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

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Vector Entomologist, Insect Genomics, RNAi , Gene-based Targeting, Pathogens, Plant trait modification, Microbiomes, Biopesticides, Microbicides

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

The Future of Food

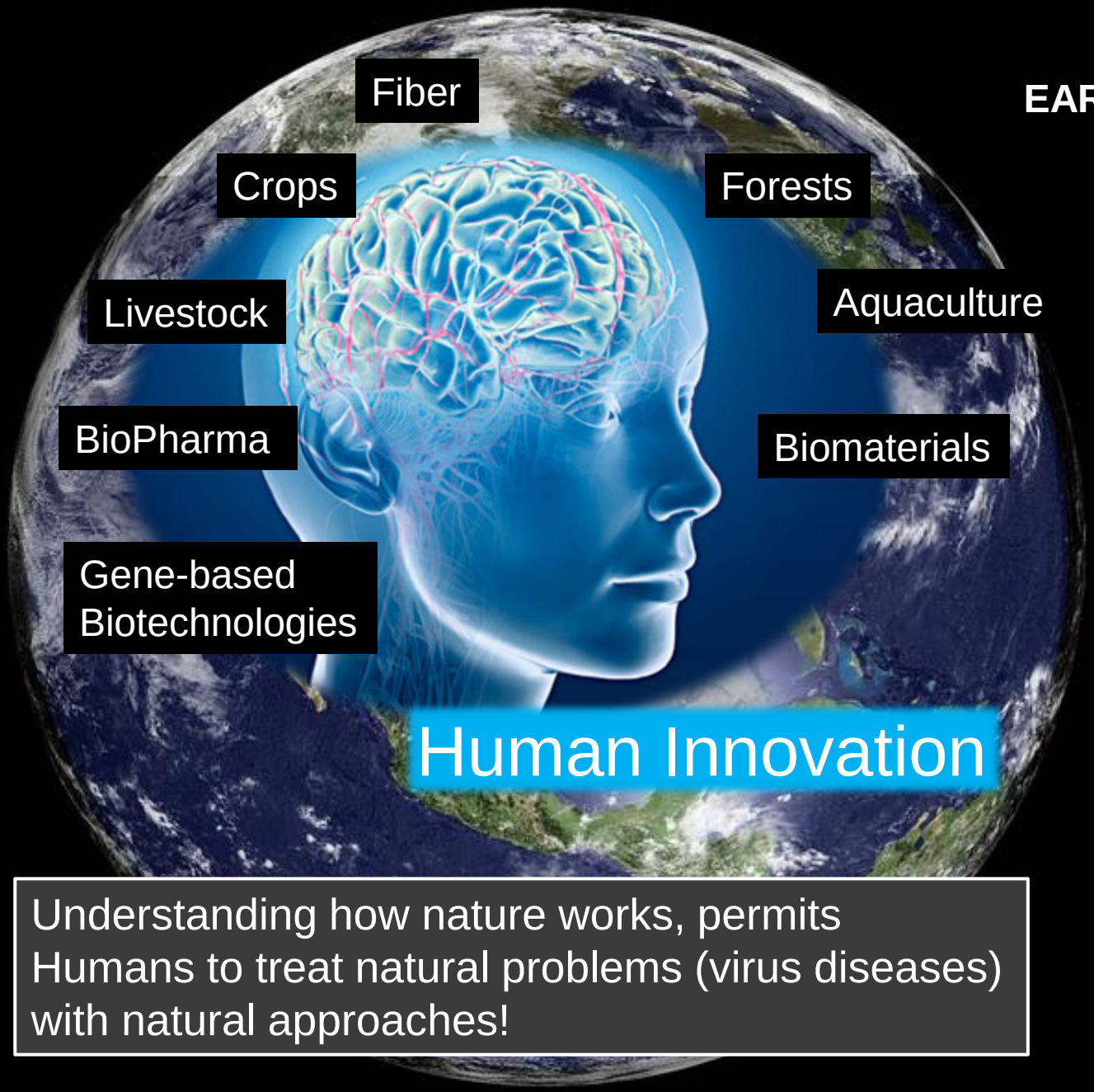
How can we do that—without overwhelming the planet?

