Mechanism for Release of Genetically-modified Citrus

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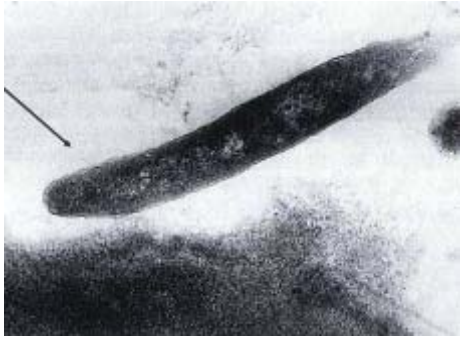
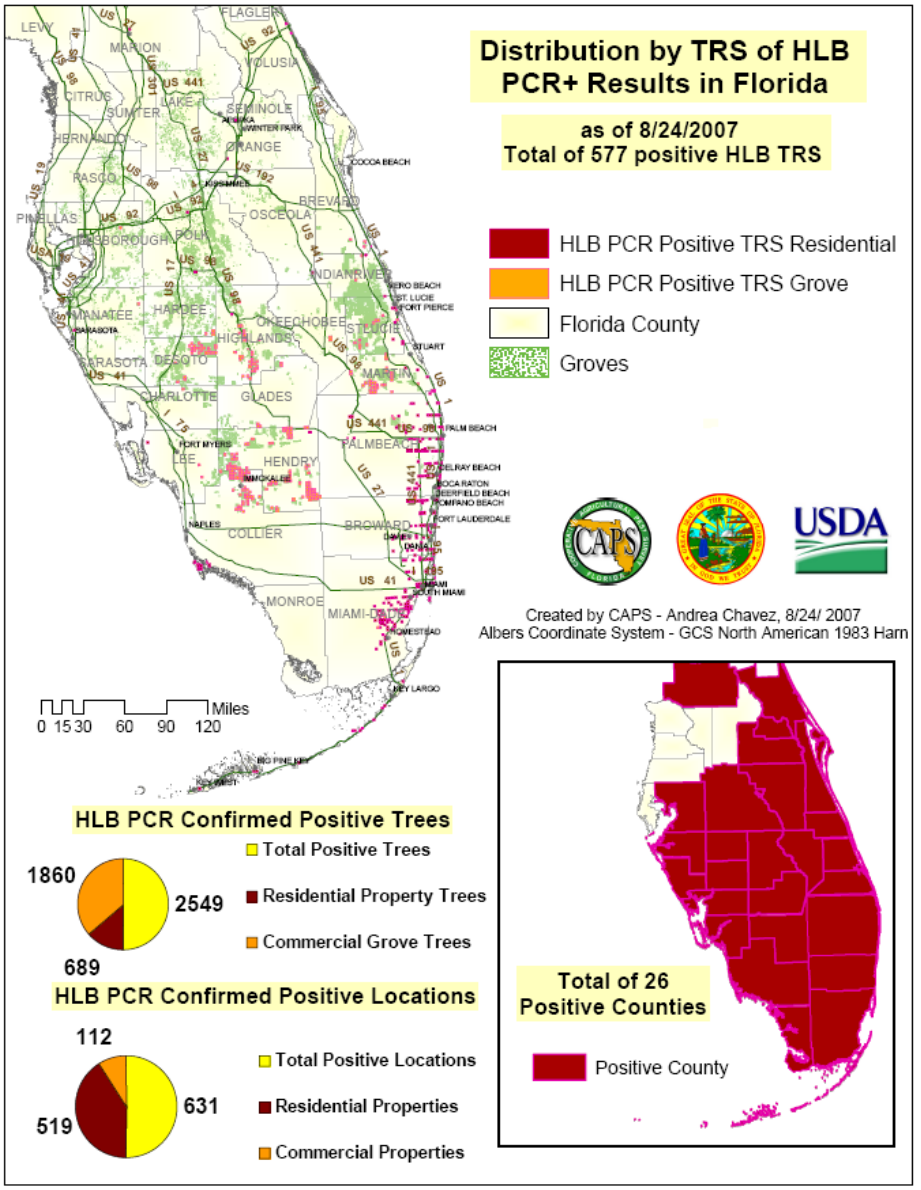
The problem…




