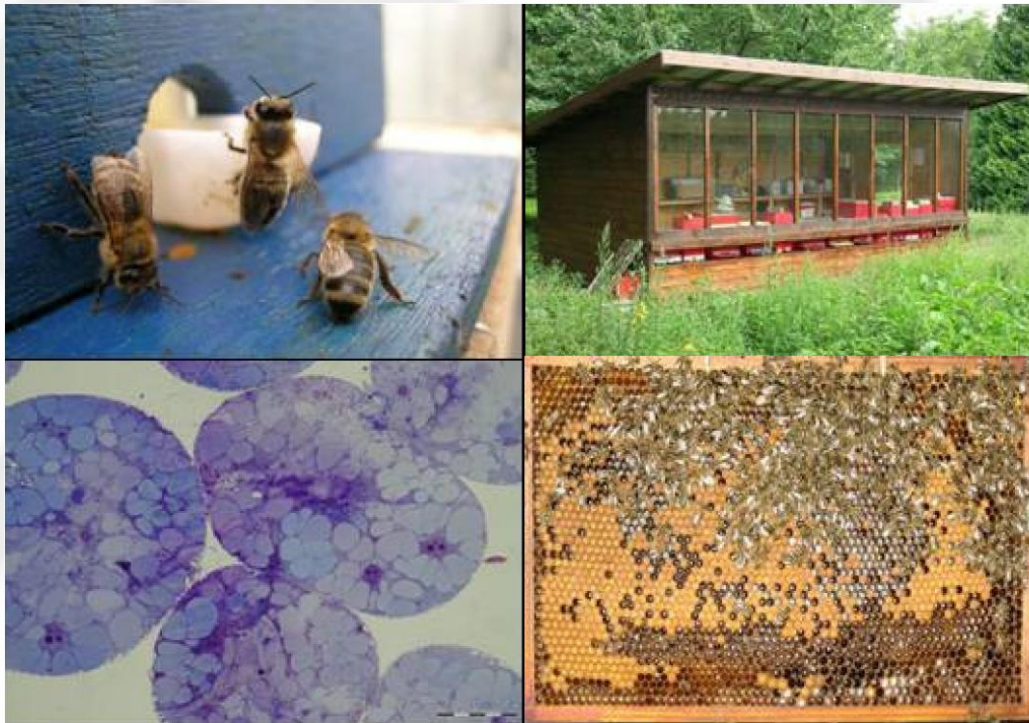


# Side effects of crop protection products on bees: identification and evaluation of the impact of sublethal effects



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IWT Landbouwkundig onderzoek



# Agriculture & Bees

Honeybees are extremely important for mankind

- Beekeepers: honey production
- Farmers: honeybee **pollination** for agriculture productivity

(representing global economic value of 153 billion € in 2005; Gallai et al., 2009)



- Importance for biodiversity: **invaluable**

# Agriculture & Bees



- Beekeepers: honey production: importance flower visits



- Farmers:
  - Importance pollination for agriculture productivity
  - But also importance crop protection against insect pests:
    - dependent on crop protection agents



Good agricultural practice: no bee toxic products during blossoming!

# Lethal effects

Easy to detect

Acute mortality



# Sublethal effects

No direct bee mortality, but behavioral disturbance (e.g. task regulation, learning, mobility, foraging, etc.)

- Contact with low concentrations toxic compounds (like residues)
- Contact with non-lethal compounds
- May harm the colony on long term

Very difficult to detect and quantify



# Sublethal effects

No direct bee mortality, but behavioral disturbances (e.g. task regulation, learning, mobility, foraging, etc.) **Very difficult to detect and quantify**

**Principal aim research project:**

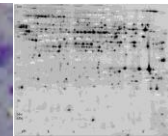
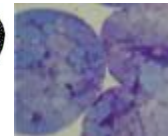
**How can we objectively identify and measure sublethal effects?**

Innovative combination of:

- Extensive field-related behavioral testing  
(observation, filming, registration behavior)

and simultaneously

- Morphological and molecular study of endogenous modifications  
(combined functional genomics/proteomics: microarray, 2-D DIGE, RT-PCR, histology)



# General outcomes

- Controlled sublethal contamination of bees (imidacloprid, indoxacarb, fenoxycarb, captan)
- Detailed description sublethal effects (foraging/flower visits, brood care, etc.)
- Datasets of differentially expressed genes and proteins
- Link behavioral effects to endogenous molecular changes

(Heylen et al., 2008, 2009, 2010, Gobin et al., 2008; Belien et al., 2009, 2010, 2012; etc.)

