Case Study 2

Simplot Innate® W8 Potato

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Note: EPA registration for W8 is pending, and nothing in this slide desk shall be construed as an offer for sale, commercialization, or promotion of W8.

innate®

Simplot

Agenda

- Simplot Plant Sciences
- Overview of W8 potato
  - Traits, benefits, and development
- Unique aspects of potatoes
- U.S. regulatory strategy for W8
- VNT1 protein
  - Production, detection and safety
- Lessons learned along the way

J.R. Simplot Company

- 10,000 employees in 14 countries
- Products marketed in 40 countries
- 1.6 billion kg frozen fries annually
- 30,000 cow/calf herds, 16 ranches
- Family owned, private company



Simplot Plant Sciences

INNOVATIVE CULTURE WITH GLOBAL ASPIRATIONS

Major Investment
Started in 2001

Based in Boise, ID
Near Simplot headquarters

People
About 107 employees, including 19 PhDs
R&D, Agronomy, Communications, Marketing, Regulatory, Commercial

Products
White Russet fresh potatoes (branded) and multiple brands of chips, starting in 2015

Late Blight Protection and Quality Benefits

- Late blight protection
  Phytophthora infestans—cause of Irish potato famine

- Reduced black spot
- Stays white when cut or peeled

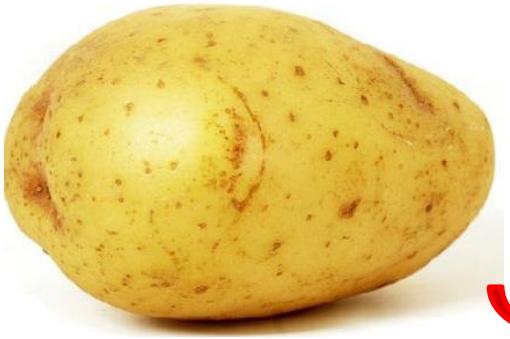
Control

W8

- Lower acrylamide potential
- Less dark color after frying

W8 Potato Has Two Inserts

Russet Burbank



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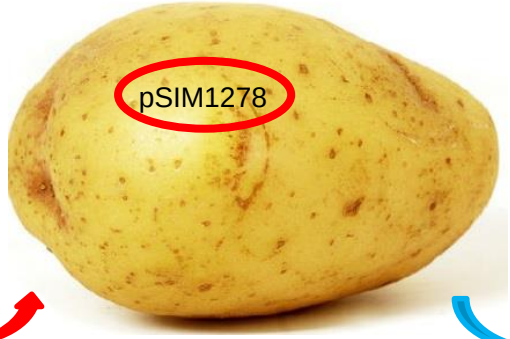
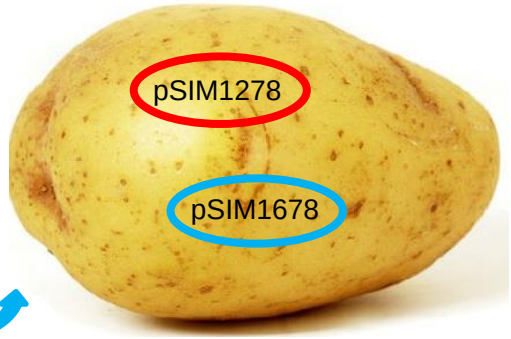


