

# Case Study 1

## Citrus Tree Defensin Protein: For Control of Citrus Greening

---

*Margaret Jones*

*September 20, 2016*

*Specialty Crop Regulatory Assistance*



United States Department of Agriculture

APHIS – Animal and Plant Health Inspection Service  
BRS - Biotechnology Regulatory Services

# Presentation Outline

---

- Citrus Unique Challenges
- Permitted Field Trials
- Petition for no longer regulated under 7 CFR 340
- Regulatory Flexibility



# Background for resistance to Citrus Greening

## Regulatory timeline

- Field tested 2009 to present
- Meetings with APHIS annually since 2008
- Citrus tristeza virus expressing antimicrobial peptides – phenotypic testing & as a curative
- EPA – Temporary Tolerance Exemption – spinach defensin 2,7 & 8



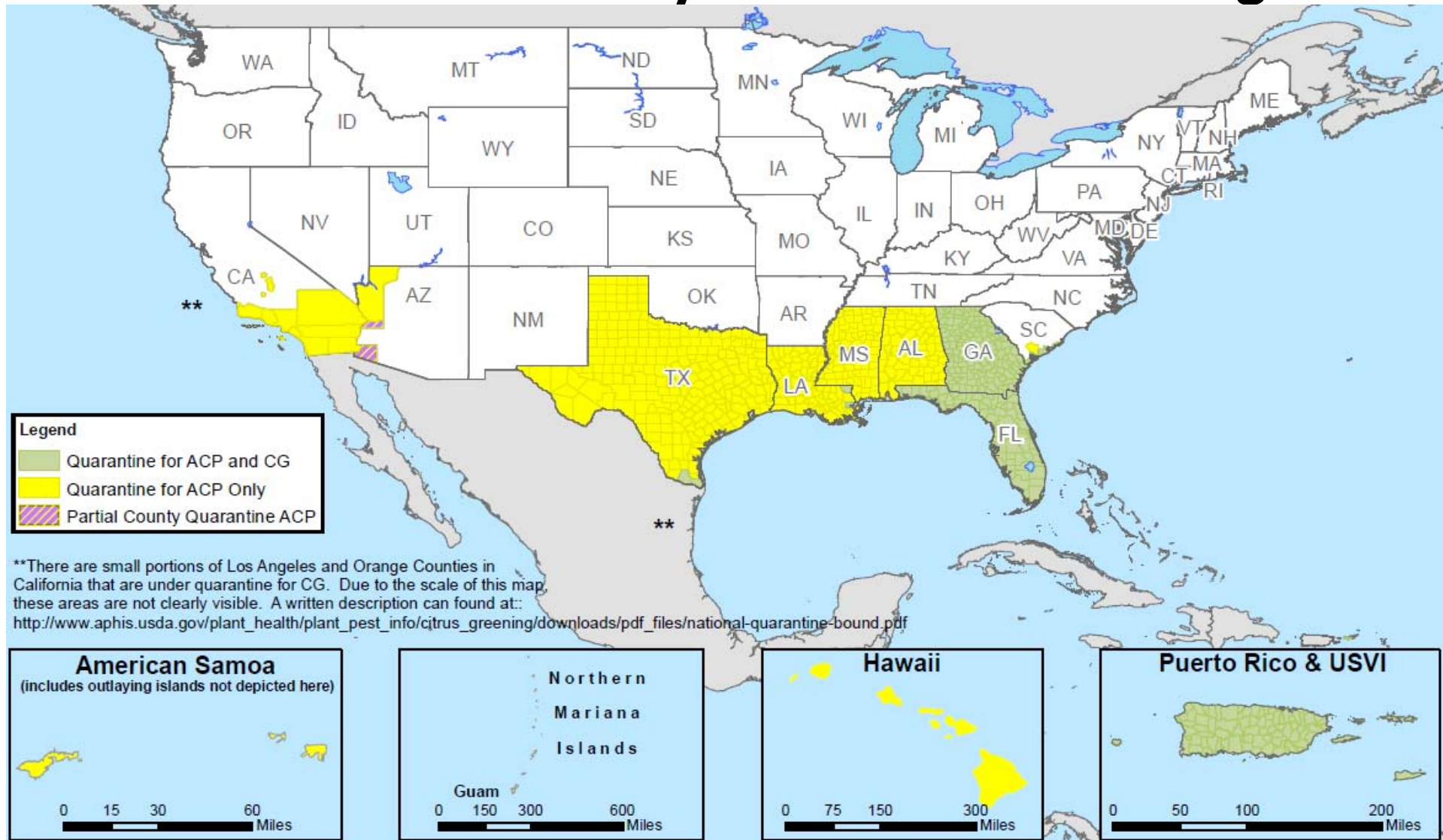
# Safeguarding U.S. Agriculture

## Trade Secrets Not Disclosed in this Presentation



United States Department of Agriculture

# National Quarantine Map (2014) for Asian Citrus Psyllid & Citrus Greening



# Citrus - Taxonomically & Biologically Diverse

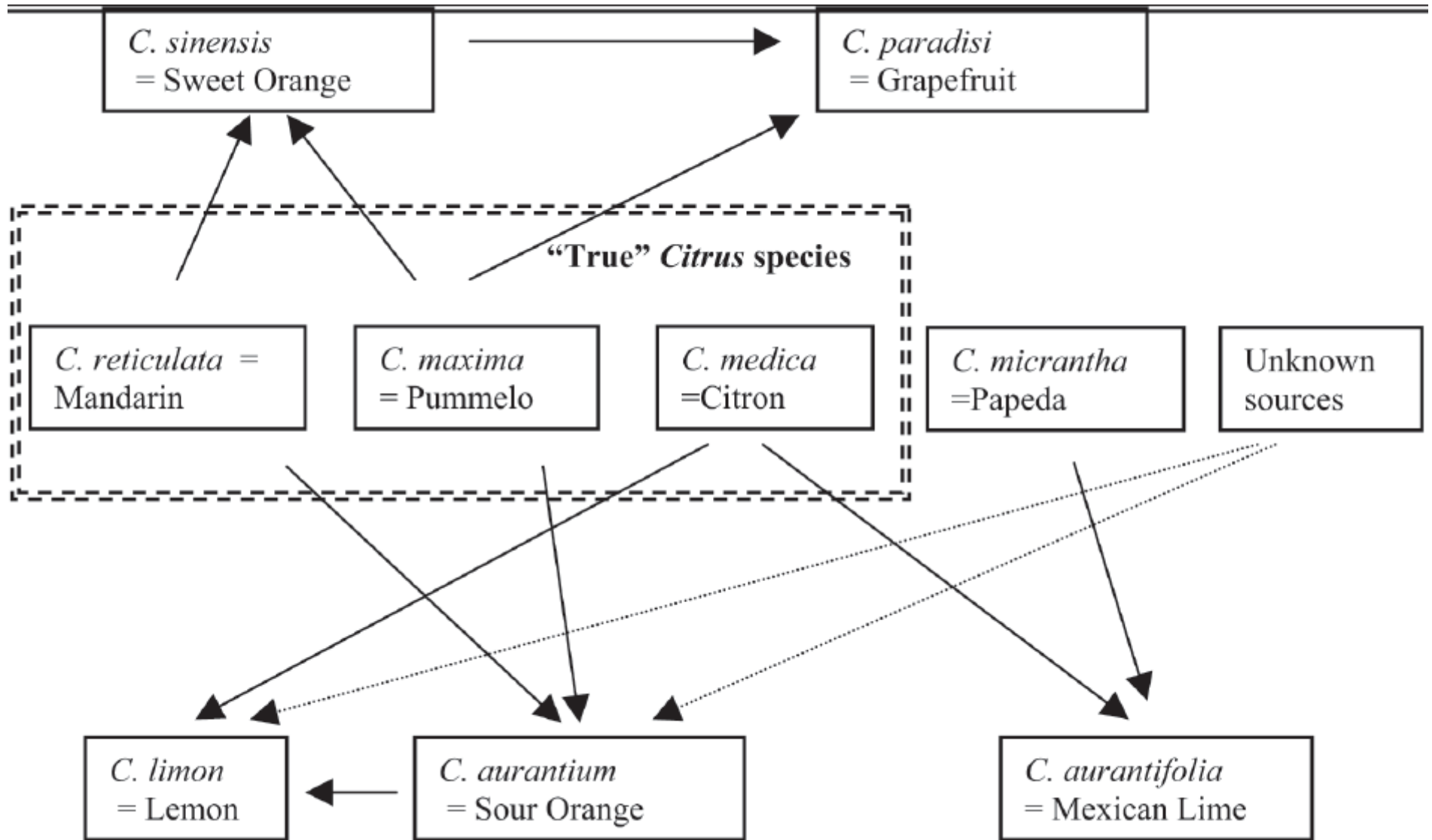


USDA ARS Scott Bauer



United States Department of Agriculture

