Advances in RNA interference Technologies
Management of Insects, Pathogens, and Plant Trait Modification

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By 2050 we’ll need to feed two Billion more people.

The Future of Food

How can we do that—without overwhelming the planet?
That’s **75 million** more people each year

About the number of people in Germany
Understanding how nature works, permits Humans to treat natural problems (virus diseases) with natural approaches!
WHY try RNA interference?

Cell Diagram: Central Dogma Molecular Biology from Nuclear DNA to Protein,

Transcription  Translation

MODULATE Protein Levels
First evidence Shown in Plants

- Introduction of a transgenic copy of the Chalcone synthase in petúnia plants resulted in a suppression of the endogenous gene.

- Co-suppression, or
- post transcriptional gene silencing
- Pathogen-derived resistance

Mechanism RNAi described in 1998.
2006 Won Nobel Prize: Andrew Fire & Craig Mello.

Napoli et al., Plant Cell 2:279, 1990
What is RNA Interference?

* RNA interference (RNAi) is the knockdown of gene expression by small RNA fragments

1998 – Mello and Fire publish a seminal Nature paper elucidating the trigger for the RNAi process

2006 – Mello and Fire awarded Nobel Prize in Physiology and Medicine

Andrew Fire
Stanford

Craig Mello
U.Mass
RNA interference across Kingdoms

Dimmer Switch
Modulation of Proteins

After Ref: Gordon & Waterhouse Nature Biotechnology 2007
To get a solution to market you need a BIG company!

1) Discussion focus on the **Science, Safety and Benefits** of **RNAi Technology**.

2) Over **15 years of data supporting safety in humans & mammals**, for oral ingestion of dsRNA’s.
Barriers to dsRNA in Humans

Over 10 years of medical research

Demonstrating rapid degradation of dsRNA when ingested or injected into Humans, and other mammals.

Supporting Safe to Humans

Very difficult to get RNAi to work in humans and other mammals.

With RNAi Technologies:

Delivery is the KEY
REAL WORLD EXAMPLE of RNAi -

Preventing Bee Mortality with RNAi product, Remebee™ – Beeologics, LLC

Bees naturally infected with Multiple Types of viruses, fungi, and mites.

Pathogens Contributes to 30-40% Honey bee losses each year!

Nitzan Paldi
Lead Scientist/CEO
Forrest Innovations
RNAi treatment saves honey bees,


Reduce Varroa Mites
Reduce Virus
Asian Citrus Psyllid and HLB

- **Diaphorina citri**, Psyllidae: Hemiptera
- **1998** detect in Florida
- Target pest- Insecticides
- Vector of pathogenic bacteria

- Huanglongbing (HLB), Citrus greening Disease
- **2005** detect in Florida
- Pathogen *Candidatus Liberibacter asiaticus*
- Losses over **$4.6 billion** and **6,000 jobs** since 2006 (Hodges 2012)

Hunter, W.B.
Good Citrus Production
Irregular Ripening
Discolored

SOUR

MISSHAPEN

Increased Fruit Drop

Cross-section

HLB Symptoms

BAD CITRUS PRODUCTION
Some of the Problems in Insect pest management:

* Broad Spectrum Insecticides kill many insect species, including beneficials.

* Development of insecticide resistance, within 2-4 years of heavy use.

* Emergence of secondary insect pests, due to loss of parasitoids and predators.

What would be a better pest management strategy?

1) Something that is more specific to the target

2) Not generate resistance development

3) Not hurt beneficials, protects Pollinators- like honey bees, Predators, and Parasitoids.
Q: What is needed?

Answer: Genetic Sequences

- Treating Plants as Patients.
- Non-transformative, Plant Delivery.
- Suppressing psyllid survival.

Researchers using for RNAi against:
- Pathogen interactions
- Feeding

Target each life stages - Topical RNAi

- Reduce Spread of Pathogen HLB
- Suppress Population
- Increase Psyllid mortality
- Decrease Psyllid Fitness

Spray or root drench into Citrus Trees

Psyllids Ingest by feeding

Targeted mRNA Can be any Life stage

Down-regulate Proteins needed by psyllids

dsRNA In plant
dsRNA In insect

Psyllids Ingest by feeding

Decrease Psyllid Fitness
Root absorption of dsRNA in water: 2008-2009
Green House Trials using Citrus Trees and Seedlings

Drench Trial applying dsRNA with

1) Bare roots and 2) Roots in soil.
   Treatment 2 g dsRNA total concentration in 1.5 L water.

2) Citrus trees absorbed dsRNA and detectable in top canopy leaves in 3-4 hrs. PCR analyses.