One example: specialized devision of labor in adult worker bees

First 20 days: active inside hive  
(brood care, cleaning, etc.)



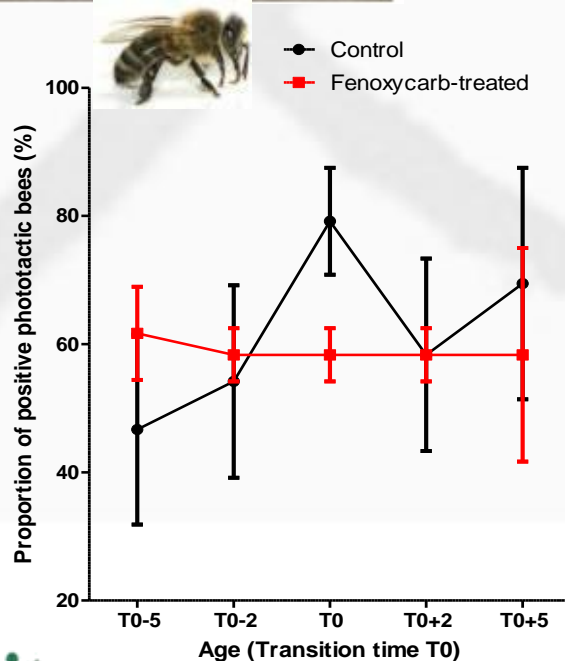
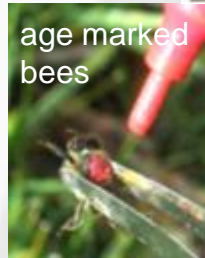
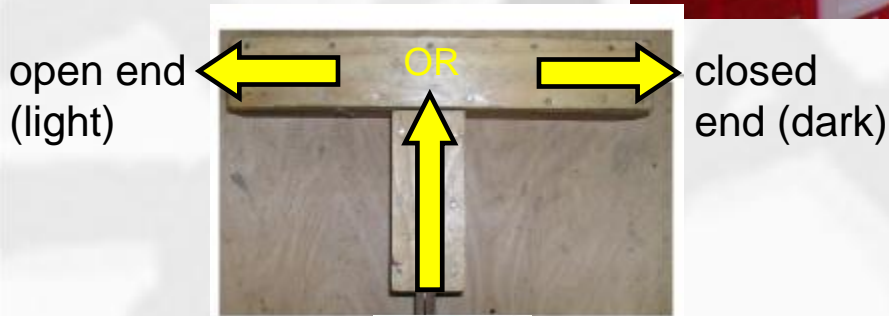
Around 21<sup>st</sup> day: task transition  
foraging  
(collecting food)