# Origins of Citrus Cultivars



Stover (2005) HortTechnology 15:501-506

# GE Citrus – Currently Authorized for Release Under APHIS Permit

Common Name	Scientific name
Sweet orange	<i>Citrus sinensis</i>
Grapefruit	<i>Citrus × paradisi</i>
Lemon	<i>Citrus × limon</i>
Key lime, Mexican lime	<i>Citrus × aurantiifolia</i>
Citrange	<i>C. sinensis x Poncirus trifoliata</i>
Trifoliolate lime	<i>Poncirus trifoliata</i>
Sunki (or sour) Mandarin	<i>Poncirus trifoliata x C. sunki</i>
Citrumelo	<i>C. maxima x Poncirus trifoliata</i>
Citrindarin	<i>C. reticulata x Poncirus trifoliata)</i>





# Citrus – Critical Biological Factors

- Long life cycle
- Reproduction by vegetative propagation
- Seeds are not generally consumed
- Bee pollinated
- Number of seeds – none/few to dozens
- Reproductive variability - zygotic or nucellar
- Can be self-incompatible, requiring pollination



# Background for Citrus Defensin

- Developed by US Sugar/Southern Gardens Citrus
- *Agrobacterium tumefaciens* transformation
- Engineered with
  - *SoD2, SoD7, SoD2/DoD7* from spinach
  - NPT II selectable marker
  - Constitutive promoters already present in deregulated products



# GE Citrus expressing the Defensin Protein

- Antimicrobial protein
- From Spinach
- Consumed by humans without report of harm



Regulated under 340 – Agrobacterium transformation



United States Department of Agriculture

# 340: Movement and Environmental Release of Regulated Material

In a manner to minimize the potential for the regulated organism to:

- Escape and disseminate in the environment
- Persist in the environment
- Produce offspring that will persist
- Significant impact non-target organisms



# USDA – What Activities are Regulated under 7 CFR 340 In GE Plant Variety Development?

Laboratory and Greenhouse (not regulated by APHIS)

USDA -APHIS



Permit & Notification

Environmental Release: Confined  
Importation & Interstate Movement: Contained



	<b>Citrus may be Moved Under Notification</b>	<b>Permit for Release</b>
	<b>Notification</b>	<b>Permit</b>
Regulatory Oversight	Familiarity & Lower Risk Expedited Permit Performance Standards	Increased data requirements and APHIS review
Does GE organism meet eligibility criteria?	Certain GE citrus movement under notification	Multi-year GE citrus confined field release
Design Protocols	Not required for movement	Always required