W8



1st transformation

pSIM1278

- Reduced black spot
- Lower free asparagine
- Lower reducing sugars

2nd transformation

pSIM1678

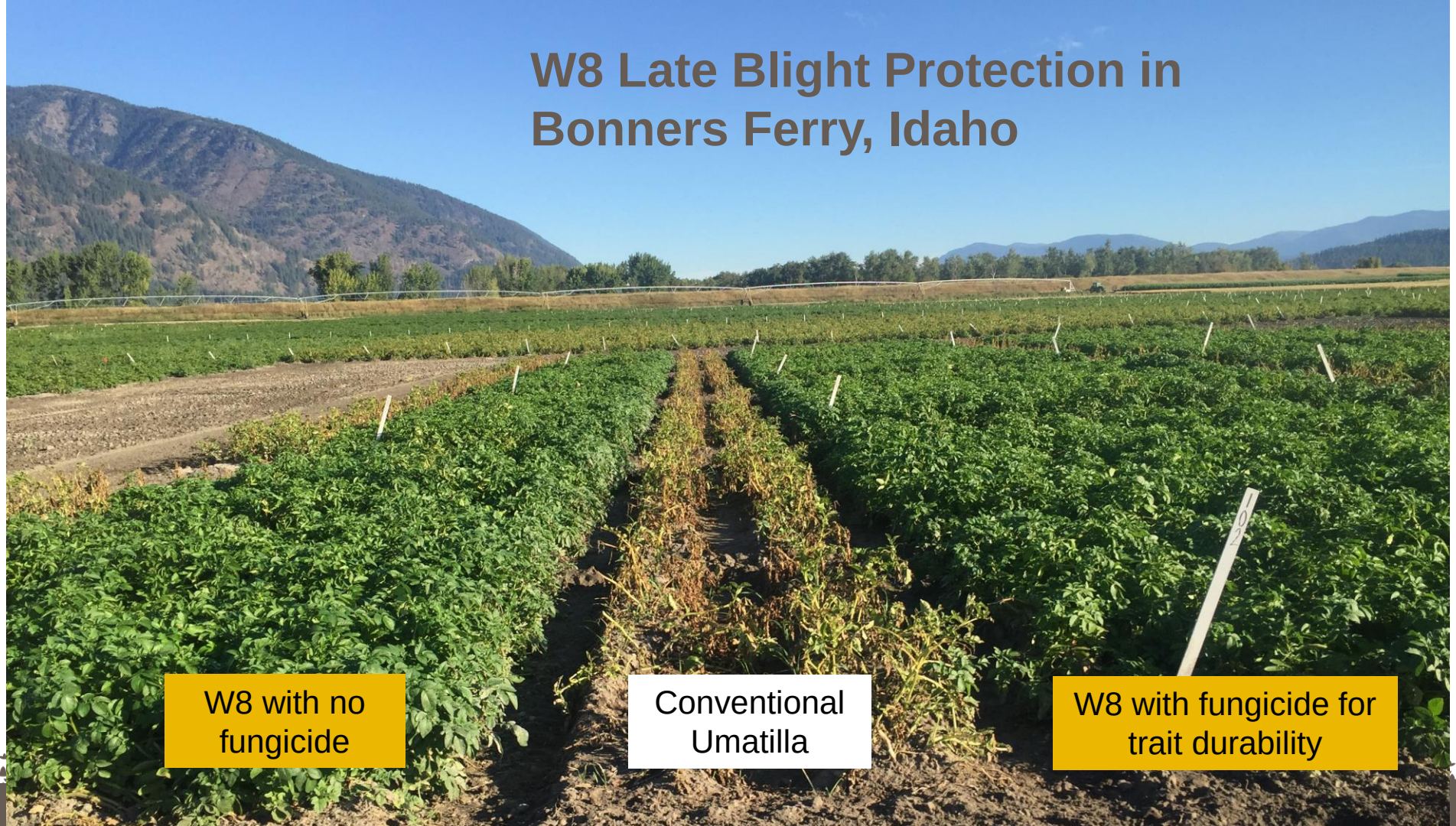
- Late blight protection
- Further lowered reducing sugars

