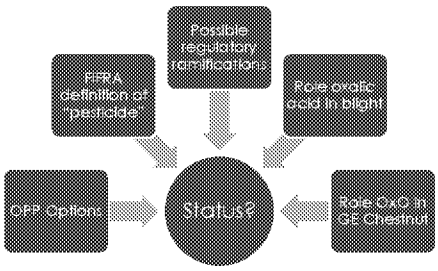
OxO Chestnut: A Pesticide Subject to FIFRA Regulation?

Considerations and Implications of a Pesticide Determination

Agenda



- Context of decision
- History of request for a determination
- How the fungal pathogen causes the chestnut blight
- How EPA interprets the definition of pesticide
- Regulatory ramifications of a decision
- OPP preferred options
- Appendices of useful information

3

Determination Context: Biotechnology and US Legal Structure

- As part of the Coordinated Framework, EPA under its FIFRA authorities regulates pesticides developed though biotechnology processes
  - Used FFDCA 408 authorities for residues

See Appendix I for information on US biotechnology oversight

- Plant-incorporated protectants or "PIPs" are an important class of biotechnology pesticides
  - A PIP is defined as a pesticidal substance that is intended to be produced and used in a living plant, or in the produce thereof, and the genetic material necessary for the production of such a pesticidal substance
- Does the OxO chestnut contain a pesticide i.e., a PIP?

See Appendix II for information on American chestnut

History of Request for a Determination

02/14/2018: Dr. Bill Powell and the American Chestnut Foundation meet with OPP staff to describe the OxO Chestnut and request a determination on whether it contains a PIP and thus is a pesticide

05/25/2018: OPP determines that OxO Chestnut contains a PIP
→ "while the direct action of the OxO active ingredient is on the oxalate molecule, the net effect of the transgene's action is to mitigate or lessen the effect of the … fungal pathogen"

06/07/2018: Dr. Powell requests a reconsideration of OPP's decision

Communication Between EPA and Dr. Powell

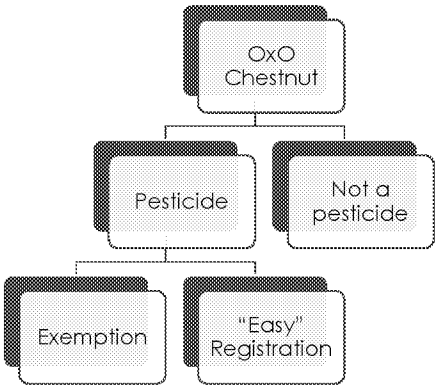
EPA's 05/25/2018 Letter

- We have discussed your request internally and determined that the OxO American Chestnut does meet the statutory definition for a pesticide under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). While the direct action of the OxO active ingredient is on the oxalate molecule, **the net effect of the transgene's action is to mitigate or lessen the effect of the *Cryphonectria parasitica* fungal pathogen and thereby reduce the impact of a pest**.
- As we have discussed previously, you may proceed with an application for a FIFRA Section 3 registration for the OxO transgenic chestnut. It may be possible to submit waiver rationale to fulfill some of the data requirements. It is also possible that you may request that the agency consider exempting this plant-incorporated protectant from FIFRA oversight by petitioning the Agency for such a regulatory action, to be accomplished through rule-making.

Dr. Powell's 06/07/2018 Response

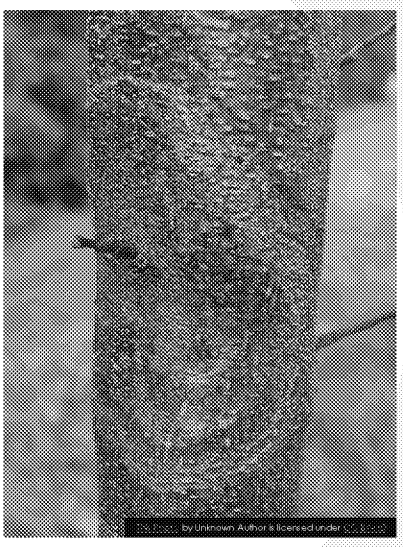
- We received your May 25 letter (copy enclosed) responding to our request and generally agree with the EPA's statement that, "[w]hile the direct action of the OxO …is on the oxalate molecule, **the net effect of the transgene's action is to mitigate or lessen *the effect of the Cryphonectria parasitica* fungal pathogen and thereby *reduce the impact* of a pest**" (emphasis added). However, we do not understand how your finding that the OxO reduces the physical effects on the tree arising from the presence of the fungus in turn leads to the conclusion that the OxO American chestnut should be regulated as a pesticide.