That's **75 million**

more people each year

About the number of people in Germany

Jack A. Bobo | Senior Advisor for Biotechnology | U.S. Department of State



EARTH

Agrarian societies

Domestication of Animals

Discovery of DNA,

Develop Computers

Genomics

Bioinformatics

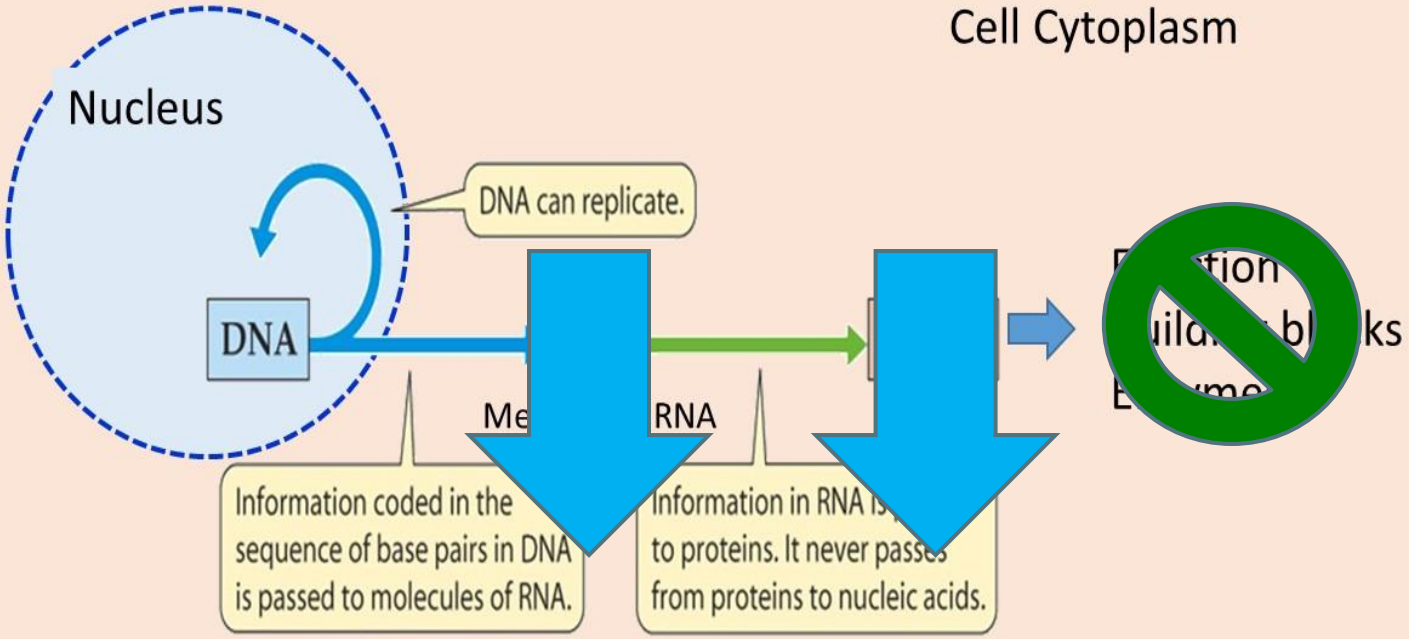
Chemical technologies

★ Cognitive computing

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**

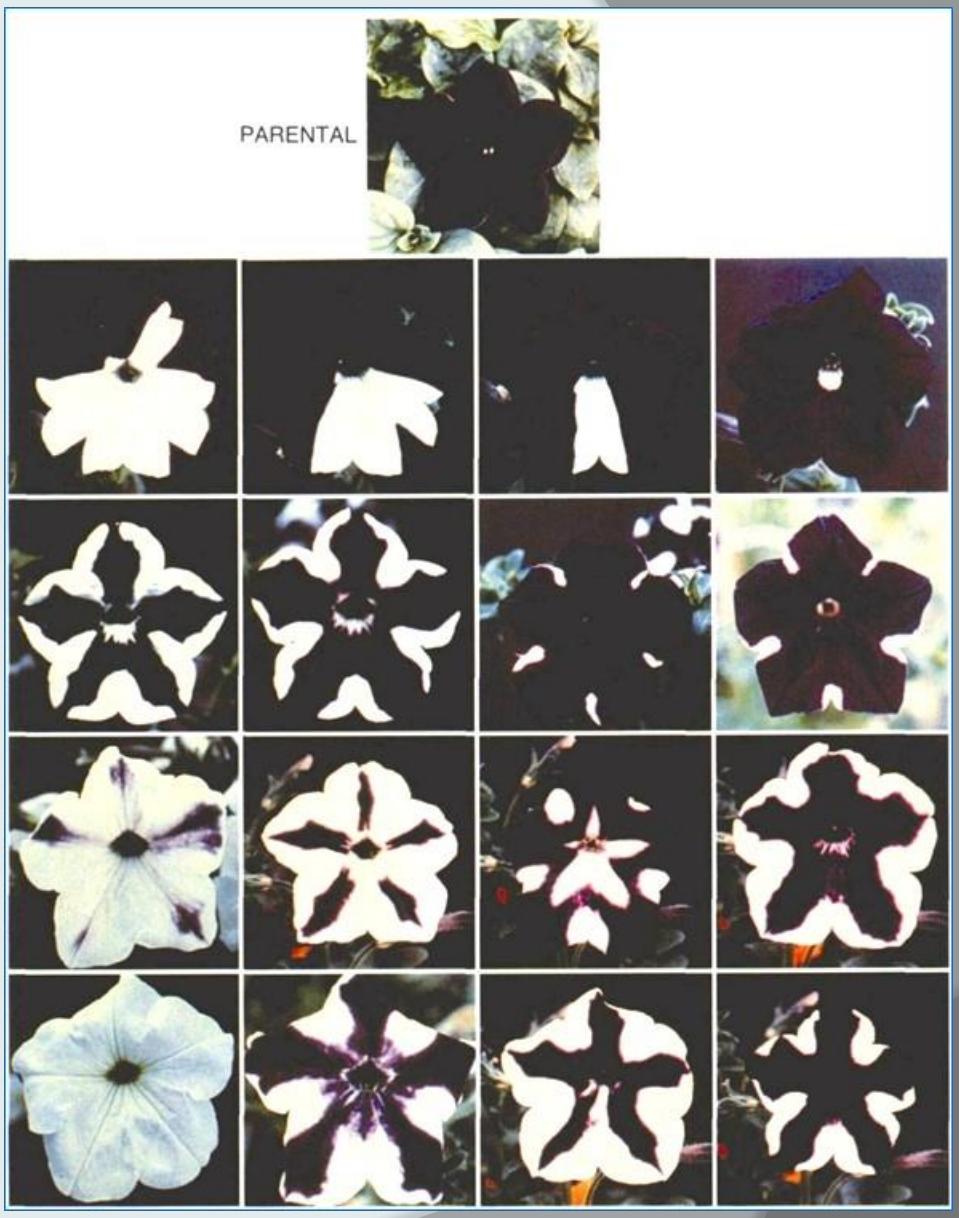
MODULATE Protein Levels

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

INSECT **dsRNA trigger** INSECT

Normal Enzyme-A expression → **Suppress** Enzyme-A

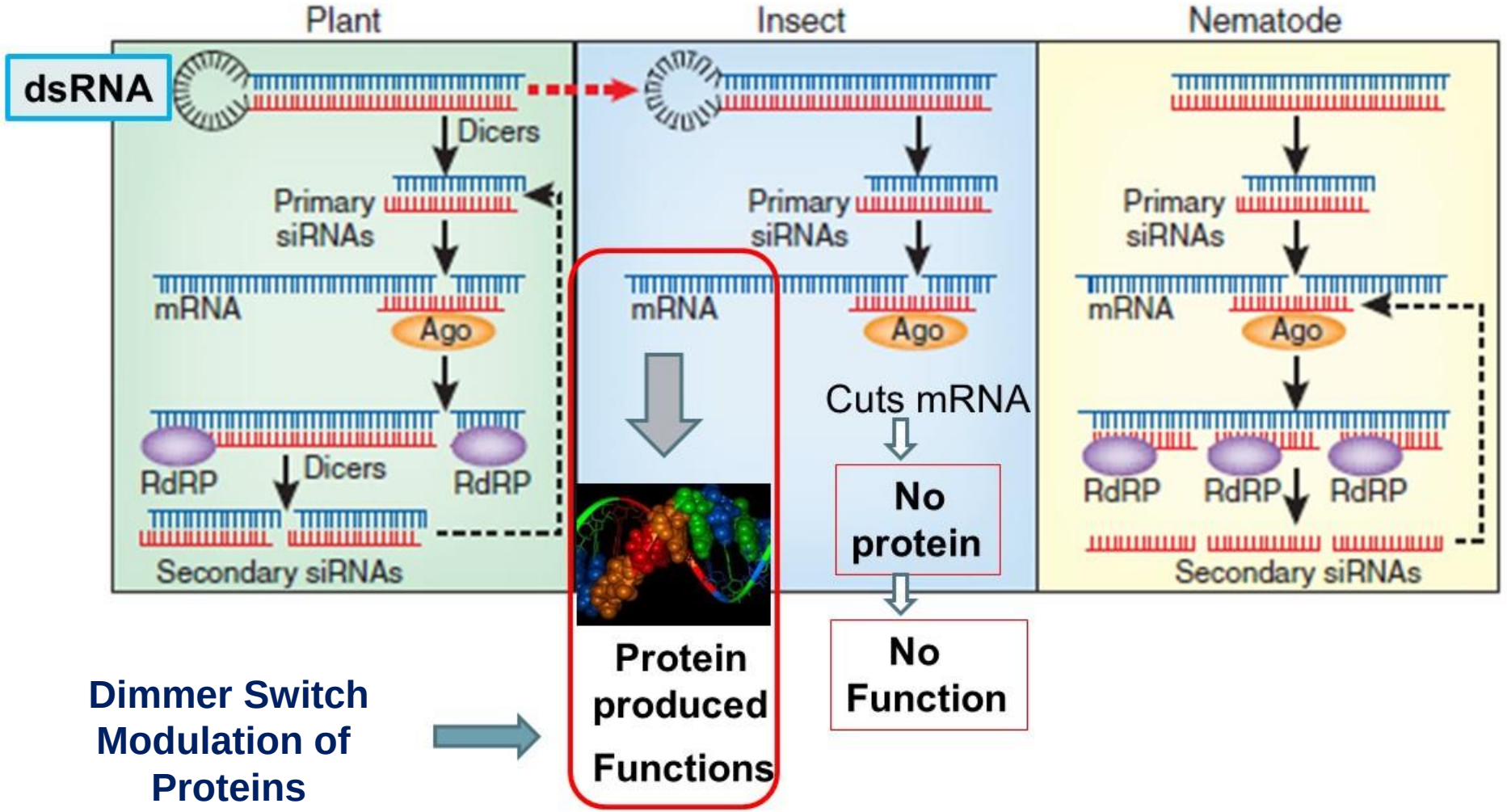
1998 – Mello and Fire publish a seminal **Nature paper** **<u>elucidating the trigger for the RNAi process</u>**

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

Research Focus is on the Technology :

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.