INNATE By Simplot

W8 Potato Genes and Traits

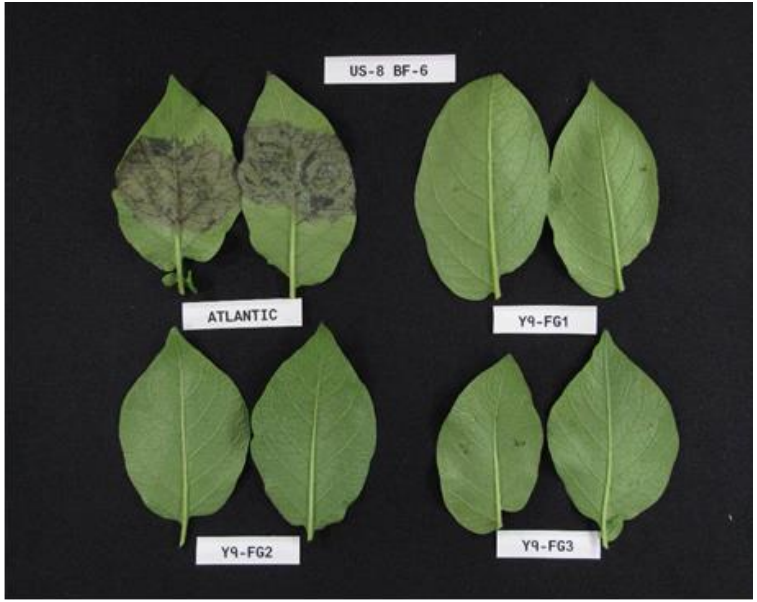
Construct	Gene	Method	Intended Trait
pSIM1278	Asparagine synthetase	RNAi	Lower free asparagine
	Polyphenol oxidase		Reduced black spot
	R1 water dikinase		Lower reducing sugars
	Phosphorylase-L		
pSIM1678	Vacuolar invertase		
	Rpi-vnt1 R-gene	Protein	Late blight protection

W8 Late Blight Protection in Bonners Ferry, Idaho



VNT1 Protein Protects Against Pathogen

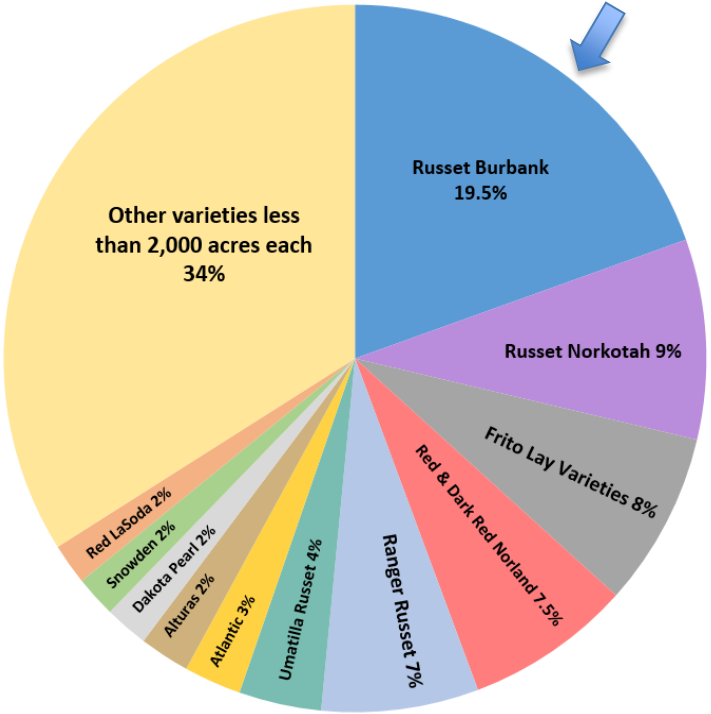
- VNT1 recognizes pathogen effector protein Avr-vnt1
- Immune response results in plant cell death
  - Hypersensitive response affects plant, not pathogen
- VNT1 at extremely low levels
- EPA has regulated as a Plant-Incorporated Protectant (PIP)