Decision Options



The Chestnut Blight

- Fungus *C. parasitica* causes a canker to develop on the tree
- Eventually the trunk or branch is "girdled" which kills the tree above the infection site
  - Fluids and nutrients cannot pass the girdling of the trunks or branches
  - All parts of the plant above the girdle die



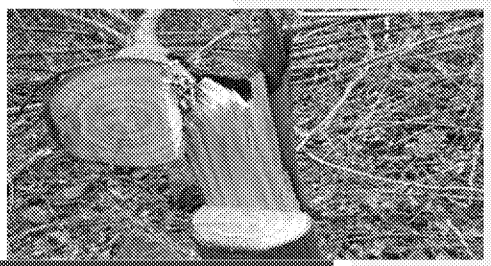
Role Oxalic Acid: How Does C. *parasitica* Cause Blight

Role oxalic acid in the blight

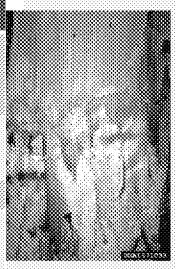
- *C. parasitica* produces a number of virulence factors necessary for successful pathogenesis on the American chestnut
- Oxalic acid production is a key virulence factor for *C. parasitica* as well as for numerous other fungal pathogens, including important agricultural pathogens such as *Botrytis cinerea* and *Sclerotinia sclerotiorum*

S. sclerotiorum (aka, stem rot, white mold)	B. cinerea (aka, gray mold)
Attacks many economically important crops, including legumes (soybean, pea, and bean), oilseeds (canola, sunflower), most vegetables, tobacco, many flowering bedding plants and stone fruits	Attacks over 200 dicotylenous plant species including vegetables (e.g., chickpeas, lettuce, broccoli, and beans), and small fruit crops including grape, strawberry and raspberry

Oxalic Acid and Chestnut Blight



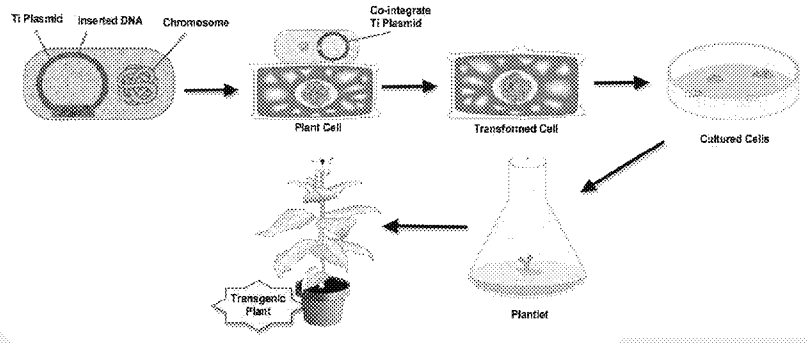
Oxalic Acid Action	Result
Oxalic acid is secreted at levels that are toxic to the cells of the tree but optimal for fungal enzymes pH in the infected area of the tree drops from about a near neutral 5.5 to a highly acidic 2.8	Kills tree cells; dead cells provide nutrients to the fungus
Inactivates tree defense systems	Tree unable to mount a defense against the fungus – e.g., cannot form a wound periderm to encapsulate fungus
Induces programmed cell death in tree tissue	Dead cells provide nutrients to fungus
Binds divalent cations (i.e., calcium) in the cell wall	Works to weaken cell walls, releasing nutrients, facilitating penetration by fungal hyphae allowing the fungus to spread rapidly on tree tissue