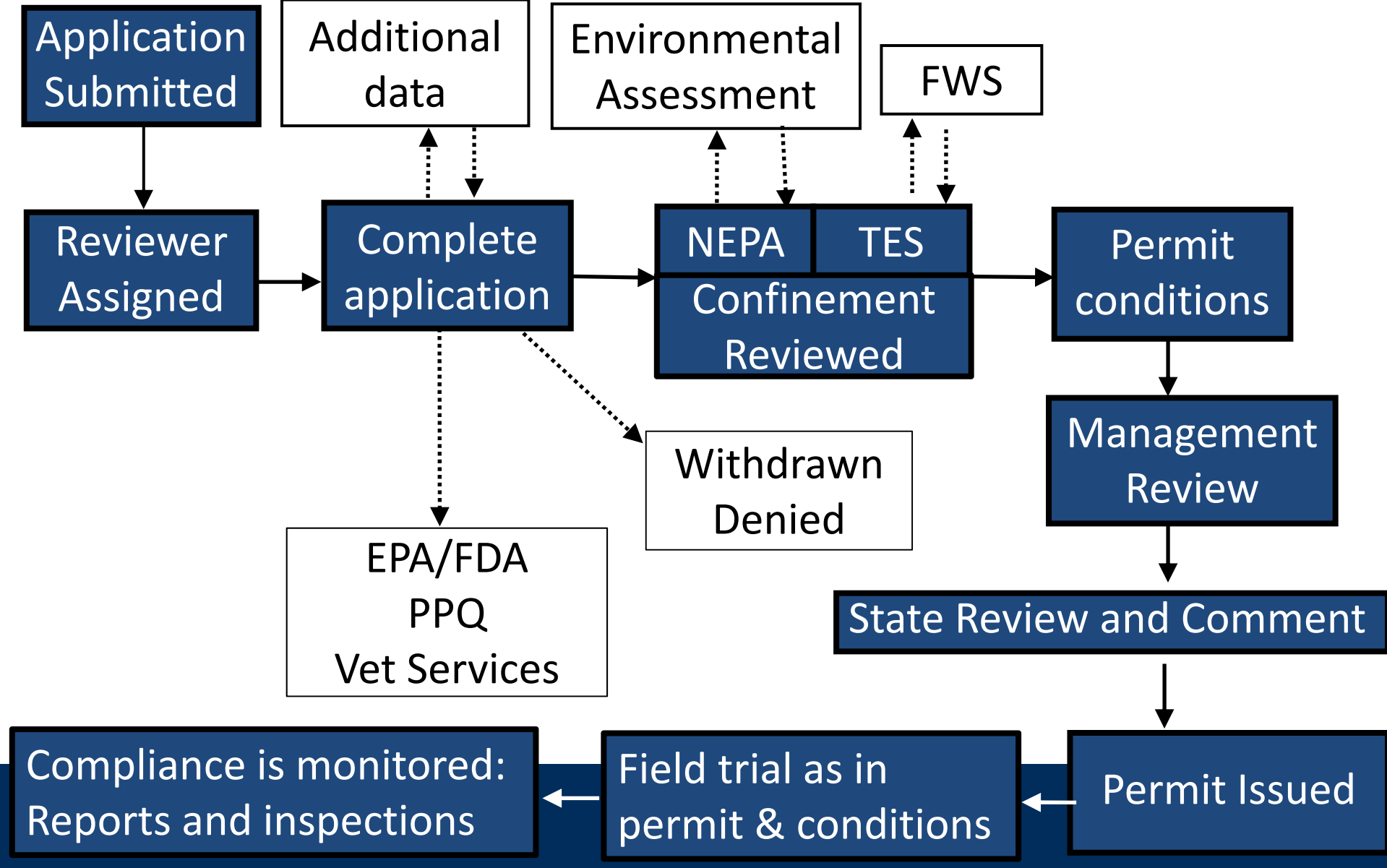
APHIS Review (days)		
	Notification	Permit
Release	30	120
Movement Interstate/Importation	10/30	60/60

Effective (years)	
Notification	1
Permit	1 – 3

15



# Permit Workflow: Major Steps

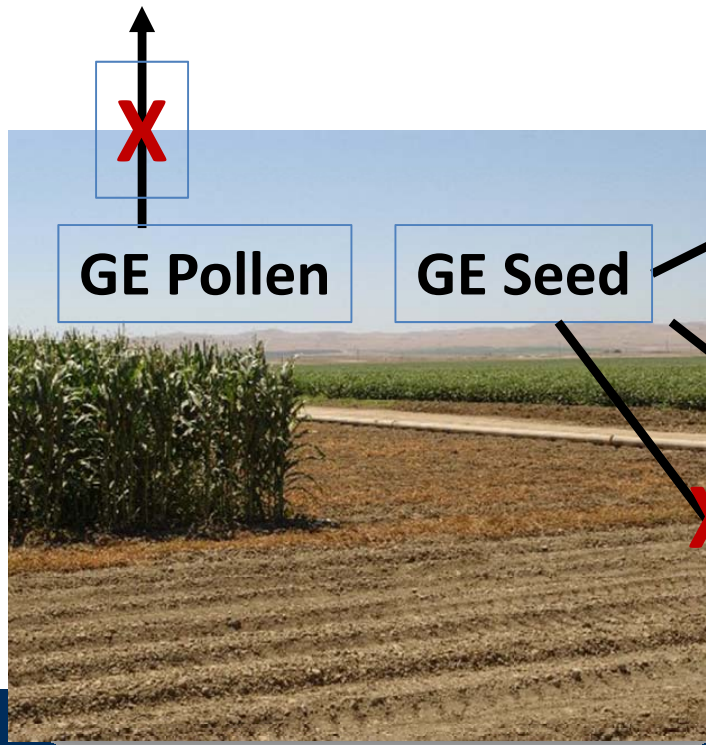




# Confined Field Trials: Keep GE And Non-GE Plants Separated And Not Persist Example: Corn

Maintain appropriate separation distance to sexually receptive plants while pollen is being produced.