Unique Aspects of Potatoes

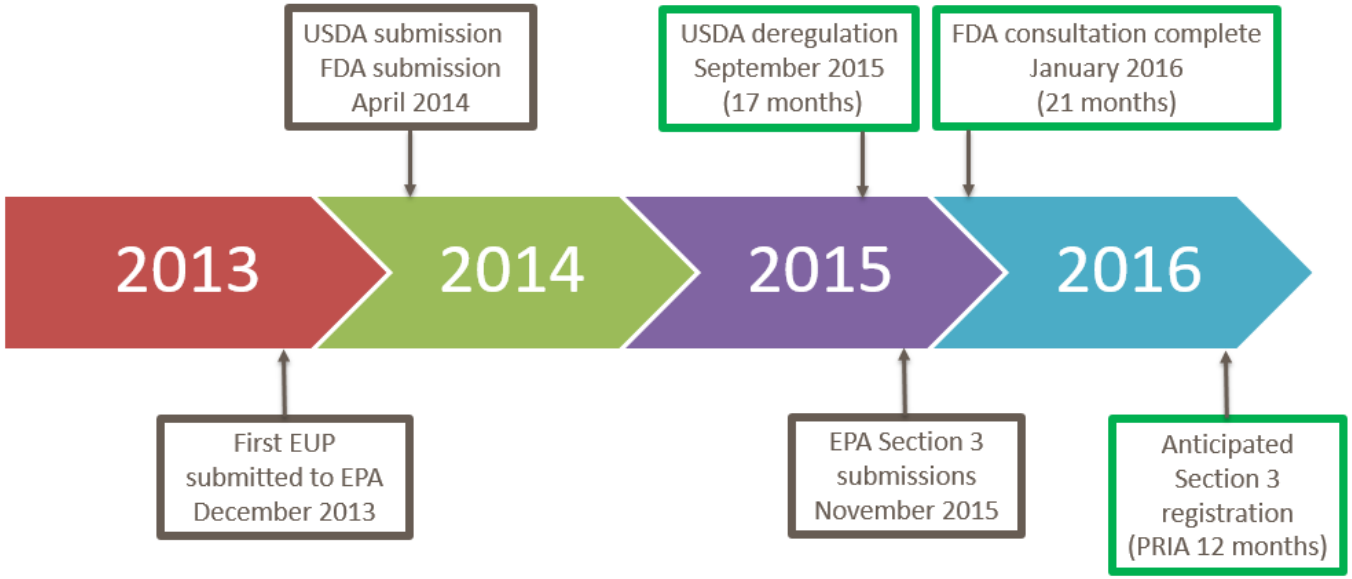
- "Seed" is whole or cut tubers
- Vegetatatively propagated
  - Unlike corn and soybean, no "generations" or breeding diagram
- Difficult to breed and backcross
  - Highly heterozygous and subject to inbreeding depression
  - Each variety must be transformed
  - Low rate of introduction of new varieties
- Tetraploid; complex genome
  - Not fully sequenced

Russet Burbank still #1 after 100 years



2014 seed production acres in U.S.

U.S. Regulatory Timeline for W8



- Submitted W8 with two additional events from same construct to EPA
  - Ranger Russet and Atlantic varieties
  - “Parent-child” submissions; saved ~$500,000 on PRIA fee

U.S. Regulatory Strategy for W8

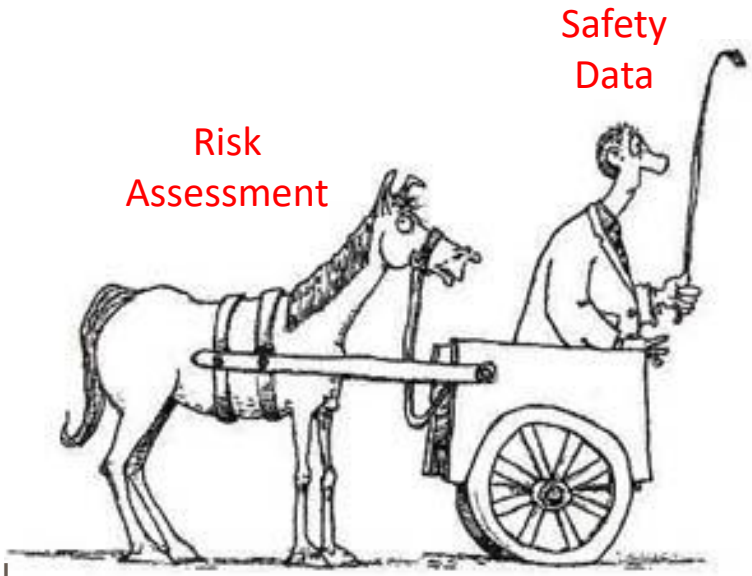
- Built off Gen1 success at USDA and FDA
  - Native *Solanum* genes; no marker or herbicide genes
  - Engage potato industry and key stakeholders
    - Effective communication strategy
- Used pre-submission consultations at all three agencies
- EPA has lead on VNT1 protein safety
  - Typical data package not feasible

