



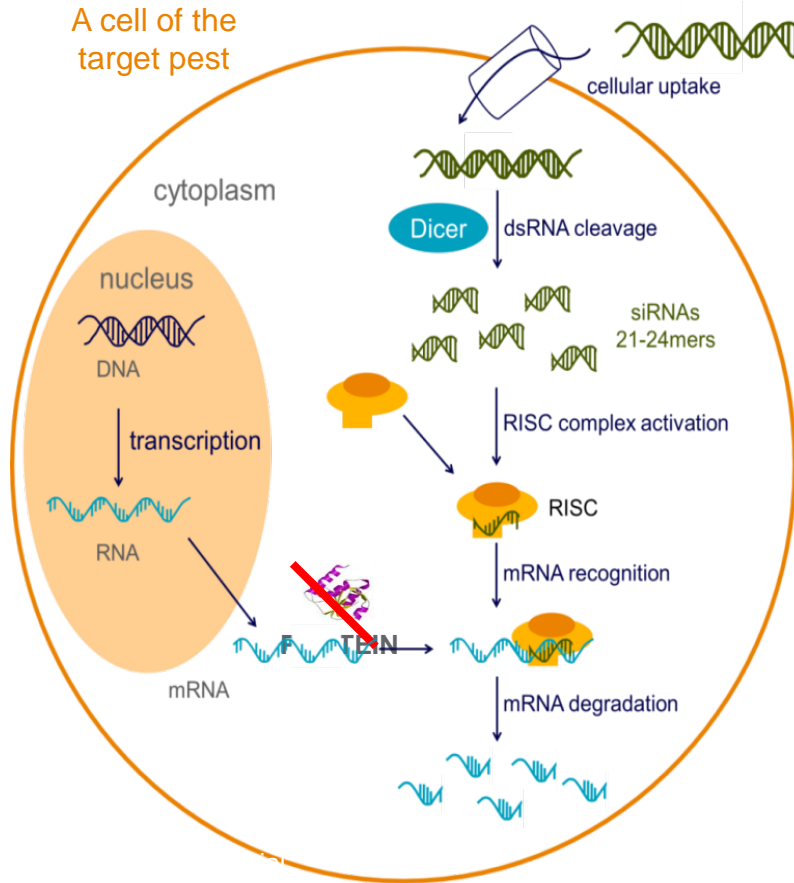
RNA-based biocontrols: een nieuwe technologie voor insectbestrijding

Geert Plaetinck

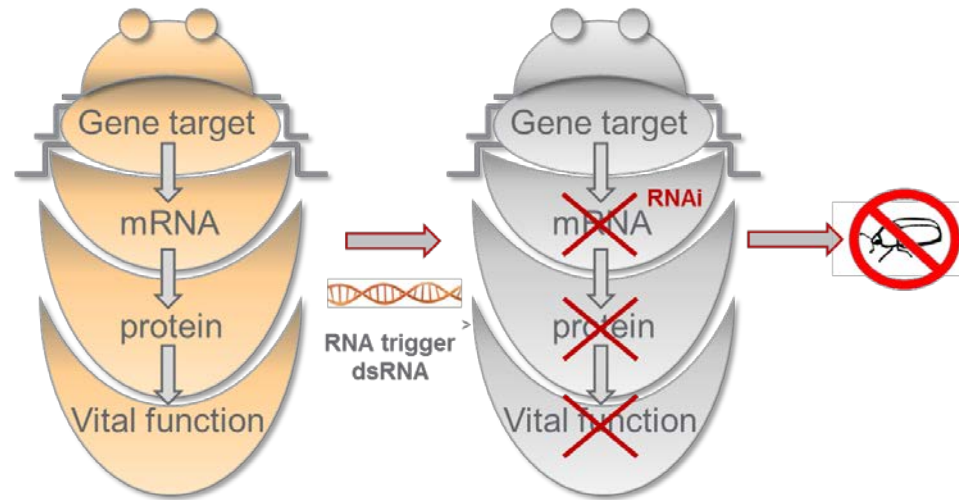
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Ede donderdag 15 juni 2017

