

# RNAi & resistance against African SP weevils



UNIVERSITEIT  
GENT

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<http://insects.ugent.be>



# GHENT



DNA/RNA  $\leftrightarrow$  protein  $\leftrightarrow$  cell  $\leftrightarrow$  organism (insect)  $\leftrightarrow$  greenhouse



# African sweet potato weevil

*Cylas puncticollis* & *C. brunneus*







- Founded in 1817. Now one of the leading institutions of higher education and research in *the Lower Countries* and in Europe. It distinguishes itself as a socially committed and pluralistic university in a broad international perspective.
- Place 85 in Shanghai ranking (Academic Ranking of 1000 World Universities); first of Belgium
- Full university with 11 faculties, 130 departments and 38,000 students.
- 5 Doctoral Schools for doctoral researchers.

**1. RNAi mechanism**

**2. Project outline with  
WPs**

**3. First results**

# The Nobel Prize in Physiology or Medicine

## 2006 – Andrew Z. Fire, Craig C. Mello



**NATURE BIOTECHNOLOGY** VOLUME 25 NUMBER 11 NOVEMBER 2007

### Control of coleopteran insect pests through RNA interference

James A Baum<sup>1</sup>, Thierry Bogaert<sup>2</sup>, William Clinton<sup>1</sup>, Gregory R Heck<sup>1</sup>, Pascale Feldmann<sup>2</sup>, Oliver Ilagan<sup>1</sup>, Scott Johnson<sup>1</sup>, Geert Plaetinck<sup>2</sup>, Tichafa Munyikwa<sup>1</sup>, Michael Pleau<sup>1</sup>, Ty Vaughn<sup>1</sup> & James Roberts<sup>1,3</sup>

### Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol

Ying-Bo Mao<sup>1,2</sup>, Wen-Juan Cai<sup>1,2</sup>, Jia-Wei Wang<sup>1,2</sup>, Gao-Jie Hong<sup>1,2</sup>, Xiao-Yuan Tao<sup>1,2</sup>, Ling-Jian Wang<sup>1</sup>, Yong-Ping Huang<sup>1</sup> & Xiao-Ya Chen<sup>1</sup>



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**MONSANTO**







**Western corn rootworm, *Diabrotica virgifera virgifera***





**Reduction in root damage**



Contents lists available at ScienceDirect

# Journal of Insect Physiology

journal homepage: [www.elsevier.com/locate/jinsphys](http://www.elsevier.com/locate/jinsphys)