Distribution by TRS of HLB PCR+ Results in Florida
as of 8/24/2007
Total of 577 positive HLB TRS
HLB PCR Positive TRS Residential
HLB PCR Positive TRS Grove
Florida County
Groves
CAPS
USDA
Created by CAPS - Andrea Chavez, 8/24/ 2007
Albers Coordinate System - GCS North American 1983 Harn
Miles
0 15 30 60 90 120
HLB PCR Confirmed Positive Trees
1860
2549
689
Total Positive Trees
Residential Property Trees
Commercial Grove Trees
HLB PCR Confirmed Positive Locations
112
519
631
Total Positive Locations
Residential Properties
Commercial Properties
Total of 26 Positive Counties
Positive County

Management strategies for HLB

- Vector control

- Clean nursery stock

- Rouging infected trees

The most viable option for dealing with citrus greening:

TRANSGENICS

Programs to develop disease resistant citrus via transgenics

- US public sector
  - ✓USDA, ARS, USHRL
  - ✓Univ. of Florida
  - ✓Texas A&M
  - ✓Univ. Calif.
- US private sector ?
- Brazil public sector
- Brazil private sector
  - ✓Allelyx

Host plant resistance is the ideal disease management strategy

- Highly effective (when available)
- Environmentally benign
- No reliance on pesticides
- Little or no additional expense to producers

Unfortunately… conventional breeding for HLB resistance is not a viable option

- No documented resistance to greening among edible citrus types
- *Citrus latipes* and *Citrus indica* may have some resistance – BUT THEY ARE NOT COMMERCIALLY ACCEPTABLE

- Time to incorporate resistance by traditional breeding is too long
- Best case scenario - 20 years
- Very unlikely to produce an acceptable product

- ***Citrus sinensis*** **is not amenable to traditional breeding.**
- **Sweet oranges are clonal propagules of an ancient natural hybrid.**
- **It has not been possible to recreate sweet oranges by breeding.**

A transgenic approach is the most viable option to develop citrus with resistance to HLB

- Many potential sources of resistance
- Change a single trait
- Much less time required than conventional breeding

Transgenics have proven successful for other fruit crops

Papayas
Papaya ring spot
Viral disease

Apples
Fire blight
Bacterial disease

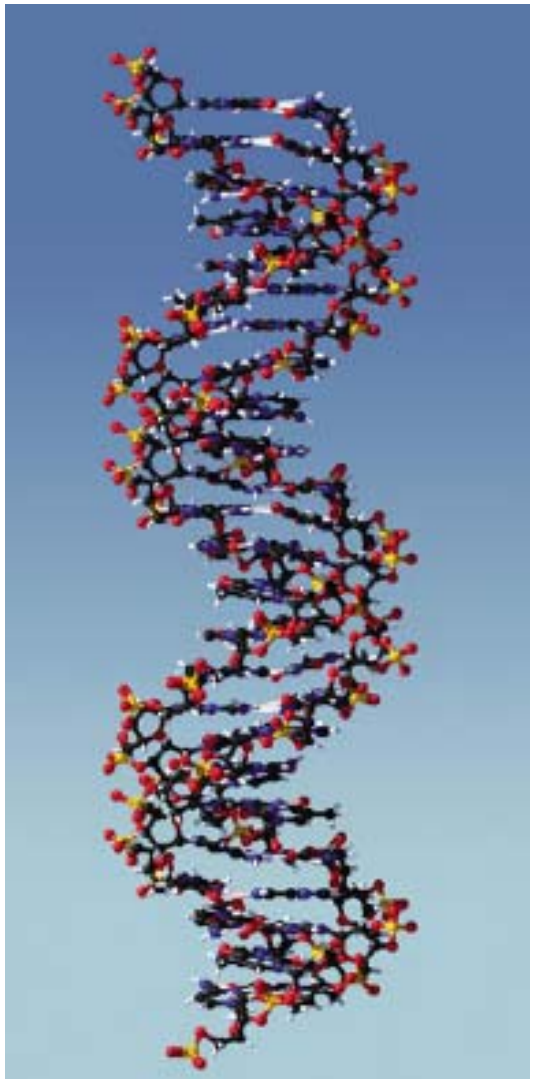
Plums
Plum pox
Viral disease

What is a “transgenic” plant?