1) Clean GE seed from planters and harvesters  
2) Keep GE separated from non-GE seeds



Next growing season remove volunteer plants before pollen or seed is produced

GE-seed from test is not used for human or animal food

# Confined Field Trials: Keep GE And Non-GE Plants Separated And Not Persist Example: Citrus

Border row of citrus trees



Restrict Use of Bee Hives



# USDA – What Activities are Regulated under 7 CFR 340 In GE Plant Variety Development?

Laboratory and Greenhouse (not regulated by APHIS)

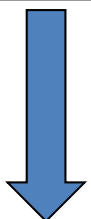
USDA -APHIS



Permit & Notification

Environmental Release: Confined  
Importation & Interstate Movement: Contained

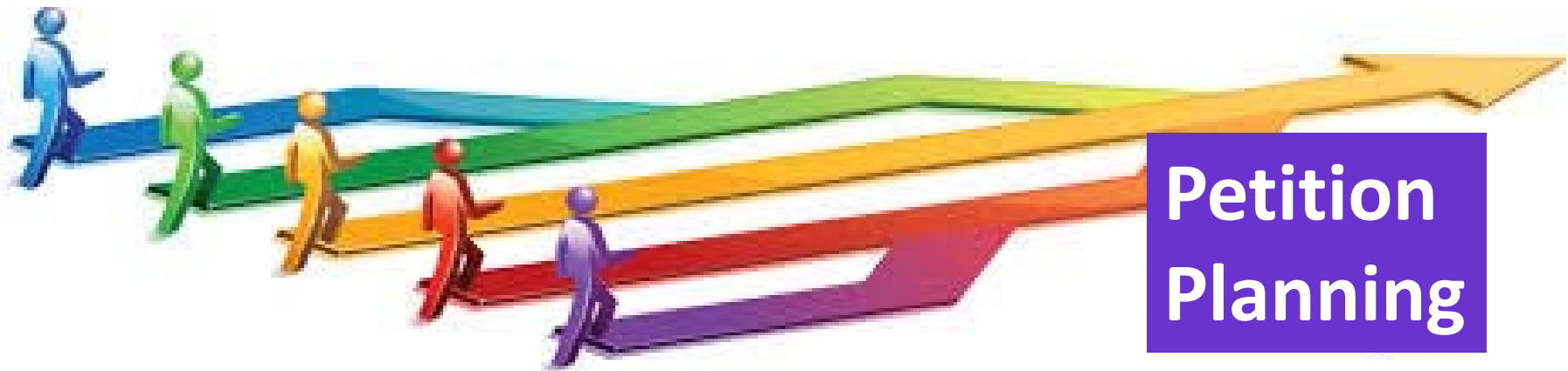
USDA -APHIS



Petition

Nonregulated status –not regulated under 340: Unconfined





- Request meeting with APHIS BRS and discuss project design
- Review a petition and submission process
- Current scientific knowledge can be referenced
- Get expert assistance when needed
- Submit a complete and well-written document
- Consider conferring with EPA and/or FDA



# Petition: Data Requirements 7 CFR 340.6(c)

- Description of the biology
- Relevant experimental data and publications.
- Genotype between the regulated article and the nonmodified recipient organism and where the developed.
- Field test reports for all trials conducted under permit or notification



# Petition: Data Requirements 7 CFR 340.6(c)

**A detailed description of the phenotype of the regulated article.**

- Plant pest risk characteristics, disease and pest susceptibilities
- Expression of the gene product, new enzymes, or changes to plant metabolism
- Weediness
- Impact on the weediness of any other plant with which it can interbreed
- Agricultural or cultivation practices
- Effects on nontarget organisms



## Petition: Data Requirements 7 CFR 340.6(c)

- Indirect plant pest effects on other agricultural products
- Transfer of genetic information to organisms with which it cannot interbreed
- And any other information which the Administrator believes to be relevant to a determination.
- Any information known to the petitioner that indicates that a regulated article may pose a greater plant pest risk than the unmodified recipient organism shall also be included.