Safety Rationale for W8

- Well characterized molecularly
- Stable during vegetative propagation
- No open reading frames with significant homology to known toxins or allergens
- Genetic elements from sexually compatible *Solanum* species
  - Down regulating endogenous potato genes
  - Small RNA is safe
- VNT1 is safe
- Nutritional composition same as conventional potatoes for key nutrients and toxins
  - Efficacy demonstrated for lower reducing sugars, lower free asparagine—not nutritionally important components of potatoes
- Agronomic and phenotypic properties same as conventional potatoes
- No weediness or gene flow concerns

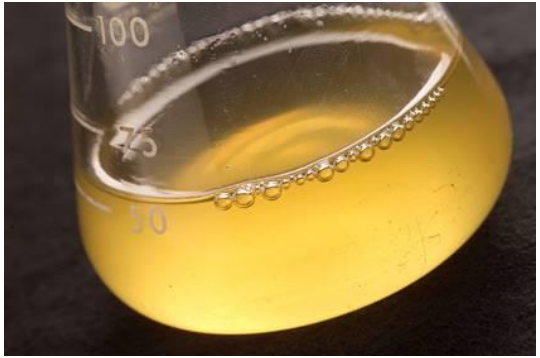
Focus on Risk Assessment

- Risk = hazard x exposure
- Let risk assessment drive safety data
  - Hazard: VNT1 and homologues have history of safe consumption → minimal hazard
  - Exposure: too low to measure → negligible exposure
- Look at others' submissions
  - Focus on what makes sense
  - Disregard what doesn't make sense
    - E.g., composition of potato vegetative material



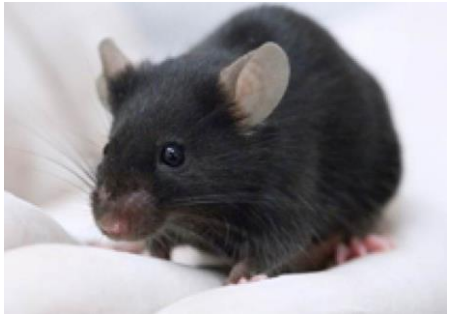
VNT1 Protein Production in Heterologous System

- Several approaches in two systems: *E. coli and N. benthamiana*
  - Able to produce both full-length and partial VNT1 in small quantities
- Purification attempts unsuccessful
- Lesson learned
  - Focus on <u>safety</u> of protein (minimal hazard and negligible exposure)



Typical Steps to Evaluate a Protein

- Using bioinformatics, assay protein for homology to known allergens and toxins
- Produce protein in heterologous system
- Establish equivalency between heterologous protein and plant-produced protein
- Using heterologous protein
  - Assess protein lability in pepsin and pancreatin (SGF and SIF)
  - Conduct mouse acute oral study with protein
- Quantify expressed protein in various tissues at multiple timepoints



VNT1 Protein Detection

- VNT1 cassette contains native promoter and terminator
- RT-qPCR used to verify gene expression

- Multiple approaches for antibody production against VNT1
  - Most sensitive polyclonal antibody used for western blot studies
    - LOD: 9 pg
    - LOQ: 30 ppb in tubers; 60 ppb in leaves
  - High cross reactivity with numerous VNT1 homologues
- VNT1 in W8 leaves and tubers too low to quantify

VNT1 expression ~1000X lower than Bt proteins!