RNAi: a natural process to regulate protein levels in the cell

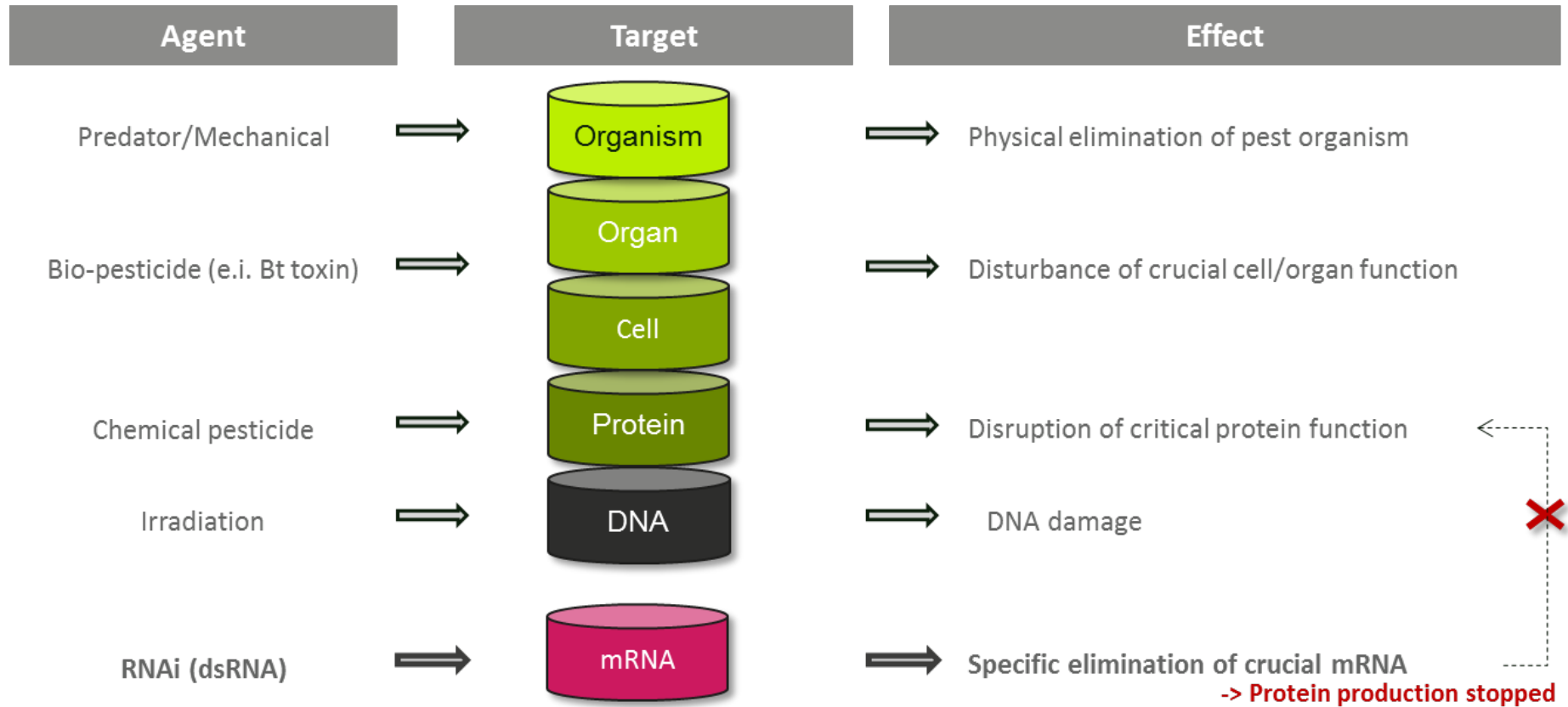


RNA-based biocontrol



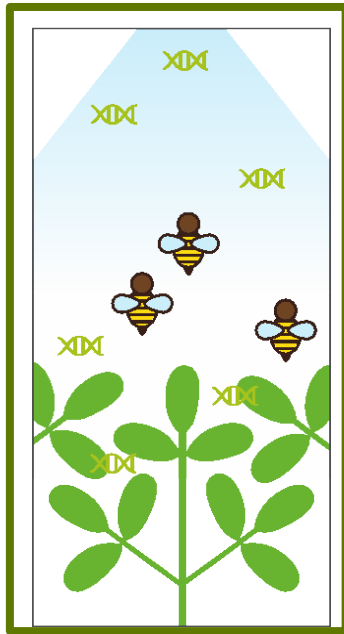
- mRNA messenger RNA
- dsRNA double-stranded RNA
- siRNA small interfering RNA
- RISC RNA induced silencing complex

RNAi: A new Mode of Action for pest control

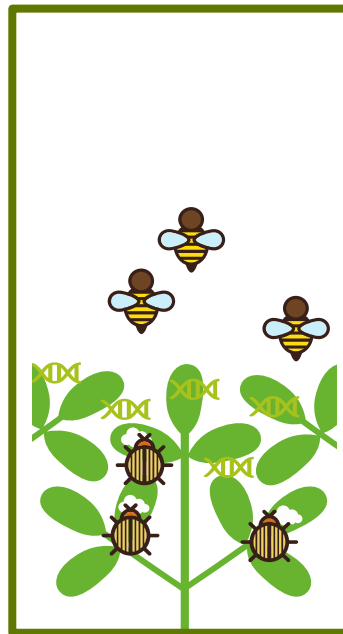


RNAi has a unique «Mode of Action» that is different from current pest control methods

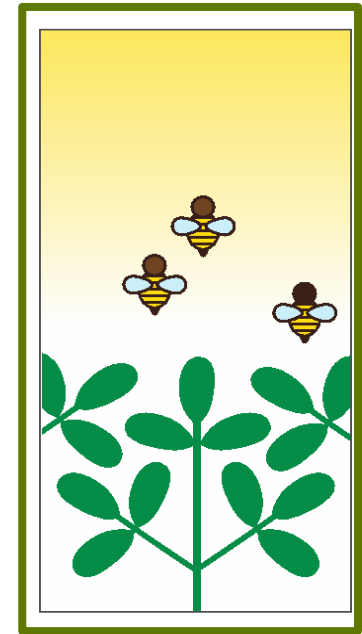
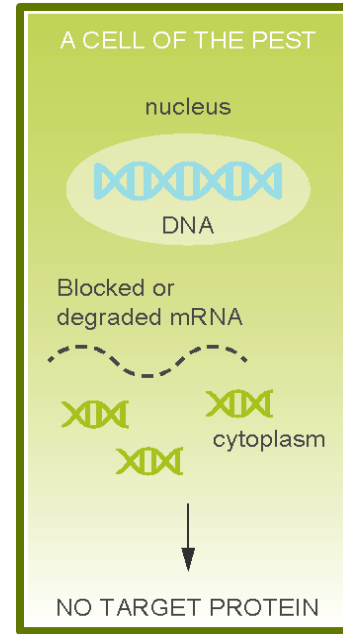
RNA-Based Biocontrols: topical application and action



The RNA-based biocontrol, which is double-stranded RNA, is sprayed onto the plant.