## Review

# Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: A review

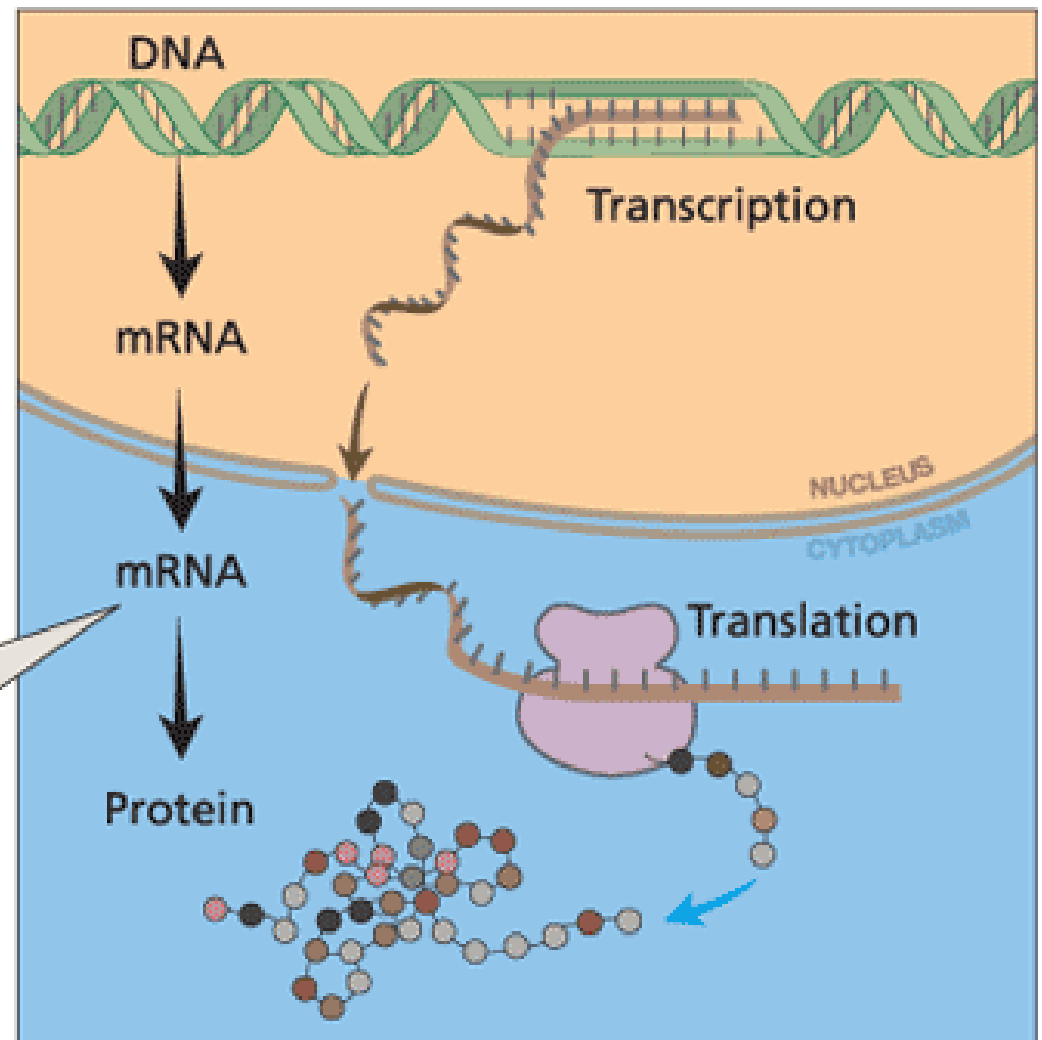
Hanneke Huvenne, Guy Smagghe \*

*Laboratory of Agrozoology, Department of Crop Protection, Ghent University, Coupure Links 653, 9000 Ghent, Belgium*



# Central dogma

The genetic information in double-stranded DNA is transcribed into single-stranded messenger RNA (mRNA) in the cell nucleus and subsequently translated into protein in the cytoplasm.



~~mRNA~~

## RNA interference

The mRNA is destroyed before it is translated into protein.



# RNAi

- **Fundamental research**  
-> *functional genomics*
- **Applications:**
  - > *insect pest control*
  - > *protection of beneficial insects (e.g. bees) against pathogenic viruses*

## RNA interference, RNAi

Double-stranded RNA triggers gene silencing.

Tailor-made ds/siRNA molecules  
-> degrade specific mRNA

Double-stranded RNA  
(dsRNA) binds to a  
protein complex, Dicer...



dsRNA



...which cleaves dsRNA  
into smaller frag-  
ments.



One of the RNA strands  
is loaded into another  
protein complex,  
RISC...



...and links the complex  
to the messenger RNA  
(mRNA) by base pairing.



mRNA is cleaved  
and destroyed.



No protein can  
be synthesized.

