Game-changing Solutions

Engineering weevil resistance in sweetpotato to benefit farmers in Africa

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Outline

1. Damages caused by weevils on sweetpotato

   Cylas puncticollis and *Cylas brunneus*

   Coleoptera: Brentidae

2. Accumulation of toxic compound in healthy-looking parts

   Lydia Wamalwa, Jesse Machuka, Baldwyn Torto, and Marc Ghislain
   CIP / ICIPE / KU

3. Engineering weevil resistance using *Bt* technology

   Lydia Wamalwa, Sandra Manrique, Jan Kreuze, Runyararo Rukarwa, Robert Mwanga, Maria Soto-Aguilar, Marc Ghislain

   CIP-ABL & BecA / NARO / DDPSC

4. RNAi against essential genes of the weevils

   Katterinne Prentice, Olivier Christiaens, Ine Pertry, Guy Smagghe

   Ghent University

5. Conclusions

   Marc Ghislain

Damages caused by weevils

The threat:

28% of crop losses every year (farmer survey in Uganda)

Losses can be up to 90% during dry periods

Impact on food security, marketability, healthiness

Solutions:

Integrated pest management practices is difficult

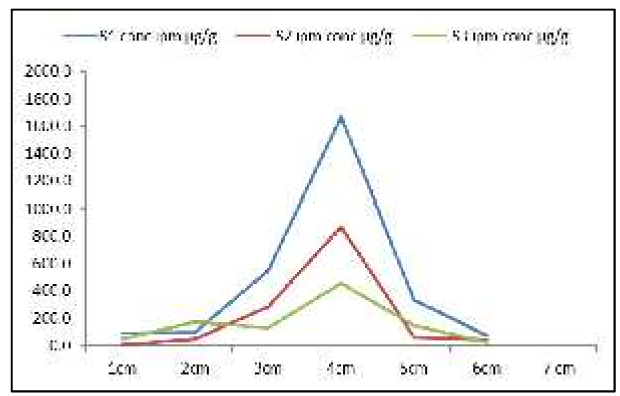
Conventional breeding not yet successful

Quickly harvest and salvage what is left of their crop

Accumulation of toxic compound

The phytoalexin, **ipomeamarone**, accumulates in the healthy-looking parts of microbially infected storage roots at levels posing health threat to farmers in SSA who consume the undamaged parts of damaged storage roots

Figure 4 in Wamalwa et al. in *Journal of agricultural and food chemistry* 63.1 (2014): 335-342



Bt sweetpotato

Cry proteins active against weevils (*Cylas puncticollis* & *C. brunneus*)

LC_{50} values (µg/ml diet) of Cry proteins against 1st instar *C. puncticollis* and 2nd instar *C. brunneus* (An LC_{50} below 1 ppm is low enough to expect high levels of toxicity when expressed in sweetpotato)

	Cylas puncticollis					*Cylas brunneus*				
Bt protein	Total n	LC_{50} Values	95% F.L	Slope (mean ± SE)	χ^2	Total n	LC_{50} Values	95% F.L	Slope (mean ± SE)	χ^2
Bt1 (ET33/34)	2760	0.417	0.394 - 0.441	2.733 ± 0.151	9.1	2760	0.458	0.411 - 0.502	2.636 ± 0.240	9.7
Bt2 (ET70)	2550	0.781	0.678 - 0.882	1.835 ± 0.095	24.0	2700	1.014	0.936 - 1.092	1.851 ± 0.091	11.3
Bt3 (Cry3Aa3)	2700	1.993	1.754 - 2.262	1.578 ± 0.131	14.2	2230	1.885	1.542 - 2.201	2.302 ± 0.204	17.0
Bt4 (Cry3Bb2)	2230	1.273	1.165 - 1.378	2.028 ± 0.101	13.9	2250	1.304	1.183 - 1.437	1.518 ± 0.116	6.4
Bt5 (Cry3Bb3)	2750	1.815	1.625 - 1.996	2.118 ± 0.138	21.0	2700	1.826	1.676 - 1.983	1.585 ± 0.097	10.2
Bt6 (Cry3Ca1)	2700	0.575	0.530 - 0.619	2.598 ± 0.112	20.6	2100	0.696	0.644 - 0.750	2.082 ± 0.119	6.5
Bt7 (Cry7A1)	2469	0.335	0.310 - 0.359	2.778 ± 0.178	8.0	2250	0.435	0.402 - 0.467	2.54 ± 0.178	3.8