The pest eats the biocontrol which is taken up into the pest's cells. The RNAi process is triggered, which stops synthesis of the essential protein in the target pest, controlling the pest before it can cause too much damage to the crop.



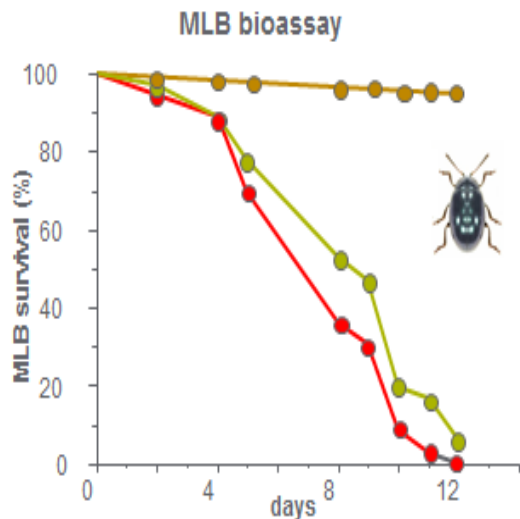
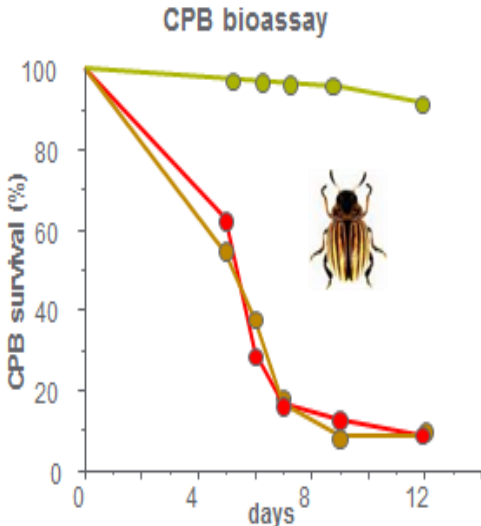
The pest is controlled and the plant matures to yield its crop. Non-target insects are not affected due to the precision of the RNA-based biocontrol.