# Risk Assessment

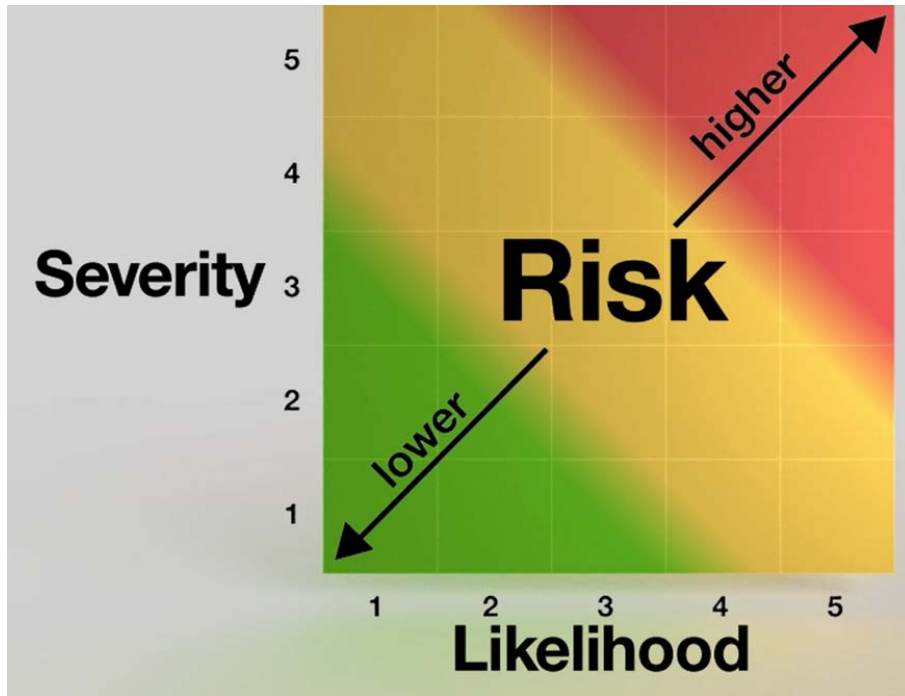
## Plant Pest Risk Assessment

Regulatory authority - poses risks to plants or plant products.

## National Environmental Policy Act (NEPA) Assessment

## Act (NEPA) Assessment

Examine the environmental impact and compared with reasonable alternatives.





# Plant Pest Risk Assessment – Topics Covered

- Plant Pest and Disease
- Beneficial Nontarget Organisms
- Weediness
- Weediness of Other Plants with which Can Interbreed
- Changes to Agriculture or Cultivation
- Horizontal Transfer



# Plant Pest Risk Assessment – Example of types of analyses in the PPRA

**Will Defensin Citrus increase the weediness of sexually compatible plants?**

- What plants citrus relatives are present in the U.S. that could interbreed?
- If hybrids can be produced, would they survive in the wild?
- Are there indications that inclusion of defensin resistance would effect fitness of any resulting hybrid progeny?



# NEPA Assessment

Alternatives



- 1) No action
- 2) Preferred Alternative
  - Alternatives Considered But Rejected from Further Consideration
  - Comparison of Alternatives