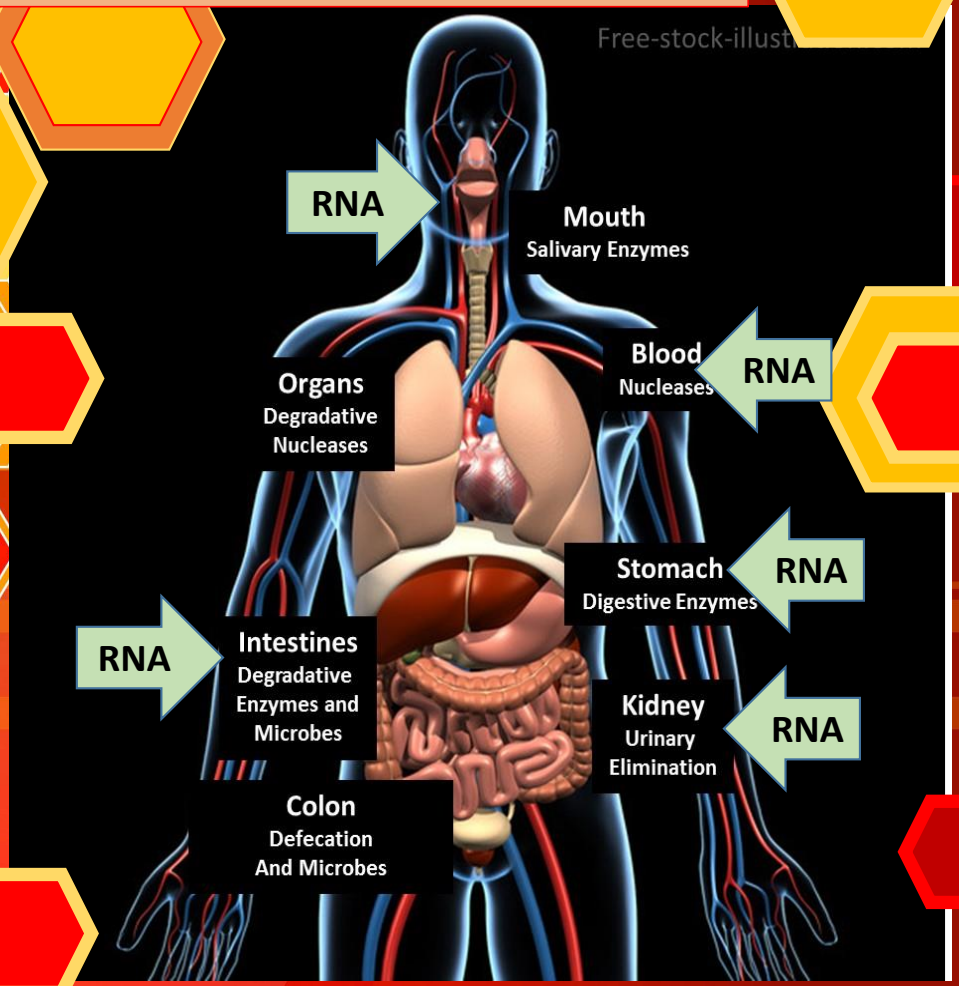
SAFE
SAFE
SAFE

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.

Petrick, Brower-Toland, Jackson, Kier, 2013. Regulatory Toxicol. Pharmacol. 66:167-176.

With RNAi Technologies:

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.

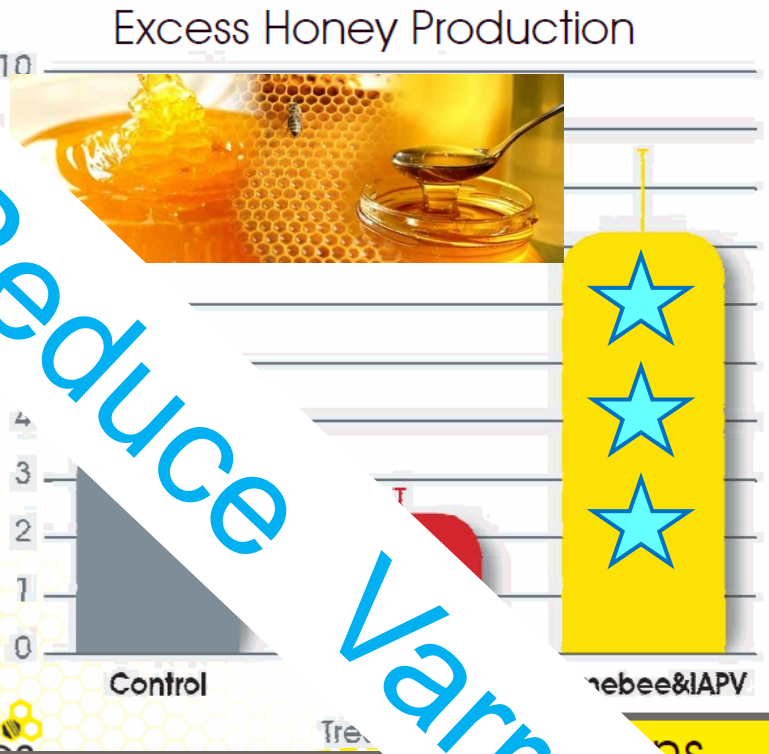
Pathogens Contributes to 30-40% Honey bee losses each year!

Nitzan Paldi
Lead Scientist/CEO
Forrest Innovations

Beeologics

2008-2010

Honey production differential



Reduce Varroa Mites

Reduce Virus

Maori et al, 2007;2

- IAPV
- RNAi tre es honey bees,
- IAPV specifi to bees protects them and prolongs their

Hunter, W., et al, 2010. Large-scale field application of RNAi technology reducing Israeli Acute Paralysis virus disease in honey bees (*Apis mellifera*, Hymenoptera: Apidae). PLoS Pathogens 6(12):e1001160. 2010.