GE Engineering of American Chestnut

Role oxalate oxidase

- The gene "OxO", which encodes for the enzyme oxalate oxidase, was isolated from wheat and engineered into chestnut



Role Oxalate Oxidase (OxO) in Chestnut

- Oxalate oxidase breaks down oxalic acid to carbon dioxide and hydrogen peroxide (H_2O_2). **This**:
  - Keeps the pH at a chestnut-friendly 5.5 rather than a *C. parasitica*-friendly 2.8
    - Chestnut enzymes, including defensive enzymes, function optimally at 5.5, *C. parasitica* enzymes work better at 2 – 3
    - Protects chestnut tissue from lower pHs that result in cell death; dead cells provide nutrients for *C. parasitica*
  - Activates defensive genes in the chestnut
    - E.g., Production of lignan for reinforcing plant cell walls, hindering fungal spread
- Presence of oxalate oxidase results in smaller cankers that do not girdle the tree allowing nutrients to flow between the canopy and the roots
  - The fungus does not grow as rapidly in the tree, as there are fewer available nutrients
  - Tree able to form a protective periderm to encapsulate the fungus

Powell Arguments That OxO Not a Pesticide

Powell Arguments	OxO Action: Technical Counter Argument
OxO not intended to kill fungus, only minimize physical damage to the tree; OxO reduces acid allowing fungus and tree to "coexist"	•OxO tips the balance in the pathogen/tree interaction in favor of the tree, slowing fungal spread, allowing encapsulation • While OxO does not kill the fungus, it does adversely affect it
Not intended to destroy the habitat where the fungus might grow	•Tree = habitat and food source. OxO limits the food source of the fungus by preventing the acid from killing tree tissue which the fungus would then otherwise eat, and reduces fungus spread, giving tree time to wall off the blight fungus and prevent further fungal spread and disease development
Does not interfere with colonization or ability of the fungus to produce spores	•Fewer resources available to the fungus, slowing fungal growth reduces numbers of spores produced
In presence of OxO, tree remains a food source for the fungus	•Living tree circulates nutrients throughout the tree, fungus can grab some but availability much reduced in absence of oxalic acid induced tissue destruction

FIFRA Definition of "Pesticide"

FIFRA definition of "pesticide"

- Section 2(u) of FIFRA, 7 U.S.C. § 136(u), defines the term "pesticide," in relevant part, as "**any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest** . . ."
- That statutory language is echoed in the regulatory definition of "pesticide" at 40 CFR 152.3
- 40 CFR 152.15 elaborates, in part, that a substance is considered to be intended for a pesticidal purpose, and thus to be a pesticide if the person who distributes or sells the substance
  - Claims, states or implies (by labeling or otherwise) that the substance can or should be used as a pesticide, or
  - Even in the absence of a pesticidal claim, **the person who distributes or sells the substance has actual or constructive knowledge that the substance will be used, or is intended to be used, for a pesticidal purpose**

General Meaning of the Term "Mitigate"

- The term "mitigate" is not defined by FIFRA or its implementing regulations
- According to "Canons of Construction (adapted from Scalia and Garner)," *available at* http://www.law.uh.edu/faculty/adjunct/dstevenson/2018Spring/CANONS%20OF%20CONSTRUCTION.pdf: "Words are to be understood in their ordinary, everyday meanings," and "[g]eneral terms are to be given their general meaning (*generalia verba sunt generaliter intelligenda*)"
- The term "mitigate" is generally defined to mean "to make less severe or painful," and synonyms include "relieve, lighten, assuage, and allay," all of which mean "to make something less grievous." *See* https://www.merriam-webster.com/dictionary/mitigate. According to Merriam-Webster, "mitigate suggests a moderating or countering of **the effect of** something violent or painful." *Id.* (last visited Feb. 5,2019; Webster's Ninth New Collegiate Dictionary 761,995 (1984))(emphases added)