Moses et al. (2010). Journal of Economic Entomology 103:1493-1502

Bt sweetpotato

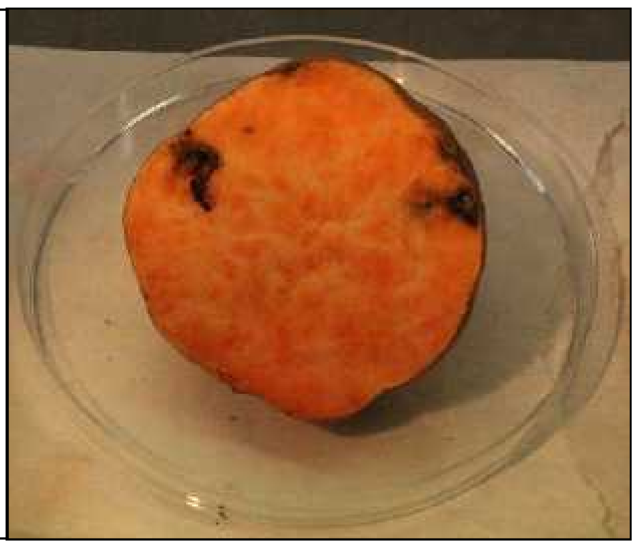
Transformation with *cry* genes:

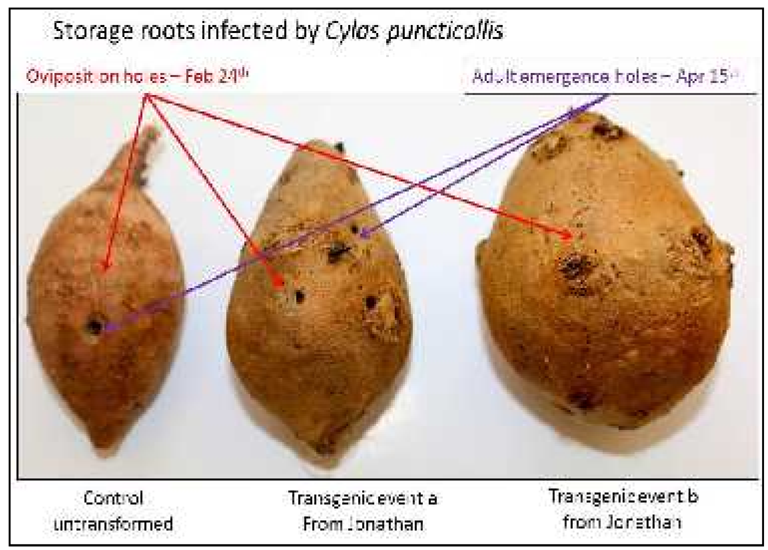
- **Sweetpotato-like *cry* gene constructs:** β-amylase: cry7Aa1, sporamin:cry3Ca1, sporamin:cryET33-34 <<< 3 μg / gr

Agrobacterium-mediated transformation:

117 transgenic events; DAS-ELISA => High Cry protein; Bioassays (oviposition to adult emergence = 6 weeks)

Transgenic event Jewel 12.1 with cry7A and ET33-34





Bt sweetpotato

Transformation with *cry* genes:

- **High expression *cry* gene constructs:** d35s promoter, 5'UTR translation enhancer, full proteins of Cry3Ca1, CryET33 and CryET34

***Agrobacterium*-mediated transformation:**

> 600 transgenic events; DAS-ELISA => High Cry protein; Bioassays

5 + 12 in ABL and BecA greenhouse respectively

More to come from DDPSC.