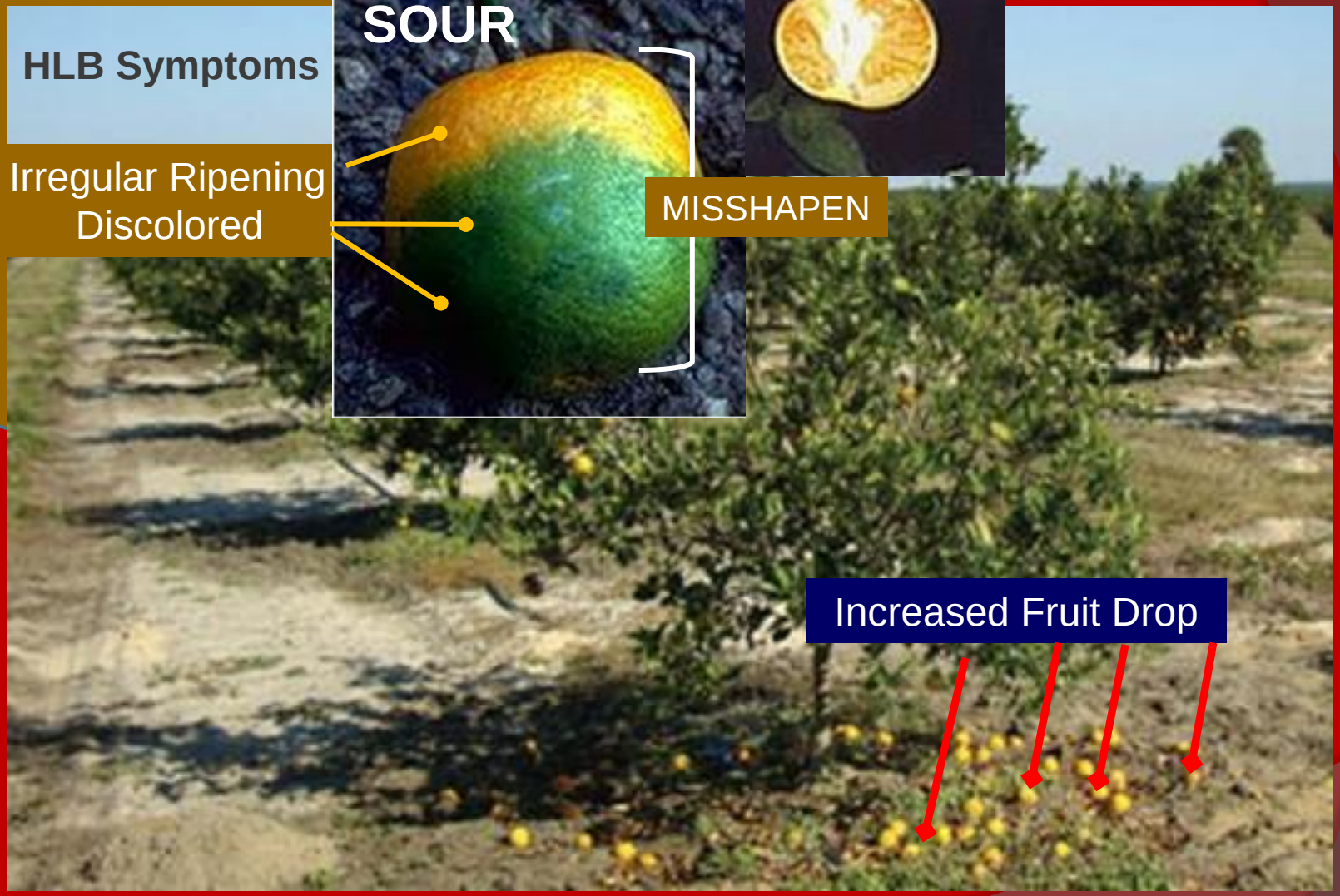
Asian Citrus Psyllid and HLB

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

Hunter, W.B.

- 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)

Good Citrus Production



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.

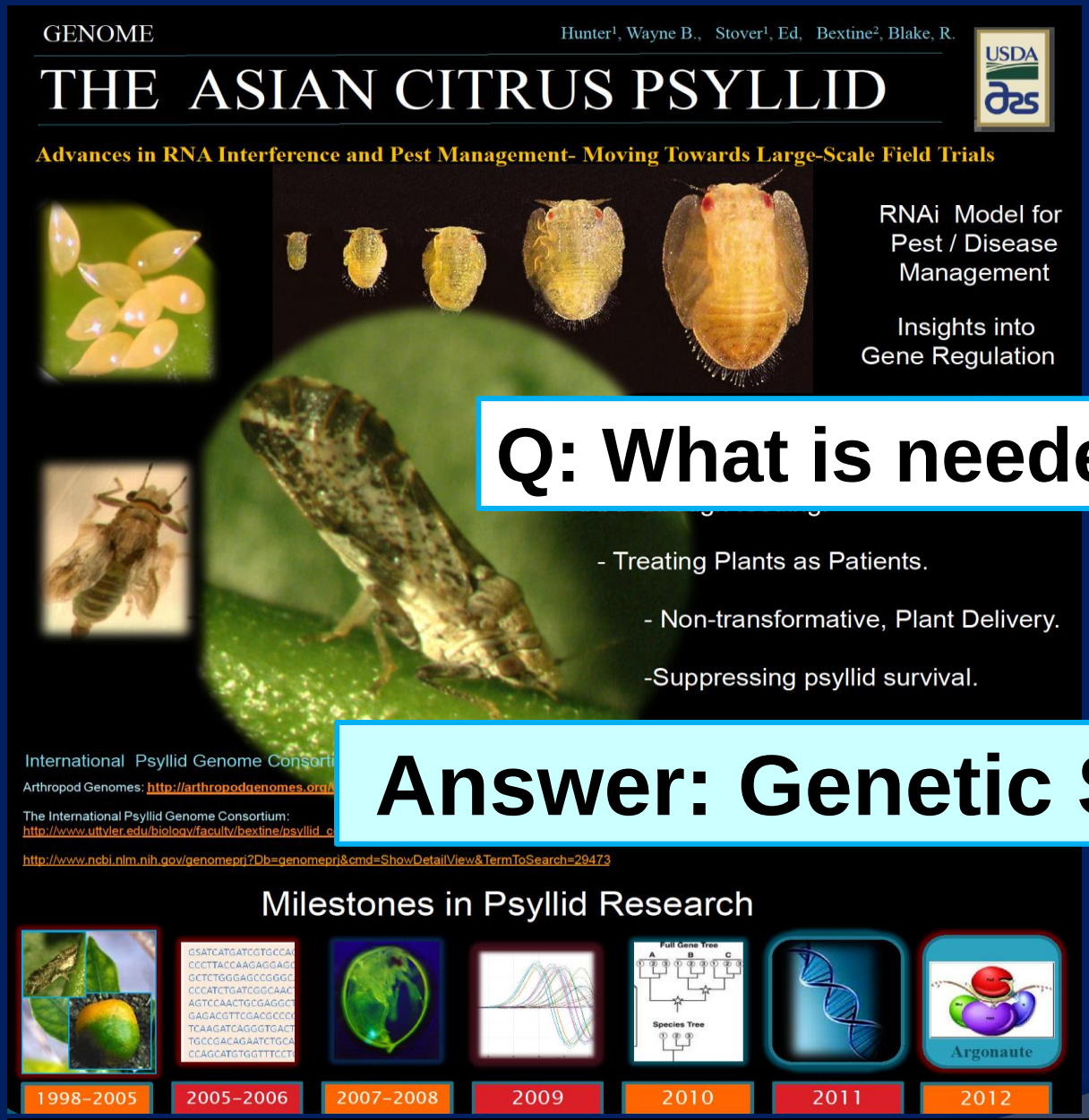
*Emergence of __secondary insect pests, due to loss of parasitoids and predators__.