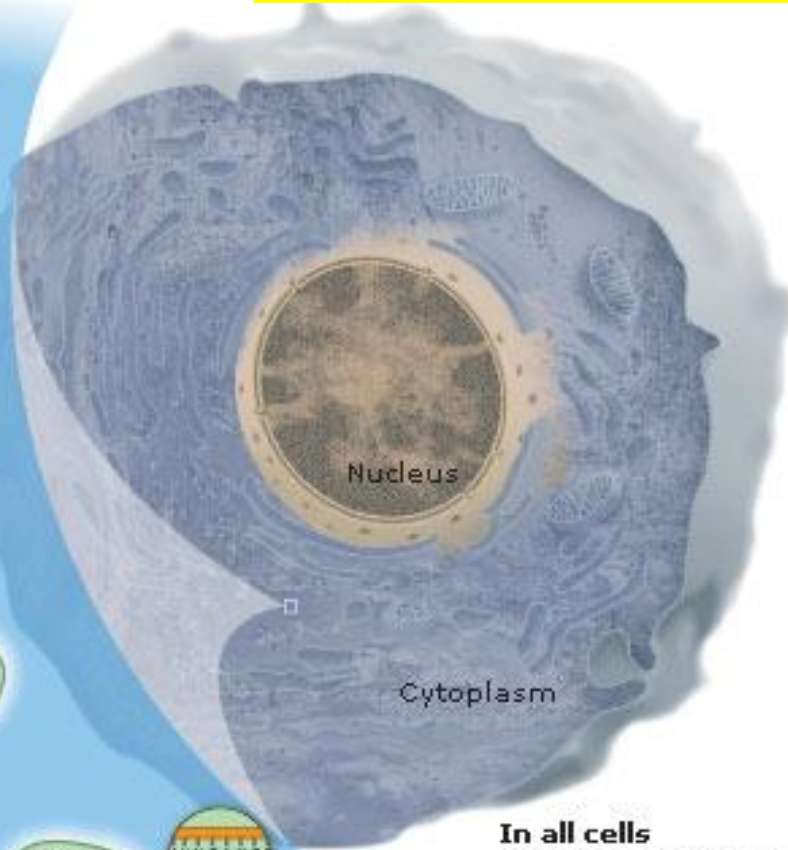


The gene  
is silenced.