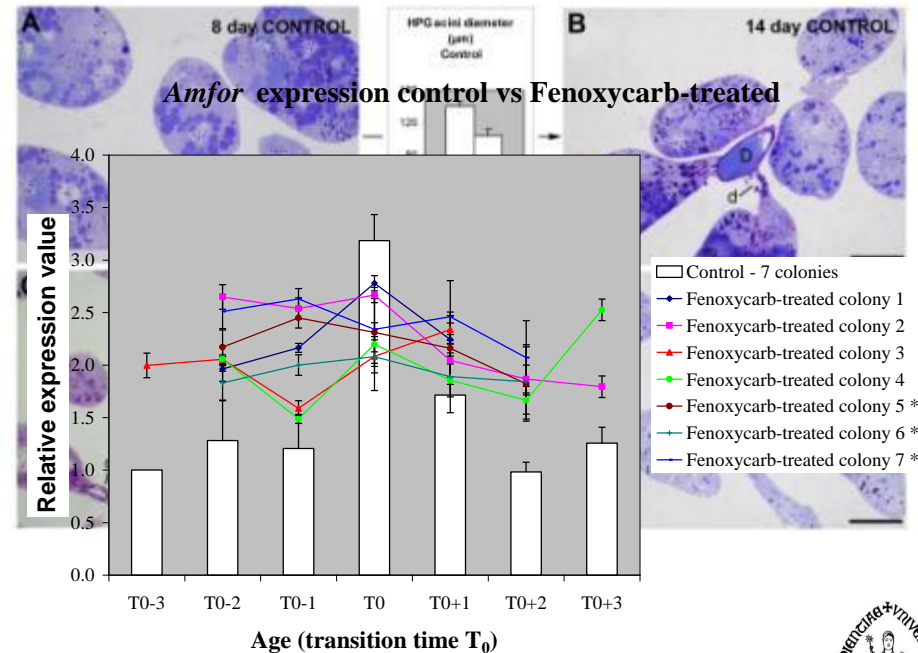
# Disturbed task regulation: onset of foraging



Imidacloprid (Confidor)  
 Indoxacarb (Steward)  
 Fenoxycarb (Insegar)  
 Untreated check



Univariate ANOVA: sign. interaction effect (P = 0.044)



# Impact on long term colony vitality

Following up of contaminated colonies by regular assessments

- Total number bees alive/dead
- Total surface capped brood
- Total weight of the colony
- etc.