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.



Hunter, W.B. 2014

Psyllid Genome Ver. 2.0
Completed 2013
(ARS, CRB, LANL)

Identified 18,600 genes
For use in RNAi research.

ts Eggs,
Nymphs, and Adults

Researchers using for RNAi against :

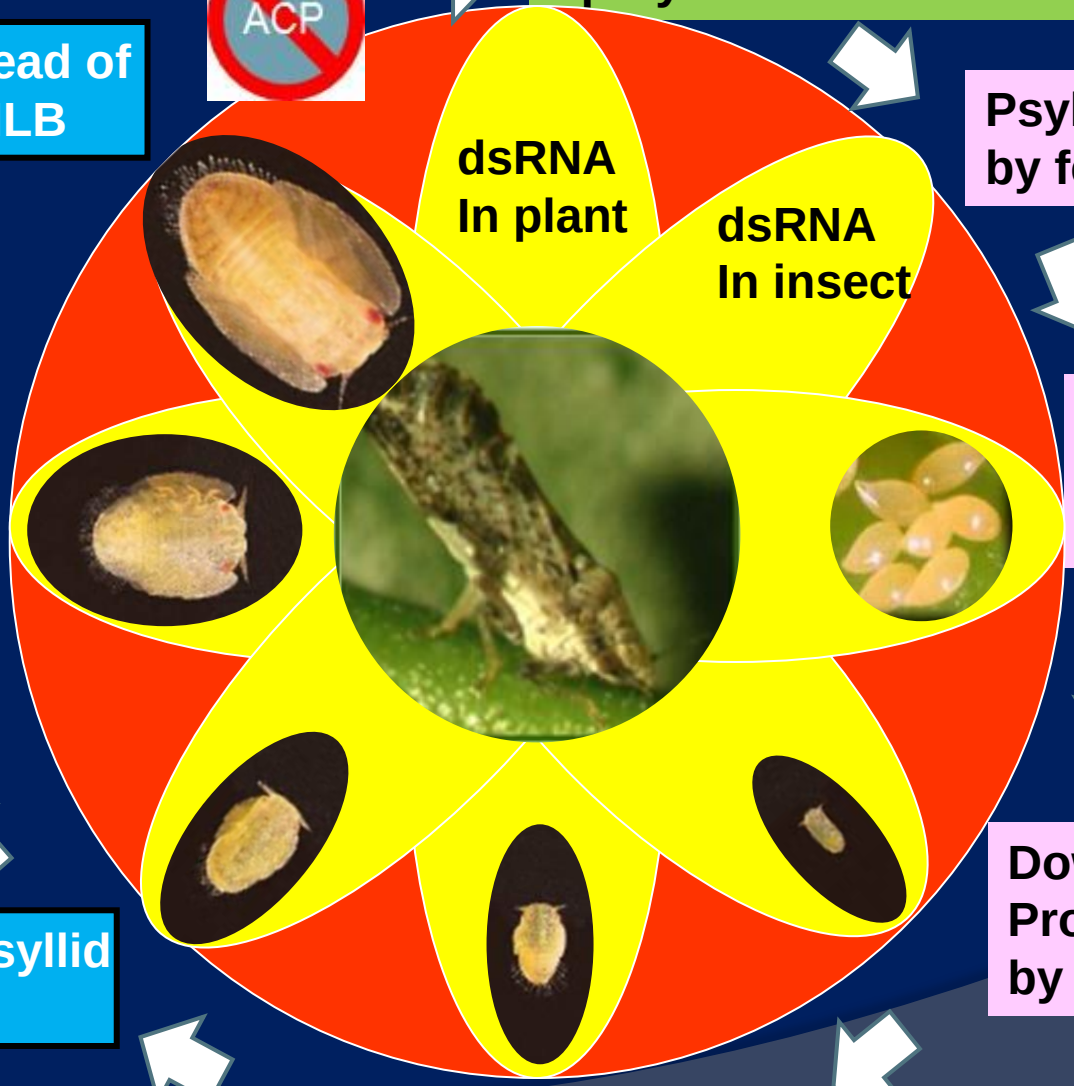
- Pathogen interactions
- Feeding

Q: What is needed?

Answer: Genetic Sequences

Reese, et al., 2013. Characterization of the Asian citrus psyllid transcriptome. Jour. Genomics 2013(2): 54-58. doi: 10.7150/jgen.7692. Intl Psyllid Genome Consortium

Target each life stages - Topical RNAi



Spray or root drench into Citrus Trees

Psyllids Ingest by feeding

Targeted mRNA Can be any Life stage

Down-regulate Proteins needed by psyllids

Decrease Psyllid Fitness

Increase Psyllid mortality

Suppress Population

Reduce Spread of Pathogen HLB

BREAKTHROUGH

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.

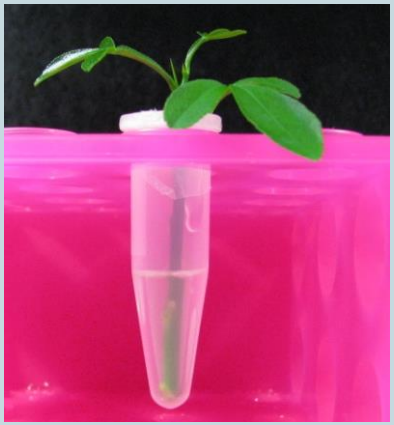
3) Detect dsRNA at 2 months post treatment.

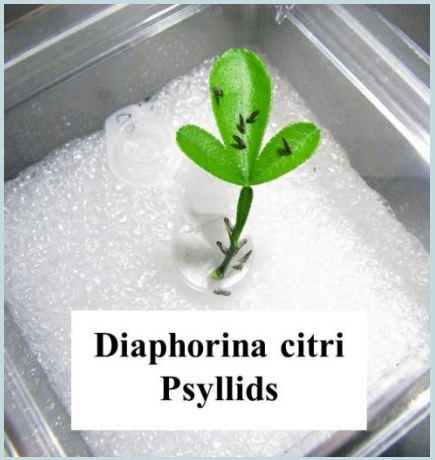
Hunter, W., Paldi, N., Glick, E., Bextine 2008,2010; 2012.