Bt sweetpotato

Variety	cry gene(s)	Event	Comments	Jun-11	Aug-11	Sep-11	Dec-14	Feb-15	Apr-15	Jun-15	End Jun-1	Jul-15
Jewel	cry7	8.7										
Jewel	cry7	8.9										
Jewel	cry7	8.12										
Jewel	cry7	8.14										
Jewel	cry7	8.17										
Jewel	cry7	8.21										
Jewel	cry3	9.1										
Jewel	cry3	9.3										
Jewel	cry3	9.5										
Jewel	cry3	9.7										
Jewel	cry3	9.11										
Jewel	cry3	9.12										
Jewel	cry3	9.13										
Jewel	cry3	9.14										
Jewel	cry3	9.15										
Jewel	ET33-34	10.1										
Jewel	ET33-34	10.11										
Jewel	ET33-34	10.12										
Jewel	ET33-34	10.14										
Jewel	ET33-34	10.15										
Jewel	ET33-34	10.16										
Jewel	ET33-34	10.18										
Jewel	ET33-34	10.19										
Jewel	cry7 cry3	11.2										
Jewel	cry7 cry3	11.3										
Jewel	cry7 cry3	11.4										
Jewel	cry7 cry3	11.5										
Jewel	cry7 cry3	11.6										
Jewel	cry7 cry3	11.7										
Jewel	cry7 ET33-34	12.1	Short tunnels, one adult dead in one tuber, one adult weak in another tuber									
Jewel	cry7 ET33-34	12.2	In Lima .1 and .2 were the same									
440167	cry7	13.1										
Huachano	cry7 ET33-34	14.1										
Huachano	cry7 ET33-34	14.1										
Huachano	cry7 ET33-34	14.4										
Huachano	cry7 ET33-34	14.5										
Imby	cry7 cry3	16.5										
Imby	cry7 cry3	16.12										
Imby	cry7 cry3	16.13										
Imby	cry7 cry3	16.24										
Imby	cry7 cry3	16.29										
Imby	cry7 cry3	16.32										
Imby	cry7 cry3	16.34										
Imby	cry7 cry3	16.36										
Imby	cry7 cry3	16.39										
Imby	cry7 cry3	16.bulk										
440167	cry7 cry3	17.2										
440167	cry7 cry3	17.5										
Jonathan	cry7 cry3	18.bulk										
Jonathan	cry7 cry3	18.X4	No damages									
Jonathan	cry7 cry3	18.X1	Dead pupae									
Jonathan	cry7 cry3	18.1										
Jonathan	cry7 cry3	18.2										
Jonathan	cry7 cry3	18.3										
Jonathan	cry7 cry3	18.5										
Jonathan	cry7 cry3	18.6	Lower adult emergence									
Jonathan	cry7 cry3	18.7										
Jonathan	cry7 cry3	18.8	No damages, no tunnels									
Jonathan	cry7 cry3	18.13										
Jonathan	cry7 cry3	18.14										
Jonathan	cry7 cry3	18.15										
Jonathan	cry7 cry3	18.17										
Huachano	cry7 cry3	23.bulk										
Jonathan	hecry7 hecry3	30.1										
Jonathan	hecry7 hecry3	30.6										
Imby	hecry3	33.6										
Imby	hecry3	33.8	No visible damages inside, no tunnels.									

Color code	
	Emerged adults >75% of control
	Emerged adults >50% of control
	Emerged adults >25% of control
	Emerged adults >0% of control
	No adult emerged

- 67 transgenic events tested resulted in 6 with significant differences.
- Under reconfirmation.

RNAi against weevils

- Rationale: We may never reach enough Cry protein in the storage root for full control of the weevils
- Our strategy: Similar to Bt (Cry3Bb1)+ RNAi (*Snf7*-RNAi) corn for resistance against western corn rootworm (*Diabrotica virgifera*)

Sequences of 24 essential genes:

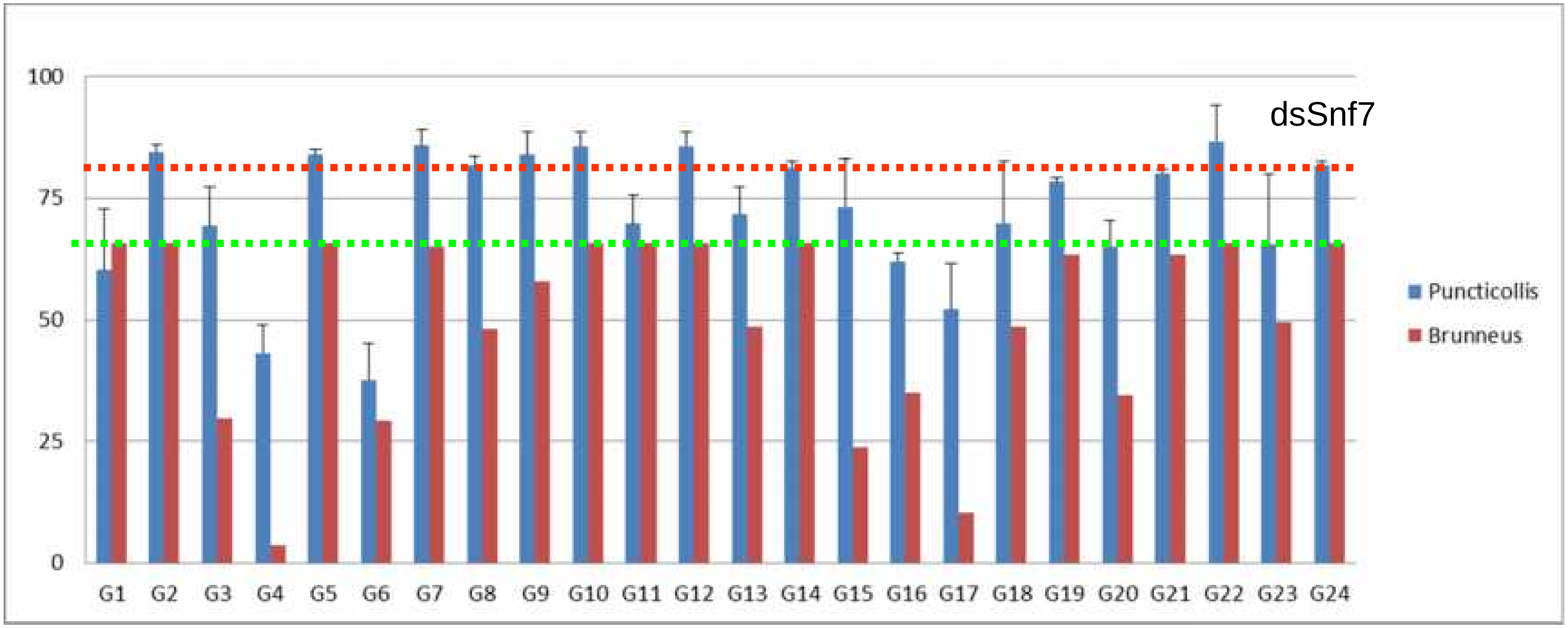
Cylas puncticollis and *C. brunneus* by RNAseq (Ghent University, Venganza ltd, CIP)

dsRNA	Gene
G1	Vha68-2 F1 ATP synthase β subunit
G2	V/A-type ATP synthase catalytic subunit A
G3	Synaptobrevin, isoform A
G4	Pfk phosphofrutokinase
G5	adenylate kinase-2
G6	Focal adhesion kinase isoform D
G7	gamma-coatomer protein, isoform C
G8	delta-coatomer protein, isoform A
G9	alpha-coatomer protein, isoform D
G10	TBP-associated factor 1, isoform D
G11	lethal (2) NC136, isoform B
G12	Proteasome 20 kD subunit
G13	DNA pol interact tpr cont. prot. 47 Kd
G14	alpha-Adaptin, isoform A
G15	Mad1
G16	Ubiquitin conjugating enzyme E2
G17	RNA pol beta subunit
G18	RNA helicase
G19	ribosomal protein S13e
G20	DNA polymerase alpha 50 kD
G21	vATPase A
G22	vATPase D
G23	RPL19
G24	Snf7

Injection against essential weevil genes

Injection of dsRNA for 24 target genes in 2nd instar larvae of *C. puncticollis* and *C. brunneus* (400 bp average, 500 ng/mg BW)

8 dsRNA as good as *Snf7* (resistance to WCR)



Mortality percentages in *C. brunneus* over the control (water) (Abbott correction) after 14 days post-injection of 2nd instar larvae with dsRNA against 24 essential genes and mean mortality percentages for selected target genes to silence in *C. puncticollis* after 14 days.