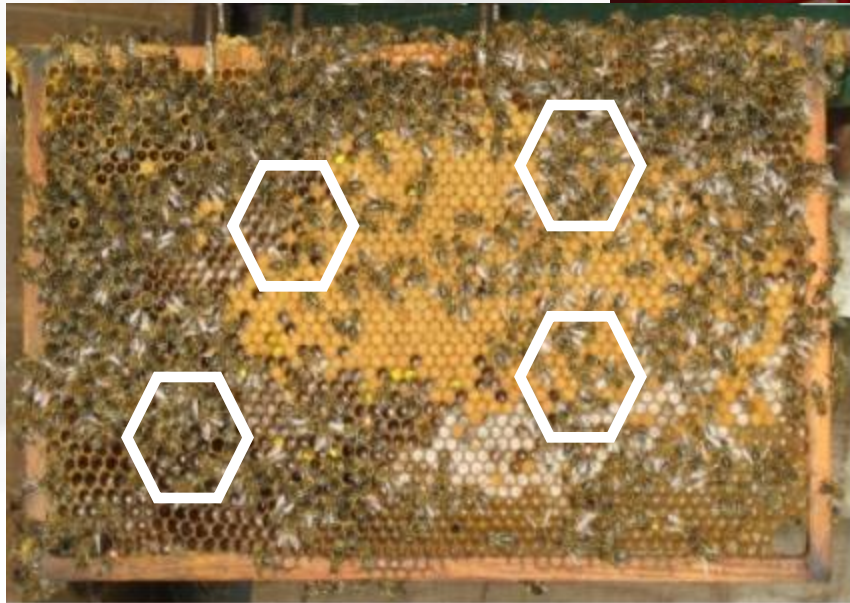


4 Indoxacarb

3 Imidacloprid

2 Fenoxycarb

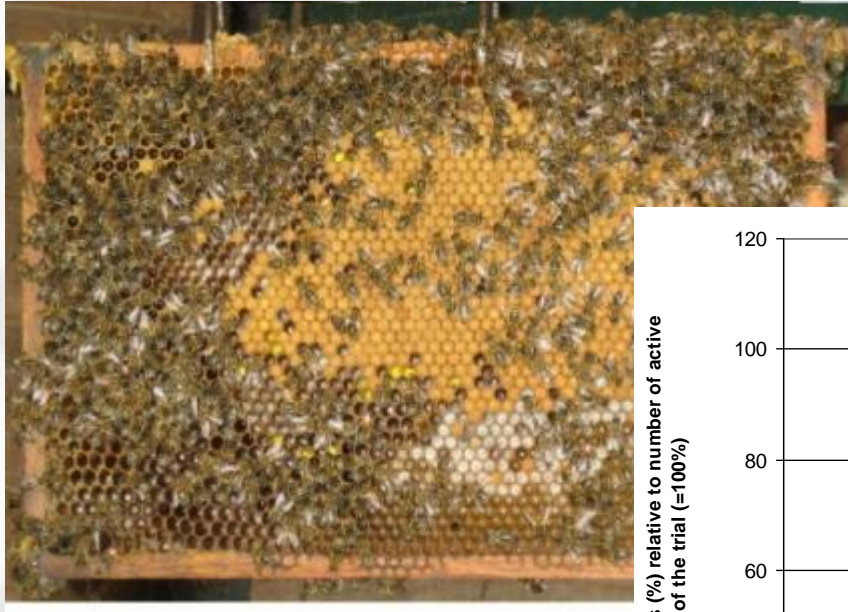
1 untreated





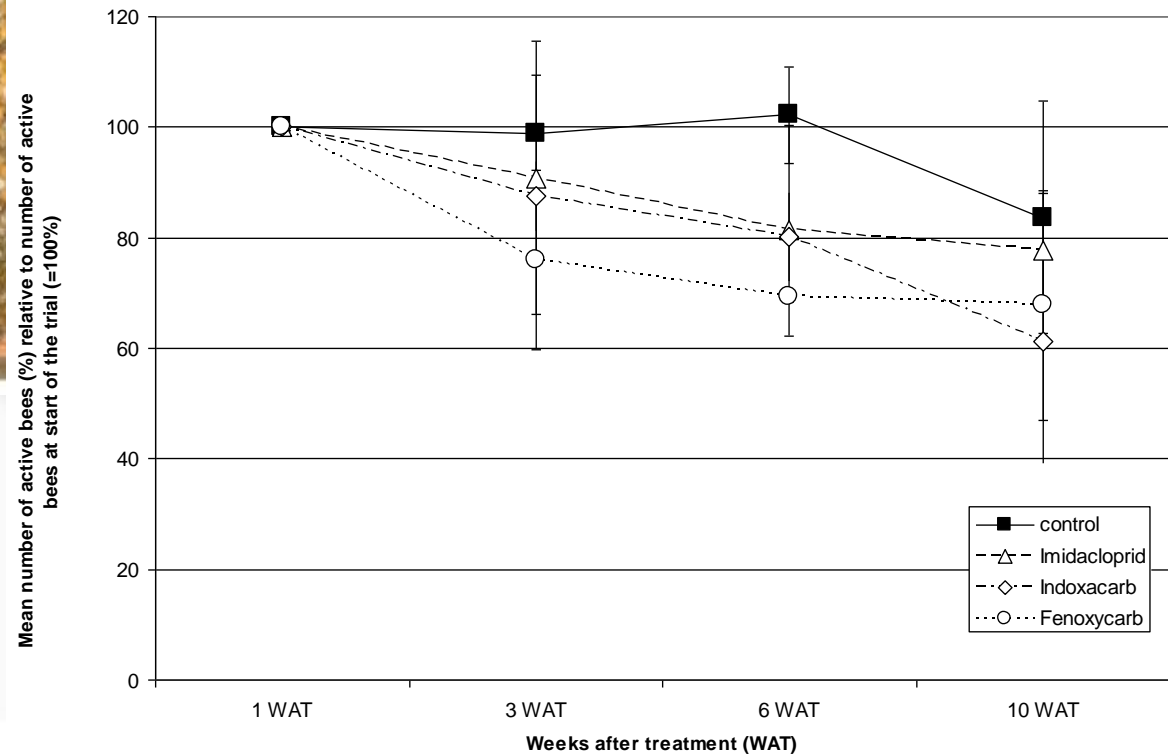
# Impact on long term colony vitality

- Total number bees alive



ANOVA analysis, Duncan multiple comparison  
6 weeks after treatment

- Fenoxycarb < untreated ( $P < 0.05$ )
- Indoxacarb < untreated ( $P < 0.05$ )
- Fenoxycarb < Imidacloprid ( $P < 0.05$ )