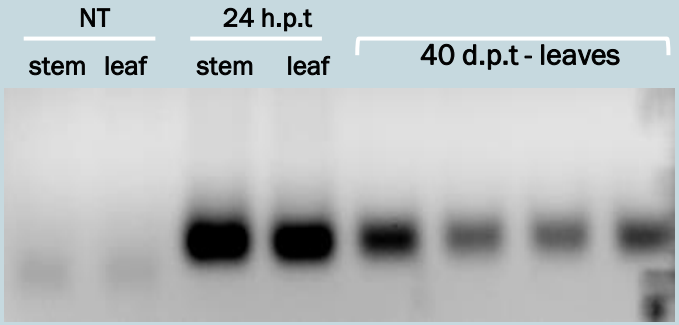
RNAi Bioassay – Feeding *plant* system

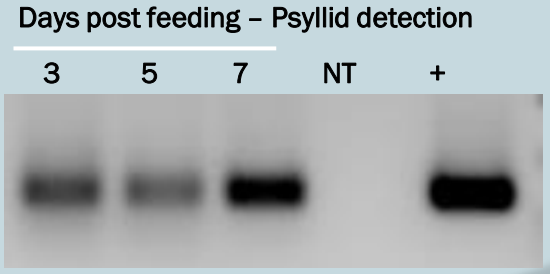
Hunter and Andrade 2013

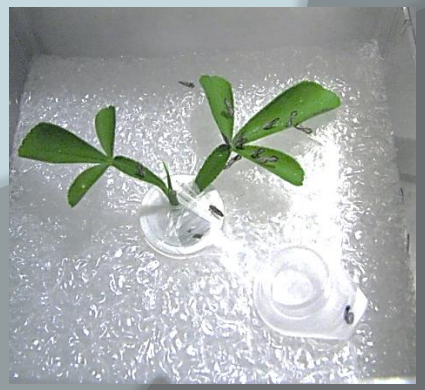
- *in plant* system:
  - Bioassay for plant-feeding Hemiptera; "Piercing-Sucking Mouthparts"
  - Plant flushes were washed and placed in water (control) or dsRNA solution;





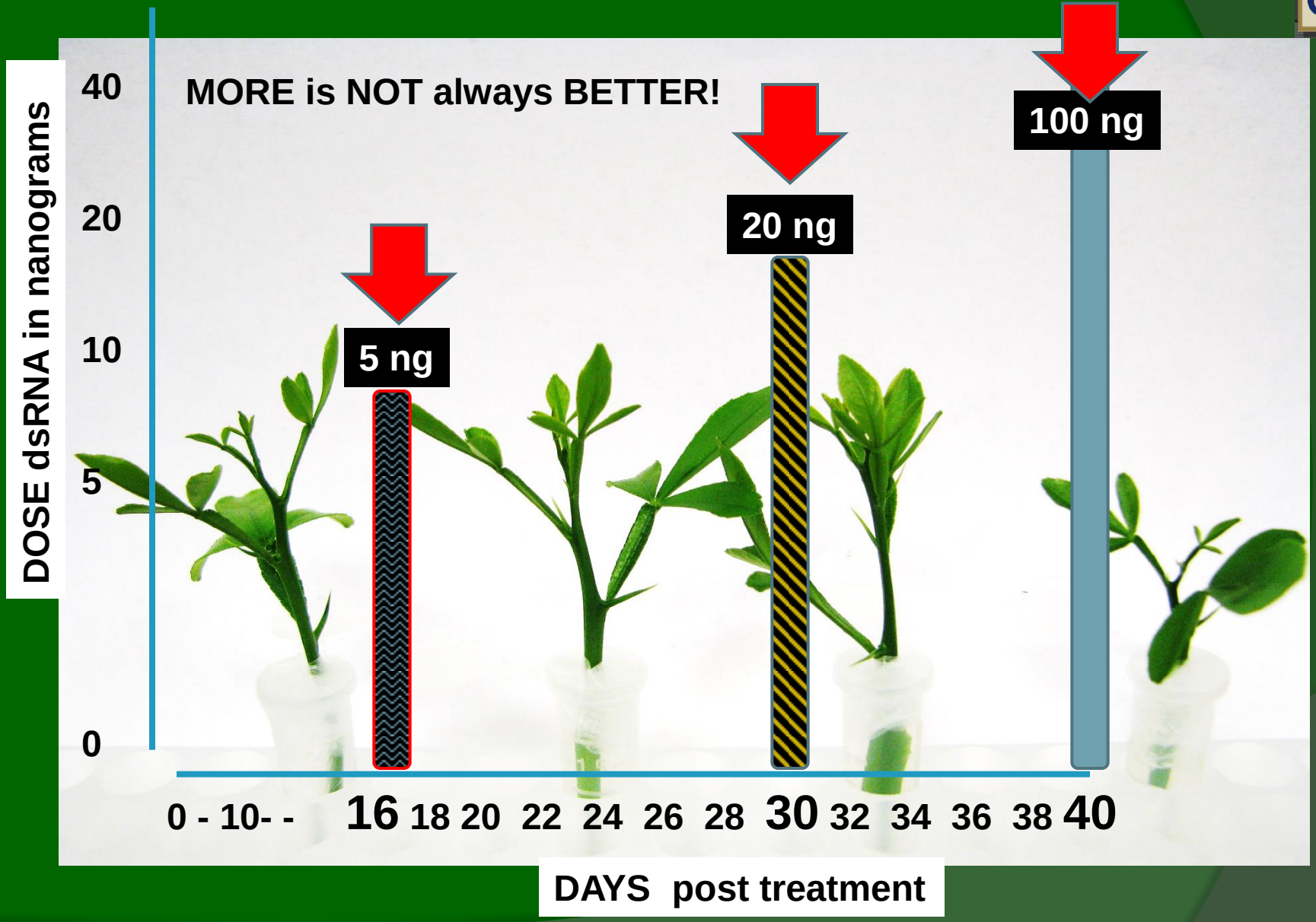






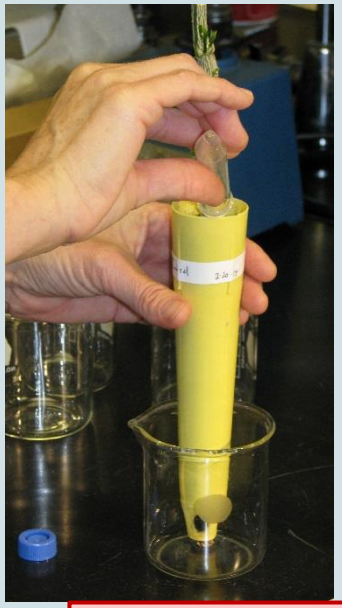
Plant tissue ~0.25 grams/ treated with 10, 50, or 100 nm dsRNA

Dosage Response of dsRNA and Efficacy in RNAi



Hunter et al, 2012

Target evalulation – “natural” system



100% Mortality Adults

Andrade, E. and Hunter, W. 2016. RNA Interference – Natural Gene-Based Technology for Highly Specific Pest Control (HiSPeC), InTech.

Highly Specific Pest Control, HiSPeC

LH dsRNA feed

ACP dsRNA feed

Reverse dsRNA feeding did *not* cause increased mortality effects.

*** Can design ONE RNAi to Reduce ONE insect!**

Specificity Tests

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

(Evans et al, 2009, USDA: Hunter, Andrade, Evans, 2014).

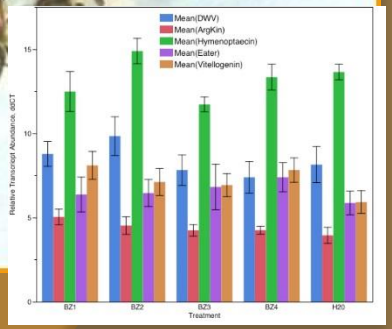
Newly emerge nurse bees. (Honey bee, *Apis mellifera* L.)

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



What are the INDUSTRY NEEDS?

