The Forest Health Initiative's Policy Response Plan

Prepared by the Institute of Forest Biotechnology – Interim v0.9

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Introduction

The Forest Health Initiative

The Forest Health Initiative (FHI) is a three-year project exploring whether there is an appropriate and valuable role for biotechnology in protecting and restoring North America's increasingly threatened forest ecosystems. The FHI as a whole believes the best way to fully explore the many scientific, environmental, social, and regulatory challenges surrounding the use of biotechnology is to develop a test tree that responds to an existing forest health threat. This tree, a transgenic American chestnut modified with genes from a Chinese chestnut, is being developed and could be tested in confined field trials to explore if it could provide a safe and effective way to rapidly achieve blight resistance. The FHI is pursuing this test tree without prejudice - it may or may not culminate in the unrestricted release of a transgenic American chestnut. This document details the FHI's interactions with policy related stakeholders and U.S. regulatory agencies in particular.

FHI Policy Group

The Policy group of the FHI investigated how to best navigate the regulatory processes in place in the U.S. that govern the use of a biotech tree that is *by design intended to flower and propagate in the open forest*. In this potential scenario a genetically engineered American chestnut would be regulated by three U.S. agencies:

1. U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) regulates all genetically engineered organisms that could affect agriculture or the environment. *All biotech trees are regulated by APHIS.*
2. U.S. Environmental Protection Agency (EPA) regulates uses of Plant-Incorporated Protectants (PIPs) for safe use in the environment. *All biotech trees that are resistant to viruses are regulated by the EPA.*
3. U.S. Department of Health and Human Services' Food and Drug Administration (FDA) regulates all human food and animal feed additives. Genetically engineering a food product is considered to be a food additive to the FDA. *All biotech trees that produce human food or animal feed are regulated by the FDA.*

While this specific application of biotechnology is intended purely for environmental and societal benefit, it does not change the fact that these agencies are designed, and required, to respond to actionable items. In other words, agencies that regulate need some *thing* to regulate. However, there may be no actionable item (a biotech tree) for years. The Policy group's task was to work within these systems to gather information that may help speed regulatory determinations.

The Policy group collaborated with the entire FHI and the three aforementioned agencies to develop this response plan for using advanced biotechnology as a tool for forest health.

Rapid Response Plan Summary

In very general terms the FHI's Policy Response Plan is to:

- Engage regulators on all aspects up to five years before a product is available
- Engage stakeholders about the forest threat and the potential of using a biotech tree
- Document intellectual property rights, the tree's biology, and the overall regulatory process
- Evaluate the environmental impact of the biotech tree

These components are discussed in more detail in the following three sections of the document. The first section is a generalized policy response plan to help guide the process of using biotechnology as a tool to combat forest health problems. The second section is a summary of how the generalized plan was put into action for the Forest Health Initiative's (FHI) first test tree, a biotech American chestnut. The last section is an appendix containing reports and useful reference material.

FHI Policy Response Plans

General Policy Response Plan

The following 8 components are integral to a policy plan that quickly responds to forest health threats with biotechnology tools. The left column shows the relative order of components grouped into phases, and when it is appropriate to start work. Relative timing of phases is important, but there is overlap and many components can happen concurrently.

Phase 1

Start 5 years prior to possible deregulation and use

1. **Open lines of communication with policy stakeholders**
   Objective: Foster open discussions to guide biotechnology efforts
   Communicating with key policy stakeholders is a critical step because it will help guide all of the following steps that have regulatory implications. In general, the earlier an initial communication is made the better. However, planning for a product that is more than five years away will make the discussion less concrete to regulating agencies.

2. **Engage a wide spectrum of additional stakeholders**
   Objective: Increase understanding between participants and stakeholders
   Creating a positive, collaborative dialogue with many types of stakeholders is critical because healthy forests are a social good. Topics should include the environmental and economic impact of the forest health threat and the corrective options available.

Phase 2

Start 3-5 years prior to possible deregulation and use

3. **Review intellectual property**
   Objective: Understand which biotechnology options are available
   Intellectual Property (IP) holders are stakeholders with legal recourse based on patents they own. IP reviews, also referred to as patent landscapes, help researchers determine what direction research is moving in the field, can help identify competitors, and identify the geographic areas where research is occurring. More specifically, a detailed review of patents can be used as an examination of potential patent problems that may interfere with future research in an area and commercialization of discoveries. However, a patent landscape is not a legal opinion on freedom to operate. Costs vary, but initial, overview IP reviews cost about $15,000.

4. **Assemble a biological dossier**
   Objective: Provide stakeholders a single biological information resource
   A detailed document covering both the forest health threat and the tree species in danger helps regulating agencies, stakeholders, and research scientists communicate more effectively by referring to common information. This information is very useful in making regulatory decisions and environmental assessments. Costs vary, but an initial, overview biological dossier costs about $15,000.

5. **Review the regulatory landscape**
   Objective: Understand the framework that regulatory agencies work within
   A review of the current state of regulations and related legal actions that control or influence the use of biotech trees for forest health is necessary before a regulatory course can be planned. This step also helps guide scientific efforts and public interactions. In the U.S., there are three government agencies that can have jurisdiction over the development and deployment of biotech trees:
   - U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS)
   - U.S. Environmental Protection Agency (EPA)
   - U.S. Department of Health and Human Services' Food and Drug Administration (FDA)

   Regulatory landscape reviews vary in cost, but a basic review costs about $15,000.

Phase 3

Start 3-1 years prior to possible deregulation and use

6. **Query agencies to define a regulatory course**
   Objective: Gather specific information about the regulatory process
   In order to develop a concrete plan to use a biotech tree for forest health, routine interactions with regulatory agencies are necessary. Since regulators are mandated to respond to discrete requests, this step will involve a number of iterative questions and answers to and from the particular agency(s) involved.

7. **Prepare an environmental report**
   Objective: Assess the risks and benefits of using the biotech tree
   The U.S. National Environmental Policy Act (NEPA) applies to all three agencies in situations that could potentially have a large effect on the environment. It is likely that NEPA will play an increasingly large role in the regulation of biotech trees in the future. Preparing an environmental report is a major step in addressing NEPA issues. In addition, this document is a useful tool to further engage regulatory and public stakeholders on project details. Costs vary, but basic environmental reports start at about $100,000.

8. **Interact with agencies on future regulations**
   Objective: Increase the effectiveness of regulations that deal with biotech trees
   Regulations change slowly over time while forest health threats move quickly. By and large, environmentally focused stakeholders believe that helping agencies evolve their regulations is critically important to ensure that the risks of using advanced biotechnologies are reduced, while the benefits from these technologies are increased.

This generalized policy response plan was synthesized during the FHI's policy work, which is ongoing, and will likely evolve as the FHI's efforts progress. To date the process has been very iterative and is a result of many collaborators from academia, industry, government, and non-profit organizations.

FHI's Current Policy Response Plan

Following the general policy response plan, the FHI's current plan is in progress as of this writing. Below is a summary of the plan, actions being taken, and recommendations for the policy component of the FHI's test species, a biotech American chestnut.

1. Open lines of communication with policy stakeholders

The Policy group started discussions with the three regulating agencies, USDA, FDA, and EPA very early in the FHI's process. On March 30, 2009 a meeting was held that included a member of the FHI that represented each arm of the initiative:

- Carlton Owen – Steering Committee
- Adam Costanza – Policy and Regulatory Committee
- John Davis and Dana Nelson – Science Committee
- John Heissenbuttel – Social and Environmental Committee
- Susan McCord – Project management

This meeting was well attended by all three agencies. It was an important step to launch the FHI into the policy arena and get early feedback from the agencies. Over the next year there were two additional dedicated policy meetings with the regulatory agencies, and one meeting at the first FHI annual meeting. Each was interactive and informative. Critical guidance from these meetings included:

- All agency representatives suggested pre-registration meetings as early as possible if deregulation is a goal.
- Develop a comprehensive review of the biology of the tree.
- Large-scale plantings of this tree would have to be registered via EPA's Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Begin a FIFRA Experimental Use Permit (EUP) early since they take at least 8 months to review. Since FIFRA considers risk and benefit tradeoffs, the social benefits of the tree are important to highlight.
- Consider 'large scale permitting' prior to deregulating to begin gathering data. Select sites that can be used long-term, and that cover the entire range of the species.
- It is very important to address NEPA requirements. Begin NEPA work as soon as possible.

Next Steps: The Policy group recommends ongoing and routine meetings with each agency.

2. Engage a wide spectrum of additional stakeholders

The Social and Environmental group of the FHI is tasked with engaging a wide spectrum of stakeholders to "better understand concerns, inform the science and regulatory efforts, and create a more informed citizenry about forest threats and opportunities to overcome these threats." The Policy group of the FHI works closely with the Social and Environmental group to make sure information is shared in a very fluid manner. Together these groups have engaged more than 25 organizations *in addition* to the regulatory agencies. The current list is available online at: http://foresthealthinitiative.org/committees.html

Next Steps: The Policy group has begun preliminary discussions with a few government agencies that do not directly regulate biotech articles. The National Park Service, Bureau of Land Management, Fish and Wildlife Service, and the Bureau of Mining and Reclamation are a few of the agencies that will have a significant role in the potential use of a biotech American chestnut on public lands. This committee recommends continuing, and expanding, discussions with such agencies. In addition, organizations that are working on similar biotech tree health problems should be engaged such as the citrus growers and the USDA's Agricultural Research Service.