RNA-based biocontrol: unparalleled pest selectivity

INTENDED TARGET BY DESIGN

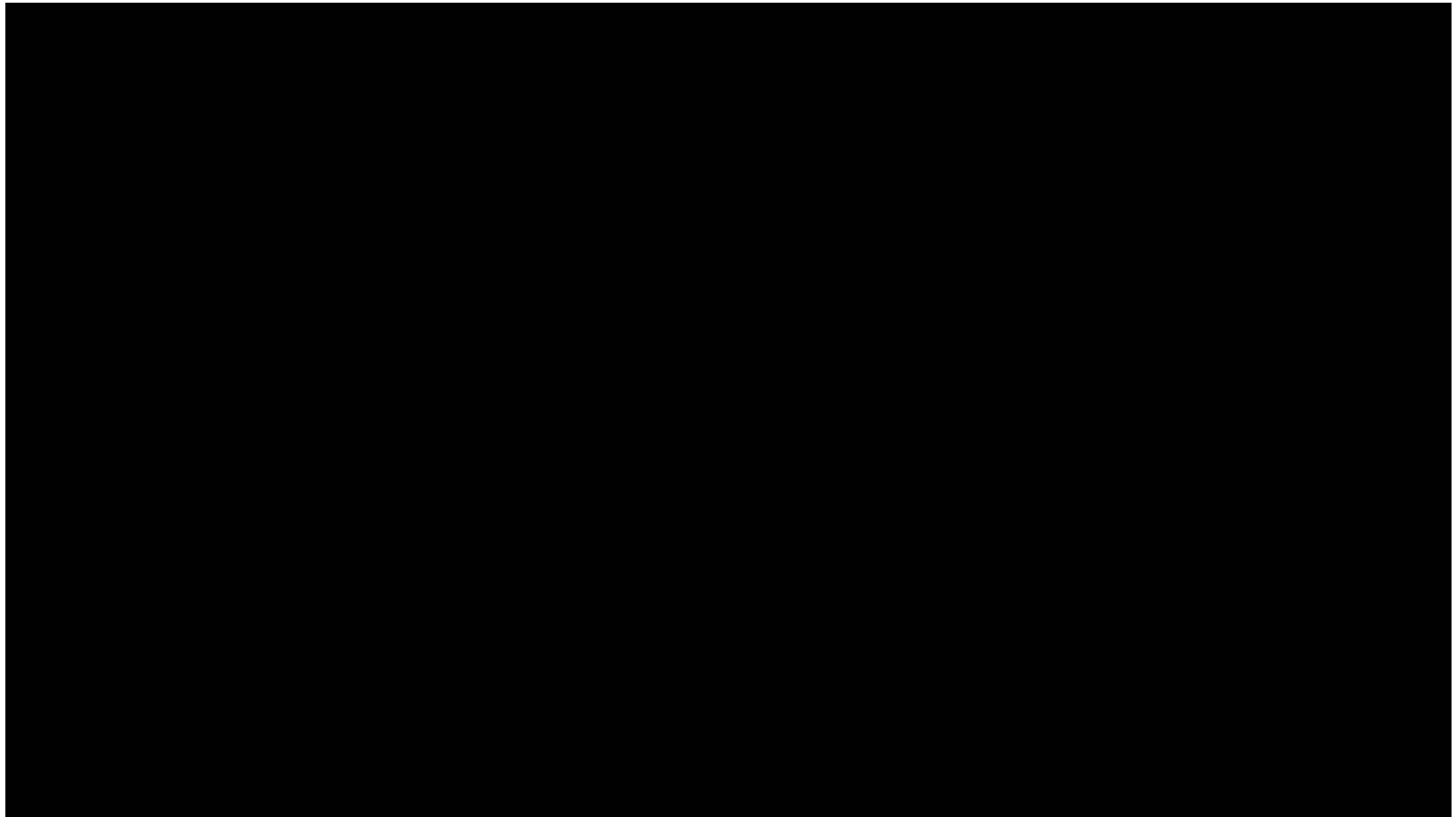
			 
INSECT TESTED	 ✓	 ✗	 ✓
	 ✗	 ✓	 ✓

- Two closely related chrysomelid beetles
 - Colorado potato beetle (CPB)
 - Mustard leaf beetle (MLB)
- Targeted the expression of a conserved gene present in both species
- Precision design:
 - CPB selective RNA-based biocontrol
 - MLB selective RNA-based biocontrol
 - RNA-based biocontrol against CPB and MLB



RNA-based Colorado Potato Beetle (*movie*)

Protection of potato plant by spray administration of dsRNA



RNA-based biocontrol protects under field conditions

● EXPERIMENTAL FIELD TRIAL CONDITIONS

- High incidence of Colorado potato beetle infestation
- Populations of Colorado potato beetle that have developed resistance against classical pesticides

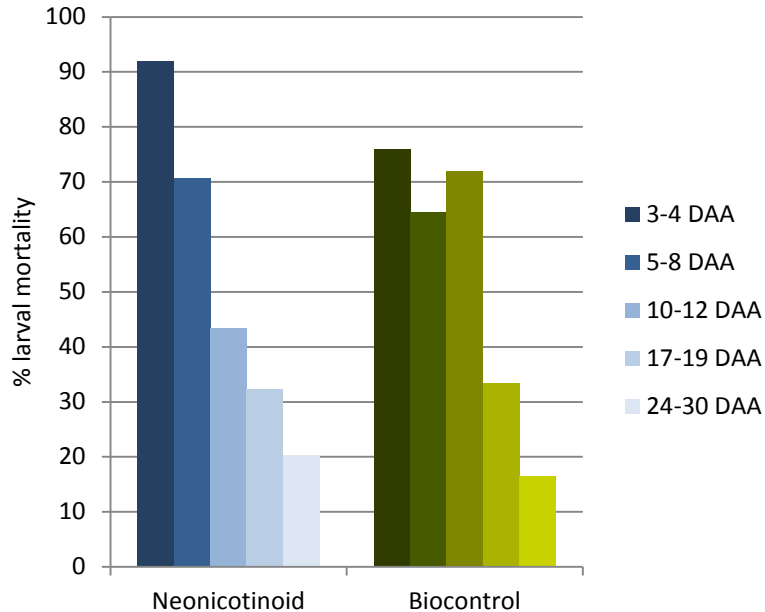
● OBSERVATIONS AND RESULTS

- Excellent protection from feeding damage in lab and field
- Equivalent control/protection to class-leading chemistry
- Effective against adults and larvae
- No impact on beneficial insects or predators of Colorado potato beetle
- No phytotoxicity
- *On planta* stability for a relevant duration in time



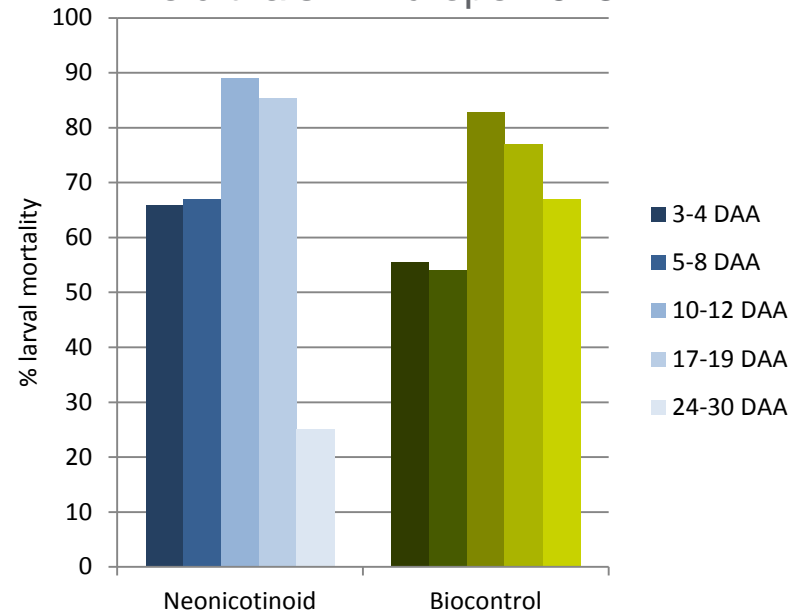
Field Performance of RNA-based Biocontrol: CPB larvae