- **A plant with one or more genes that have been transferred into it from another organism.**

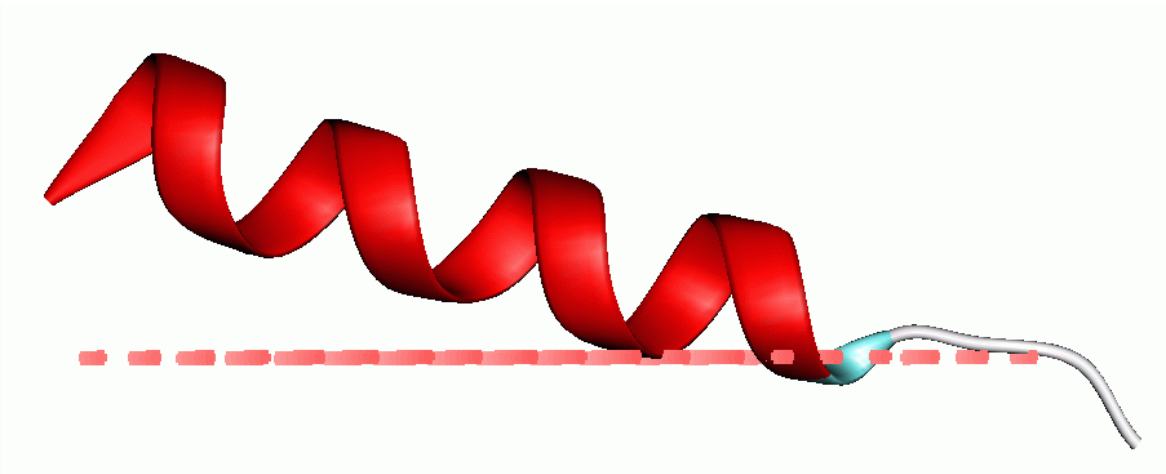
What is a “gene”?

- The basic biological unit of heredity.
- A segment DNA needed to contribute to a function.
- Most genes encode proteins.



What is a “protein”?

- A molecule composed of amino acids linked together in a particular order specified by a gene’s DNA sequence.
- Some proteins have ANTIBACTERIAL activity.



What genes should be transferred into citrus?

- **Antibacterial peptides** (small proteins)
- Found in many species

  > 700 have been identified in numerous organisms
- Some common characteristics

  small (30 – 50 amino acids)

  multiple lysine and arginine residues

  amphipathic nature (+ and -)
- Mechanism of action not clearly understood

Examples of antibacterial peptides:

- **Magainins - secreted by the skin of *Xenopus laevis***

- **Cecropins - haemolymph of *Hyalophora cecropia***
  - ***Attacin, Sarcotoxin, Diptericin***

- **Defensins - from various plant species**

Fig. 3. Resistance to rice blast disease in transformant by introduction of defensin gene
A: Non-transformant. B: Transformant. C: Non-transformant, not infected.

Brazilians have reported on transgenic 'Hamlin'

J. Amer. Soc. Hort. Sci. 131(4):xxx–xxx. 2006.

Attacin A Gene from *Tricloplusia ni* Reduces Susceptibility to *Xanthomonas axonopodis* pv. *citri* in Transgenic *Citrus sinensis* 'Hamlin'