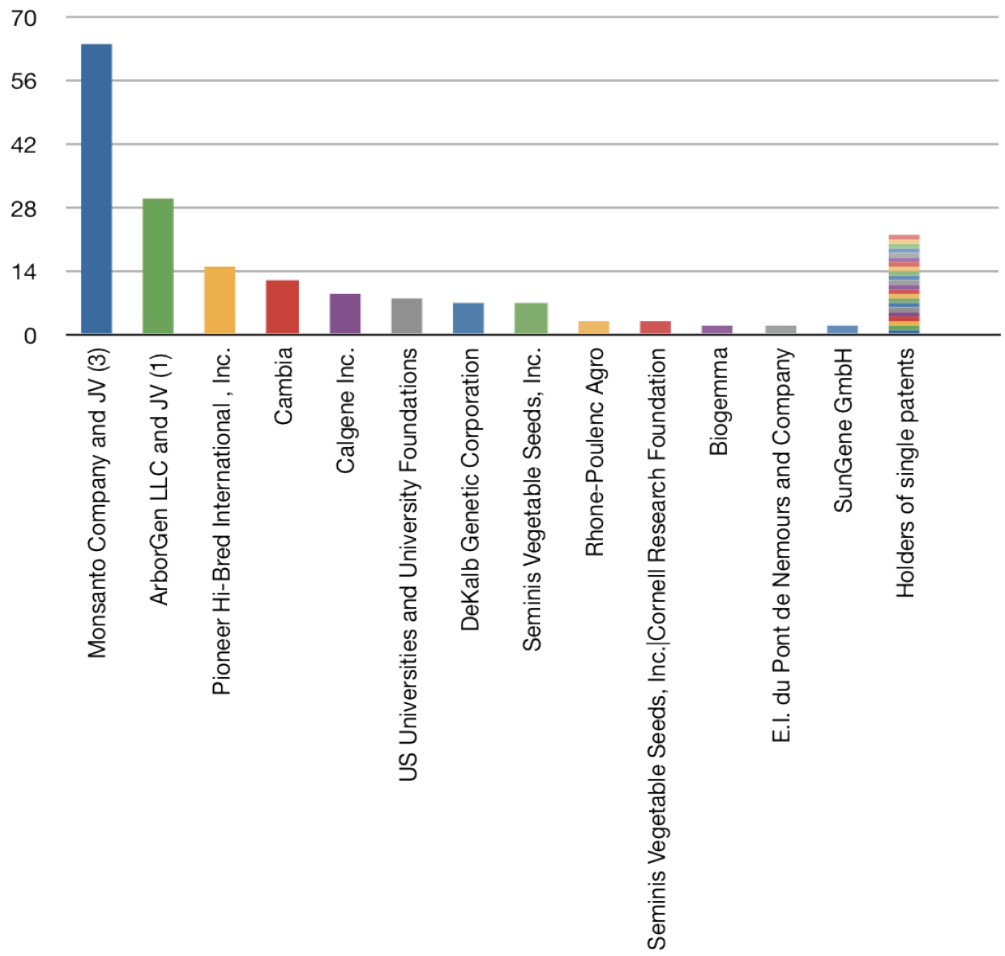
3. Review intellectual property

Ms. Lori Knowles of the Health Law Institute in Alberta, Canada was commissioned to perform an intellectual property review on the potential use of certain genetic constructs in the biotech American chestnut. The goal of this review is to allow the FHI science teams to gain a better understanding of the direction research is moving in the field, who is conducting that research, and where that research is being conducted. In addition, the IP study will identify parties who hold relevant intellectual property and identify opportunities for the creation of strategic relationships or negotiations with patent holders.

In summary, the results of this review show that:

Based on keyword searching of patent databases in the broadly-defined field of agrobacterium-moderated transformation of plants the dominant holder of patents are Monsanto Technology Inc. and Arborgen LLC. There is evidence of a trend towards consolidation of related patents held by these two companies, especially as they emerge as the dominant players over the past half decade. (See Chart 1) The shift from a more numerous and international portfolio of patent holders ten to twenty years ago to a more consolidated field may be, in part, a function of the number of different species originally subject to transformation work for trait selection, and the development in the field of new methods of agrobacterium transformation.

Chart 1 – Plant Agrobacterium Transformation Patent Holders

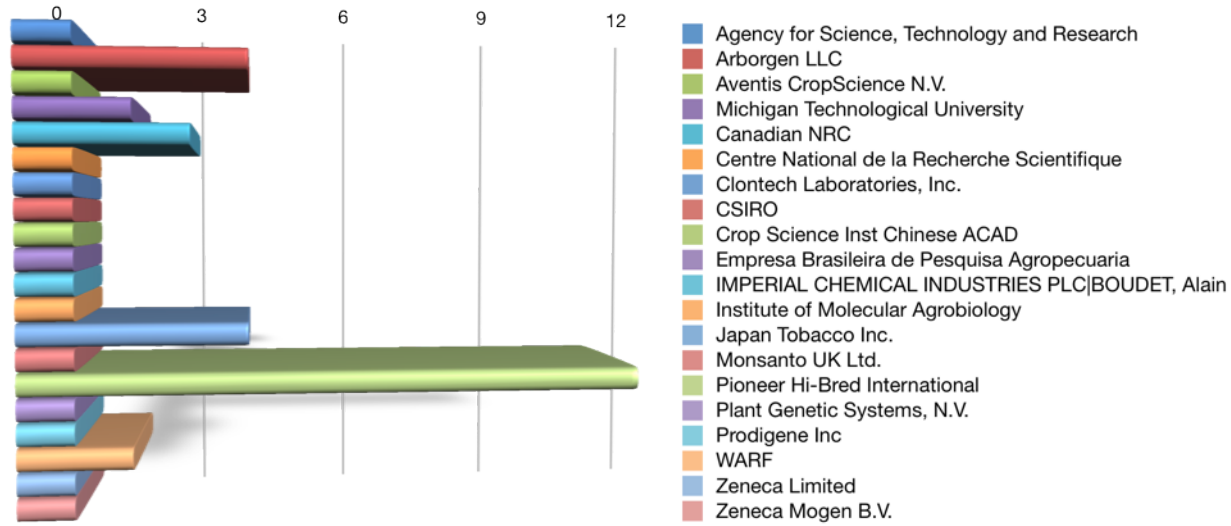


Over the last 5 years the number of patents issued (or that are published) has diminished. This may be evidence of a maturing field, and also the result of a shift from patents focused on

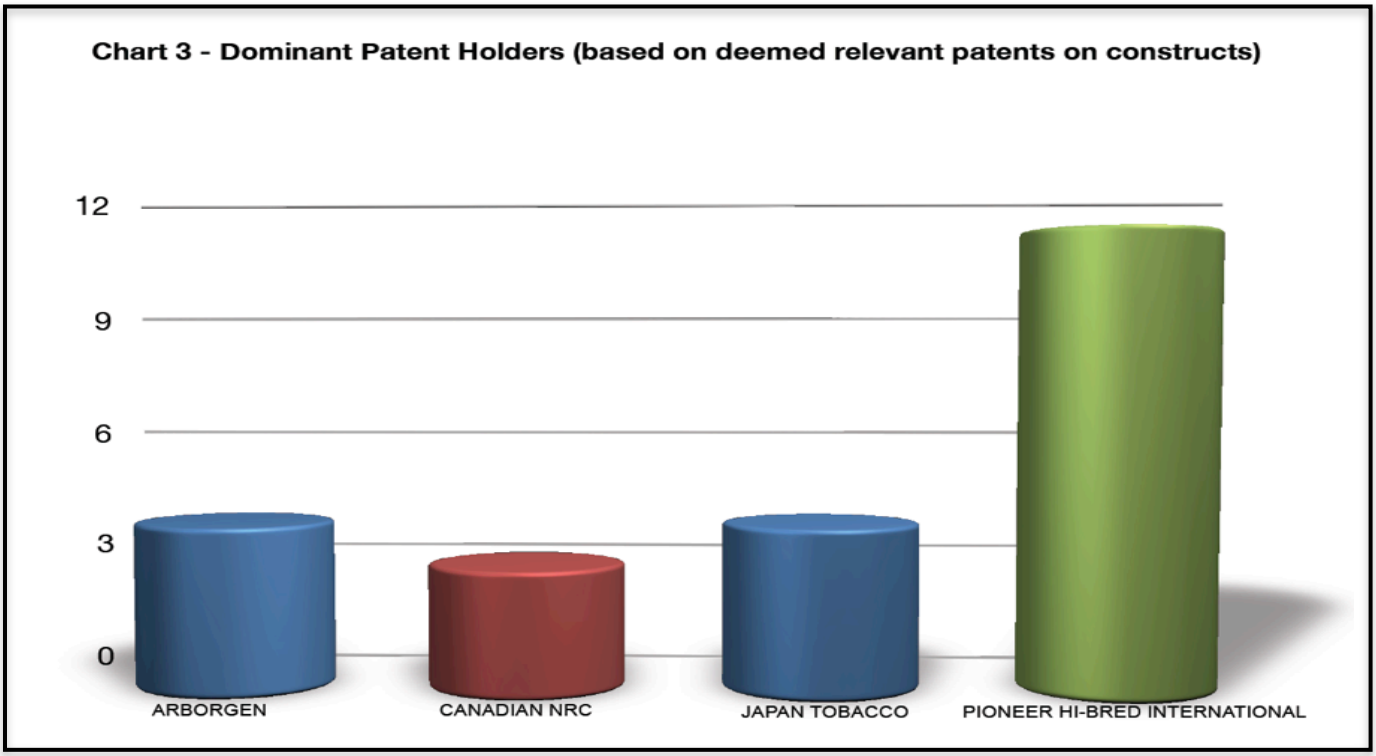
specific species of plants as subjects of patents, to a narrower focus on innovations and improvements of those methods of transformation. In addition, concern about the commercial viability of genetically modified crops given political or popular reticence to adopt these products may have had a chilling effect on research, although this has not been empirically validated in this study.