EPA's Stated Interpretation of the Term "Mitigate"

- EPA has issued guidance regarding the meaning of the term "mitigate," in the form of a "fact sheet" entitled "Determining If a Cleaning Product Is a Pesticide Under FIFRA." The fact sheet is available at https://www.epa.gov/pesticide-registration/determining-if-cleaning-product-pesticide-under-fifra, and is cited in both the "Label Review Manual" and the "Pesticide Labeling Questions and Answers"
- The fact sheet states that it "describes the Agency's **longstanding interpretation** of the statutory and regulatory language applicable to products … that claim, state or imply … that they mitigate a pest." The fact sheet states: "**A product need not act directly on a pest in order for it to mitigate the pest and be considered a pesticide** [see: 1995 Administrative Law Judge decision for Aquarium Products, Inc]. For example, **claims that a product affects the habitat or food source of a pest are considered to be 'mitigation' claims** against that pest." (Emphases added)

Ex. 5 Deliberative Process (DP)

Dr. Powell's Description of OxO Chestnut Purpose Meets 40 CFR 152.15

- 40 CFR 152.15 elaborates, in part, that a substance is considered to be intended for a pesticidal purpose, and thus to be a pesticide if the person who distributes or sells the substance
  - Claims, states or implies (by labeling or otherwise) that the substance can or should be used as a pesticide, or
  - Even in the absence of a pesticidal claim, the **person who distributes or sells the substance has actual or constructive knowledge that the substance will be used, or is intended to be used, for a pesticidal purpose**
- "American chestnut (*Castanea dentata*) was transformed with a wheat oxalate oxidase (oxo) gene in an effort to degrade the oxalic acid (OA) secreted by the fungus *Cryphonectria parasitica*, **thus decreasing its virulence."**
- "By degrading the OA secreted by C. parasitica, **the speed of mycelial fan progression could be reduced**, thus providing the transgenic trees more time to form a complete wound periderm **to wall off the blight fungus and prevent further disease development."**
  - Bo Zhang, Allison D. Oakes, Andrew E. Hexhouse, Kathleen M. Bates, Charles A. Maynard, and William A. Powell A threshold level of oxalate oxidase transgene expression reduces *Cryphonectria parasitica*-induced necrosis in a transgenic American chestnut (*Castanea dentata*) leaf bioassay. Transgenic Res 2013; (22)5: 973-982

Regulatory Ramifications of Non-Pesticidal Determination

- Deployment possibly hindered by legal challenges from chestnut enthusiasts, and others, who have concerns about OxO Chestnut project
  - In GMO sphere, challengers sue Coordinated Framework agencies on interpretations of statutory language and agency application of procedures
  - The American chestnut was a culturally important tree and food source for many Native Americans, and some are wary of genetically altering such a species
- EPA review could strengthen government position should there be a legal challenge
- Potential impact on OPP programs: Challenges possible from registrants of currently registered pesticides or future products; e.g., to:
  - PIPs
  - Anti-microbials
  - Chemicals/Biochemicals
- Could potentially be broad as we know of no pesticide that kills all members of the target pest population

Examples: Currently Registered Pesticides

Anti-Microbial Pesticides	Chemical Pesticides
Bacteriostats or fungistats inhibit growth/reproduction but do not necessarily kill the bacteria or fungi Mildewstats and products that make claims for mildew prevention also fall in the category of inhibiting growth but not killing Products with pesticidal removal claims also fall in the category of mitigating but not killing "Cleans away, washes away or removes biofilm or scum" "Cleans or remove the habitat where biofilm, germs, allergens or microorganisms can hide, thrive or grow"	"Systemic acquired resistance" chemicals are registered anti-fungals that do not directly affect the fungal pathogen itself but rather elicit a response in the plant by mimicking plant chemical signals that activate plant defenses such as production of thicker cell walls and anti-fungal proteins E.g., Acibenzolar – enhances the plants' ability to defend against/resist the pest E.g., Aliette IGRs E.g., methoprene, pyraxifen Feeding inhibitors Unknown modes of action