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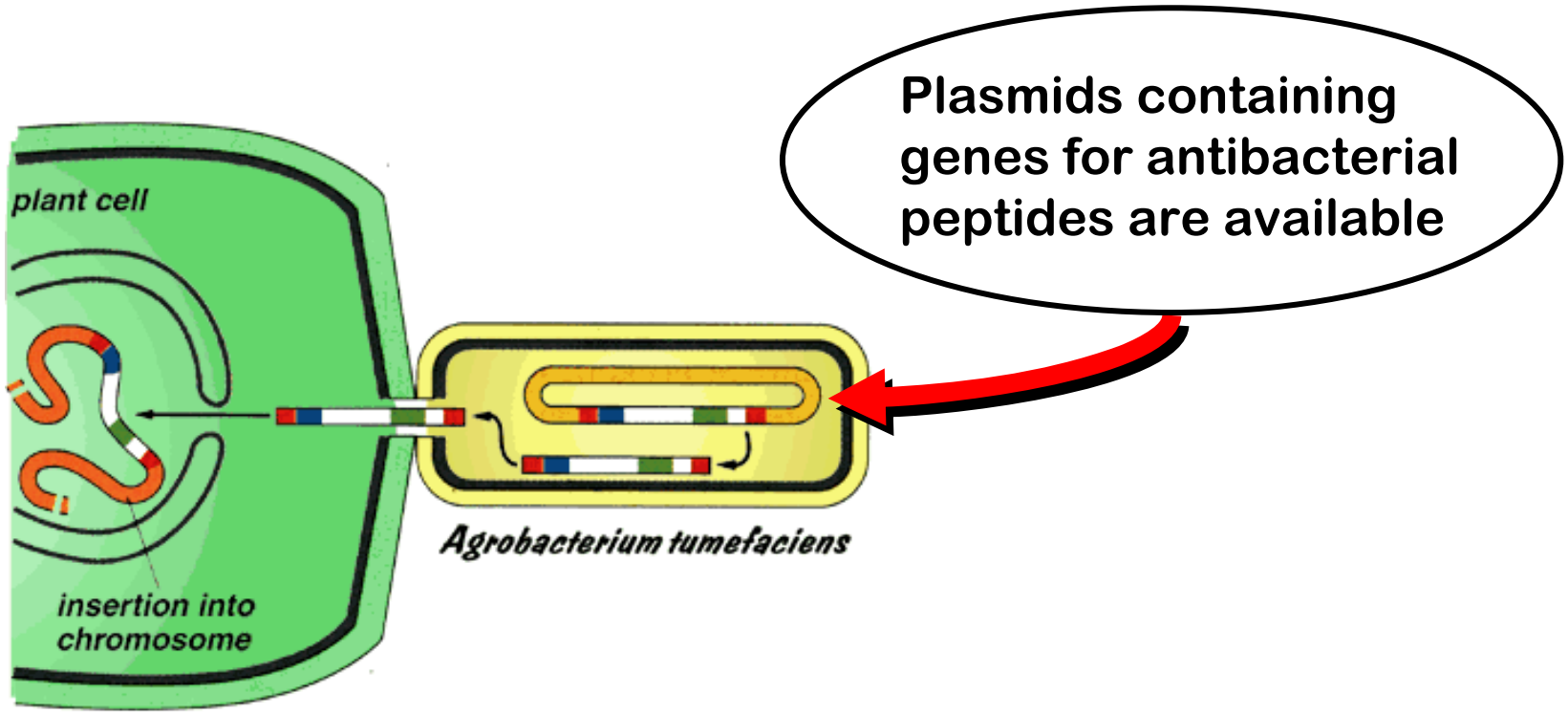
Antimicrobial peptides are a good option because…

- They have proven successful
- They are **single gene** products
- They are robust (resistance is unlikely)

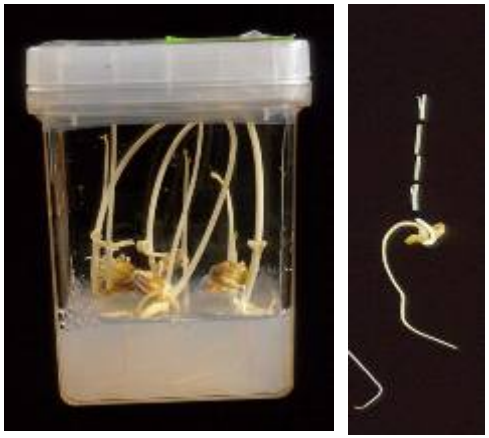
How are genes transferred from one organism to another?