In response to information from members of the science team (Powell Nairn laboratories), searches of patents held on particular constructs yielded narrower, more focused view of the patents deemed relevant to the FHI research. Analysis by title and abstract of the approximately 380 patents narrowed results to 41 potentially relevant patents. Applicants and assignees of these patents are based primarily in Europe, Japan, Canada, and the US. (See Chart 2) Despite the international spread of relevant scientific research there appears to be little evidence of multinational or multi-center patent filings between laboratories, as evidenced by the inventor names on the patents. With a few exceptions, it appears that research in this area is largely conducted by isolated teams. In addition the results of this study indicate that few patents are held outright by US public research institutions or universities.

Chart 2 - Relevant Patent Holders of Constructs



Of the 41 potentially relevant patents there emerge 4 dominant patent holders for construct-based searches; Pioneer Hi-Bred International Inc; Arborgen LLC; Japan Tobacco Inc; and the Canadian National Research Council (see Chart 3). Of these 4 patent holders, the patents held by the Canadian National Research Council and Japan Tobacco Inc., are close to expiration. To date 20 of the 41 patents identified have been tagged as clearly relevant, while the others are possibly relevant or may be relevant in the future. Additional patent claims analysis is suggested to definitively determine relevance, but this research will require an update and input from the FHI science team.



Next Steps: In the past month the FHI science team has created a list of 42 candidate genes that are either part of or soon to be part of FHI transformational research. Science team leaders anticipate that a similar list of constructs will be forthcoming before the end of the year. Additional intellectual property review of these constructs is deemed necessary to determine where potential lies for future obstacles that require either workarounds or licenses, and where future collaborations may lie.

These constructs should be cross-referenced with the work outlined above, and searched in the USPTO database. If potential applications or future research may impact the Canadian market a search of the Canadian Patent Office is indicated. Interviews with the science team will further clarify the potential relevance of any patents identified through research, with emphasis on identifying research that is not being conducted to help eliminate patents from the final results. Examination of applicants, assignees and inventors can help identify potential allies, partners and mentors and lay the groundwork for building constructive relationships to further the goals of the FHI.

4. Assemble a biological dossier

Dr. Douglass F. Jacobs of Purdue University, and Dr. Dana Nelson of the U.S. Forest Service were commissioned to create a dossier that detailed biological information of the American chestnut. This information will be useful to regulating agencies that might be asked to make determinations on the risks and benefits of growing a biotech American chestnut in the open environment. Our discussions with regulatory agencies confirm that this dossier will be helpful, but may need to be expanded if additional characterization of the tree is necessary. The introduction to the preliminary biological dossier is below, and the complete document is included in the Appendix.

Synthesis of American chestnut (Castanea dentata) biological, ecological, and genetic attributes with application to forest restoration

Douglass F. Jacobs[1], Harmony J. Dalgleish[1], C. Dana Nelson[2]

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Introduction

American chestnut (*Castanea dentata* (Marsh.) Borkh. once dominated the much of the eastern deciduous forests of North America during the early 1900's (Russell, 1987), with a natural range exceeding 800,000 km^2 (Braun, 1950) (Figure 1).

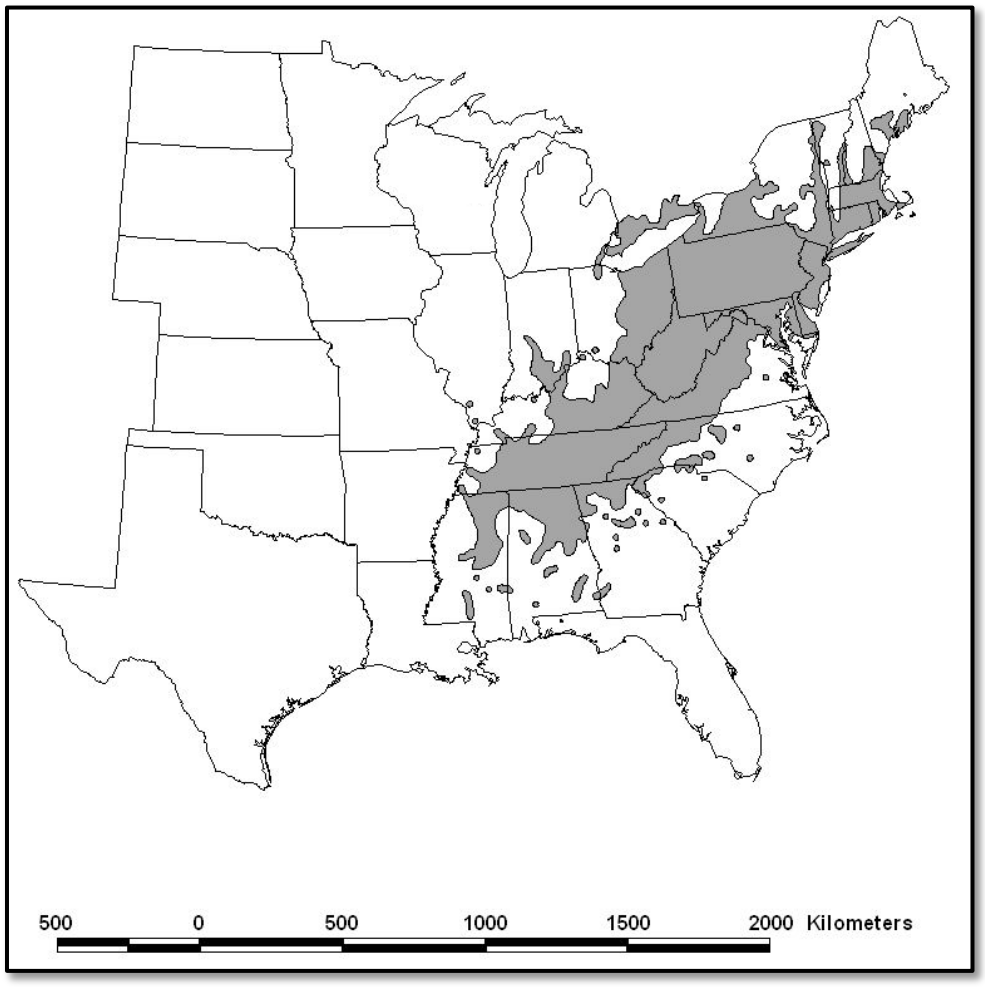

Figure 1 - Original natural range of *Castanea dentata* in eastern North America, as adapted from Little (1977).

Castanea dentata was a dominant tree throughout much of its range, comprising between 25-50% of the canopy (Braun, 1950; Foster et al., 2002; Russell, 1987; Stephenson, 1986). Particularly in the Appalachian region, *C. dentata* filled an important ecological niche (Ellison et

al., 2005; Youngs, 2000). The wood of *C. dentata* was characterized by a straight grain, which was strong and easy to saw or split and lacked the radial end grain found on many hardwoods. It was also extremely resistant to decay (Youngs, 2000), thereby serving for many specialty uses including telephone poles, posts, and railroad ties, as well as construction lumber, siding, and roofing (Smith, 2000; Youngs, 2000). Due to the high tannin content, both the wood and bark were used to produce tannin for leather production. The nuts, which are edible raw or roasted, were collected throughout the fall to provide a dietary supplement and were also used as a commodity for sale or trade on U.S. streets (Steer, 1948; Youngs, 2000).

