GHENT UNIVERSITY

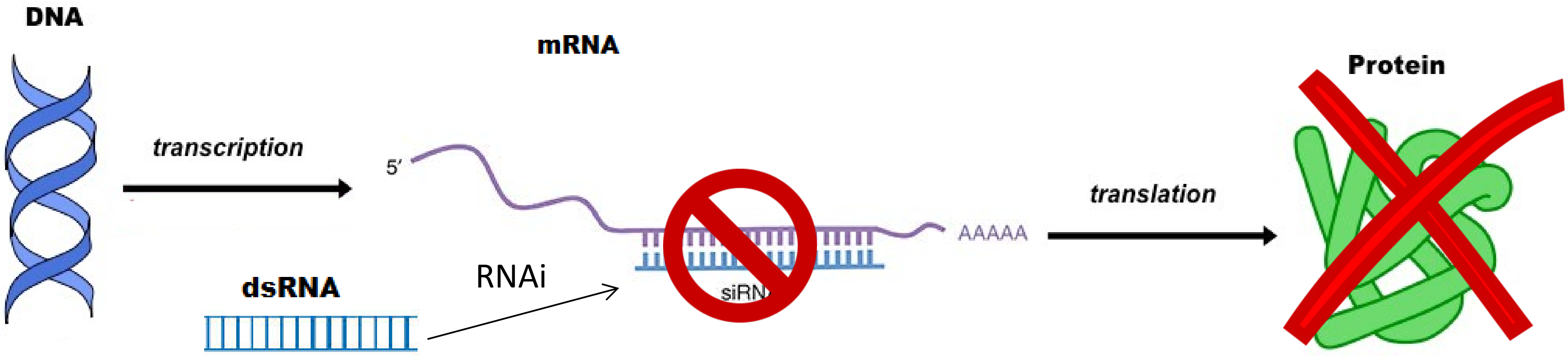
FACULTY OF BIOSCIENCE ENGINEERING

RNAi as an insect pest control strategy: challenges and biosafety considerations

Dr. ir. Olivier Christiaens

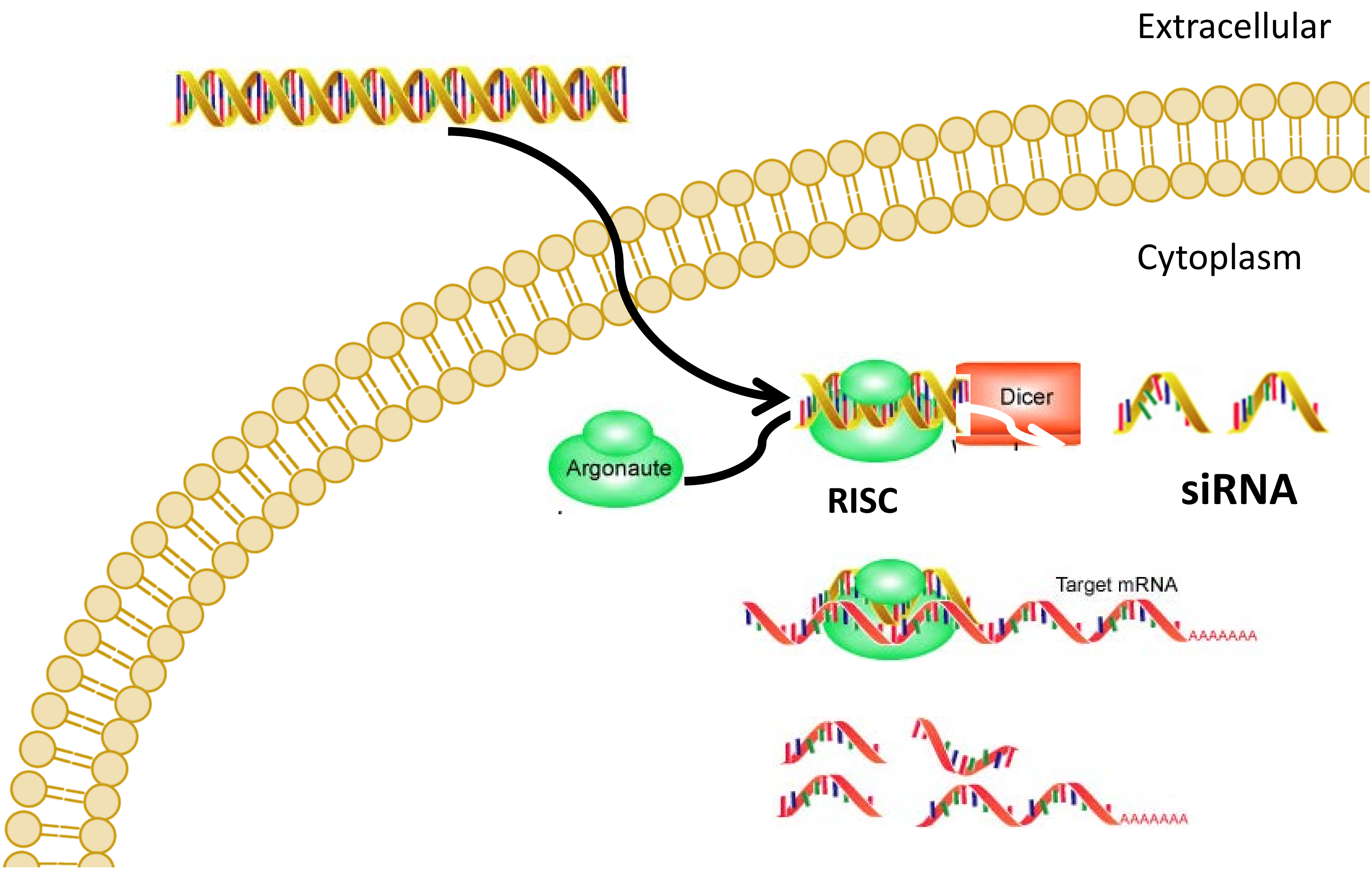
Introduction to RNAi

Basic principle



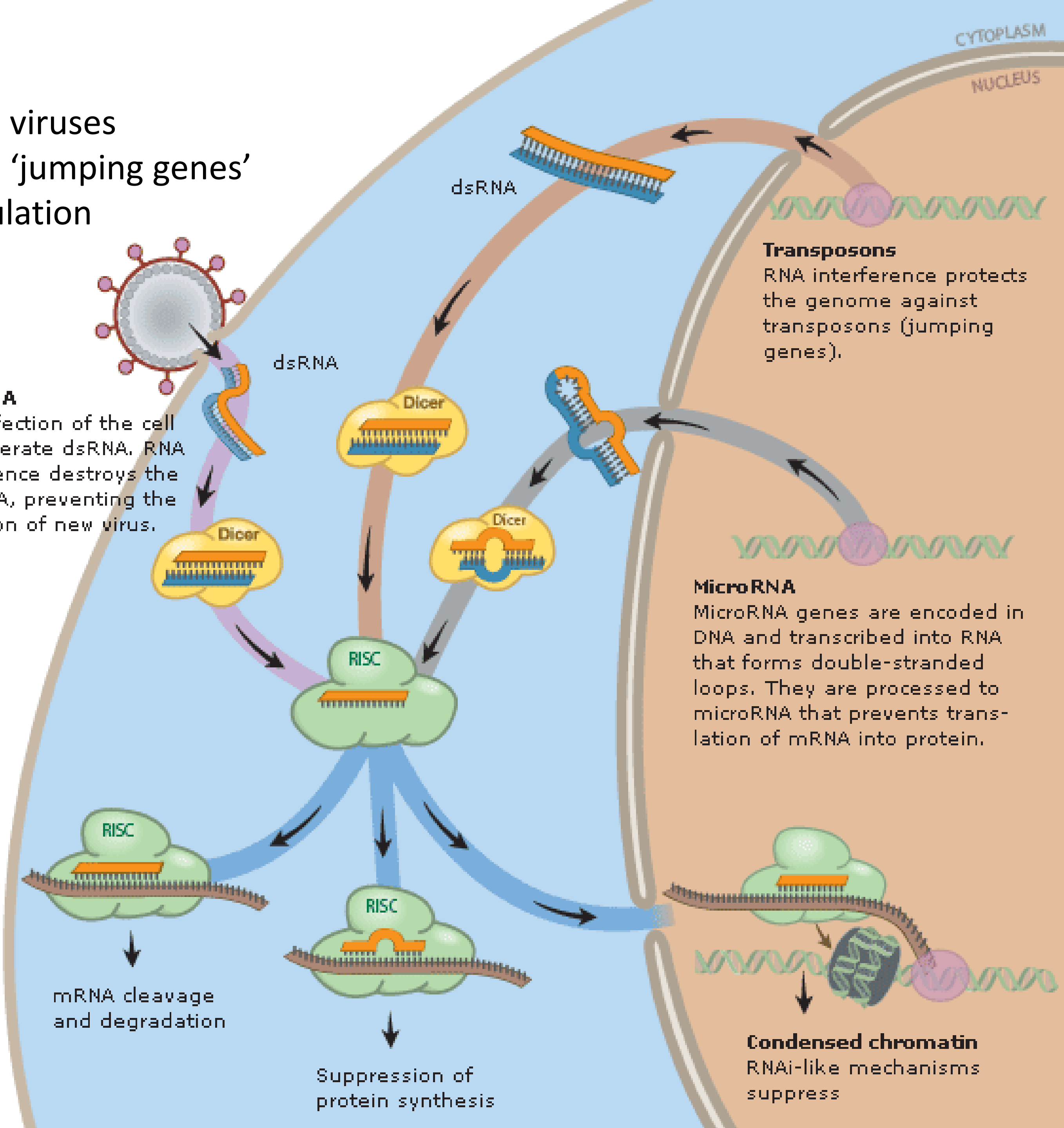
Introduction to RNAi

Molecular mechanism of the siRNA pathway



Cellular functions:

- Protection against viruses
- Protection against 'jumping genes'
- Internal gene regulation