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

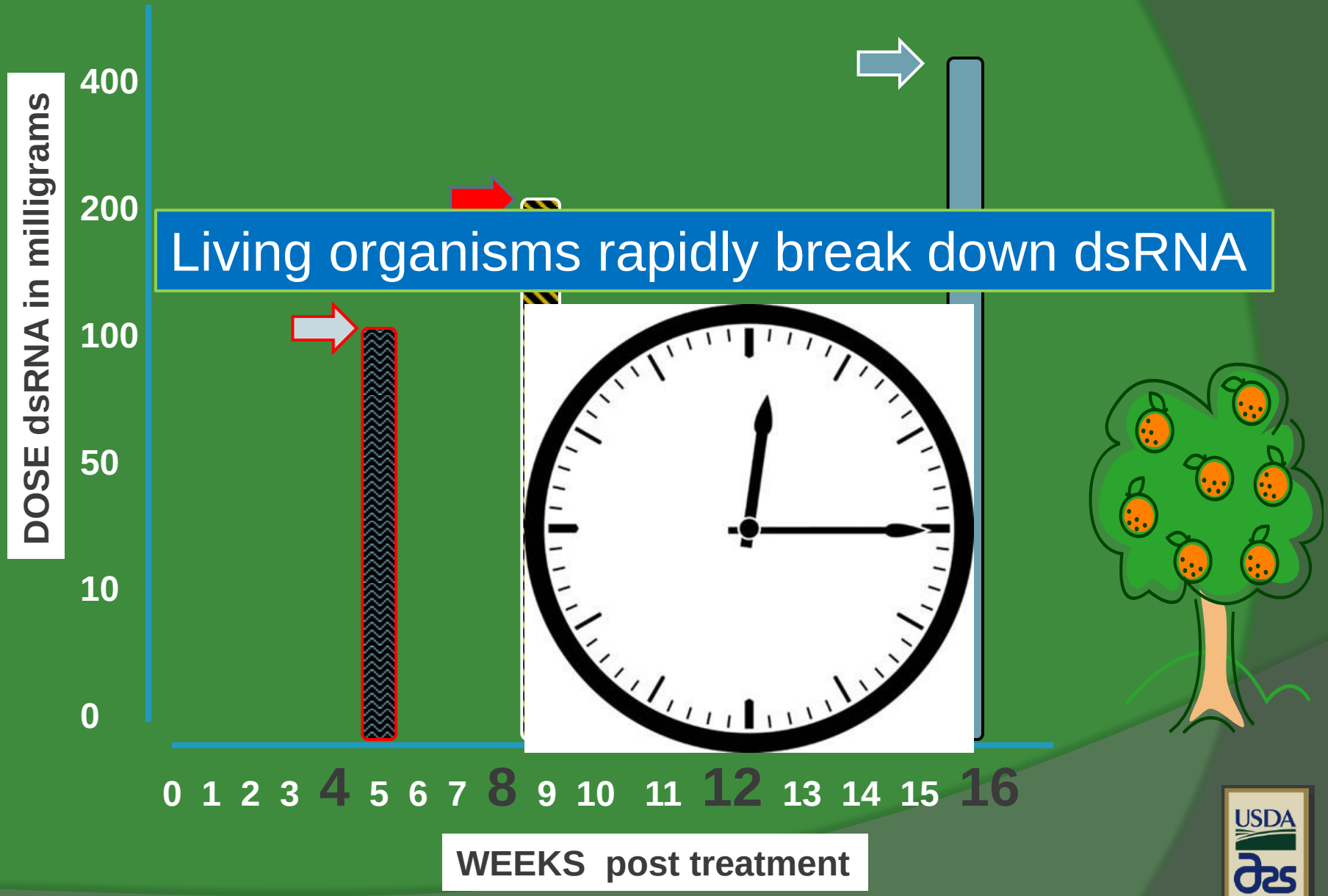
Environmental Fate

Preliminary results
dsRNA degrades and
Undetectable in 48 hrs

SOIL analyses:

Brian Pearson, Univ. FL

Dosage Response of dsRNA and Persistence in Trees



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).

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 F*ield Trials*)

BIG interest by all major Agro-Industries

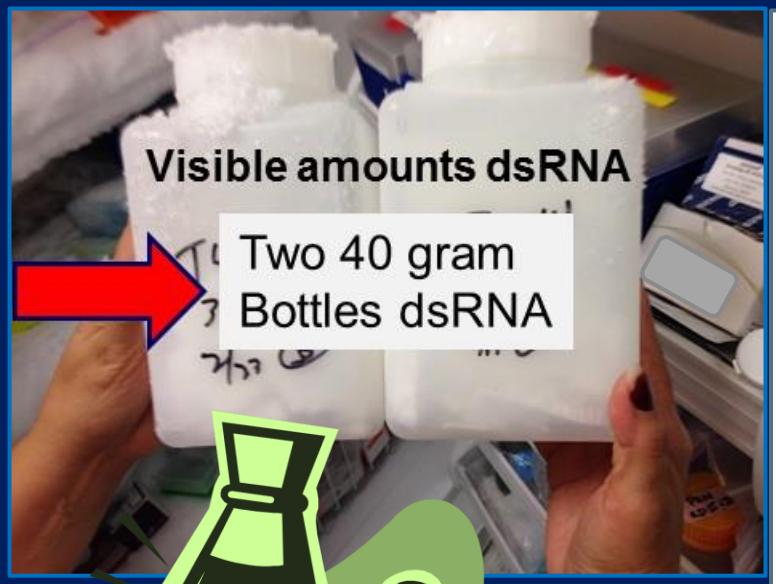
Established Regulatory Pathway by Agencies

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**

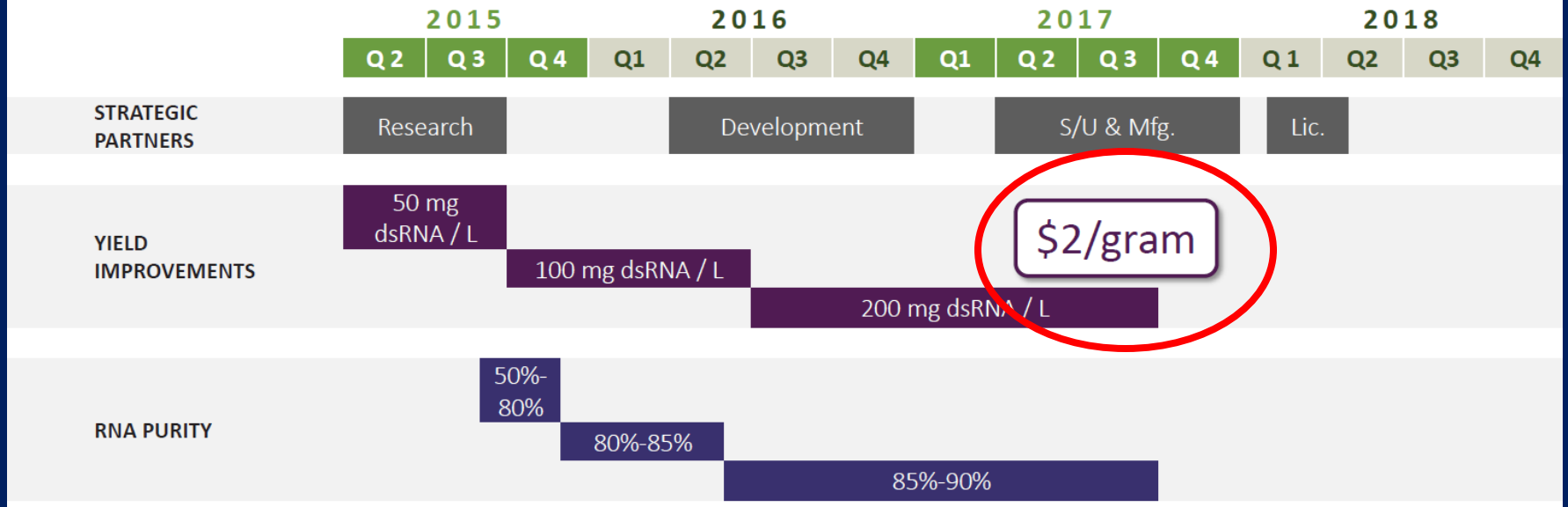
….costs keep dropping!