Agrobacterium **– a plant pathogen with the capacity to transfer DNA**

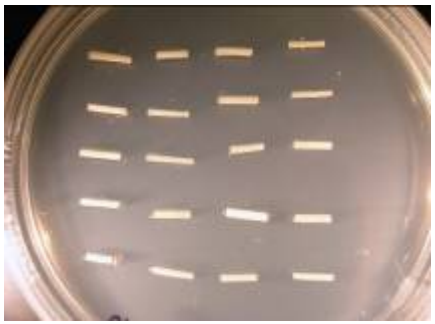


Citrus Transformation

Transformation



Selection



Regeneration

Evaluation

Infrastructure is in place

Laboratories

Growth room

Growth chambers

Greenhouses

Secure farm sites with ample space

Science is most likely easier than implementation…

“Transgenic crops face a daunting array of pre-commercialization regulatory requirements and post-commercialization market restrictions”

Bradford et al. 2005 Nature Biotechnology

Obstacles to commercialization of transgenic citrus

- Regulatory
- Intellectual Property
- Social

These obstacles are not insurmountable, but require large amounts of money to overcome.

Reasons for regulating transgenic crops

- Human safety
- Protect the environment
- Avoid fraud
- Social/ethical
- Product/process
- Public concern

Potential human health hazards

- New allergens or toxins
  - edible product
  - pollen
- Plieotropic effects of new protein combinations

Potential environmental hazards

- Movement of transgene/expression in a different organism
- Hazards associated directly or indirectly with the plant as a whole
- Non-target hazards outside the plant
- Resistance evolution in targeted pest

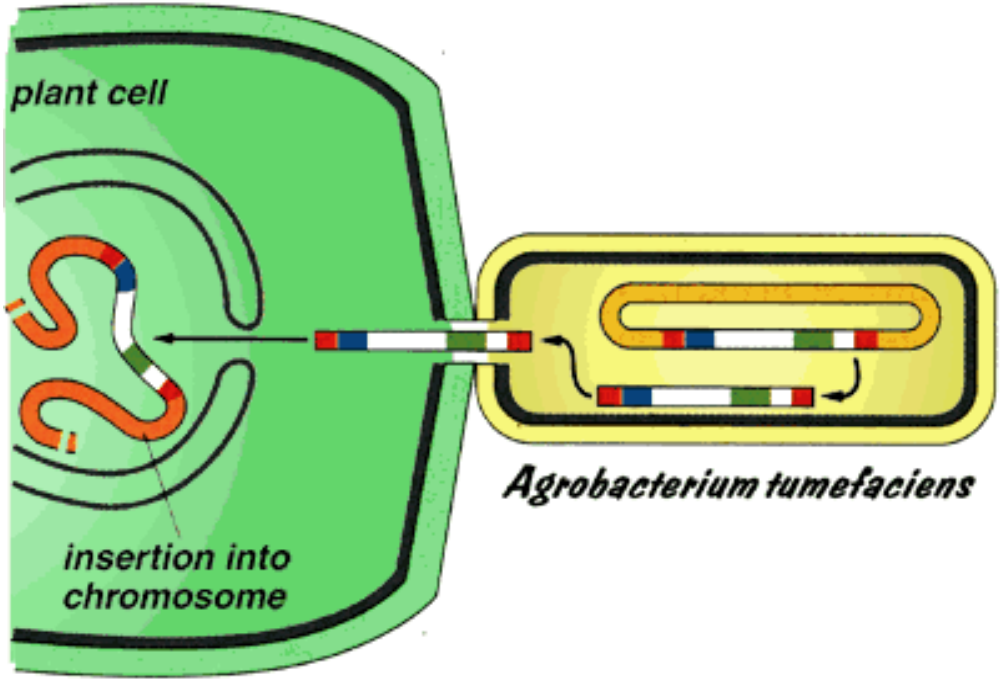
US Agencies with Regulatory Authority for Transgenic Crops

- **USDA-APHIS**
  - ✓Release into the environment
  - ✓Interstate transport
  - ✓Import
- **EPA**
  - ✓Plants expressing a “pesticide”
- **FDA**
  - ✓Food-safety