Introduction to RNAi

Applications

- Functional genomics

Knockdown of *laccase-2* in *T. castaneum*

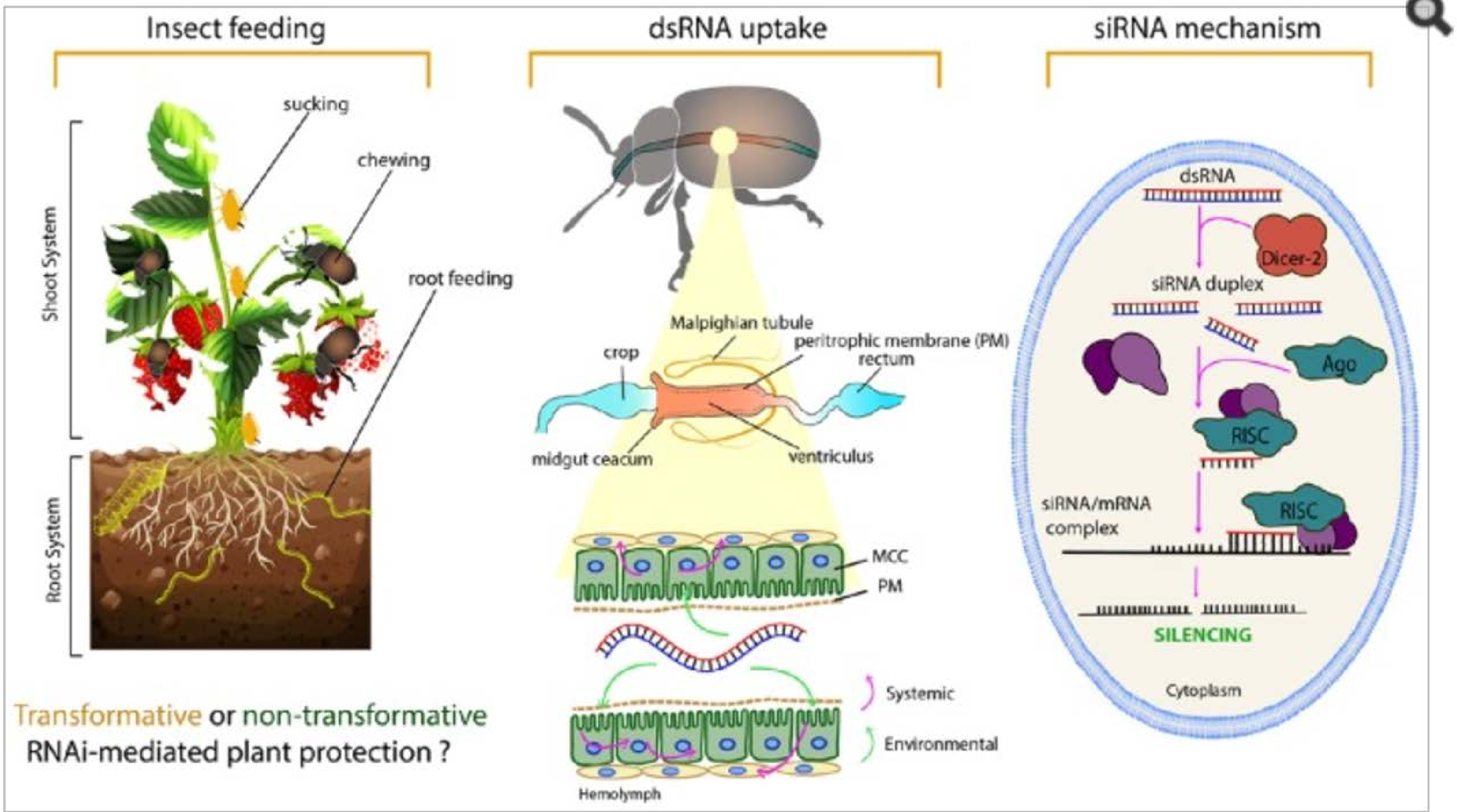
Arakane *et al.*, 2005, PNAS 102

Knockdown of *laccase-2* in *T. castaneum*

RNAi of the gene *Laccase 2*, which expresses a phenoloxidase in larvae of *Tribolium castaneum*, prevents tanning after the pupal (*a*) and imaginal (*b*, *c*) molts (2). In each photograph the control is shown at the left and the RNAi knockdown at the right. Photos courtesy of Yas Arakane.

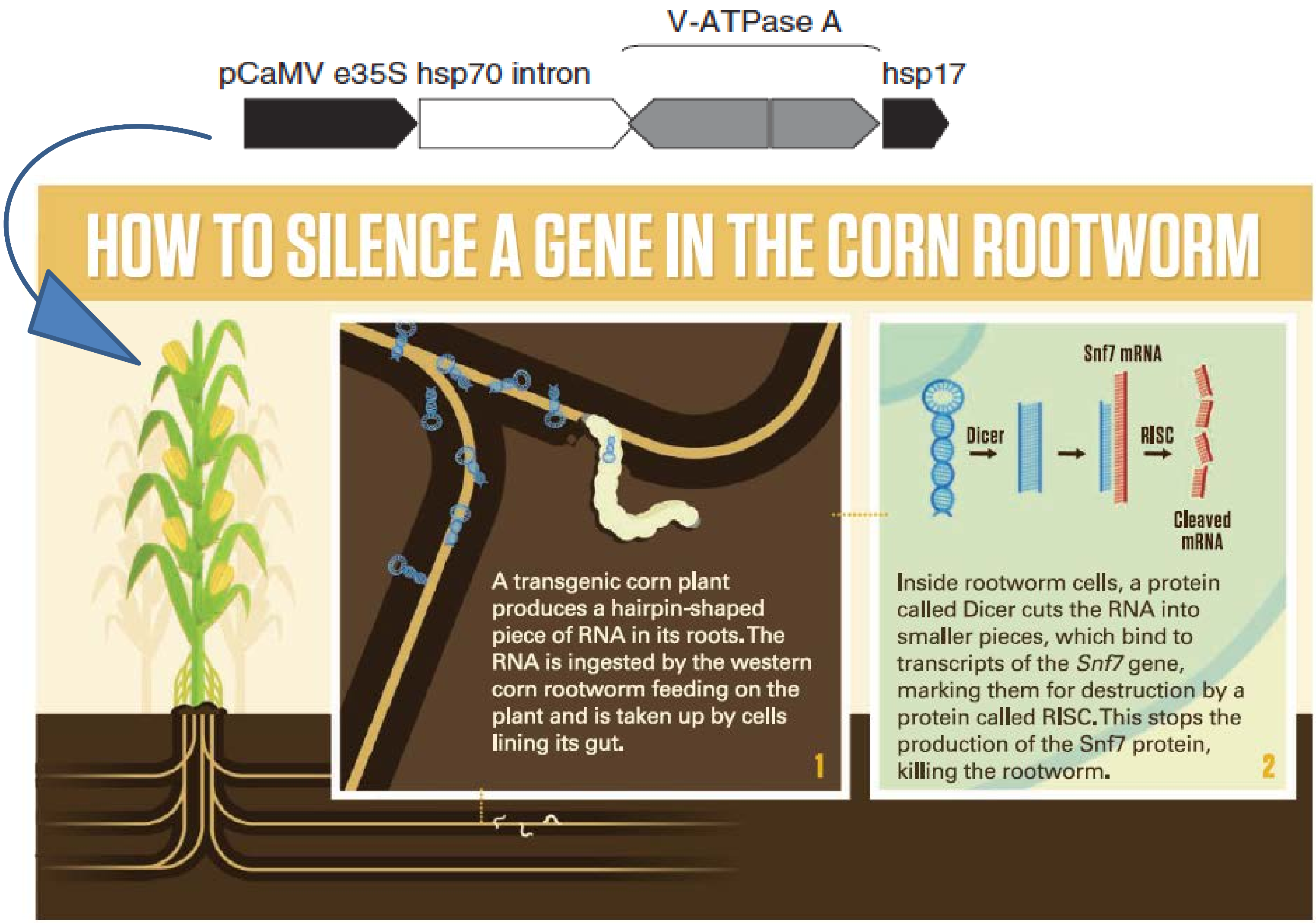
RNAi as a crop protection technology

Silencing essential genes to cause toxicity in insect pests



RNAi as a crop protection technology

First proof of concept: Baum et al., 2007



Possible Applications

2. Crop protection

Baum *et al* (2007), Nature Biotechnology 25

Possible Applications

2. Crop protection

Monsanto SmartStax Pro (MON87411 X DAS-59122-7)

- Cry3Bb1
- Cry34Ab1/Cry34Ab2
- Glyphosate resistance
- dsRNA targeting the snf7 gene
- Approval for cultivation in USA, Canada and Brazil

ELSEVIER
Regulatory Toxicology and Pharmacology
Volume 81, November 2016, Pages 77-88

Ecological risk assessment for DvSnf7 RNA: A plant-incorporated protectant with targeted activity against western corn rootworm

Pamela M. Bachman, Kristin M. Huizinga, Peter D. Jensen, Geoffrey Mueller, Jianguo Tan, Joshua P. Uffman, Steven L. Levine

Transgenic Res. 2013; 22: 1207–1222. PMCID: PMC3835954
Published online 2013 Jun 8. doi: 10.1007/s11248-013-9716-5

Characterization of the spectrum of insecticidal activity of a double-stranded RNA with targeted activity against Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte)

Pamela M. Bachman,[1] Renata Bolognesi,[2] William J. Moar,[1] Geoffrey M. Mueller,[1] Mark S. Paradise,[1] Parthasarathy Ramaseshadri,[2] Jianguo Tan,[1] Joshua P. Uffman,[1] JoAnne Warren,[1] B. Elizabeth Wiggins,[2] and Steven L. Levine[1]

Author information ► Article notes ► Copyright and License information ►

Pest Manag Sci. 2017 Sep;73(9):1883-1899. doi: 10.1002/ps.4554. Epub 2017 Mar 17.

Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management.

Head GP[1], Carroll MW[1], Evans SP[1], Rule DM[2], Willse AR[1], Clark TL[1], Storer NP[2], Flannagan RD[1], Samuel LW[1], Meinke LJ[3].

RNAi as a crop protection technology

Challenges

Delivery

- Genetically modified plants: regulation, public opinion

- Virus-mediated delivery
- Sprayable biopesticide: Cost? Stability of dsRNA in the field?
- Non-GM in planta applications (stem injection, root drenching)

RNAi as a crop protection technology

Challenges

Efficiency of (oral) RNAi in arthropods:

- Very variable

  Coleoptera > Diptera, Hemiptera, Orthoptera > Lepidoptera

- Multiple factors

  dsRNA stability in the digestive system

  Cellular uptake efficiency

  Viral infections

  RNAi effector gene repertoire

  dsRNA design

RNAi biosafety considerations

RNAi-mediated crop protection: potential

- Molecule ubiquitously present in nature
- Highly species specific in theory
- Persistency in the environment

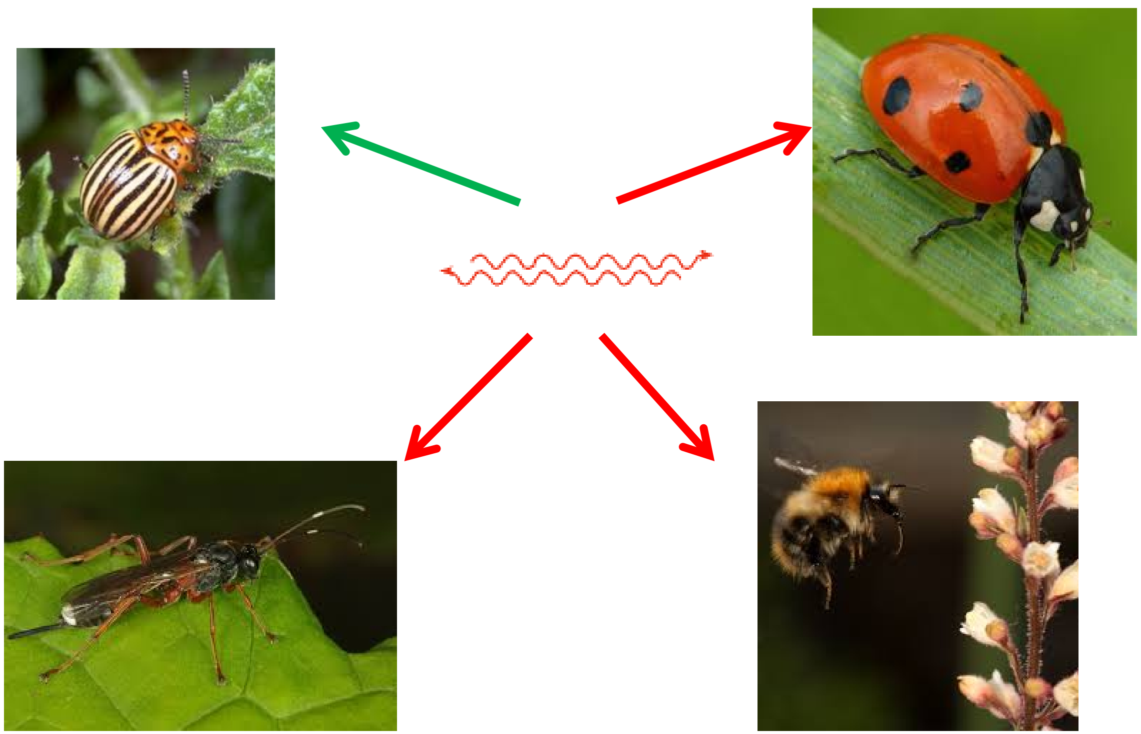
Potential environmental risks associated with RNAi technology

- Gene silencing in non-target organisms
- Environmental fate of dsRNA
- RNAi machinery saturation?
- Immune stimulation?

RNAi biosafety considerations

Gene silencing in non-target organisms

- Knockdown of any gene in a non-target organism
- Due to sequence homology
- dsRNA design is important