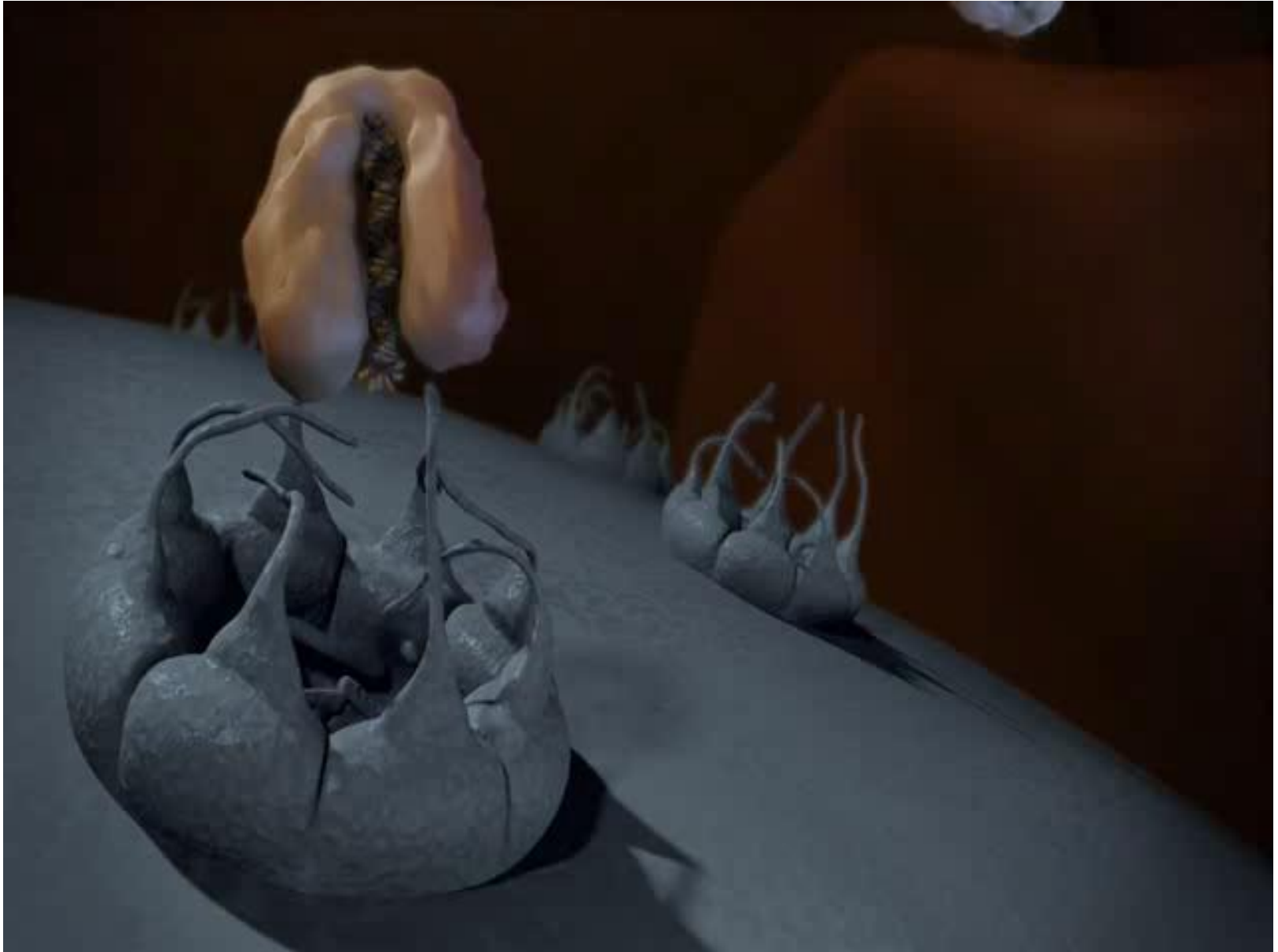


### In all cells

RNA interference occurs  
in the cytoplasm in plants,  
animals and humans.



**RNAi : a process where dsRNA silences the complementary mRNA**





1. RNAi mechanism
2. Project outline with WPs
3. First results

# PROJECT DETAILS:



- North-South project
- Collaboration CIP, UGent (Prof. Guy Smagghe, Prof. Godelieve Gheysen, Dr. Marc Ghislain)
- 4 years; started March 2013
- 4 WPs:
  - *WP1-Biology and maintenance of colony of African SP weevils *Cylas puncticollis* and *C. brunneus* in the laboratory*
  - *WP2-Transcriptome analysis and selection of best candidate genes in *Cylas puncticollis* and *C. brunneus* for RNAi*
  - *WP3-Transformation of African cultivars of sweetpotato with best candidate(s) of insect-specific dsRNA*
  - *WP4-Testing the efficacy of transformed African sweetpotato cultivars to resist against an infection of African SP weevils of *Cylas* sp.*

- 1. RNAi mechanism**
- 2. Project outline with WPs**
- 3. First results**



# We started

- 2+2 yrs sandwich PhD grant North-South by UGent
- Collaboration with CIP in Uganda/Kenia, and Venganza



Katterinne Prentice

**WP1-Biology and maintenance of colony of African SP weevils *Cylas puncticollis* and *C. brunneus* in the laboratory**

- Start with weevils from NaCRRI lab in Uganda
- Colony at Ghent University on fresh sweet potatoes. Controlled incubators Sanyo at 25°C 75% RH, 16L:8D
- Also attempts to keep colony on artificial diet

## **WP2-Transcriptome analysis and selection of best candidate genes in *Cylas puncticollis* and *C. brunneus* for RNAi**

- Sequencing at Genomic Service of North Caroline State Univ., USA
- Gene annotation at NCSU and UGent
- Selection of c.20 essential target genes (+ housekeeping genes for qPCR)

	Gene	C. p.	C. b.
1	Vha68-2 F1 ATP synthase beta subunit	X	X
2	V/A-type ATP synthase catalytic subunit A	X	X
3	Synaptobrevin, isoform A	X	X
4	Pfk phosphofructokinase		X
5	adenylate kinase-2	X	X
6	Focal adhesion kinase isoform D		X
7	gamma-coatomer protein, isoform C	X	
8	delta-coatomer protein, isoform A	X	
9	alpha-coatomer protein, isoform D	X	
10	TBP-associated factor 1, isoform D	X	X
11	lethal (2) NC136, isoform B	X	X
12	Proteasome 20 kD subunit	X	X
13	DNA polymerase interactin tpr containing protein of 47 Kd, isoform B	X	X
14	alpha-Adaptin, isoform A	X	X
15	Mad1	X	X
16	Ubiquitin conjugating enzyem E2	X	
17	RNA pol beta subunit	X	X
18	RNA helicase	X	X
19	ribosomal protein S13e	X	X
20	DNA polymerase alpha 50 kD		X
21	vATPase A		X
22	vATPase D	X	X
23	RPL19		X
24	Snf7		