VNT1 Protein Safety Weight of Evidence

1. VNT1 gene source has history of safe use
   - Same gene found in South American potatoes
2. R-proteins, such as VNT1, are ubiquitous in plants
   - One of the largest gene families known in plants
     - Apple: 900 genes
     - Grape: 459 genes
     - Potato: 435 genes
     - Rice: 400 genes
     - Soybean: 319 genes
3. VNT1 activates endogenous immune response pathway
   - Hypersensitive response is existing mechanism common in plants
   - Not a toxic mode of action to the pest
4. VNT1 lacks significant homology to known allergens and toxins

VNT1 Protein Safety Weight of Evidence

5. VNT1 has identity to proteins with history of safe consumption
   - Potato: 77-100%
   - Tomato: 75-77%
   - Pepper: 74%

6. Low dietary exposure to VNT1
   - R-proteins low expressing; 18 ppt in leaves (Bushey et al., 2014)
     - VNT1 <100 ppb in W8 tubers
   - Dietary assessment of VNT1 consumption
     - 95% percentile potato consumers
     - Highest consumption group (children ages 1-2)
     - 100% W8 potatoes
     - VNT1 compared to average daily protein intake

Negligible VNT1 exposure

0.000714 mg/kg bw/d

0.00021% of daily protein consumption

Lessons Learned Along the Way

Lessons Learned Along the Way

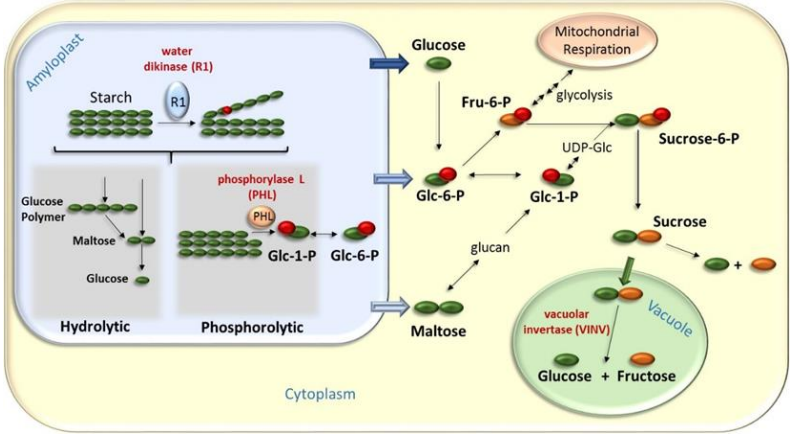
1. Customize the submission for each agency

	USDA	FDA	EPA
Molecular: structure, backbone, stability	Yes	Yes	Yes
Allergen and toxin homology of novel proteins	Yes	Yes	Yes
Open reading frame analysis	No	Yes	No
Agronomic field data; responses to biotic stress	Yes	No	No
Nutritional composition data	Yes	Yes	No
Detection method	No	No	Yes
Efficacy information	Ask	Ask	Yes for PIP

Lessons Learned Along the Way

2. Weave the safety data into a simple, compelling story

3. Focus on readability
   - Simple tables and graphics
   - Bullet points, white space



4. Regulatory process not easy for first timers
   - USDA petitions online; transparent process
   - FDA Notes to File can be helpful
   - EPA process most opaque (PRIA, forms, data requirements, processes)