Cryphonectria parasitica (Murr.) Barr., an aggressive diffuse canker disease (Anagnostakis, 1987), rapidly annihilated American chestnut throughout its range (Roane et al., 1986). The introduced pathogen was believed to have been imported on *Castanea* spp. seedlings from Asia and the disease was first discovered in 1904 on infected chestnut trees at the Bronx Zoological Park in New York City (Roane et al., 1986). By 1950, the disease had spread throughout the range of *C. dentata*, and by 1960 had killed an estimated 4 billion trees; essentially extirpating the species as a canopy tree (Anagnostakis, 1987; Hepting, 1974; McCormick and Platt, 1980). Since the discovery of chestnut blight, many groups have worked to develop blight-resistant *C. dentata* through several strategies including biocontrol of the fungus, breeding and selection of large surviving *C. dentata* trees, inter-species backcross breeding with resistant Asian chestnut species, and genetic modification. In the 1980's, a breeding program was initiated by a non-profit group, the American Chestnut Foundation (TACF), to restore *C. dentata* to its native range (Burnham, 1988). TACF has made steady progress toward developing a blight-resistant backcross chestnut tree that possesses the phenotypic characteristics of *C. dentata* (i.e., morphological, phenological) yet with blight resistance conferred through initial hybridizations with Chinese chestnut (*C. mollissima* Blume) (Diskin et al., 2006; Hebard, 2006). Simultaneously, scientists are working to impart disease resistance to *C. dentata* using advanced biotechnology (Merkle et al. 2007). Thus, a large-scale re-introduction program is imminent (Jacobs, 2007).

Scientific emphasis over the last 30 years has focused on breeding for blight resistance. Because *C. dentata* disappeared decades before the development of modern principles of forest ecology (Paillet, 2002), our knowledge of basic biological and ecological characteristics of the species is rudimentary (Jacobs, 2007; Paillet, 2002). Much of our understanding regarding establishment and growth of *C. dentata* originated from historical observations or growth of stump sprouts (Paillet, 1982; Paillet, 1984; 2002). Recently, here has been increased prioritization for research examining *C. dentata* establishment and growth in planted and natural forests (Jacobs, 2007). This progress, combined with continued advances in genetic technologies for production of blight-resistant *C. dentata* trees for reintroduction, indicates the need for an updated critical synthesis to aid in further developing protocols for disease resistance breeding and subsequent germplasm deployment. The purpose of this review paper is to synthesize the current state of knowledge regarding 1) *C. dentata* biology and natural history 2) the development of blight-resistant *C. dentata* trees and 3) the ecology of *C. dentata*. These knowledge areas as well as understanding of their considerable overlap will contribute to the formulation of a restoration plan for the ecologically and socially important *C. dentata* (Figure 2).

> *Next Steps:* The Policy group recommends ongoing interactions with the three regulatory agencies to determine if this dossier is sufficient for their needs, or additional information is necessary. Making the biological dossier available online is also recommended.

5. Review the regulatory landscape

There is one overarching and three specific regulations that apply to the widespread use of a biotech American chestnut. The U.S. Coordinated Framework for Regulation of Biotechnology[1] is the legal foundation by which each agency has the authority to regulate a biotech American chestnut.

- The National Environmental Policy Act (NEPA) is a complicated set of rules that have strong implications for most large environmental efforts. Our review shows that addressing NEPA will be required if a biotech tree is regulated by any of the above agencies and if the agencies will be petitioned to deregulate the tree (a.k.a. grant it a 'non-regulated status'). In other words, if a biotech American chestnut is developed for forest health, and the goal is to bring the species back to our forests by letting it grow and multiply naturally, then NEPA must be addressed. However, it is not an automatic certainty that NEPA has to be addressed if there is no plan to deregulate the tree.

- The U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) and its Biotechnology Regulatory Services (BRS) oversee safe cultivation and environmental introductions of all biotech trees[2]. Our review confirms that any American chestnut developed using any transgenic approaches would be regulated by APHIS in every instance. Recent lawsuits against APHIS reinforce this assertion.

- The Environmental Protection Agency (EPA), via the Federal Insecticide, Fungicide, and Rodenticide Act, oversees the safe use of pesticides and has jurisdiction over all biotech trees and products that have Plant-Incorporated Protectants (PIPs)[3]. Our review shows that the EPA would currently regulate a biotech American chestnut if all of the following criteria were met:
  1. The tree is regulated by APHIS
  2. The tree has PIPs
  3. The tree is used in the open environment in a planting area larger than 10 acres

  Following these criteria, the EPA would regulate the American chestnuts being researched by the FHI if they were widely used in the open environment at almost any forest-sized scale. However, there may be clarifications to these criteria if the biotech tree was developed using cisgenic approaches (DNA from trees that could naturally breed). Additional information about this recent possibility is in the Appendix.

- The U.S. Food and Drug Administration (FDA) regulates via the Food, Drug, and Cosmetic Act. The FDA oversees food safety, labeling, and has jurisdiction over biotech trees that produce edible food[4]. Our review shows that the FDA would regulate a biotech American chestnut if the following criteria were met:
  1. The tree would be regulated by APHIS
  2. The tree would produce food edible by humans or livestock

  Following this criteria, the American chestnuts being researched by the FHI would be regulated by the FDA if they were used in the open environment and produced nuts.

A recent review by Tom Redick of the Global Environmental Ethics Counsel explores the interactions between these regulations and the impact that current lawsuits may have on the future of these regulations. A coy of his paper, Navigating Existing US regulations on Forest Biotechnology Research, is in the Appendix.

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[1] United States Federal Register, June 26, 1986, 51 FR 23302. U.S. Regulatory Agencies Unified Biotechnology Website: http://usbiotechreg.nbii.gov

[2] http://www.aphis.usda.gov/biotechnology/brs_main.shtml

[3] http://www.epa.gov/pesticides/biopesticides/pips/index.htm

[4] http://www.fda.gov/food/biotechnology/default.htm

Next Steps: The Policy group recommends creating informational material that diagrams the overall regulatory framework that a biotech tree developed for forest health is subject to. This material could be used to educate decision makers on the need to either simplify the regulations such trees are subject to, or create another mechanism that allows more rapid use of biotechnology for forest health.

6. Agency Q&A to define a regulatory course

The Policy Committee of the FHI, is asking the following questions to the three US agencies with potential regulatory authority over biotech forest trees. These questions are asked in the context that:

1. These questions and answers are based on the FHI's initial test tree, a transgenic American chestnut – *Castanea dentata* – that has been genetically modified for resistance to the Chestnut Blight.

2. There may or may not be a petition in the future by the FHI or another entity to deregulate this test tree, or otherwise gain regulatory approval to plant the tree in an unconfined environment - allowing it to propagate unconstrained.

3. The FHI fully understands that it is impossible to outline a strict policy course of action for any potential use of biotechnology because regulations, public sentiment, and biotechnology science are constantly evolving.

4. This Response Plan, and the answers to these questions, relate to current situations only, and are meant to guide efforts, not dictate them.

Questions to all three agencies

1. Regarding FHI's current process. This document outlines the process the FHI has taken with each agency to date. The following questions are intended to provide additional process guidance to the FHI.
    a. For the General Policy Response Plan detailed in this document, what would you recommend changing or adding considering that the goal is to address the most important policy related components, timing, and predominant interactions in order to rapidly and effectively respond to forest health threats with biotechnology tools.
    b. For the FHI's Current Policy Response Plan, is the information provided by each step sufficient to meet its intended objective?

2. Regarding established regulatory guidelines.
    a. Is there a standard set of questions that your agency recommends we ask ourselves (FHI participants) that will help guide research necessary to deregulate a biotech forest tree.
        i. If there is a standard set of questions, can you please provide them?
        ii. If there is not a standard set of questions, what do you recommend the FHI do in order establish a standard set of questions to help guide the potential use of biotechnology as a forest health tool?
    b. What standard set of questions should we (FHI participants) ask your agency?
        i. If there is a standard set of questions, can you please provide them?
        ii. If there is not a standard set of questions, what do you recommend the FHI do in order establish a standard set of questions to help guide the potential use of biotechnology as a forest health tool?

Questions to USDA APHIS

1. With the understanding that responding to forest health threats rapidly is important, under current APHIS regulations, what field testing approach would allow the FHI to most quickly start gathering the scientific information necessary for APHIS to grant a petition for non-regulated status for the use of a biotech forest tree for forest health purposes?

Questions to EPA

1. If there is a gene that is in the natural tree population, and this gene expresses a trait that protects the plant from a disease, (such as in Chinese chestnut) and this gene is then inserted, via genetic engineering, into the tree's close, breeding relative (such as the American chestnut), would the genetically engineered tree be regulated under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)? For example if a gene that causes a transgenic trait of interest is in the genome of the tree or a close relative, and possibly has a modified expression profile; are toxicology, gene flow, or non-target effect studies required? If so, what specific kinds of data are needed, at what depth and time frame?

Questions to FDA

1. If the transgenic trait of interest is in the genome of the tree or a close relative, but the trait is not expressed in the nut, would the biotech tree be of concern to the FDA?

Next Steps: The Policy group recommends following up with each agency to get complete answers to these questions.

7. Prepare an environmental report

An environmental report for a biotech American chestnut will help identify some of the U.S. National Environmental Policy Act (NEPA) requirements and points of contention. The report would be similar to an Environmental Assessment (EA) in scope, as opposed to a costly and time-consuming Environmental Impact Statement (EIS). While an EIS is often the final review document for environmental risk and benefit assessments, it is not necessarily more useful than a thorough environmental report, or EA. Some points made by regulatory agencies that could be addressed in and environmental report include:

- Detail the social benefits that could come from this biotech tree.
- Review the allergen potential for genes of interest since the chestnut is edible.
- Review the potential toxicity of the tree pollen to bees.
- Detail any long-term testing of the trees and how it will be accomplished.