Field trials in North America 2015



• Average of 3-5 individual trials conducted in North America

Field trials in Europe 2015



• Average of 4 individual trials conducted in Europe

RNA-based Biocontrol shows comparable level of control to commercial chemical insecticides (at registered field rate) against CPB larvae and adults.

DAA = Days After foliar Application

CPB-targeted RNA-based biocontrol is selective

Species	Common name	Order	Biological Activity
<i>Spodoptera littoralis</i>	Cotton leafworm	<i>Lepidoptera</i>	No
<i>Plutella xylostella</i> *	Diamondback moth	<i>Lepidoptera</i>	No
<i>Bemisia tabaci</i> *	Silverleaf whitefly	<i>Hemiptera</i>	No
<i>Aonidiella aurantii</i>	Red scale	<i>Hemiptera</i>	No
<i>Aphis craccivora</i>	Cowpea aphid	<i>Hemiptera</i>	No
<i>Myzus persicae</i> *	Green peach aphid	<i>Hemiptera</i>	No
<i>Nilaparvata lugens</i> *	Brown plant hopper	<i>Hemiptera</i>	No
<i>Orius laevigatus</i>	Flower bug	<i>Hemiptera</i>	No
<i>Aphidius colemani</i>	Parasitic wasp	<i>Hymenoptera</i>	No
<i>Apis mellifera</i> *	Honey bee	<i>Hymenoptera</i>	No
<i>Typhlodromus pyri</i>	Predatory mite	<i>Acarina</i>	No
<i>Tetranychus urticae</i>	Spider mite	<i>Trombidiformes</i>	No
<i>Frankliniella occidentalis</i>	Western flower thrip	<i>Thysanoptera</i>	No
<i>Callosobruchus maculatus</i>	Cowpea weevil	<i>Coleoptera</i>	No
<i>Diabrotica undecimpunctata</i> *	Spotted cucumber beetle	<i>Coleoptera</i>	No
<i>Diabrotica barberi</i>	Northern corn rootworm	<i>Coleoptera</i>	No
<i>Diabrotica balteata</i>	Banded cucumber beetle	<i>Coleoptera</i>	No
<i>Diabrotica virgifera virgifera</i> *	Western corn rootworm	<i>Coleoptera</i>	No
<i>Phaedon cochleariae</i>	Mustard leaf beetle	<i>Coleoptera</i>	No
<i>Leptinotarsa decemlineata</i> *	Colorado potato beetle	<i>Coleoptera</i>	Yes

} Beneficials

Family: *Chrysomelidae*

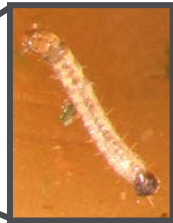
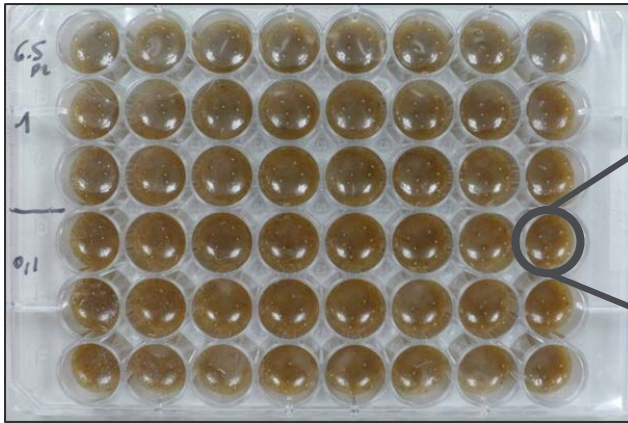


Sharing data via Open Data Institute: <http://www4.syngenta.com/how-we-do-it/research-and-development>