RNAi biosafety considerations

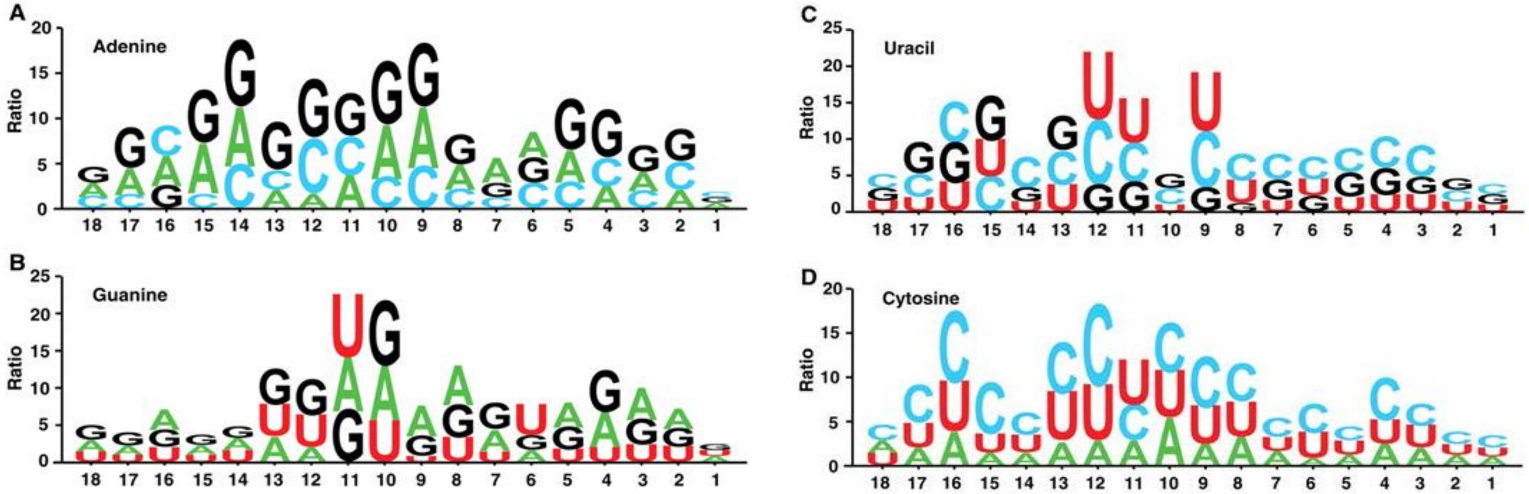
Gene silencing in non-target organisms

How specific is RNAi?

- No real consensus (rules for miRNA are clear, not for siRNA)
- Some mismatches could be allowed depending on:

  Location in the siRNA: seed region vs non-seed region

  Type of mismatch

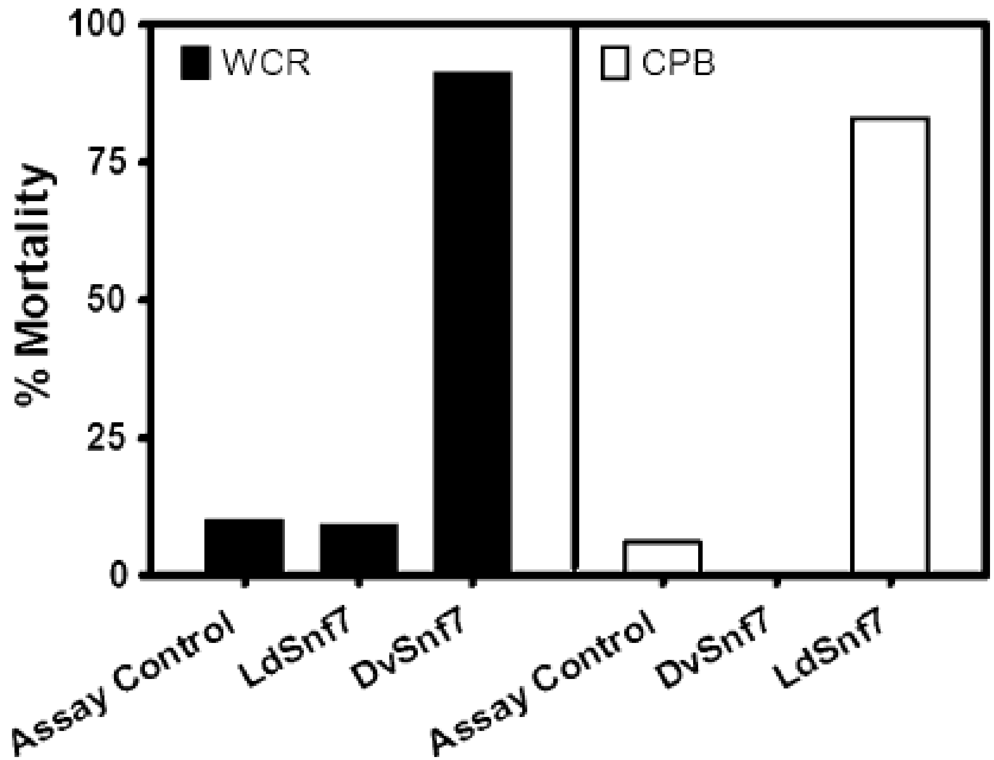


Huang et al., 2009

RNAi biosafety considerations

Gene silencing in non-target organisms

How specific is RNAi?



Bachman et al., 2013 – Transgenic Res

RNAi biosafety considerations

Gene silencing in non-target organisms

How specific is RNAi?

Order	Family	Subfamily	Species	Bioassay duration (days)	Endpoints	LC_{50}^{a} or no observed effect concentration[b] (ng/mL or g diet)
Coleoptera	Chrysomelidae	Galerucinae	*D. virgifera virgifera*	12	Survival	1.2[a]
	Chrysomelidae	Galerucinae	*D. undecimpunctata howardi*	12	Survival	4.4[a]
	Chrysomelidae	Chrysomelinae	*L. decemlineata*	12	Survival, Growth	5,000[b]
	Tenebrionidae	Tenebrioninae	*T. castaneum*	30	Survival, Growth	5,000[b]
	Coccinellidae	Coccinellinae	*C. maculata*	24	Survival, Growth, Development	3,000[b]
	Coccinellidae	Epilachninae	*E. varivestis*	28	Survival, Growth, Development	3,000[b]
	Carabidae	Harpalinae	*P. chalcites*	35	Survival, Growth, Development	5,000[b]
Hemiptera	Anthocoridae	Anthocorinae	*O. insidiosus*	9	Survival, Growth, Development	5,000[b]
Hymenoptera	Eulophidae	Entedoninae	*P. foveolatus*	21	Survival	3,000[b]
	Pteromalidae	Pteromalinae	*N. vitripennis*	20	Survival	5,000[b]
Lepidoptera	Noctuidae	Noctuinae	*S. frugiperda*	8	Survival, Growth	500[b]
		Heliothinae	*H. zea*	12	Survival, Growth	5,000[b]
	Crambidae	Pyraustinae	*O. nubilalis*	12	Survival, Growth	5,000[b]
	Bombycidae	Bombycinae	*B. mori*	14	Survival, Growth	5,000[b]

Bachman et al., 2013 – Transgenic Res

RNAi biosafety considerations

Gene silencing in non-target organisms

How specific is RNAi?

