$3,000,000,000,000 in losses
Agricultural Pests and Impacts

- Insects
- Fungi
- Invasive Plants
- Viruses
- Protists
- Bacteria
Integrated Pest Management

- Crop rotation
- Soil preparation
- Biocontrol
- Chemical Pesticides
- Monitoring/Recording
- Timing
- Alternative strategies
Integrated Pest Management

- Crop rotation
- RNA Pesticides
- Soil preparation
- Timing
- Biocontrol
- Monitoring/Recording
- Chemical Pesticides
RNA Interference (RNAi)

siRNA
RNA Interference (RNAi)
RNA Interference (RNAi)

Target messenger RNA
RNA Interference (RNAi)
Central Dogma of Molecular Biology

- **Gene**
  - **DNA**
  - **Messenger RNA**
  - **Translation**
  - **Protein**

Transcription

Translation
Colorado potato beetle
RNA-based insecticides

Zhang et al. 2015. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. *Science*. 347 (6225), 991-994
Survivorship (%)

Days of Feeding

0 5

Wildtype  dsRNA Treated

Adapted from Zhang et al., 2015
RNAi impacts plants, fungi, insects, animals, and protists.
RNAi-based Pesticides

Benefits
• Highly specific
• Potentially less hazardous
• Resistance evasion

Current Limitations
• Cost of siRNA synthesis
• Synthesis scale
• Prediction of effective targets
Fusarium graminearum spores
F. graminearum Infection Cycle
Deoxynivalenol (DON)
RNA-Based Fungicides?
Transgenic Plant

- Reduced scale up cost
- siRNA toxicity to consumers?
- Portable to other plants?
- New target = new transgenic strain

Topical Application

- Higher scale up cost
- siRNA removed by washing
- Portable
- Easy to use new targets
**MET13 knockout mutant**

Wildtype: 
- Met 

MET13 KO mutant: 
- Met 

Frandsen R.J.N. *et al.*, 2010
methylenetetrahydrofolate reductase 1
methylene tetrahydrofolate reductase 1
✓ Analyzed each sequence in order to predict potential off target effects
Collaboration

Agriculture and Agri-Food Canada

Agriculture et Agroalimentaire Canada

Lethbridge Research Station
Spore Production

10 nM siRNA
Expected result in untreated

Expected result in treated

+Met
Day 3
RNAi Fungicides

siRNA Synthesis
Design Principles – RNA synthesis strategy

- Modular
- Scalable
- Generally recognized as safe (GRAS)
- Standard lab equipment
- Reusable reagents and components
- Cost-effective
Theophylline-dependent Aptazyme

Win, M.N., Smolke C.D. 2007
Ribozyme Affinity Purification (RAP) Device

BL21(DE3) *Escherichia coli*
Ribozyme Affinity Purification (RAP) Device

MS2 Protein Module

Aptazyme module

His tag

MS2 Coat Protein
Ribozyme Affinity Purification (RAP)
Modular RNA part assembly

MS2 Protein Module

Aptazyme module

Constant Oligos

Gene-specific oligos

Extension

Digestion + Ligation

New RNA Part
Ribozyme Affinity Purification Testing

MS2 Protein Module

Aptazyme module

Strong RBS

Weak RBS
MS2 Expression Testing

Low RBS

Time post induction

0 h  0.5 h  1 h  2 h  3 h

No detectable MS2

High RBS

Time post induction

0 h  0.5 h  1 h  2 h  3 h

MS2 (% total protein)

MS2

MS2

No detectable MS2
MS2 Purification

Bound MS2 Protein (ηmol/g E. coli)

Low RBS

High RBS

0.0

0.05

0.1

0.15

0.2

0.25

0.3

0.22 ηmol

0.28 ηmol

High RBS

Total Protein

Soluble Protein

High RBS

ηmol
Aptazyme RNA Purification

Yield Aptazyme RNA = 0.28 \( \eta \text{mol/g E. coli} \)

<table>
<thead>
<tr>
<th>Input</th>
<th>Flow</th>
<th>Wash</th>
<th>Bound RNA</th>
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(252 nt)

(229 nt)

Bound MS2 Protein (\( \eta \text{mol/g E. coli} \))

- Low RBS: 0.22 \( \eta \text{mol} \)
- High RBS: 0.28 \( \eta \text{mol} \)
Theophylline cleavage assay
Design Principles – RNA synthesis strategy

- Modular
- Scalable
- Generally recognized as safe (GRAS)
- Standard lab equipment
- Reusable reagents and components
- Cost-effective
RNA Affinity Purification - Cost Analysis

Cost 10 μmol siRNA ($ CAD)

- RAP: $284.00
- RAP MS2*: $28.40
- RAP MS2* Apt*: $14.20
- Chemical: $355.00
- Chemical + HPLC: $155.00
Submitted Constructs

Strong RBS

Weak RBS

BBa_K1791001

BBa_K1791002

BBa_K1791000
Future Directions of RNAiCare

• Target *F. graminearum* with dsRNA in absence of methionine
• Test efficacy of RNAi pesticides in other pest eukaryotes
• Improve RAP procedure (increased MS2 yield and aptazyme activity)
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