* Reported sensitivity to eRNAi

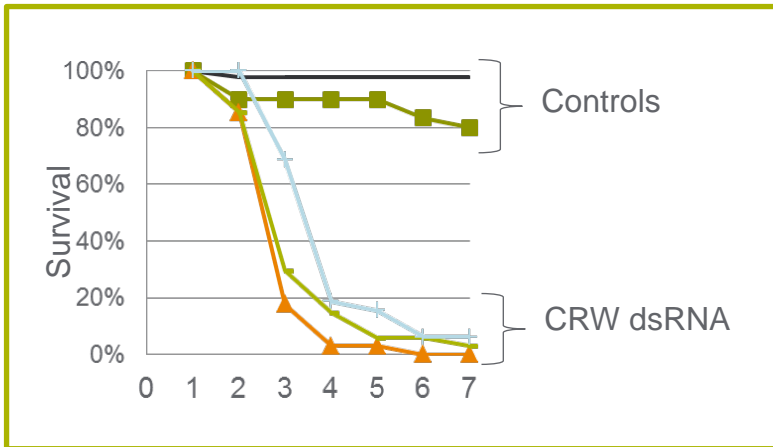
Corn rootworm: Target gene selection

Plate based bioassay on artificial diet



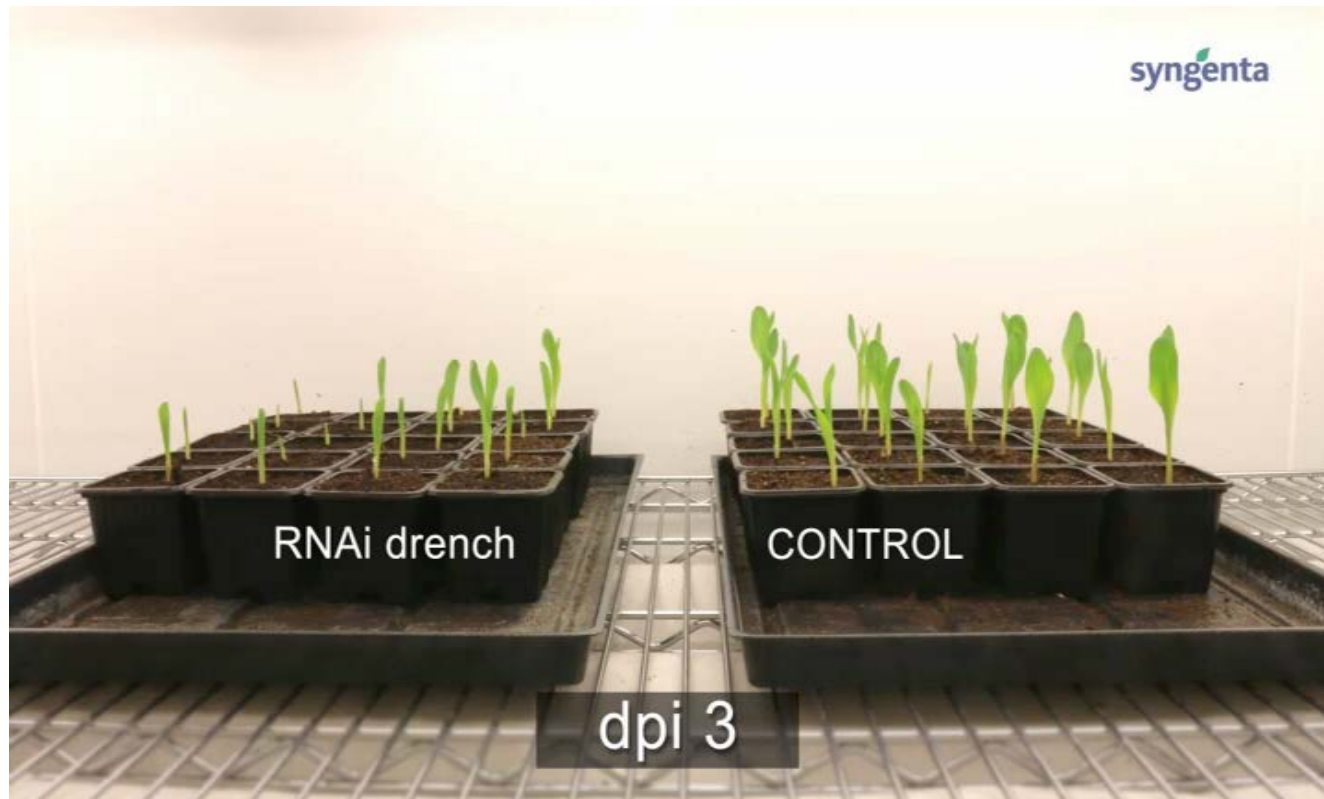
Selection criteria:

- Dose
- Speed-to-kill
- Spectrum (WCRW, NCRW, SCRW)



RNA-based biocontrol of Corn Rootworm (*movie*)

Protection of corn plant by soil administration of dsRNA



Dpi: days post infestation

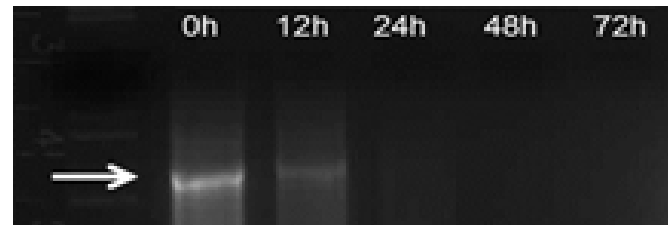
RNA-Based Biocontrol for CRW

Protection of corn plant by active ingredient (AI)

Protection of corn against WCRW, (*Diabrotica virgifera*) by unformulated active ingredient (AI), applied as a drench



It works.... but:



...dsRNA is rapidly degraded in live soil

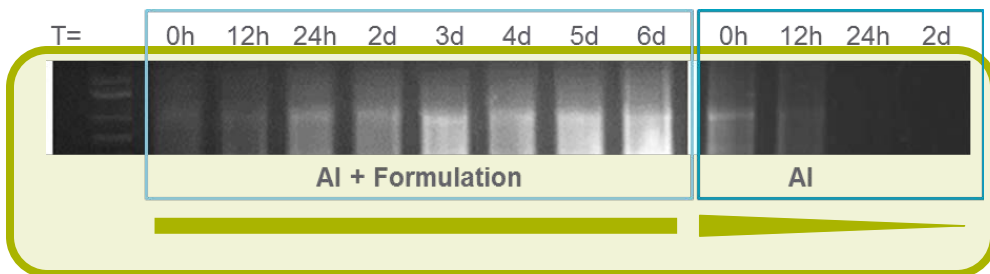
Major loss of AI in soil is due to enzymatic (RNase) activity from:

- Soil borne microorganisms
- Residual RNases

Formulation technology to extend in soil activity of the AI

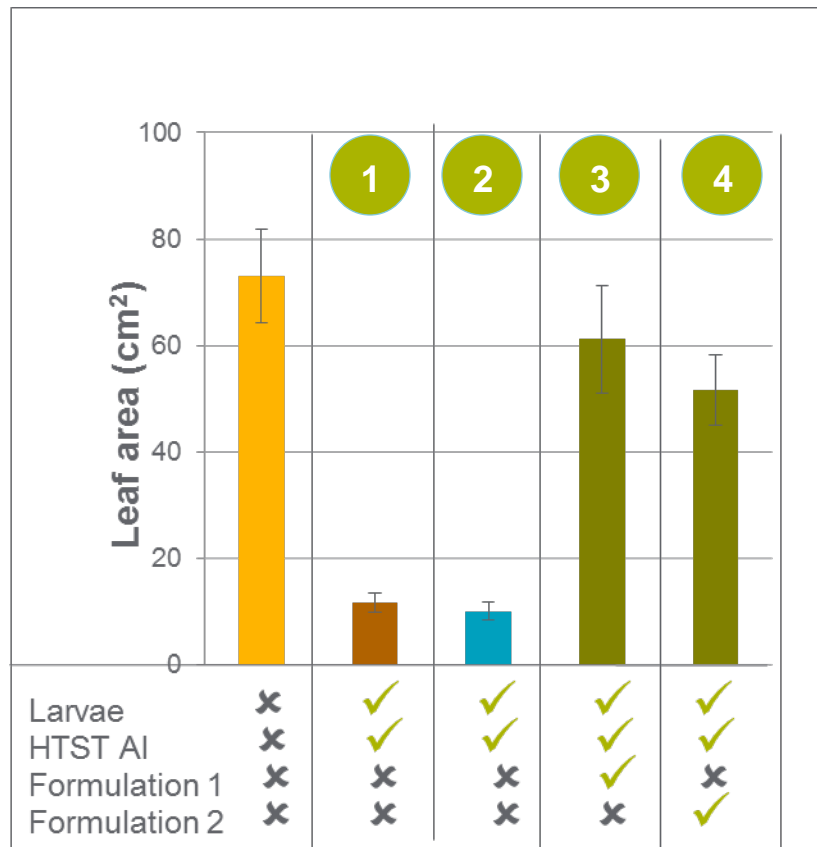
Analytical assay:

AI formulation leads to improved stability in soil environment



Biological assay:

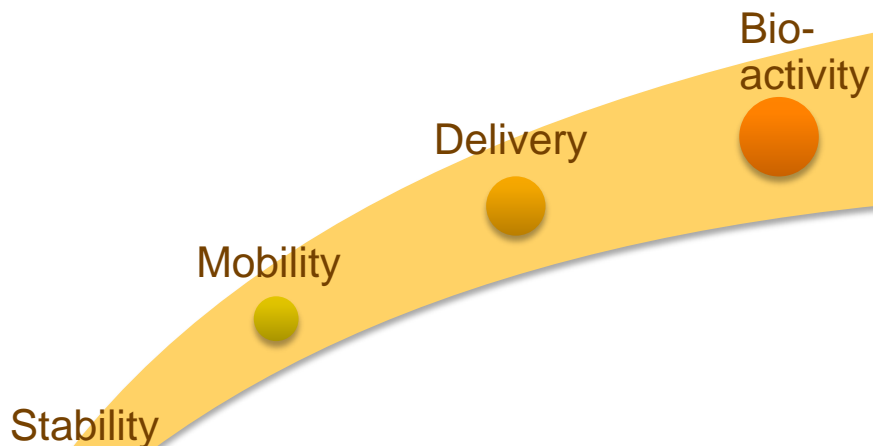
Formulation improves soil persistence (7 days) & leads to plant protection in pot assays



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Challenges in bringing the technology from the lab to the field

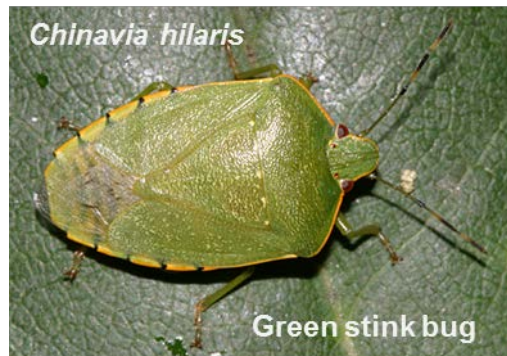
Increase our understanding of CRW feeding behavior and fate of the AI in soil conditions