RNAi Bioassay – Feeding *plant* system

➢ *in plant* system:
  • Bioassay for *plant*-feeding Hemiptera; “*Piercing-Sucking Mouthparts*”
  • Plant flushes were washed and placed in water (control) or dsRNA solution;

![Image of plant and insect]  

Plant tissue ~0.25 grams/ treated with 10, 50, or 100 nm dsRNA
Dosage Response of dsRNA and Efficacy in RNAi

MORE is NOT always BETTER!

Hunter et al, 2012
Target evaluation – “natural” system

Andrade, E. and Hunter, W. 2016. RNA Interference – Natural Gene-Based Technology for Highly Specific Pest Control (HiSPeC), InTech.
Reverse dsRNA feeding did not cause increased mortality effects.

* Can design ONE RNAi to Reduce ONE insect!

Hunter, WB., Andrade, E. Specificity of RNAi, 2014;

Andrade, E. and Hunter, W. 2016. RNA Interference – Natural Gene-Based Technology for Highly Specific Pest Control (HiSPeC), InTech. Online.
Specificity Test Bioassay RNAi by oral feeding trial

Newly emerge nurse bees. (Honey bee, *Apis mellifera* L.)

Double-blind study, water, GFP, and Dc-dsRNA’s 10 ng dsRNA in sucrose solution ingested.

**Sequence Specific = Specie Specific**

Bees marked and put back into hive for 15-20 d. Open and record number of survivors.

**No negative effects observed on Bees**
Environmental Fate

Preliminary results
dsRNA degrades and
Undetectable in 48 hrs

Brian Pearson, Univ. FL

SOIL analyses:

Delivery by sprays as
Foliar and on Soils

Soil Drench: Small Citrus Seedlings in 1 gal pots:
Treated 200 mg dsRNA, 300 mL water
Detectable dsRNA in leaves at 4 hrs, and 5 weeks post treatment

What are the INDUSTRY NEEDS?
Dosage Response of dsRNA and Persistence in Trees

Living organisms rapidly break down dsRNA
Field Trial Delivery Systems for dsRNA to Citrus Trees – 2012-16.  
6 yr old Valencia trees, Collaboration: USDA- Shatters, Hall, and Andrade- EMBRAPA, Brazil

Preliminary Data

Topical Sprays worked!
30 d post treatment RNA detected in citrus leaf tissues

Systems Biology Approaches:

“Provides a Roadmap as a vision of integrating the many diverse components of agroecosystems, into a systems-level understanding.

Includes the environment, all the macroorganisms, and microorganisms (Beattie and Buchanan, 2015).

June 2014 Field Tree RNAi Trial. Delivery Systems.

Delivery trial: Foliar sprays
Root drench
Trunk Injections
Future directions:

RNAi Technology  Environmentally benign, Non-GMO
Management--

- Target only insect pests, Safe for Beneficials
- Overcome Insecticide Resistance
- Reverse Herbicide tolerance in weeds
- Plant/Crop modification in the field! “Traits on Demand!”

Falling prices in RNAi production (more Field Trials)

BIG interest by all major Agro-Industries

Established Regulatory Pathway by Agencies

RNAi as Topical Application, Non-transgenic

RNAi currently being used in….

….Transgenic plant modes of applications
Advances in Production = Shrinking Costs Towards Commercial Product

2008. $575,000 to make 40 grams {using Kits} (if treating 100 trees) cost $5,745/ tree.

2010. Method Developed - Beeologics, LLC. $250,800 for 40 grams dsRNA, for 100 trees $2,500 per tree.

2012. Another production breakthrough - Reduced cost to $4 per tree.

2014. Field trial test of RNAi, effective dose: Costs $1 per tree.

2015. Improved production & Delivery: Cost $0.50 per tree

...costs keep dropping!
These statements are not an endorsement by the USDA-ARS. Other products or services may be suitable.
Biotechnology working to develop crops needing:
LESS Water,
LESS Fertilizer
LESS Insecticides

Maize breeding
Increased Plants, Ear size and yields

Food Security
Better Environment
More Nutritious
Modify Plant Traits
Non transgenic

RNAi

SOUR

RNAi

SWEET

DELICIOUS

APPLES

Bitter melon

BE DELICIOUS
Plant Improvement, Trait Modification

Seedy → RNAi → Seedless

See a Problem

Make a Solution!
Other uses for RNAi strategies

INNOVATION

Medical or Chemical Production
Produced Using
Commercial Kits

$30,000 USD 2009

200 mg dsRNA

Produced Using Commercial Company

~$150 2016

Maria Gonzalez,
Biological Science Technician, USDA, ARS, Ft. Pierce, FL
Global Mega Trends
Safe tools for a Sustainable Future