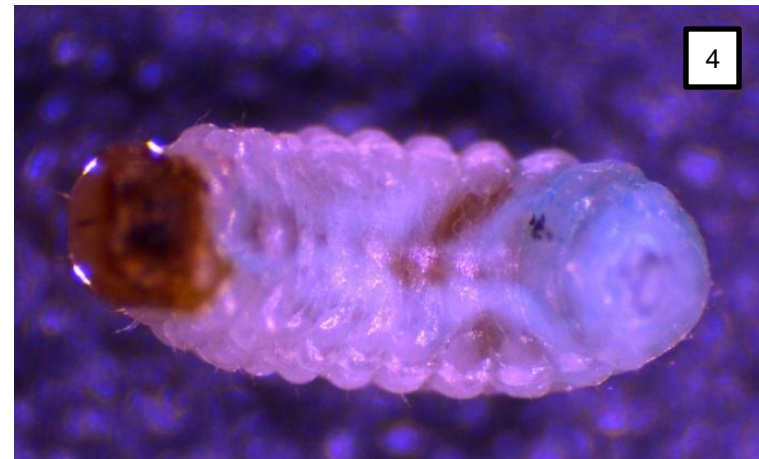
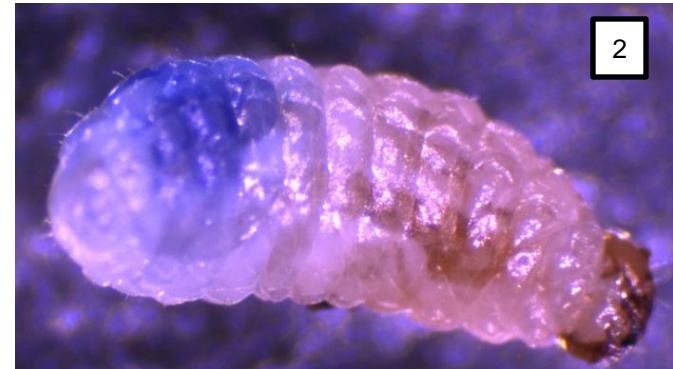
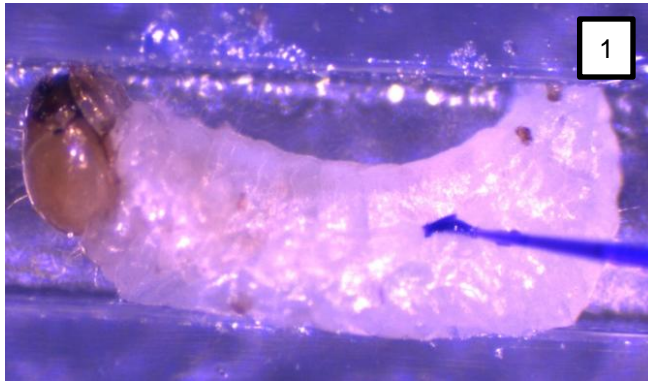
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- Synthesis of dsRNAs for these c.20 target genes
- Screening insecticide activity of c.20 dsRNAs in insect bioassays by nano-injection