An environmental report begins the process of addressing NEPA requirements and it is a useful tool to further engage regulatory and public stakeholders on project details.

Next Steps: The Policy group recommends the FHI develops an environmental report if a biotech American chestnut is planned for widespread, deregulated, use in forests.

8. Agency interaction on future regulations

U.S. regulatory systems change over time. Whether the pace of change is fast enough is up for debate, but they do change nonetheless. A current example is the EPA's Proposal to adjust the data requirements for cisgenic biotech products:

"EPA has forwarded to the Secretaries of Agriculture and Health and Human Services a draft proposed rule that would codify data requirements that specifically address the registration data needs of plant-incorporated protectants (PIPs)… EPA will propose to exempt cisgenic PIPs from registration to encourage research and development of useful biotechnology and reduce the number of PIPs seeking registration..."[5]

The EPA's cisgenic proposal is not the only possible change to biotech regulations. The following synopsis from Tom Redick goes into detail on other regulatory adjustments underway.

USDA's Overhaul of Biotech Crop Regulations

May 2011 Synopsys by Tom Redick

In 2009, the U.S. Department of Agriculture (USDA) reopened its "Proposed Rule for Biotechnology Regulations" under 7 CFR §340.6[6] (over 6,000 comments were received). USDA has recently (April 2011) announced that it would finally issue its revised rules, possibly expanding its authority beyond the limited "plant pest" review of 7 CFR §340.6. Under this limited scope, the only "plant" that is a plant pest would be a "parasitic plant", so the USDA cannot deny or limit approval for biotech crops simply due to economic impacts to other crops.

The impending regulatory overhaul, however, could include "noxious weed" authority that covers "indirect" effects to the environment (a potentially broad regulatory review approximating the Environmental Impact Statement (EIS) done for Roundup Ready® Alfalfa). Proposals to broaden the scope of §340.6 to reflect the Plant Protection Act's broad delegation of authority were supported by the biotech industry and various other commenters[7] who suggested that the "noxious weed" provided USDA more oversight authority for various impacts of biotech crops.[8] At the same time, some commenters warned that regulatory oversight of economic impacts would exceed USDA's authority in a manner that could violate US obligations under international agreements.[9]

USDA's regulatory overhaul of §340.6 has to take into account the parallel court proceedings involving the National Environmental Policy Act (NEPA). In Geertson v Monsanto (the Roundup Ready® Alfalfa case), the US Supreme Court interpreted NEPA to require USDA to assess economic impacts of biotech crops. While rejecting a lower court's nationwide injunction as excessive, the US Supreme Court also suggested USDA had the ability to issue a "partial" deregulation (with various conditions on the location and manner of planting), USDA did an Environmental Impact Statement (EIS) for Roundup Ready® Alfalfa as required, issuing a nationwide approval (with no restrictions designed to prevent economic impacts to non-GMO, export and organic producers of alfalfa). USDA was sued again in California District court. Another court-ordered injunction could issue against USDA, sending it back to the regulatory drawing board. Monsanto's Roundup Ready® beets are also being litigated, and USDA chose to follow the US Supreme Court's suggested "partial" deregulation (with various conditions on the location and manner of planting).[10]

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[5] U.S. Docket Identification (ID) number EPA-HQ-OPP-2009-0499

[6] The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) is responsible for the Federal Plant Pest Act (7 U.S.C. 150aa–150jj) and the Plant Quarantine Act (7 U.S.C. 151–167), regarding "plant pests". APHIS regulations (7 CFR part 340) regulate releases of biotech crops into the environment. Under 7 CFR §340, a "Petition for determination of nonregulated status" allows a person to petition APHIS to evaluate a submission showing the crop does not present a plant pest risk.

[7] United States Department of Agriculture, Proposed Rule of Importation, Interstate Movement, and Release into the Environment of Certain Genetically Engineered Organisms, Docket ID: APHIS-2008-003; See also, USDA Reopens Public Comment Period on "Proposed Rule for Biotechnology Regulations, (January 15, 2009) available at http://www.aphis.usda.gov/newsroom/content/2009/01/biotecreg.shtml (last visited December 28, 2009)

[8] Comments of Michael Wach, Biotechnology Industry Organization to APHIS Docket No. 2008-0023 (November 24, 2008)

[9] Id. Citing World Trade Organization and International Plant Protection Convention standards on weed seeds and economic impacts.

[10] Center for Food Safety v. Vilsack, No. C 08-00484 JSW, 2009 WL 3047227 (N.D. Cal. Sept. 21, 2009) (APHIS required to prepare EIS for GMO sugar beets), also available at http://truefoodnow.files.wordpress.com/2009/09/9-21-09-order-re-cross-msjs1.pdf. (last visited December 28, 2009)

Next Steps: The Policy group recommends supporting the EPA's Recommendation to treat cicgenic events different from non-cisgenic transgene events. The group also recommends closely following the other potential changes in each agency and providing forest health related biotech tree information when it is appropriate.

Overall FHI Policy Recommendations

On the whole, there are some large-scale policy related items that should be addressed if a biotech tree is developed for forest health purposes and is intended to be used in the forest. This chain of events implies that the tree is genetically engineered, is granted unrestricted use by each regulatory agency, and that the tree will flower and reproduce in the wild. These events are not trivial from a regulatory standpoint. In order to use a biotech tree for forest health, the Policy Group recommends that the following steps be taken in addition to those previously noted:

- Develop a framework to use biotech American chestnut trees responsibly on public lands to prepare for the possibility that the FHI deems these biotech trees can be safely restored to our nation's forests.

- Begin long-term ecological field tests as soon as possible to begin gathering the data agencies require to make a determination that the trees are safe to use.

- Plan large-scale field tests that can be used as a surrogate for unrestricted forest use in the event that regulatory approval is not granted quickly.

- Begin discussions with stakeholders that could break logjams in agencies. Biotech trees are relatively new and those developed for forest health are unprecedented on the natural landscape. Current regulations are working to catch up with the social and environmental imperatives that the FHI represents. Since forest health threats are not waiting for regulations to evolve, creative solutions that speed the responsible use of biotech trees for forest health are needed. These stakeholders and unique solutions should be catalyzed early.

Summaries of recommendations from each policy rapid response component

1. Open lines of communication with policy stakeholders
   - Accomplished, but continue to meet with agencies on a routine basis.
2. Engage a wide spectrum of additional stakehodlers
   - Ongoing with the Social and Environmental group. Continue to collaborate with that group, expand discussions to additional government agencies and affiliated organizations.
3. Review intellectual property
   - Preliminary results available, discuss additional IP review needs.
4. Assemble a biological dossier
   - Preliminary dossier complete, recommend agency discussions to determine need to expand; put dossier online.
5. Regulatory landscape review
   - Review complete, recommend creating informational material about the regulatory process to inform stakeholders and advance regulatory processes.
6. Query agencies to define a regulatory course
   - Ongoing, recommend continuing the process.
7. Prepare and environmental report
   - Not started, recommend completing if a biotech chestnut is planned to be used for forest health in the open environment.
8. Agency interaction on future regulations
   - Review complete, interactions not started. Recommend supporting EPA's rule changes for cisgenics; support other rule changes that benefit forest health.

Appendix

Online Resources

Forest Health Initiative
Main Site: http://foresthealthinitiative.org

USDA APHIS Information
Main Site: http://www.aphis.usda.gov
Biotechnology Information: http://www.aphis.usda.gov/biotechnology/index.shtml
Biotechnology Regulatory Services: http://www.aphis.usda.gov/biotechnology/brs_main.shtml

EPA Information
Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA):
http://www.epa.gov/agriculture/lfra.html

FDA Information
Main Site: http://www.fda.gov/Food/Biotechnology/default.htm
Presentations from Mary Ditto detailing FDA's biotechnology role:
- www.bigmap.iastate.edu/symposium/ditto.pdf
- www.bigmap.iastate.edu/symposium/ditto2%20ppt.pdf

NEPA Information
Citizen's guide to NEPA: http://ceq.hss.doe.gov/publications/citizens_guide_to_nepa.html

Institute of Forest Biotechnology
Main site: http://forestbiotech.org
Responsible Use: Biotech Tree Principles: http://www.responsibleuse.org

Component #4 – Assemble a biological dossier

Synthesis of American chestnut (*Castanea dentata*) biological, ecological, and genetic attributes with application to forest restoration

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Introduction

American chestnut (*Castanea dentata* (Marsh.) Borkh. once dominated the much of the eastern deciduous forests of North America during the early 1900's (Russell, 1987), with a natural range exceeding 800,000 km^2 (Braun, 1950) (Figure 1). *Castanea dentata* was a dominant tree throughout much of its range, comprising between 25-50% of the canopy (Braun, 1950; Foster et al., 2002; Russell, 1987; Stephenson, 1986). Particularly in the Appalachian region, *C. dentata* filled an important ecological niche (Ellison et al., 2005; Youngs, 2000). The wood of *C. dentata* was characterized by a straight grain, which was strong and easy to saw or split and lacked the radial end grain found on many hardwoods. It was also extremely resistant to decay (Youngs, 2000), thereby serving for many specialty uses including telephone poles, posts, and railroad ties, as well as construction lumber, siding, and roofing (Smith, 2000; Youngs, 2000). Due to the high tannin content, both the wood and bark were used to produce tannin for leather production. The nuts, which are edible raw or roasted, were collected throughout the fall to provide a dietary supplement and were also used as a commodity for sale or trade on U.S. streets (Steer, 1948; Youngs, 2000).