Research & Development

Breakthrough$ in Production Method$

APSE **Path Forward**

License / Co-Development Business Model



JOHN **KILLMER,** *CEO*

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CONTACT

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

Food Security

Better Environment

More Nutritious

Modify Plant Traits
Non transgenic

SOUR

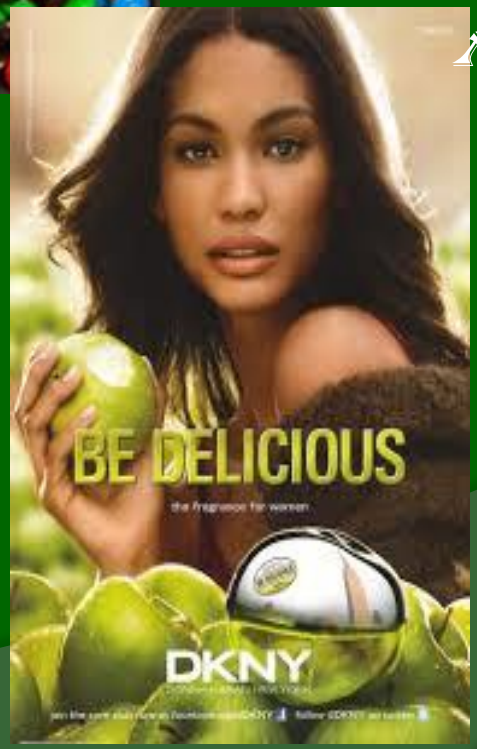
RNAi

SWEET

DELICIOUS APPLES

Bitter melon

RNAi



Plant Improvement, Trait Modification

Seedy

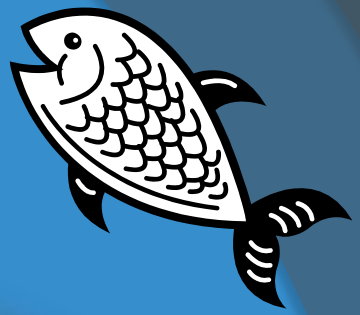
RNAi

Seedless

See a Problem

Make a Solution!

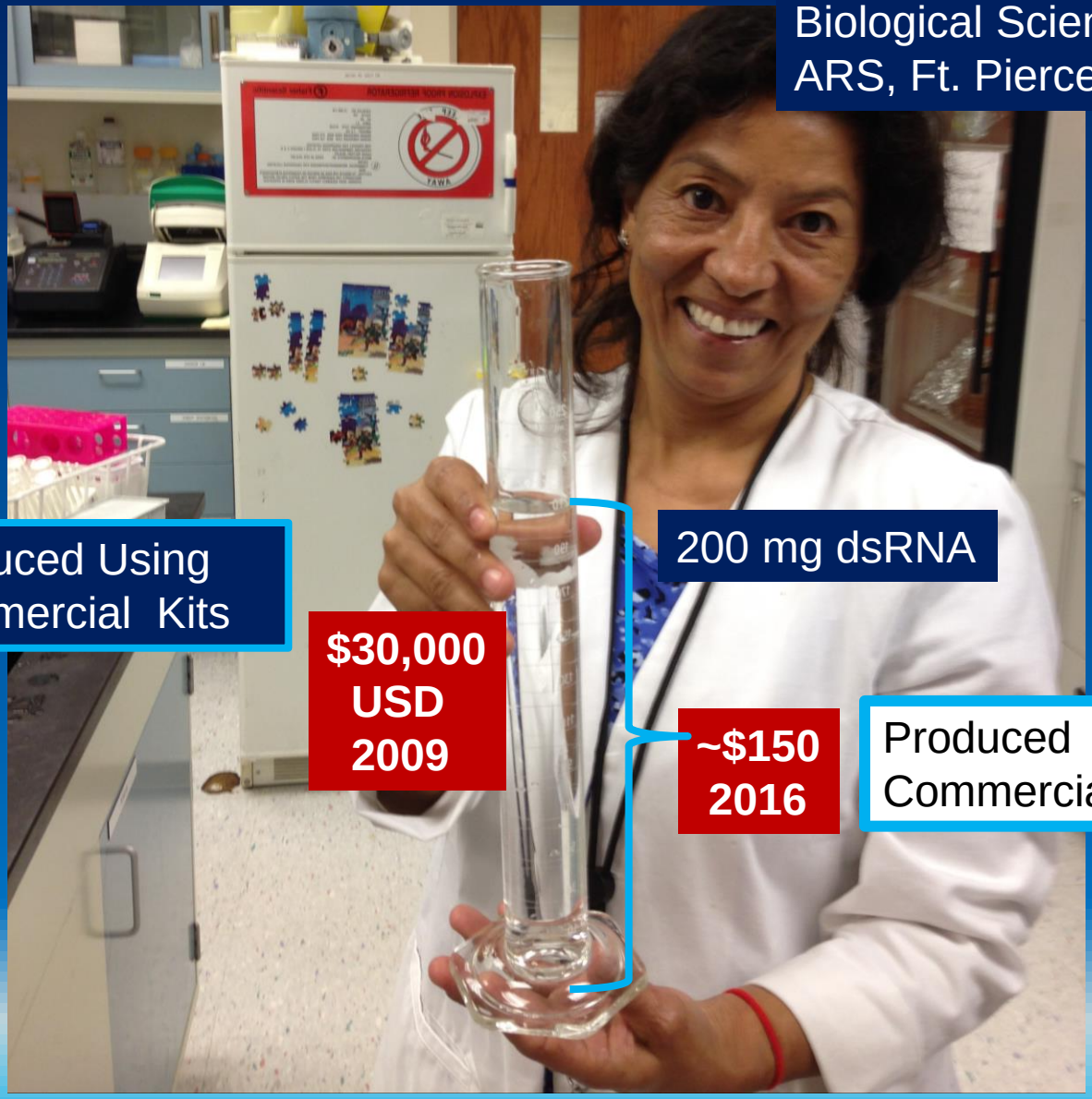
Other uses for RN gies



INNOVATION

Medical or Chemical Production

Maria Gonzalez,
Biological Science Technician, USDA, ARS, Ft. Pierce, FL



Produced Using Commercial Kits

$30,000 USD 2009

200 mg dsRNA

~$150 2016

Produced Using Commercial Company

Global Mega Trends

Safe tools for a Sustainable Future