Table 2 Percent identity, numbers of single nucleotide polymorphisms (SNPs) and number of 21 nt matches of *Snf7* orthologs from ten Coleoptera in the families Chrysomelidae and Tenebrionidae

Species	Subfamily, Tribe	Percent identity to DvSnf7 dsRNA	No. SNPs	No. 21 nt matches (or longest contiguous sequence)
D. virgifera virgifera	Galerucinae, Luperini	100	0	221
D. undecimpunctata howardi	Galerucinae, Luperini	98.8	3	186
A. vittatum	Galerucinae, Luperini	95.0	12	69
C. trifurcata	Galerucinae, Luperini	90.8	22	18
G. calamriensis	Galerucinae, Galerucini	90.8	22	3
A. lacertosa	Galerucinae, Alticini	81.7	44	0, (17 nt)
C. quadrigemina	Chrysomelinae, Chrysomelini	82.1	43	0, (19 nt)
M. ochroloma	Chrysomelinae, Chrysomelini	79.6	49	0, (19 nt)
L. decemlineata	Chrysomelinae, Chrysomelini	78.3	52	0, (14 nt)
T. castaneum	Tenebrioninae, Tribolini	72.1	67	0, (11nt)

The inclusion of a representative of the Tenebrionidae is to provide context for the degree of sequence divergence outside the Chrysomelidae

Bachman et al., 2013 – Transgenic Res

RNAi biosafety considerations

Gene silencing in non-target organisms

How specific is RNAi?

Table 3 Summary of results from heterospecific *Snf7* dsRNAs fed to WCR in 12-day continuous exposure bioassays

Species from which dsRNA was synthesized	dsRNA concentration fed to WCR (ng/mL)	Control survival %	dsRNA treatment survival %	Statistical significance	Activity	21 nt matches
A. vittatum	1,000	90	8	$p < 0.05$	Active	Yes
C. trifurcata	500	76	15	$p < 0.05$	Active	Yes
G. calamariensis	5,000	89	13	$p < 0.05$	Active	Yes
M. ochroloma	500	76	72	$p > 0.05$	Not Active	No
C. quadrigemina	5,000	94	88	$p > 0.05$	Not Active	No

The concentrations of heterospecific dsRNAs were prepared at 100–1,000 times the conspecific dsRNA 12-day LC_{50} for WCR (as reported in Bolognesi et al. 2012)

Bachman et al., 2013 – Transgenic Res

RNAi biosafety considerations

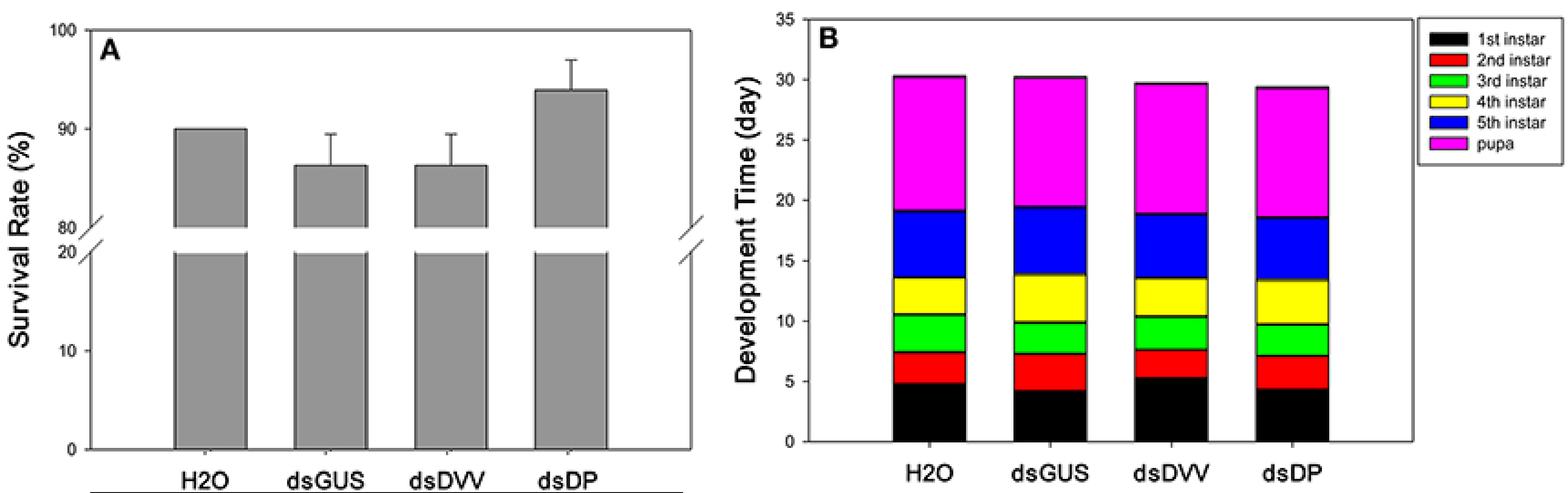
ORIGINAL RESEARCH ARTICLE

Front. Plant Sci., 22 February 2017 | https://doi.org/10.3389/fpls.2017.00242

Dietary Risk Assessment of *v-ATPase A* dsRNAs on Monarch Butterfly Larvae

Huipeng Pan[1,2], Xiaowei Yang[2], Keith Bidne[3], Richard L. Hellmich[3], Blair D. Siegfried[4] and Xuguo Zhou[2*]

- Worst case scenario:
  - Very conserved gene
  - Very conserved region
  - 1600 x Diabrotica virgifera LC50



RNAi biosafety considerations

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- Worst case scenario:
  - Very conserved gene
  - Very conserved region
  - 1600 x Diabrotica virgifera LC50

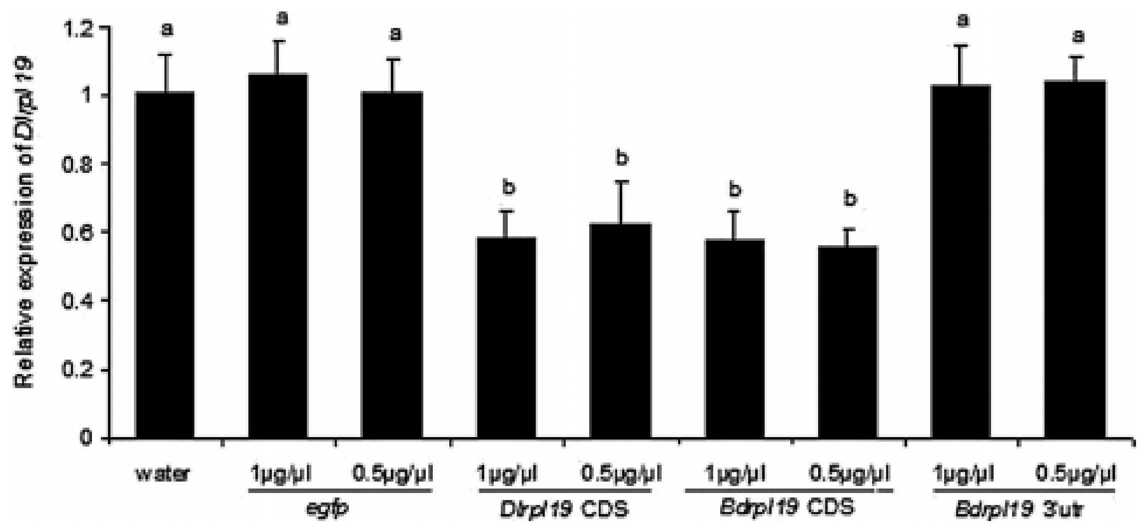
- RNAi efficiency in Lepidoptera is a factor here