# Nano-injection apparatus

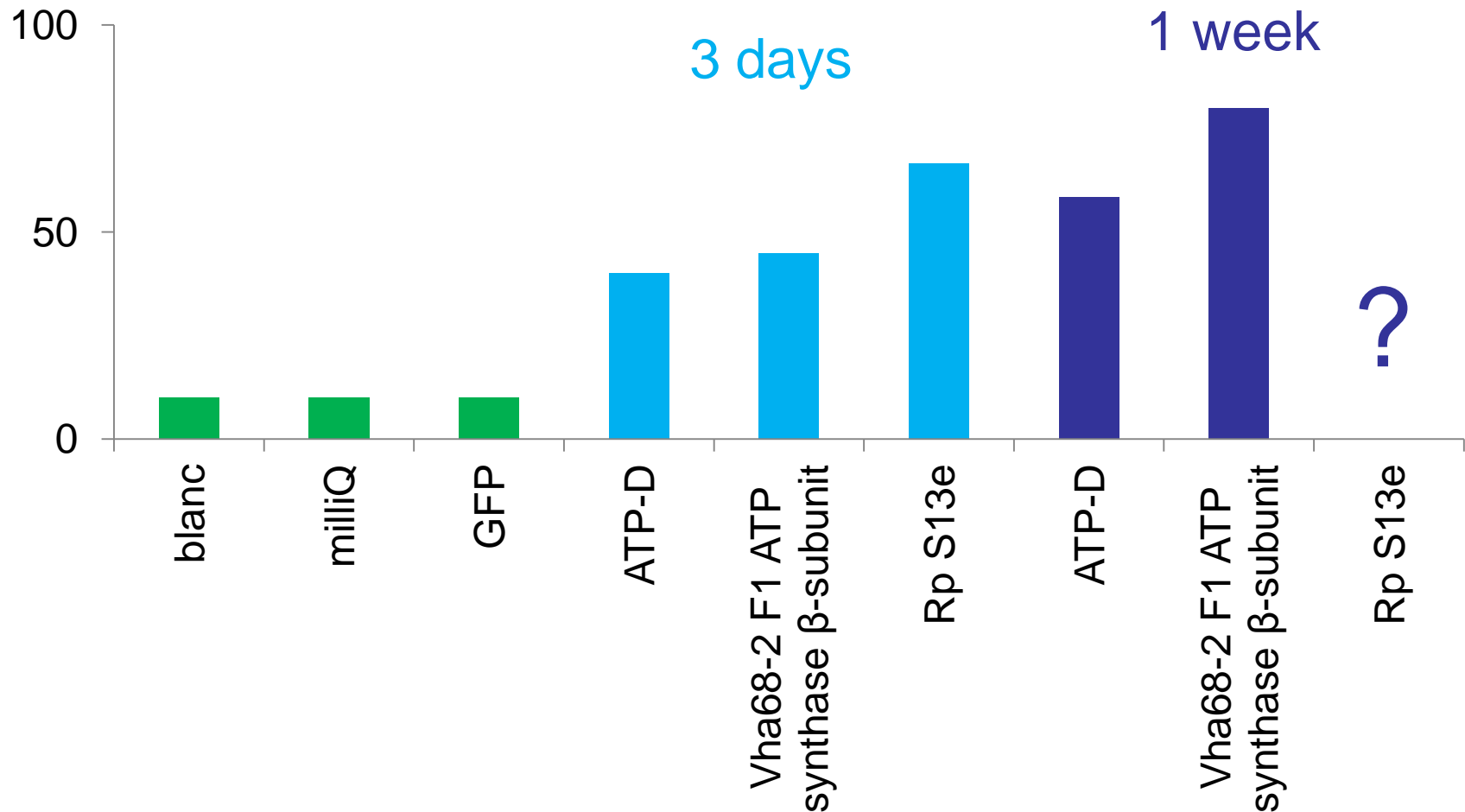


# *Cylas puncticollis*, early 3<sup>rd</sup> larval instar



**(1)** Injection with blue dye. **(2)** after 0-1 min. **(3)** 1 h **(4)** 1 day.

# First data with % insect mortality by nano-injection



- dose dsRNA injected = 100 ng/mg
- total N=190 third instars C.p.
- no info on transcription silencing yet



Blanc water  
control



dsRNA Rp S13e



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- Synthesis of dsRNAs for these c.20 target genes
- Screening insecticide activity of c.20 dsRNAs in insect bioassays by nano-injection
- Phenotypes are focusing on lethal effects, larval development, growth and reproduction
- Analyze efficacy of transcription silencing by qPCR
- Selection of c.5 best candidates
- Testing insecticide potency of c.5 best dsRNAs by feeding
- Testing insecticidal potency of combinations
- Testing insecticide potency of c.5 best dsRNAs with insects collected from field

**WP3-Transformation of African cultivars of sweetpotato  
with best candidate(s) of insect-specific dsRNA**

***->later in 2014***



# Thank you!

- Many enthusiastic MSc (87) and PhD students (18 finished and 22 ongoing)

## **Funding:**

- Belgian Fund for Scientific Research (FWO)
- Special Research Fund of Ghent University



# RNAi in crop protection

# Science

16 August 2013 | \$10

**Smarter Pest Control**

 AAAS