# NEPA Environmental Assessment: Scope

## **Agricultural Production**

Areas of Citrus Production

Agronomic Practices

Organic and Specialty Citrus

## **Physical Environment**

Soil, Water, Air

## **Biological Resources**

Climate Change

Animal & Plant Communities

Soil Microorganisms

Biological Diversity

Gene Movement



# NEPA Environmental Assessment: Scope (cont.)

## Human Health

Public Health

Worker Health and Safety

## Animal Health

Animal Feed

Livestock Health

## Socioeconomics

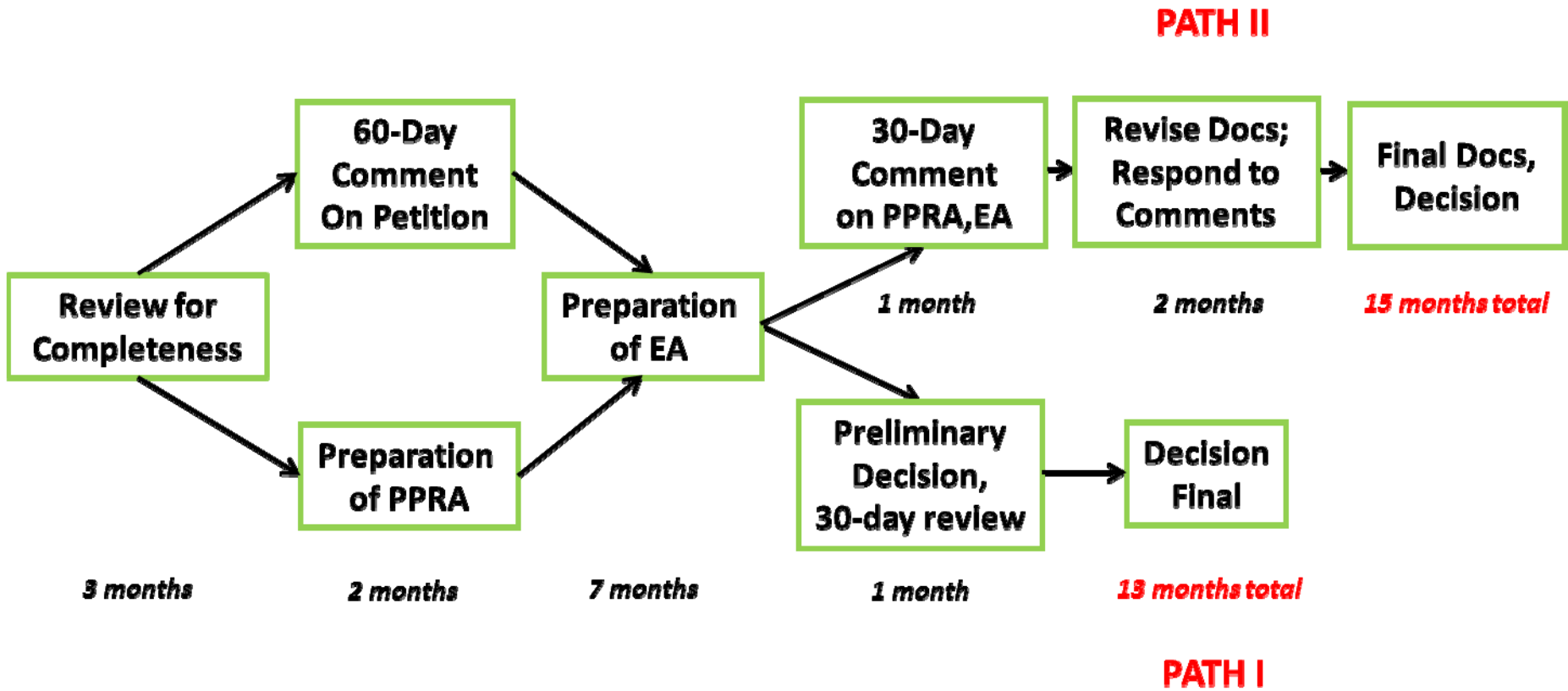
Domestic Economic Environment

Trade Economic Environment





# Petition Process Steps



# Extension Process

Extend non-regulated status from a previously de-regulated organism (called the antecedent)

## Advantages for Applicant

- Reduced data package
- Recent extensions completed in 5-7 months



# BRS Guidance Documents

## Notification

[https://www.aphis.usda.gov/biotechnology/downloads/notification\\_guidance\\_0311.pdf](https://www.aphis.usda.gov/biotechnology/downloads/notification_guidance_0311.pdf)

## Permit

[https://www.aphis.usda.gov/biotechnology/downloads/permit\\_guidance.pdf](https://www.aphis.usda.gov/biotechnology/downloads/permit_guidance.pdf)

## Petition

[https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/ct\\_new\\_users\\_petitions](https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/ct_new_users_petitions)





# Guidance Documents

## **Appendix I: Molecular Genetic Characterization**

<https://www.aphis.usda.gov/brs/canadian/usda03e.pdf>

## **Appendix II: Environmental Characterization Data for Transgenic Plants Intended for Unconfined Release**

<https://www.aphis.usda.gov/brs/canadian/appenannex2e.pdf>



# Additional Information



[Biotechquery@aphis.usda.gov](mailto:Biotechquery@aphis.usda.gov)

*We are here to facilitate the process*



United States Department of Agriculture

APHIS Biotechnology Regulatory Services