RNAi biosafety considerations

Gene silencing in non-target organisms

How specific is RNAi?

Fig. 4 Expression levels of *Dlrpl19* after feeding *D. longicaudata Dlrpl19* CDS dsRNA, *Bdrpl19* CDS dsRNA or *Bdrpl19* 3′UTR dsRNA. Feeding *D. longicaudata Bdrpl19* CDS dsRNA or *Dlrpl19* CDS dsRNA significantly decreased *Dlrp19* gene expression when compared to adults fed *Bdrpl19* 3′UTR dsRNA, egfp dsRNA or RNA-free water after 24 h



The effects of RNA interference targeting *Bactrocera dorsalis* ds-*Bdrpl19* on the gene expression of *rpl19* in non-target insects

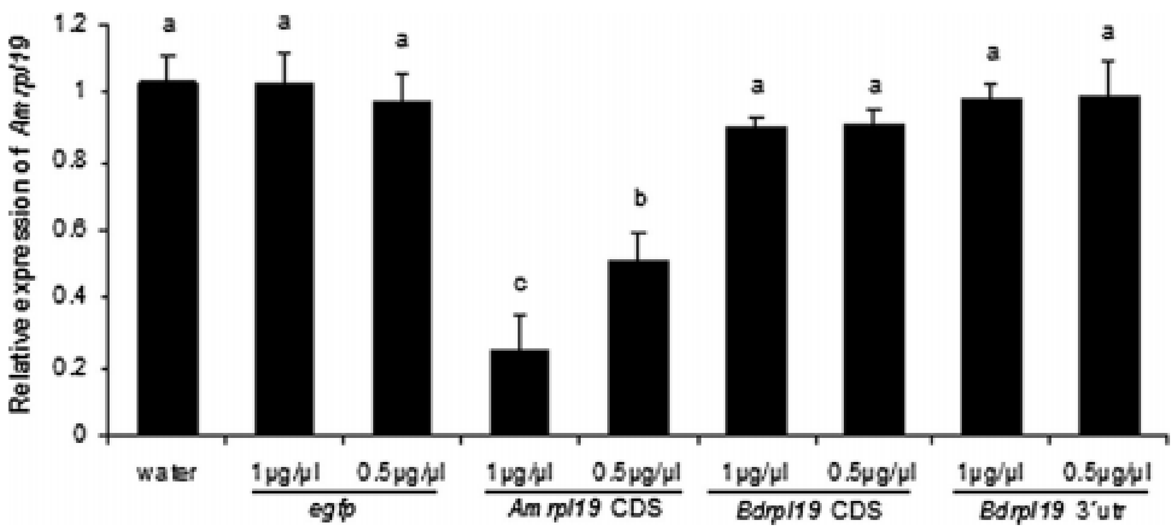
Aie Chen · Weiwei Zheng · Wenping Zheng · Hongyu Zhang

RNAi biosafety considerations

Gene silencing in non-target organisms

How specific is RNAi?

Fig. 5 Expression levels of *Amrpl19* after feeding *A. mellifera Amrpl19* CDS dsRNA, *Bdrpl19* CDS dsRNA *or Bdrpl19* 3′UTR ds*RNA*. *Amrpl19* CDS dsRNA significantly decreased the *Amrpl19* level when compared with *Bdrpl19* CDS dsRNA, *Bdrpl19* 3′UTR dsRNA, egfp dsRNA and RNA-free water after 24 h



The effects of RNA interference targeting *Bactrocera dorsalis* ds-*Bdrpl19* on the gene expression of *rpl19* in non-target insects

Aie Chen · Weiwei Zheng · Wenping Zheng · Hongyu Zhang

RNAi biosafety considerations

Bioinformatics useful in risk assessment?

- Knowledge on the ecosystems and species present

  What insects are present in a certain crop?

  Will these insects be exposed to the dsRNA?

  What kind of amount of dsRNA can we expect these species to take in?

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External scientific report

Establishing a database of bio-ecological information on non-target arthropod species to support the environmental risk assessment of genetically modified crops in the EU

Michael Meissle, Fernando Álvarez-Alfageme, Louise A. Malone, Jörg Romeis

First published: 13 September 2012 Full publication history

View issue TOC
Volume 9, Issue 9
September 2012
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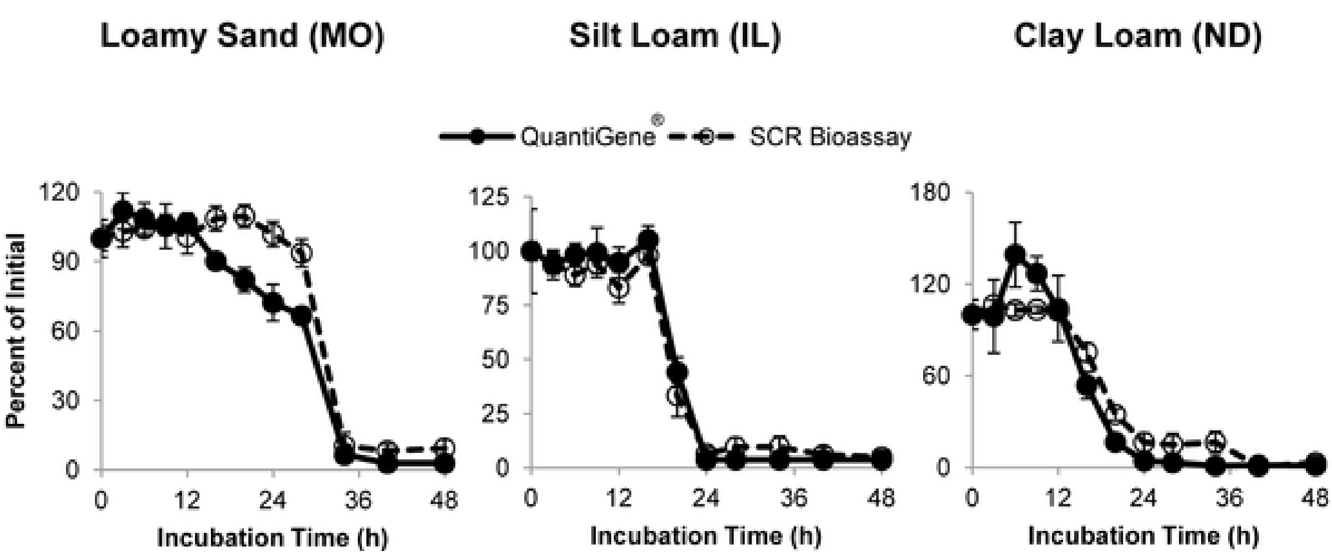
Biosafety and Risk Assessment