Example: VNT Potato Registered 2017

Consideration	OxO Chestnut	VNT Potato
Engineered into plant	yes	yes
Active ingredient	Oxalic oxidase, a plant defense protein from wheat	R factor vnt, a plant defense protein from a potato wild relative
Mode of action	Breaks down oxalic acid producing H_2O_2	Vnt reacts to substances produced by the fungus and elicits plant defenses including H_2O_2 production
Result of modification	By breaking down oxalic acid prevents fungus from lowering pH, turning off plant defenses, etc., slowing/preventing fungal growth	Ability of fungus to spread into plant tissues reduced, slowing/preventing fungal growth
Current status	?	Registered

OPP Preferred Options

If It is a Pesticide

Exemption	"Easy" Registration
●Exempt this specific product (OxO - Chestnut) from FIFRA requirements, including from FIFRA section 5 and 3 requirements, compliance requirements (section 7, 8 and labeling), and maintenance fees ●States have not in the past placed regulatory requirements on EPA exempted PIPs	BPPD has already registered a tree product, HoneySweet Plum, and addressed in the registration issues of "open" distribution and fees, similar to OxO Chestnut: ●Long-lived tree, not an annual ●Developer not a commercial entity ●"Open" distribution desired by developer

Status OxO Chestnut in Federal Coordinated Framework

See Appendix I for information on US biotechnology oversight

EPA

- EPA would under FIFRA section 3 establish a registration process to speed-up/simplify registration
- Estimated time before a decision is reached – 4-6 months versus 12 month average time
- EPA could exempt the OxO chestnut from FIFRA and FFDCA 408 requirements
- Estimated time before an exemption could be published in final – 1 ½ years

USDA Determination

- OxO Chestnut is subject to USDA Plant Pest Act regulations
- Estimated time before a decision is reached – 2 years

FDA Food Safety Assurance

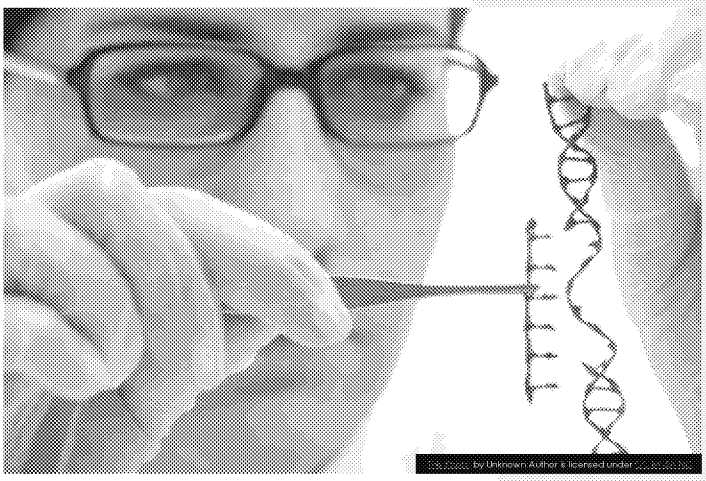
- OxO Chestnut is subject to FDA voluntary review process to ensure that food/feed from the chestnut is as safe as food/feed already on the market
- Special provisions may apply as the OxO gene was isolated from a known allergenic source - wheat

Appendices

- I. Biotechnology and the Federal Oversight System
- II. American Chestnut

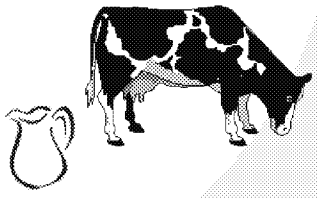
Appendix I

- Biotechnology and the Federal Oversight System



What is Biotechnology?