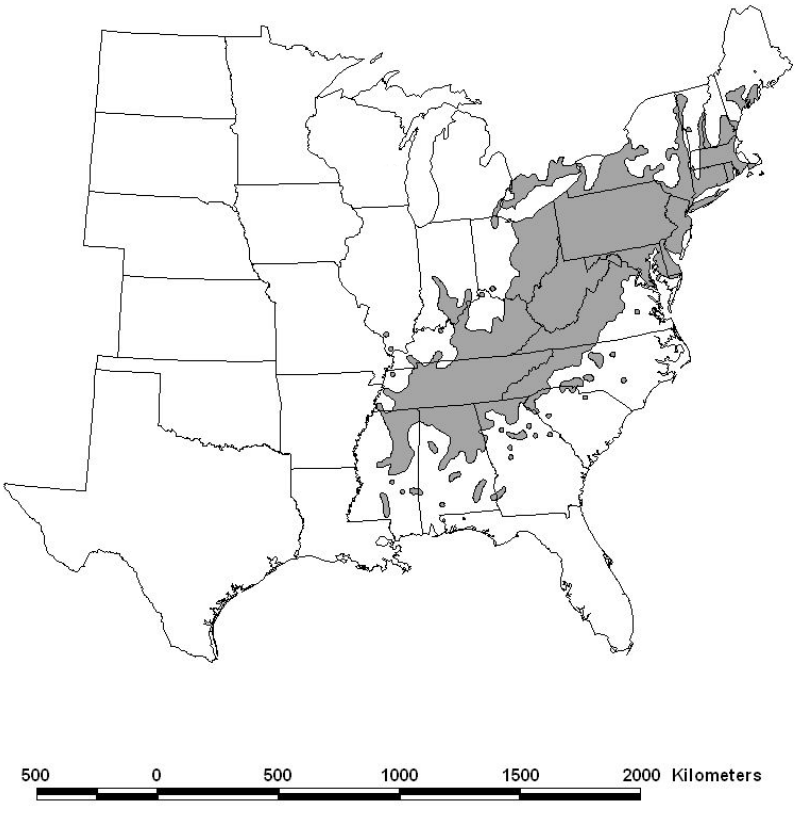

Figure 1: Original natural range of *Castanea dentata* in eastern North America, as adapted from Little (1977).

Cryphonectria parasitica (Murr.) Barr., an aggressive diffuse canker disease (Anagnostakis, 1987), rapidly annihilated American chestnut throughout its range (Roane et al., 1986). The introduced pathogen was believed to have been imported on *Castanea* spp. seedlings from Asia

and the disease was first discovered in 1904 on infected chestnut trees at the Bronx Zoological Park in New York City (Roane et al., 1986). By 1950, the disease had spread throughout the range of C. dentata, and by 1960 had killed an estimated 4 billion trees; essentially extirpating the species as a canopy tree (Anagnostakis, 1987; Hepting, 1974; McCormick and Platt, 1980). Since the discovery of chestnut blight, many groups have worked to develop blight-resistant C. dentata through several strategies including biocontrol of the fungus, breeding and selection of large surviving C. dentata trees, inter-species backcross breeding with resistant Asian chestnut species, and genetic modification. In the 1980's, a breeding program was initiated by a non-profit group, the American Chestnut Foundation (TACF), to restore C. dentata to its native range (Burnham, 1988). TACF has made steady progress toward developing a blight-resistant backcross chestnut tree that possesses the phenotypic characteristics of C. dentata (i.e., morphological, phenological) yet with blight resistance conferred through initial hybridizations with Chinese chestnut (C. mollissima Blume) (Diskin et al., 2006; Hebard, 2006). Simultaneously, scientists are working to impart disease resistance to C. dentata using biotechnology (Merkle et al. 2007). Thus, a large-scale re-introduction program is imminent (Jacobs, 2007).

Scientific emphasis over the last 30 years has focused on breeding for blight resistance. Because C. dentata disappeared decades before the development of modern principles of forest ecology (Paillet, 2002), our knowledge of basic biological and ecological characteristics of the species is rudimentary (Jacobs, 2007; Paillet, 2002). Much of our understanding regarding establishment and growth of C. dentata originated from historical observations or growth of stump sprouts (Paillet, 1982; Paillet, 1984; 2002). Recently, here has been increased prioritization for research examining C. dentata establishment and growth in planted and natural forests (Jacobs, 2007). This progress, combined with continued advances in genetic technologies for production of blight-resistant C. dentata trees for reintroduction, indicates the need for an updated critical synthesis to aid in further developing protocols for disease resistance breeding and subsequent germplasm deployment. The purpose of this review paper is to synthesize the current state of knowledge regarding 1) C. dentata biology and natural history 2) the development of blight-resistant C. dentata trees and 3) the ecology of C. dentata. These knowledge areas as well as understanding of their considerable overlap will contribute to the formulation of a restoration plan for the ecologically and socially important C. dentata (Figure 2).

Part 1: Castanea dentata Biology and Natural History

Taxonomy and Natural Range

Castanea dentata belongs to the Beech family, Fagaceae, and the chestnut genus, *Castanea* (Mill.) Closely related tree species include the European chestnut (*C. sativa* Mill.), Chinese chestnut (*C. mollissima* Blume), and Japanese chestnut (*C. crenata* Siebold & Zucc.) (Lang et al., 2006). *Castanea dentata* is morphologically distinguished by its the larger and more widely spaced saw-teeth on the edges of its leaves (i.e., *dentata*). Chinkapin (*C. pumila* (L.) Mill.), which exists as a spreading shrub or small tree, is also native to the eastern U.S. This species consists of two varieties, known as the Ozark chinkapin (var. *ozarkensis* (Ashe) Tucker) and chinkapin (var. *pumila*), which vary in form, habitat, range, and susceptibility to chestnut blight.

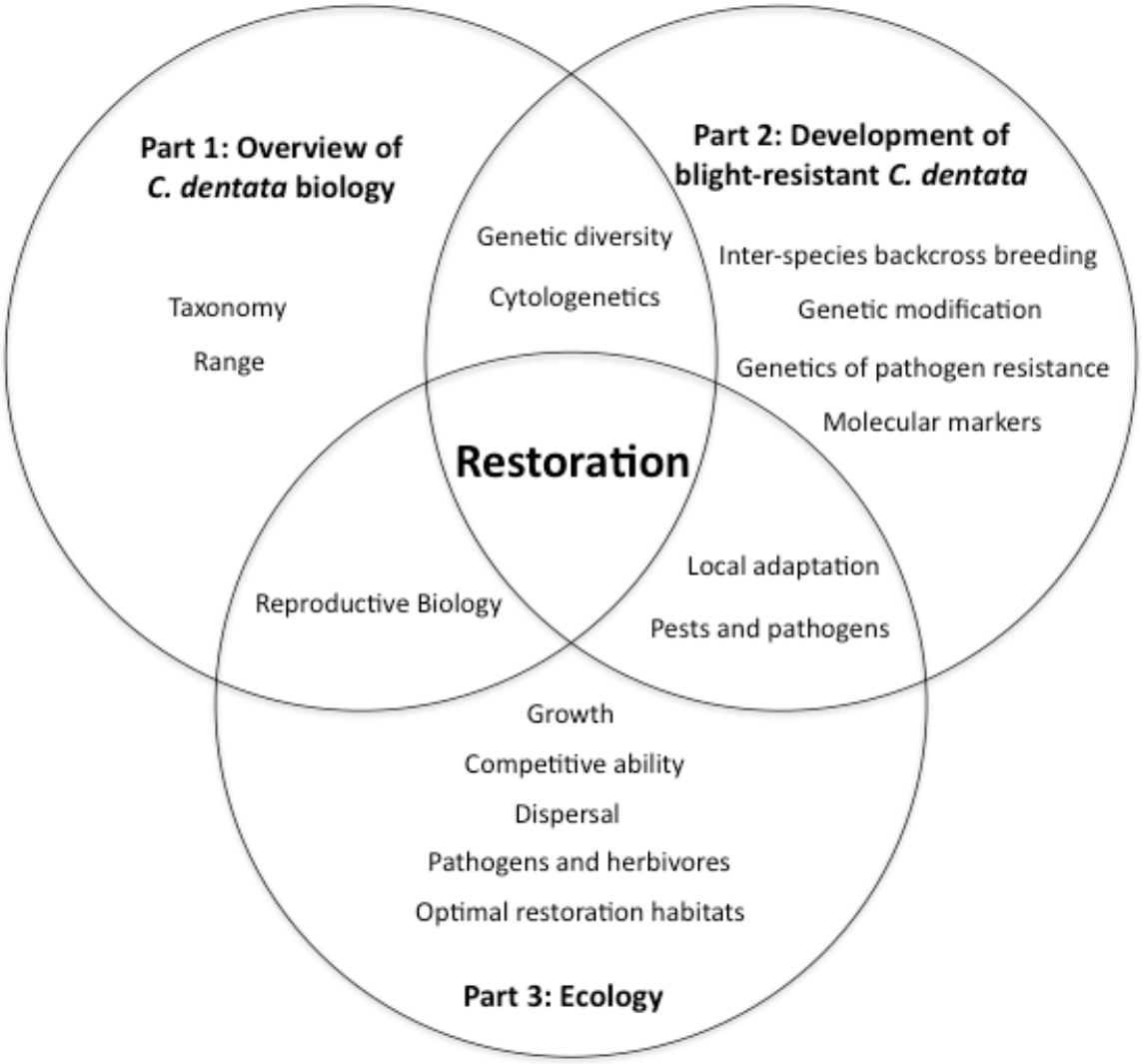

Figure 2: The three parts of this paper correspond to three overlapping spheres of knowledge that will influence the potential success of *Castanea dentata* restoration.