Ingestion against essential Weevil genes

Ingestion of dsRNA:

(Ghent University)

3 essential genes repeatedly the best target (soaking, artificial diet with synthtis or bacterial produced dsRNA)

Proteasome 20kD
Ribosomal protein S13e
Snf7

(Left) Normal pupal development. **(Right)** Dead pupae after 14 days treated with dsRNA against Ribosomal protein s13e (G19)

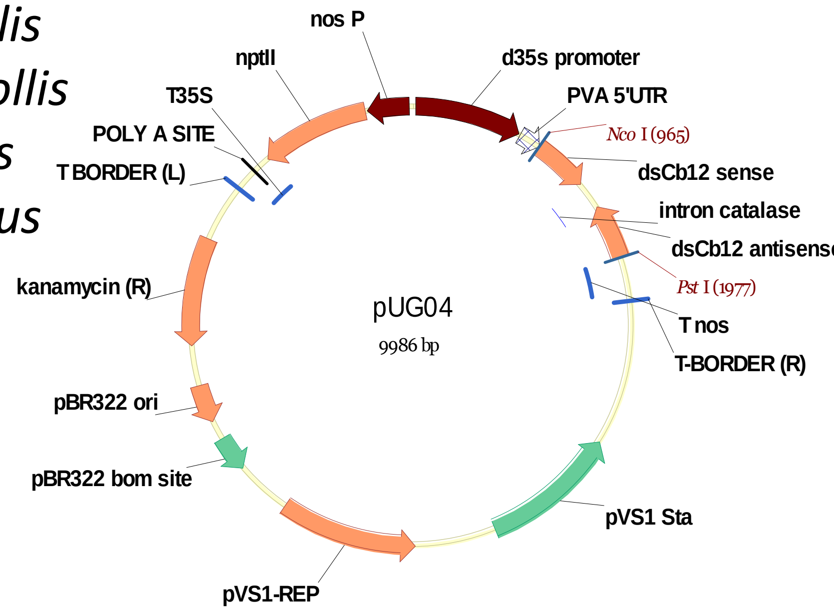
dsRNA	Gene
G1	Vha68-2 F1 ATP synthase β subunit
G2	V/A-type ATP synthase catalytic subunit A
G3	Synaptobrevin, isoform A
G4	Pfk phosphofrutokinase
G5	adenylate kinase-2
G6	Focal adhesion kinase isoform D
G7	gamma-coatomer protein, isoform C
G8	delta-coatomer protein, isoform A
G9	alpha-coatomer protein, isoform D
G10	TBP-associated factor 1, isoform D
G11	lethal (2) NC136, isoform B
→ G12	Proteasome 20 kD subunit
G13	DNA pol interact tpr cont. prot. 47 Kd
G14	alpha-Adaptin, isoform A
G15	Mad1
G16	Ubiquitin conjugating enzyme E2
G17	RNA pol beta subunit
G18	RNA helicase
→ G19	ribosomal protein S13e
G20	DNA polymerase alpha 50 kD
G21	vATPase A
G22	vATPase D
G23	RPL19
→ G24	Snf7

RNAi by genetic transformation

Work plan for next 18 months:

<u>4 gene constructs</u> (Ghent University):

dsSn7 (Cp24) hairpin – *C. puncticollis*
dsProt20Kd (Cp12) hairpin– *C. puncticollis*
dsCp24-Cp12 fusion hairpin– *C. puncticollis*
dsProt20Kd (Cb12) hairpin – *C. brunneus*
dsCp24-Cb12 fusion hairpin – *C. brunneus*



<u>Genetic transformation</u> of Johnathan (12 months) at CIP ABL

<u>Bio-assays</u> (6 months) at BecA

Conclusions

Combination of Bt and RNAi will hopefully be successful and durable to engineer weevil resistance into sweetpotato

Expected benefits: yield increase, food security, health benefits

Biotechnology and Breeding