Success depends on finding solutions for specific problems related to the soil environment:

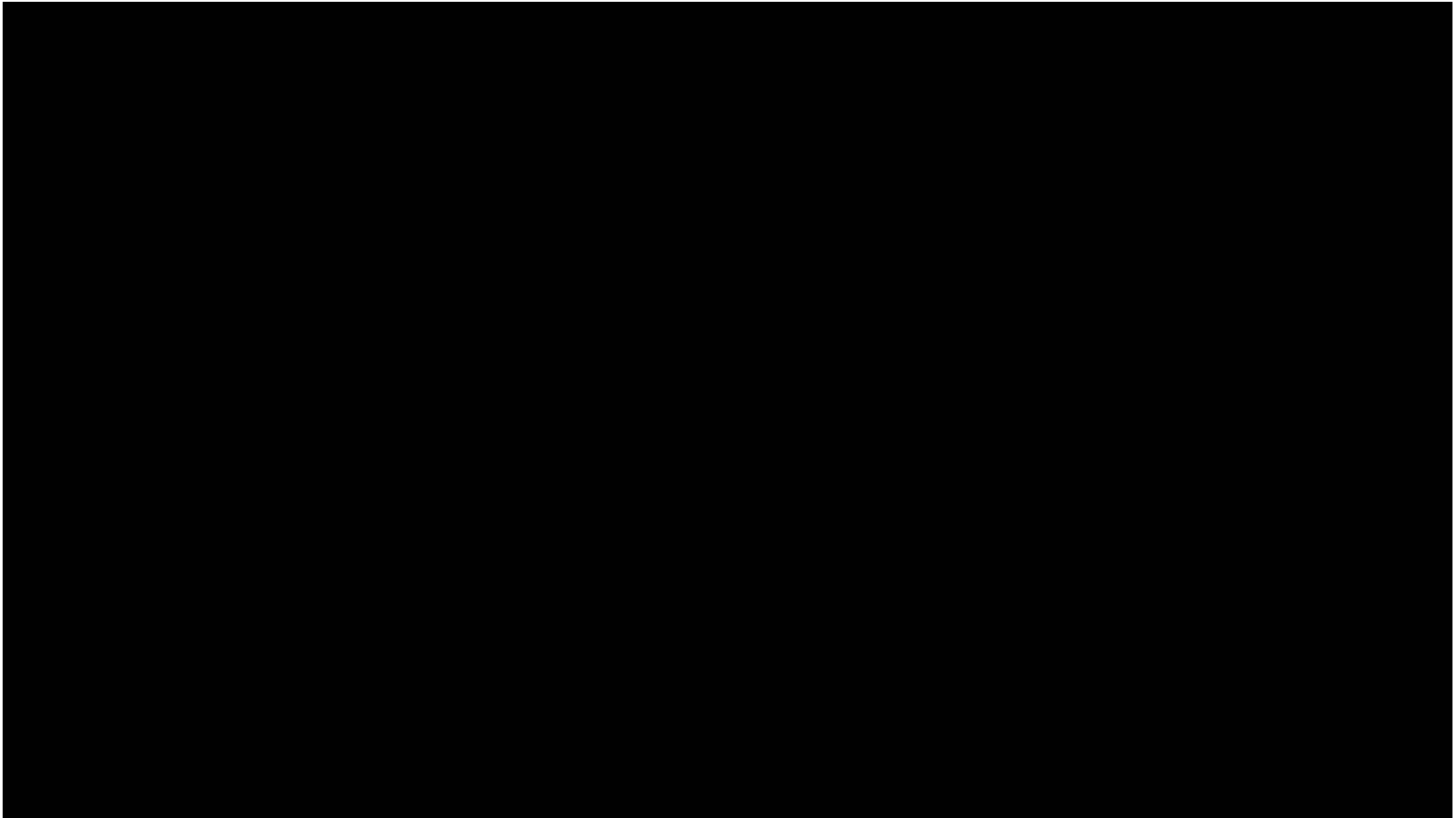
- Protecting the stability of the biocontrol exposed to degradation in the soil
- Ensuring the mobility and delivery to the place where the insect is feeding
- Developing formulations while maintaining the bio-activity

Multiple stink bug species are economically important across various geographies and crop systems



Broadening the technology focus to major pests (*movie*)

Protection of 2 week old soybean plants against Southern green stink bugs, *Nezara viridula*, through a sprayed, **formulated** RNA-based biocontrol



Further discovery effort required to translate the technology from the lab to the field

RNAi Platform, collaborating across sites and functions



Sharing data in an open and transparent way

- Syngenta is committed to being transparent in how RNA-based biocontrols are developed and to periodically make our data available, to maximize the potential benefits for people, farmers and the environment
- We are sharing our early stage non-target insect screening data for our lead product as Open Data in order to engage in a new type of dialogue with scientists and researchers.
 - We partner with the Open Data Institute to publish our data to the best practice standards in the industry.
 - <http://www4.syngenta.com/how-we-do-it/research-and-development>



Bringing plant potential to life