The pre-blight distribution of *C. dentata* in North America ranged from Alabama north to Maine, and west through Ohio and Tennessee, and north into Ontario (Little, 1977; Russell, 1987) (Figure 1). The species frequently dominated upland habitats composed of non-calcareous, acidic to moderately acidic (pH 4-6), and moist but well-drained sandy soils (i.e., submesic or subxeric sites) in mixed forests (Abrams and Ruffner, 1995; Burke, 2011; Russell, 1987; Stephenson et al., 1991). *Castanea dentata* has also been documented as a lesser component of many forest types, varying in soil characteristics and landscape position (Abrams and Ruffner, 1995; Fei et al., 2007; Whitney and DeCant, 2003). However, the range was notably truncated in areas of high pH, limestone-derived soils (Russell, 1987); additionally, frost sensitivity (Parker et al., 1993) may have limited its proliferation at higher latitudes in some northern forests (Russell, 1987). Susceptibility to frost may have also restricted its spread in ravines or valleys over portions of its range, though its prominence in riparian zones of pre-blight stands in southern Appalachia has been reported (Vandermast and Van Lear, 2002).

Evidence suggests the dynamic nature of the pre-blight range of C. dentata during post-glacial expansion. The range expansion of C. dentata during the Holocene from glacial refugia was the most recent of wind-pollinated trees (Paillet, 1982; Paillet, 2002; Russell, 1987). An outbreak of the introduced soil borne oomycete pathogen, Phytophthora cinnamomi Rands, during approximately 1825-1875 may have been responsible for permanently retracting the southern

portion of the range of C. dentata, which once extended as far south as Florida (Anagnostakis, 2001; Crandall et al., 1945). In the in the late 1800's most C. dentata in the Piedmont region of North Carolina had disappeared, while its natural range was still expanding before the introduction of the blight in other areas (Russell, 1987). For example, C. dentata was still spreading northwestward into Michigan at the time of blight introduction (Brewer, 1995).

Castanea dentata is still a common component of eastern North American forests, but nearly all individuals are sprouts that originated from blight-killed trees (Russell, 1987; Stephenson et al., 1991). Cycles of sprouting, infection, dieback, and re-infection may persist for decades (Paillet, 1984), yet sprouts rarely exceed small tree size or grow to reproductive maturity (Paillet, 2002). The species is now classified as endangered in its native range in Canada, as well as in the U.S. States of Kentucky and Michigan; it is listed as being of special concern in Tennessee and Maine.

Reproductive Biology

Castanea dentata is a monoecious, self-incompatible species (Clapper 1954a; Russell, 1987) that generally flowers from June to July (Paillet, 2002; Horton 2010). The male and female inflorescences differ with males being unisexual and proximally located on the shoot and the females being bisexual (i.e., pistillate proximal, staminate distal) and distally located on the shoot (Jaynes 1974). Although the male inflorescence has characteristics of an insect pollinated form, most evidence (Clapper 1954a) suggests that wind-pollination is the primary mechanism (Jaynes 1974). Flowering after leaf out reduces the dissemination distance of chestnut pollen compared to other spring flowering wind-pollinated species (Paillet, 2002). Summer flowering, however, helps to promote consistent seed crops because flowers are not susceptible to late-spring frosts (Horton 2010). In addition pollen release typically occurs in two phases, effectively extending pollination time, with the unisexual inflorescences releasing pollen somewhat before female receptivity and the bisexual inflorescences somewhat after receptivity (Clapper 1954a). Self-incompatibility and short distance of pollen dissemination requires that trees be within about 100 m for successful pollination (Paillet, 2002). Fertilization produces one to three large nuts encapsulated in a spiny bur (i.e., involucre).

Though seed production was more consistent from year-to-year than in many oak (*Quercus* L.) species (Dalgleish and Swihart, *in press*), heavy seed consumption by wildlife, insects, and livestock likely reduced seedling establishment (Dalgleish et al., *in review*; Steele et al., 2005). Thus, sexual reproduction may have contributed only nominally to historical regeneration of *C. dentata*, suggesting that regeneration success may have largely been dependent upon its capacity to sprout vigorously from the root collar following disturbance (Paillet, 2002; Russell, 1987). Sprouts have been reported to reach 2-3 m height in the first year and trees aged > 100 years still commonly retain sprouting ability (Russell, 1987). Thus, even when reproduction by seed is limited or absent, *C. dentata* can maintain itself in a stand and even increase in volume and density through sprouting (Paillet, 2002). Historical foresters noted the rarity of *C. dentata* reproduction by seed and silvicultural operations were specifically designed to promote *C. dentata* regeneration by sprouting (see citations in Paillet, 2002).

Within *Castanea* spp. interspecies crosses can be made between all species within both of the multiple species subgenera (Castanea or true chestnuts and Balanocastanon or chinkapins) (Jaynes 1964). The third subgenera, Hypocastanon, contains only a single species (*C. henryii*) of Asian origin. Crosses between subgenera are also possible but with lesser success rates. In all interspecies crosses at least partial incompatibilities (i.e., reduced seed set compared to within species crosses) have been observed when using various pairs of trees (Jaynes 1964). This indicates wide variability within species for factors controlling sexual compatibility. *C. dentata* appears most compatible with *C. sativa* with some levels of partial incompatibility observed with *C. mollissima, C. seguinii,* and *C. crenata*. *C. dentata* is compatible with its allopatric chinkapin congener *C. pumila* var. *pumila*, with too little information on the other chinkapin species to draw conclusions (Jaynes 1964). *C. dentata* also appears compatible with *C. henryii*. Clearly *C. dentata* is able to outcross with a variety of *Castanea* species both more and less closely related based on taxanomic classification provided by the subgenera rankings (Jaynes 1974).

Genetic Diversity and Population Structure

Studies have estimated genetic diversity in *C. dentata* and *C. mollissima* (the primary species used in backcross breeding efforts) using protein (isozymes) and non-coding (i.e., neutral) DNA markers (Dane et al., 1999; Huang et al., 1994; Huang et al., 1999; Lang and Huang, 1999; Tanaka et al., 2005; Villani et al., 1991). Isozyme studies show *C. dentata* to contain low to moderate levels of genetic diversity relative to other species with large geographic ranges and similar life history traits (Dane et al., 2003). In most direct comparisons with other *Castanea* species *C. dentata* exhibits the least diversity (0.151-0.183, range mean expected heterozygosity over loci) and *C. mollissima* the most (0.305-0.311). It remains unclear whether the low genetic diversity (as measured by expected heterozygosity) predisposed *C. dentata* to rapid population decline in response to the blight epidemic or whether is a consequence of blight-induced population decline (Dane et al., 2003). An apparent consequence of the blight and *C. dentata's* resiliency through stem-collar sprouting is the moderately high level of observed heterozygosity relative to what might be expected for such decimated populations. The most persistent genotypes tend to be more heterozygous than average although seedling reproduction is rare due to the blight and other competing environmental factors (Stilwell et al., 2003). Another possible source of persistence is somatic mutation towards blight resistance, since the source of the sprouts is comprised of only a few cells (Anagnostakis and Hillman, 1992).

Most of the genetic variation observed in *C. dentata* resides within populations (>~90% for isozymes; >~95% for DNA markers), with evidence of clinal trends in overall allele diversity and allele frequencies for some loci (Huang et al., 1994; Kubisiak and Roberds, 2006). More isozyme diversity is apparent in the southern parts of the *C. dentata* and *C. mollissima* ranges, with amounts gradually declining to the north. Some exceptions have been noted in both species with lesser diversity found in some lower and intermediate latitude populations of *C. dentata* (Huang et al. 1998) and much higher amounts found in *C. mollissima* populations in the Changjian River region, Shennongjia district (Huang et al., 1994; Lang and Huang, 1999). Two apparent clinal trends in allele frequencies have been noted in *C. dentata* suggesting the possibility of two glacial refugia (Huang et al. 1998)—one south of the Appalachian spine (towards the Gulf of Mexico) and one to the east of the southern part of the Appalachians (towards the Atlantic Ocean). Additional neutral DNA markers and population sampling strongly supported the southwest to northeast clinal trend in decreasing genetic diversity with no indication of regional boundaries (Kubisiak and Roberds, 2006). They also found low but positive correlations between genetic and geographic distances, suggesting that *C. dentata* currently consists of a single metapopulation established by high gene flow and genetic drift and apparently maintained by persistence of a large sample of the pre-blight genotypes.