- Any technique that uses living organisms (or parts of organisms) to make or modify products, to improve plants or animals, or to develop microorganisms for specific uses

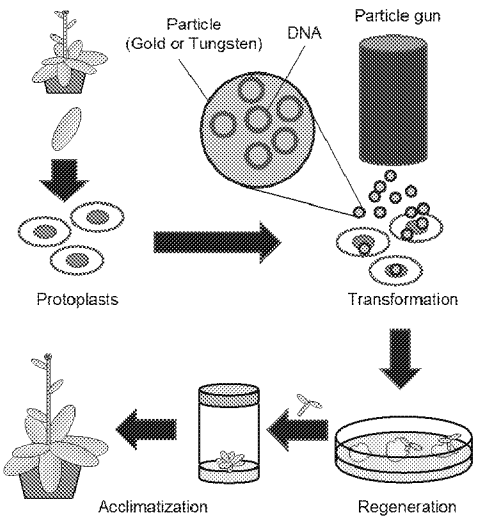


What is the Science Underlying Modern Biotechnology?

- All organisms have the information needed to run their life processes
  - E.g., respiration, digestion, reproduction, development, repair, defense
- This information is encoded in 4 bases, called nucleotides, that can be thought of as an alphabet. The sequence in which the 4 bases are strung together encodes information much like sentences do, and the whole of the information possessed by the organism can be thought of as a book
- Because all organisms on Earth encode genetic information in the same way, information from one organism can be moved into another organism
  - E.g., the information for producing the insecticidal protein "Bt" can be moved from the bacterium, *Bacillus thuringiensis*, into the genome of a plant such as cotton, where the protein continues its insecticidal function

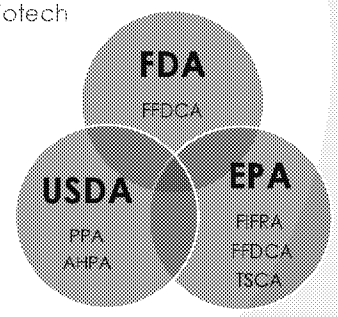
Creating a Biotech Product: Gene Gun Method

- Several methods can be used to move genetic information from one organism to another
- Pictured here is a "ballistic" technique in which genetic material, "DNA," is isolated from an organism, e.g., Bt, and coated onto a particle
- Those particles are then blasted into the individual cells that were prepared from a living plant as "protoplasts"
- The "blasted" protoplasts are then "regenerated" into a whole plant that now contains new genetic information, e.g., Bt



US Regulation of Biotechnology Products

- 3 Federal agencies regulate biotechnology products under the Coordinated Framework for Regulation of Biotechnology (CF)
- Based on laws written prior to GE biotech
  - Product specific
- Form jurisdictional network
- Evolution in regulation anticipated
  - Agencies adapt to novel products

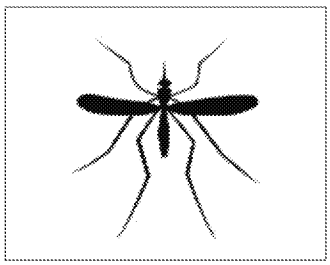


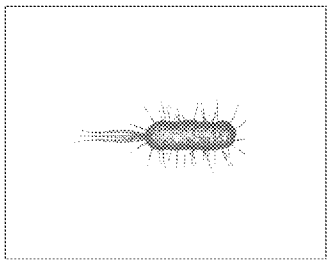
Roles in the Coordinated Framework

- FDA determines whether foods/feed grown from crops modified by modern biotechnology are as safe as their conventional counterparts
- USDA is responsible for protecting agriculture from pests and disease
- EPA's OPP regulates the use of pesticides
  - FIFRA creates framework under which EPA, through a registration process, regulates the development, sale, distribution, use of pesticides regardless of how made or mode of action
    - Pesticide: Any substance intended for preventing, destroying, repelling or mitigating any pest; or plant regulator; or nitrogen stabilizer
  - Under FFDCA 408, EPA is responsible for determining the amount of pesticide residue that may be present in food or feed to protect against dietary risk
- EPA's OPPT regulates "new chemical substances" not regulated as foods, drugs, cosmetics, pesticides, etc.
  - Current biotech regulations address intergeneric microorganisms