5. Don’t be afraid to propose a regulatory package that looks different from standard

Lessons Learned Along the Way

6. Regulators are there to help—consult with them formally and informally

7. Check in on the review process regularly
   – Ask for help understanding the process and next steps

8. Inform industry stakeholders of commercial intentions and timelines
   – Can be invaluable advocates

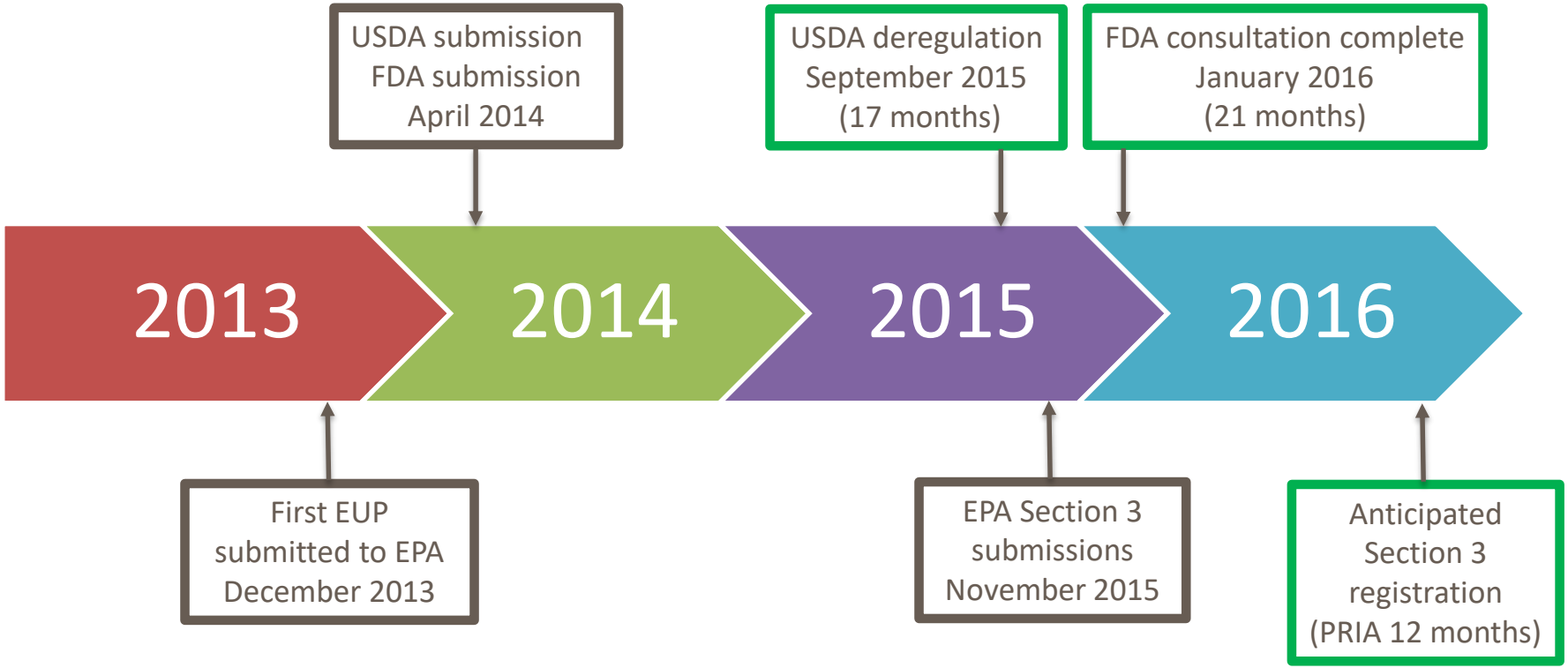
9. Ask regulators for feedback on submissions; say thank you

EPA Learnings

1. Plan for EUP long before anticipating going over 10 acres
   - PRIA timeline is ~7-11 months
2. First new trait and mode of action set the path
   - First late blight trait
   - First R-protein
   - Consider stewardship carefully
3. Think about detection method and Product Use Guide early
4. Consider IPM and trait durability with commercial business
   - Investigation of unexpected damage in the field
   - Process to confirm pathogen has developed resistance

Questions and Discussion

Appendix Slides


USDA submission
FDA submission
April 2014
USDA deregulation
September 2015
(17 months)
FDA consultation complete
January 2016
(21 months)
2013
2014
2015
2016
First EUP
submitted to EPA
December 2013
EPA Section 3
submissions
November 2015
Anticipated
Section 3
registration
(PRIA 12 months)