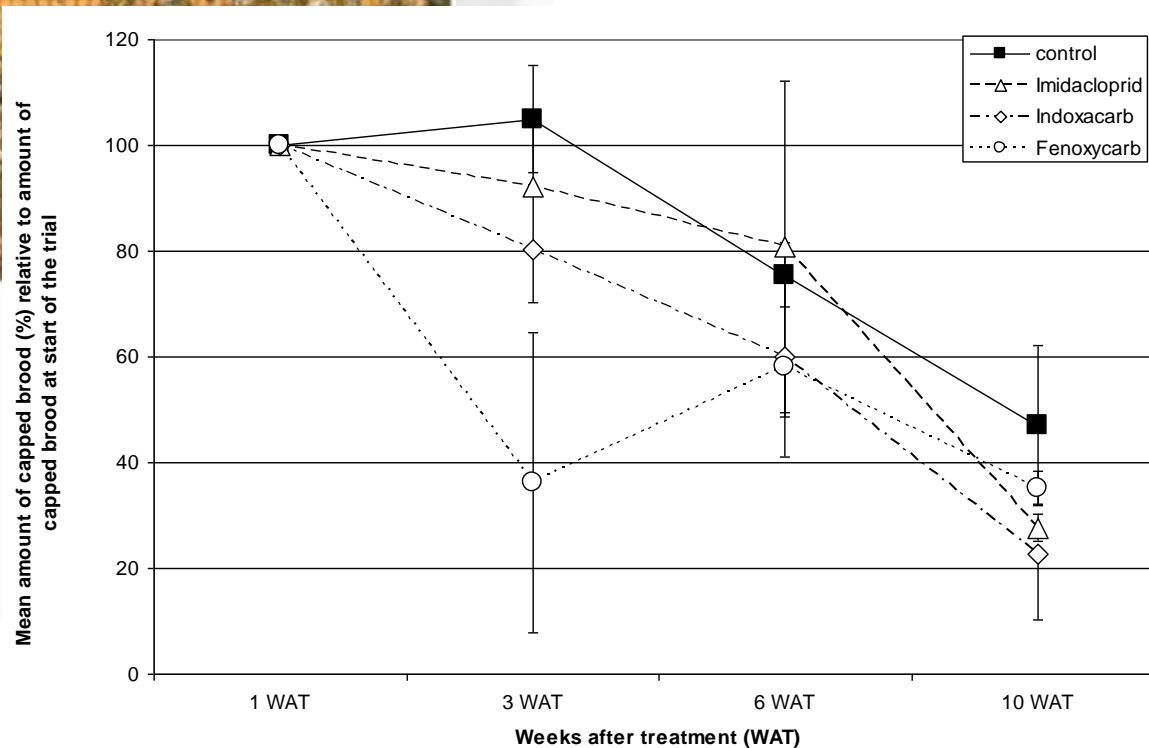
# Impact on long term colony vitality

## - Brood development



ANOVA analysis, Duncan multiple comparison  
3 weeks after treatment

- Fenoxycarb < untreated ( $P < 0.05$ )
- Fenoxycarb < Imidacloprid ( $P < 0.05$ )
- Fenoxycarb < Indoxacarb ( $P < 0.05$ )



# Conclusions

Sublethal effects  
behavior disturbance



Physiological/Molecular changes

glands  
gene expressions  
proteins



First insight into molecular mechanisms responsible for sublethal behavior disturbances

E.g. Objective detection: ***Amfor* gene expression** to measure abnormal onset foraging

No negative effects on long term colony vitality  
(sublethal doses corresponding to their use in good agricultural practices)

Importance of good understanding between fruit growers and beekeepers  
in order to avoid toxic (lethal) contaminations

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