Agrobacterium-mediated transformation

USDA – APHIS

- Plant Pest Act
- Notification and Permitting
- Non-regulated status



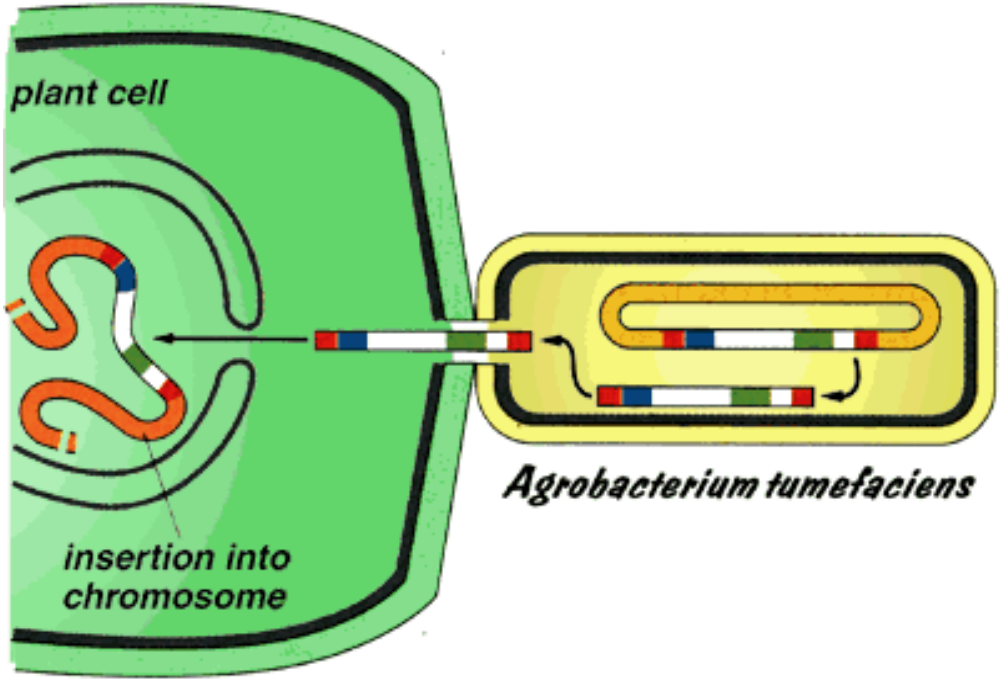
Agrobacterium – is (was) a plant pathogen

Novel genes – may be derived from plant pathogens

Agrobacterium-mediated transformation

US – EPA

- FIFRA
  - ✓Pesticides
  - ✓Environmental
- FFDCA
  - ✓Food safety



Plant-Incorporated Protectants (PIPs)