Types of Modern Biotechnology Products Regulated by OPP

- Pesticidal substance and genetic material necessary to produce it engineered into mosquito for population control
- PIPs – pesticidal substance and genetic material necessary to produce it engineered into plant
- Genetically engineered microorganisms





OPP's Regulation of Pesticides Engineered into, Produced and Used in Plants

- For plants, EPA considers a pesticide the pesticidal substance introduced into the plant and the genetic material necessary to produce the pesticidal effect
- The Agency refers to this mixture as a "plant-incorporated protectant" or a "PIP"
  - Under 40 CFR 174, a PIP is a "pesticidal substance that is intended to be produced and used **in a living plant**, or in the produce thereof, and genetic material necessary for production of such a pesticidal substance..."
- A PIP also includes any inert ingredient contained in the plant or produce thereof
  - Any substance intended to confirm or ensure the presence of the active ingredient
    - For crop plants inert ingredients tend to be substances that can be used for "selecting" a plant line that contains the active ingredient, e.g., a substance conferring herbicide or antibiotic tolerance
- Definitions can be found at Title 40 of the US Code of Federal Regulations at part 174.3
  - Regulations are at 40 CFR 174

Examples of PIPs: Actual and Hypothetical Products (Registered Product Starred)

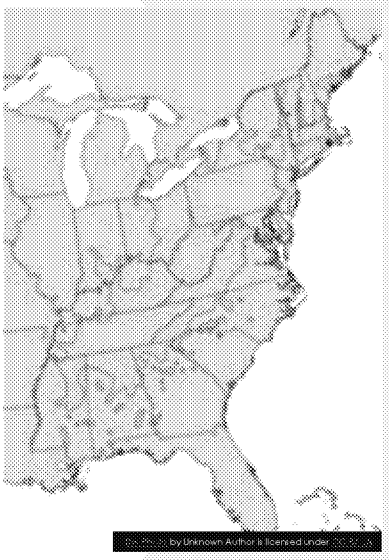
Function	Type of Product That Could Be a PIP
Insecticide*	Plant-produced protein that is toxic to insects eating a plant part, e.g., to a caterpillar
Insecticide*	Plant-produced double stranded ribonucleic acid (dsRNA) that is toxic to insects eating a plant part, e.g., to a beetle larva (RNAi product)
Fungicide*	Plant-produced substance that protects against fungi, e.g., R gene
Bacteriocide	Plant-produced substance defending against bacterial infection by inactivating a bacterial virulence factor, e.g., OCTase
Virucide*	Plant-produced genetic material that triggers the plant to respond to an infecting virus by chopping up the virus' genetic material, e.g., dsRNA (RNAi product)
Herbicide	Plant-produced substance that inhibits growth of other plants, e.g., juglone
Plant regulator	Plant-produced plant hormone, e.g., abscisic acid

Appendix II

- The American Chestnut

American Chestnut (Castenea dentata)

- Until a century ago, the American chestnut was the cornerstone tree species of eastern North America making up about a quarter of the trees in the eastern forests
- This chestnut tree, which can be thought of as the sequoia of the east, was one of the tallest trees in the forest, dominating a range of 800,000 kilometers from Mississippi to Maine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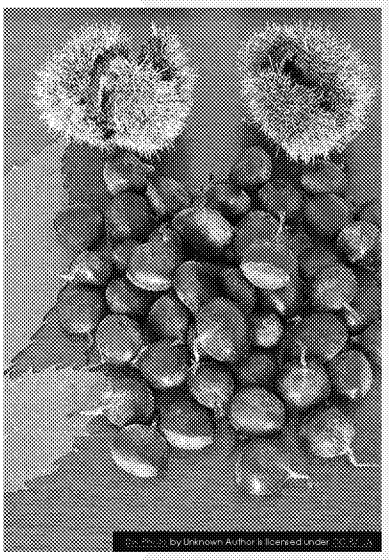
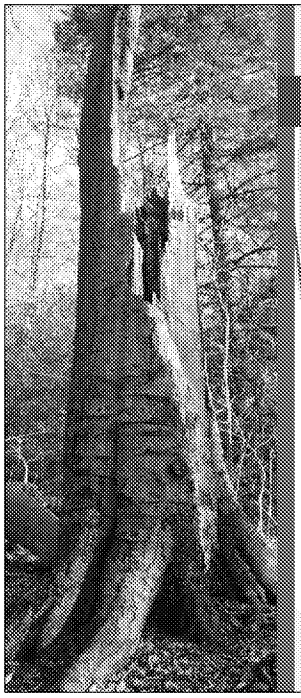
Economic Value