Potential risks associated with RNAi-based (crop protection) technology

Environmental fate of dsRNA

Dubelman et al., 2014 - PLOS One

- Investigated the persistency of dsRNA in the soil
- 3 soil types



DsRNA persistency in soil + lyophilized corn tissue

RNAi biosafety considerations

Potential risks associated with RNAi-based (crop protection) technology

Saturation of RNAi machinery

- Number of RISC complexes is limited in a cell
- Uptake of large amounts can saturate RNAi machinery
- Sequence-independent
- Potential effects on efficiency of internal gene regulation?
- Potential effects on ability to fight off viral infections?

Biosafety and Risk Assessment

Potential risks associated with RNAi-based (crop protection) technology

Immune stimulation

- In mammals: long dsRNA fragments can incite an immune response when taken up into the cell
- Will this affect the fitness of other organisms as well?
- In insects: unknown
- More research necessary

Insects **2013**, *4*(1), 90-103; doi:10.3390/insects4010090 Open Access

Article

Non-Target Effects of Green Fluorescent Protein (GFP)-Derived Double-Stranded RNA (dsRNA-GFP) Used in Honey Bee RNA Interference (RNAi) Assays

Francis M. F. Nunes [1,†,*] ✉, Aline C. Aleixo [1,†,*] ✉, Angel R. Barchuk [2] ✉, Ana D. Bomtorin [1] ✉, Christina M. Grozinger [3] ✉ and Zilá L. P. Simões [4] ✉

Biosafety and Risk Assessment

Conclusions

- RNAi seems promising, compared to current pest control methods
- Bioinformatics: useful tool, but not enough
- DsRNA disappears quickly from the environment
- Gaps in our knowledge
- Level of exposure to siRNAs and dsRNAs must be carefully considered
- Each specific event will have to be tested for effects in non-target organisms:
    - Effects on plant
    - Effects on agroecosystem
    - Effects on humans after ingestion of specific siRNA/dsRNA

Biosafety and Risk Assessment

2014: RNAi workshop in Brussels organized by EFSA

- Experts, regulatory bodies, industry and NGOs were invited
- Three break-out sessions:
    1) Molecular characterization
    2) Food/feed risk assessment
    3) Environmental risk assessment
- Report: http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2014.EN-705/epdf

European Food Safety Authority

2015: Calls for Tenders

EcoMole™ IMG

EXTERNAL SCIENTIFIC REPORT

APPROVED: 16 may 2017

doi:10.2903/sp.efsa.2017.EN-1246

Literature review of baseline information to support the risk assessment of RNAi-based GM plants

Jan Paces[1], Miloslav Nic[2], Tomas Novotny[2], Petr Svoboda[1]

[1] Institute of Molecular Genetics of the Academy of Sciences of the Czech Republic (IMG)

[2] EcoMole Ltd.

Biosafety and Risk Assessment

EFSA Tender on Environmental Safety

Contract – OC/EFSA/GMO/2015/02 - Literature review of baseline information on RNAi that could support the food/feed and environmental risk assessment of RNAi-based GM plants;

Lot 2 – Literature review of scientific information on RNAi to support the environmental risk assessment of RNAi-based GM plants” (EFSA-Q-2016-00329)

- Goal: Gather knowledge on RNAi in invertebrate species

- Consortium for the ERA lot:
    - UGENT (Dr. Olivier Christiaens and Prof. Guy Smagghe)
    - ENEA (Prof. Salvatore Arpaia and Dr. Isabella Urru)
    - ABI (Prof. Kaloyan Kostov and Dr. Teodora Dzhambazova)
    - JT Environmental Consultants (Dr. Jeremy Sweet)

efsa European Food Safety Authority

Biosafety and Risk Assessment

EFSA Tender on Environmental Safety

- Systematic literature search (+/- 13.500 papers retrieved)
- Review the available literature
  - Cellular uptake mechanisms in invertebrates
  - RNAi efficiency
  - Risks for non-target organisms
  - Exposure routes
  - Available genomic data
- Expected publication of ERA lot: early 2018

efsa
European Food Safety Authority

Biosafety and Risk Assessment

Further references

- EPA White Paper on RNAi technology as a pesticide (2013)
- Roberts AF, Devos Y, Zhou X, Lemgo G (2015) Biosafety research for non-target organism risk assessment of RNAi-based GE plants. *Frontiers in Plant Science*, 6:958,doi:10.3389/fpls.2015.00958
- Casacuberta JM, Devos Y, du Jardin P, Ramon M, Vaucheret H, Nogué F (2015) Biotechnological uses of RNA interference in plants: risk assessment considerations. *Trends in Biotechnology*, 33: 145-147
- Ramon M, Devos Y, Lanzoni A, Liu Y, Gomes A, Gennaro A, Waigmann E (2014) RNAi-based GM plants: food for thought for risk assessors. *Plant Biotechnology Journal*, 12: 1271-1273
- Lundgren JG, Duan, JJ (2013). RNAi-based insecticidal crops: potential effects on nontarget species. Bioscience 63:657-665

Thank you for your attention!

Dr. Ir. Olivier Christiaens
Lab for Agrozoology
Ghent University – Belgium
olchrist.christiaens@ugent.be

UNIVERSITEIT GENT DARE TO THINK

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Faculty of Bioscience Engineering