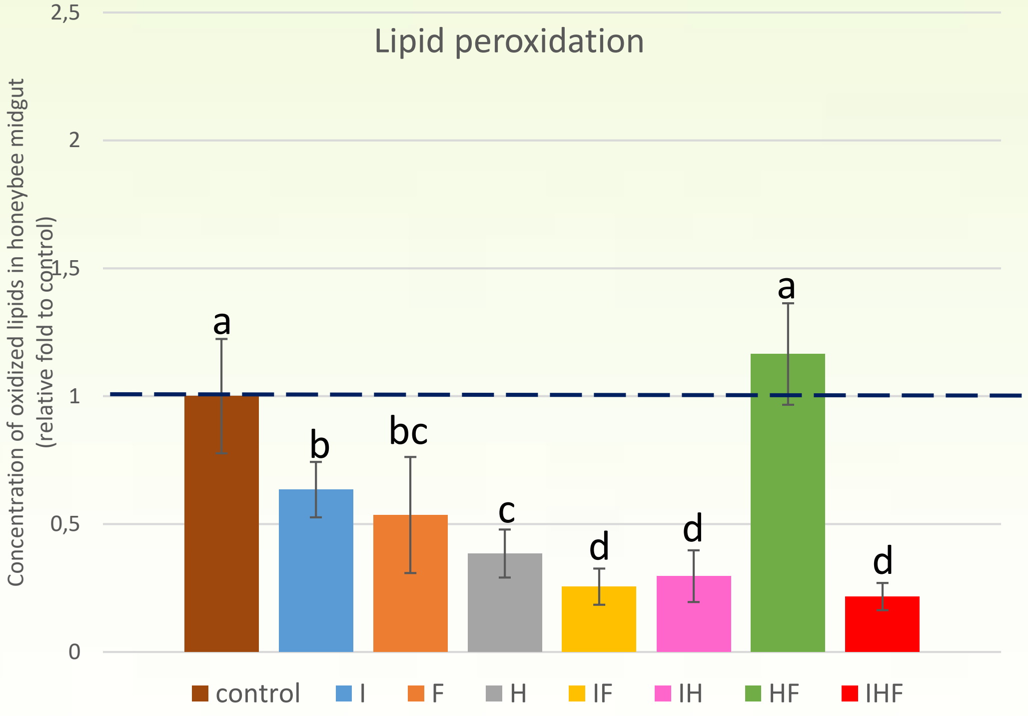
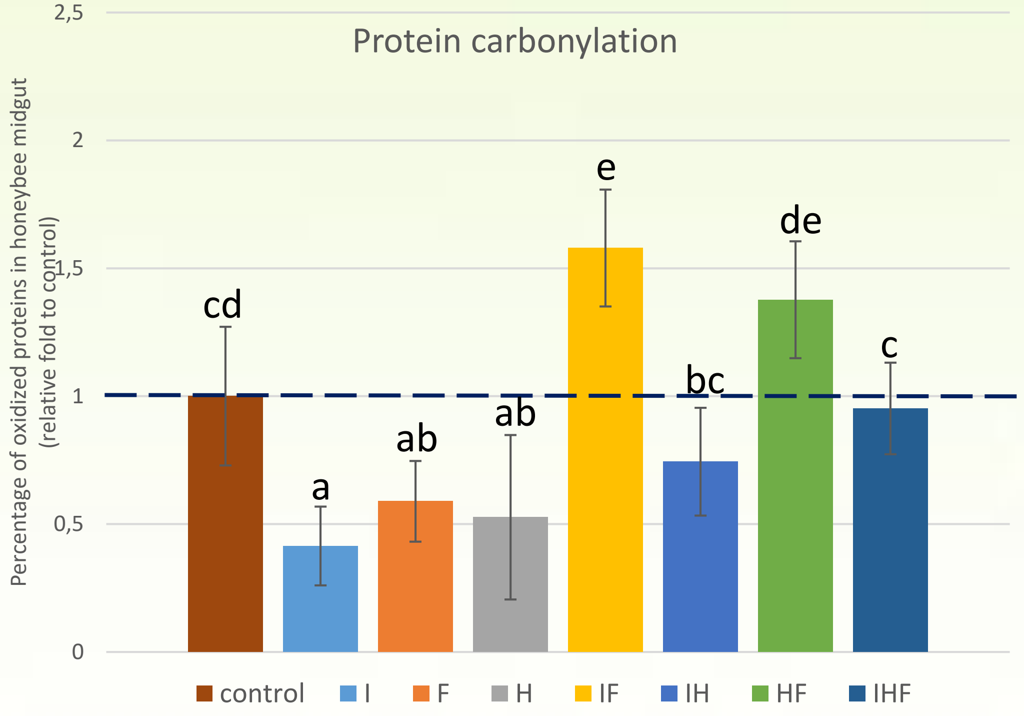
- Complex pattern of modulation
- Mixtures induce lower modulations of enzymes compared with the 1 µg/L concentration
- Hypotheses
  - The oxidative stress was of lesser importance ?
  - Did a recovery occur ?

The Stress damage ?
Effects of oxidative stress on biological molecules at 0.1 µg/L



The lipid peroxidation as reflected by TBARS decrease except with the HF



The protein oxidation as reflected by the protein carbonylation decrease except with the IF and the HF

Conclusion

- Environmental concentrations of pesticides decrease the survival rate of winter honeybees
- Pesticide combinations have higher effect than pesticides alone
- The highest effects are not systematically observed at the highest doses
  - The effects are higher at intermediate doses
  - The effects of the highest dose are similar to the effects of the lowest dose
- The increase of the number of substances dose not systemically induce higher toxicity
- Disruption of oxidative balance
  - Induction of oxidative stress
  - Evident for unitary, binary (IH, IF) and ternary (IHF) mixtures
  - Less evident for HF mixture
- Mode of action
  - The effects are systemic and can be sometimes tissue-specific
  - The substances might share one or several common metabolic targets
  - Is the oxidative stress involved in the toxicity of the mixtures?

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Thank You
For Your Attention !