- American chestnut trees produced a stable annual crop of edible nuts consumed by numerous species
  - Several associated species went extinct when the American chestnut population crashed
- Chestnuts were an important source of food for humans – with the nation producing an annual crop of 20 million pounds prior to introduction of the chestnut blight – and were used to produce several foodstuffs including gluten-free flour and beer
- Few sources of timber were more valuable than the wood harvested from American chestnut; it was naturally rot resistant, straight-grained and easy to split
  - Pesticide treated wood appeared at the same time as American chestnut populations reached virtually non-existent levels





The Chestnut Blight

- *C. parasitica* introduced from Asia in the early 1900s laid waste, except for a few isolated stands, to nearly the entire population of an estimated 4 billion American chestnut trees
- The first signs were identified in 1904 in American chestnut trees at the Bronx Zoo in New York
  - infections were then detected almost simultaneously along the east coast
- The fungus probably hitched a ride on nursery or lumber imports from Asia
- Spreading through wind and rain, the spores infect trees through bark wounds and breaks. Cankers develop, quickly encircling a branch or trunk cutting off the supply of water and nutrients from the soil

Why Develop a GE American Chestnut

- *C. parasitica* can be found on a number of other species in the forests, so a tree that is able to tolerate or resist the fungus is needed for restoration efforts
  - Although the roots of many of the chestnut trees felled by *C. parasitica* are still alive under the soil, only a few of the shoots sent up by these roots live long enough to become sexually mature before being attacked by the fungus and dying
- Researchers have been trying since the 1930s to develop a *C. parasitica* resistant American chestnut through conventional breeding with Chinese chestnut which has some natural ability to resist *C. parasitica*
  - They have had some limited success
    - The phenotype of the Chinese chestnuts differ from the phenotype of the American chestnut – the Chinese chestnuts were bred for thousands of years as low-growing orchard trees; the American chestnut is a massive wild timber tree (could grow to more than a 100 feet high with a trunk diameter of more than 14 feet)

Restoration Goals: A 100 Year Project

- Develop a chestnut with the American phenotype but resistant to the chestnut blight
- Capture at least some of the genetic diversity of the gene pool represented by the root systems still viable beneath the soil
  - To restore a wide-ranging species such as the American chestnut, it is important to ensure access to the genetics still existing in the roots of trees adapted to the local environment within the range
- Restore this species back to its natural range. Spread a blight resistant American chestnut throughout the Eastern forests to return the American chestnut to the forests as a major canopy tree

Unique Considerations Presented by OxO Chestnut

- Developer/distributers not commercial interests
  - i.e., OxO Chestnut not being developed for profit, at least not initially, although at some point because of the usefulness of the wood and the chestnuts, orchards may arise in future
- Goal is free distribution of OxO Chestnut tree
  - Restore American chestnut back to its natural range
  - Capture at least some of the genetic diversity of the gene pool represented by chestnut root systems still viable beneath the soil
- Trees present a different paradigm than annual row or field crops
  - Long-lived (hundreds of years)
  - American chestnut a massive wild timber tree (could grow to 120 feet high with a trunk diameter of 30 feet)
  - Most PIPs registered to date are annuals, and while a PIP in a long lived tree could be removed from the food supply if a problem develops this would also present a different removal paradigm
  - Represents an attempt to return to an ecology not seen in the US for 100 years