Limited to the pesticidal protein, not whole plant

Food Safety of All Transgenic Crops

FDA

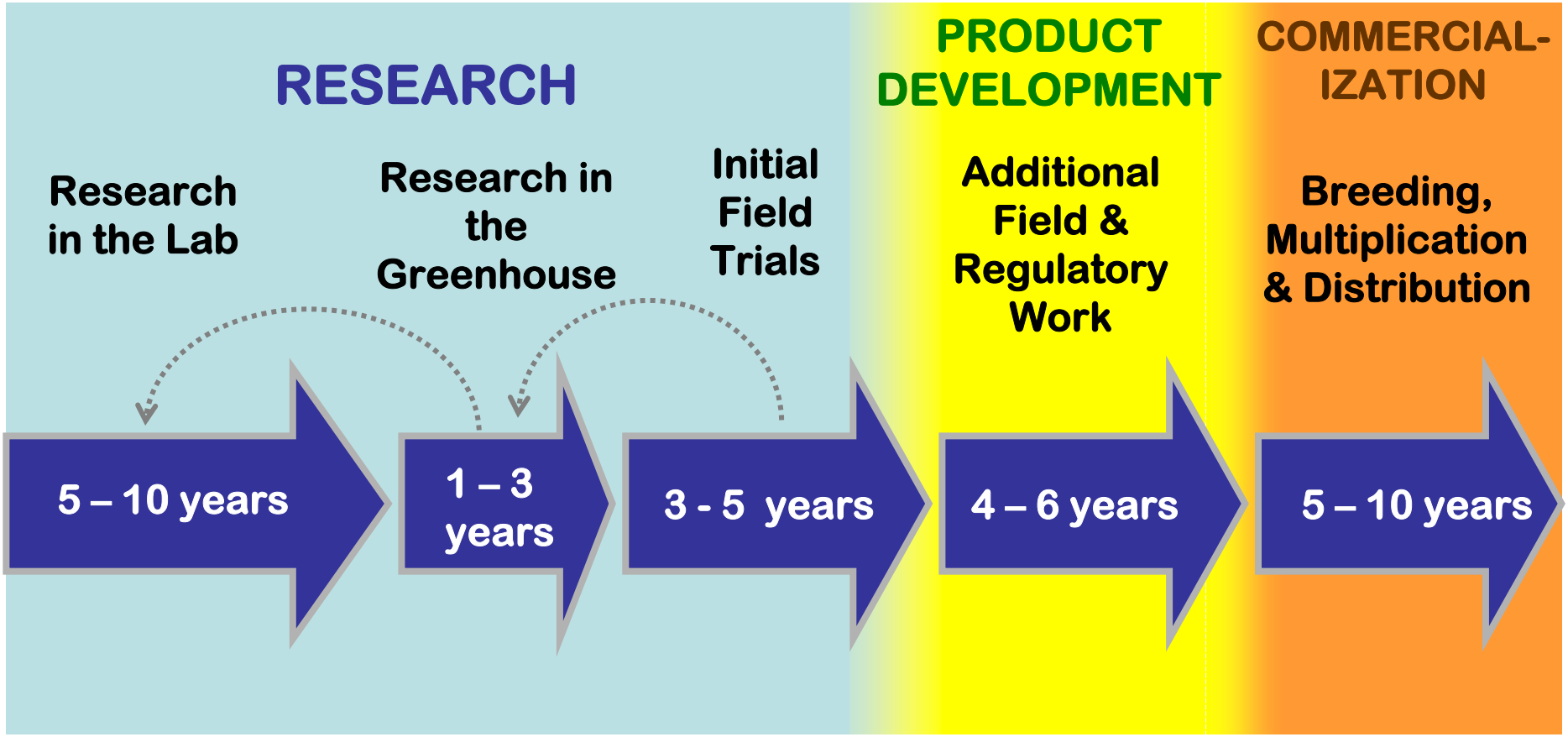
- FFDCA
- Not the PIP
- Not "food additives"
- Voluntary consultation process
- Culminates with "no further questions…at this time"

What are the compliance costs for regulatory approval of new transgenic crops?

- Information not readily available
- Estimates for transgenic maize
  - ✓Insect-resistant: $7-15 Million
  - ✓Herbicide-tolerant: $6-14 Million

These costs will most likely decrease as more and more GM-crops are developed

Timeline for Development of Transgenics (Sexually propagated crops)



----------18 – 34 years----------

All *commercial* transgenic crops have been developed by large and mid-sized corporations

Exceptions:

- **Virus-resistant papayas**
  - **Commercialized in Hawaii**
- **Virus-resistant plums**
  - **Non-regulated status**
  - **Not yet commercialized**

Comparing Oranges, Papayas and Plums

Papayas	Plums	Citrus
Specialty crop	Specialty crop	Specialty crop
Very small acreage	Small acreage	Large acreage
Viral disease	Viral disease	Bacterial disease
Infrequent in diet	Infrequent in diet	Frequent in diet
Hawaii	California/US	Florida /California

Timeline for GM Papayas

1985 – Effort to develop GM-papaya begins

1989 – Transformation begins

1991 – 1st transformant identified

1992 – 1st field trial / PRSV found

1994 – PRSV eradication abandoned

1995 – Commercial field trial success

1996 – Parent line deregulated by APHIS

Timeline for GM Papayas

1997 – Deregulation by EPA / FDA consultation completed

1999 – Widespread commercial planting

Timeline for GM Plums

1989 – Work initiated on development of transgenic plums

1996 – 1st field trials established in EU and US (under APHIS permit)

2002 – Field trails confirm resistance

2003 – Consultation with regulators begins

2006 – App. for non-regulatory status

2007 – Non-regulated status approved

Time frame for producing transgenic citrus

- 12 months – Transformed plants
- 18 months – Transformants propagated
- 24 months – Disease screening
- 36 months – Field ready transgenics



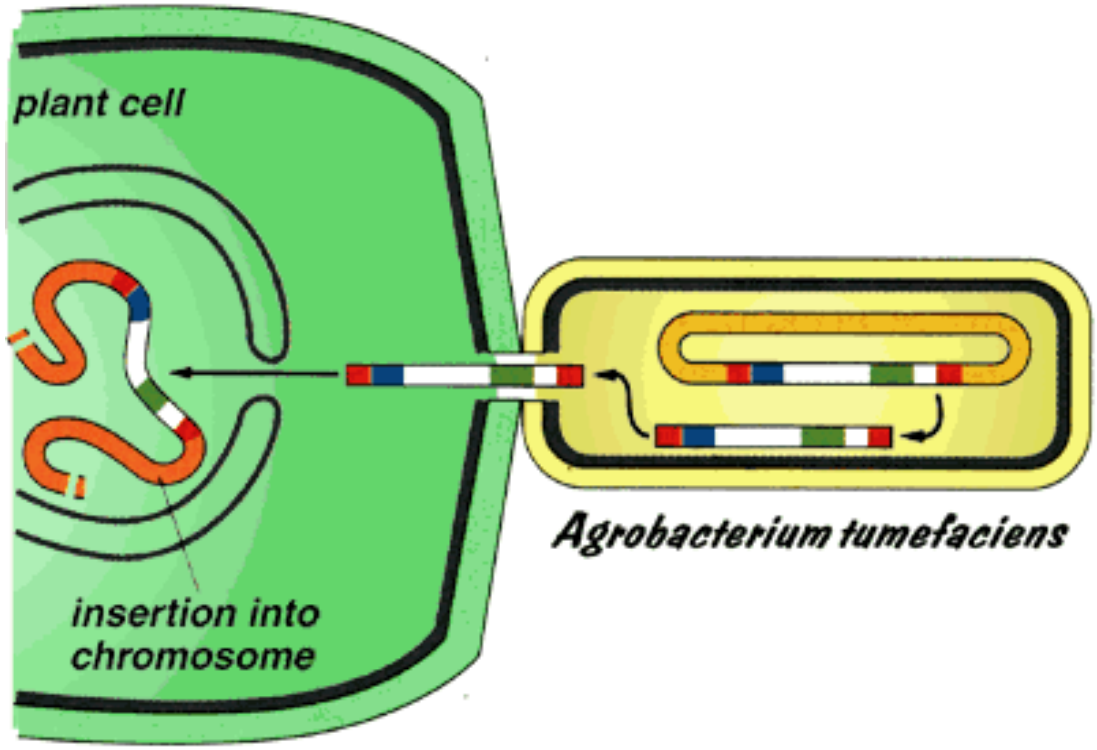

Intellectual Property

- Four firms (and subsidiaries)
  - ✓Bayer Cropscience
  - ✓DuPont
  - ✓Monsanto
  - ✓Syngenta
- Own or co-own 80% of all biotech traits that have received regulatory approval

Agrobacterium –mediated transformation

Numerous patents

- **Transformation system**
- **Genes of interest**



Will the public accept transgenic citrus?

- Not a question for researchers to answer.
- Evidence suggests increasing acceptance of other GMOs.
- Brazil is moving ahead with transgenic citrus.
- Given the choice between transgenic citrus or no citrus?

Transgenic citrus regulatory discussions

- Sept 17-18
- Meeting in Beltsville, MD
- Citrus researchers, industry representatives, regulators
- Develop a plan for moving forward with transgenic citrus

Summary

- Transgenic citrus offers the best strategy for dealing with HLB
- Significant regulatory and social obstacles
- Experience with papayas and plums suggests that obstacles can be overcome