Cytogenetics

Castanea spp are diploid with haploid (n) and monoploid (x) numbers of 12 chromosomes (2n=2x=24) (Jaynes, 1962). Estimates of the genome size of *C. sativa* include 0.98 pg (943 Mbp) (Barow and Meister, 2003) and 0.81 pg (774 Mbp) (Kremer et al., 2010) per 1C or haploid content, making the average chromosome length around 70 Mbp (or about one-half the size of the *Arabidopsis* genome). Genome size estimates for *C. dentata* and *C. mollissima* (Kremer et al., 2010) are closer to the lower figure for *C. sativa* and a whole genome sequencing project for *C. mollissima* is underway (J. Carlson, personal communication). Standard root tip (mitotic) cytology has been practiced for some time (e.g., Jaynes, 1962; McKay, 1942), but only recently have techniques, including fluorescent in situ hybridization (FISH), improved to the point of developing chromosome-specific karyotypes. Chromosomal locations of the ribosomal DNA loci (18S-26S and 5S) have been established as well as the presence of the Arabidopsis telomere repeat sequences (Islam-Faridi et al., 2009). Anthers and their microspore mother cells are extremely small (pollen grain diameter ~ 14 um) making meiotic stage cytology difficult (Dermen and Diller, 1962; Jaynes, 1962), although recent progress has been reported with *C. mollissima* x *C. dentata* hybrids (Islam-Faridi, unpublished data). Evidence for translocations and inversions with respect to these two species were suspected based on genetic linkage map data (Kubisiak et al., 1997;

Sisco et al., 2005) and supported by species crossability studies (Jaynes, 1964) and recent cytogenetics (Islam-Faridi et al., 2008). Further resolution is needed to determine the effect of these rearrangements on the ability of interspecies backcross breeding programs to introgress *C. mollissima* resistance genes into *C. dentata* (Ellingboe, 1994). It should also be noted that some isozyme loci are found to be present in *C. mollissima* and absent in *C. dentata* and vice versa (Dane et al., 2003), suggesting that post-divergence deletions and insertions will provide additional interesting genetic variation within interspecies backcross breeding populations (described below).

Part 2: Development of Blight Resistant Castanea dentata

Four main approaches have been pursued to develop blight resistant *C. dentata*: i) inoculation of *C. dentata* with hypovirulent strains of the blight fungus, ii) intra-species breeding from large surviving *C. dentata* trees, iii) inter-species breeding with Asian chestnut species, and iv) genetic modification of *C. dentata*. (i) Hypovirulence, the reduction in virulence of the blight fungus caused by a virus, has effectively controlled blight infection in many areas of Europe (Milgroom and Cortesi, 2004). The discovery of hypovirulent strains of the blight in *C. dentata* populations outside the native range in Michigan fueled hopes for using hypovirulence to control blight throughout North America (MacDonald and Double, 2005). However, with few exceptions in North America, the hypovirulent strains of the fungus fail to spread within and among trees in a population, severely limiting the use of hypovirulence viruses as biocontrol agents (Griffin, 2000; MacDonald and Double, 2005; Milgroom and Cortesi, 2004). (ii) Within *C. dentata*, low levels of blight resistance at very low frequencies suggest that if this phenotype is genetically based then within species recurrent selection and breeding may produce populations with resistance levels adequate for forest planting (e.g., Griffin et al., 1983). The American Chestnut Cooperators Foundation (ACCF) is actively pursuing an intra-species breeding program with a sizeable base of large surviving *C. dentata* trees (http://ipm.ppws.vt.edu/griffin/accf.html). ACCF programs in Tennessee (Thor, 1978), West Virginia (Given and Haynes, 1978), and Virginia (Griffin, 2000) are identifying large surviving *C. dentata* trees, screening their progeny (open- and control-pollinated) for resistance, resistance, and selecting/grafting the most resistant progeny for producing improved seed orchards and for use as breeding parents in subsequent cycles of screening and selection. (iii) The inter-species breeding program has been spearheaded by The American Chestnut Foundation (TACF) and has emphasized a backcross breeding program for American chestnut using blight-resistant Asian *Castanea* species, including Japanese and (primarily) Chinese chestnut. (iv) Genetic modification methods aim to identify the specific genes responsible for blight resistance in Asian chestnut species and insert them into the genome of American chestnut. Below we provide further details on (3) and (4). By no means are these four approaches mutually exclusive and only time will tell the relative effectiveness of these approaches toward producing viable blight-resistant populations capable of surviving and sexually reproducing in forest conditions and developing into timber quality trees.

Inter-species Breeding for Blight Resistance

Chestnut breeding in the eastern U.S. began as early as 1894 with (van Fleet, 1914) work at Beltsville, Maryland. The USDA breeding program began under van Fleet in 1909 in direct response to the chestnut blight epidemic, with an important experimental test site at Glenn Dale, Maryland, added in 1911. The primary goal of the USDA breed program was producing blight resistant forest trees for timber, tannins, and wildlife as well as orchard trees for nuts (Clapper, 1954). Van Fleet first observed blight in his material in 1907, causing him to terminate work on *C. dentata* (= *C. americana*) and concentrate on Asian chestnuts and chinkapins. By 1925 the USDA program was being led by G.F. Gravatt and R.B. Clapper, when Clapper's first *C. dentata* x *C. mollissima* hybrid crosses were made. Clapper led the program through 1949 when F.H. Berry and J.D. Diller assumed responsibility. In 1960 the USDA program was discontinued (Berry, 1978) with some materials transferred to the Connecticut breeding program.

Chestnut breeding work at the Connecticut Agricultural Experiment Station (CAES) began in 1930 with A.H. Graves working at the Brooklyn Botanical Garden and conducting field tests near

Hamden, Connecticut, through 1962. Following Graves in 1962, R.A. Jaynes lead the CAES breeding program until 1983 when S.L. Anagnostakis assumed responsibility through to the present (http://www.ct.gov/caes/cwp/view.asp?a=2815&q=376752). Work at CAES was highly collaborative with the USDA program, using similar strategies of species hybridization and resistance testing in anticipation of finding and cloning the ideal combination of resistance from Asian chestnut species and fast growth and forest tree form from *C. dentata*. One extensive forest test planting of CAES hybrid material was made between 1969 and 1975 at the Lesesne State Forest in Virginia (Jaynes and Dierauf, 1982). Most trees planted were open-pollinated seeds/seedlings of selected first- and second-generation hybrid parents (with resistance sources from *C. molissima* and *C. crenata*), thus comprising third and fourth generation trees where selection for blight resistance had been practiced. By 1980, eleven of the nearly 12,000 planted trees were selected and being propagated into two orchards in Connecticut and Virginia. However, Jaynes and Dierauf (1982) concluded that adequate field resistance was not obtainable among trees that are predominantly (presumably >50%) *C. dentata*. Later Anagnostaksis (2001) found this strategy limiting in terms of producing timber quality forest trees and is now actively backcrossing both *C. mollissima* and *C. crenata* sources of resistance to *C. dentata* as originally outlined by Burnham et al. (1986), discussed below.

In the early 1980s, a backcross breeding program was proposed to introgress blight resistance genes from Asian chestnuts into *C. dentata* (Burnham, 1981; Burnham et al., 1986). The specific steps include making three backcross generations with selection for resistance at each generation to insure retention of Asian resistance genes, intercrossing the selected BC3 trees to produce BC3F2 populations fully segregating (i.e., all of homozygote and heterozygote classes) for resistance, selecting in the BC3F2 populations for high resistance (i.e., tree being homozygous for the Asian alleles at all resistance genes), and establishing the selections in seed orchards to produce planting stock for forest planting. In this selection program, two types of orchards are maintained, isolated from each other—Type A and Type B. In the Type A orchard only backcross progeny are grown, exposed to blight, susceptible trees removed, resistant (i.e., moderately resistant due to heterozygous state of resistance genes) trees used for control-crossing to *C. dentata* to form next generation backcross or open-pollination among selected backcrosses to produce segregating F2 population. Seeds from the open-pollination in the Type A orchard are planted in the Type B orchard and again exposed to blight, susceptible and intermediate resistant trees are removed, highly resistant trees are allowed to open-pollinate each other to produce highly resistant backcross bred *C. dentata* nuts for planting in the forest.

The American Chestnut Foundation was founded to breed *C. dentata* capable of surviving and reproducing in the forest using the backcross breeding method detailed by Burnham (Burnham et al., 1986; Ellingboe, 1994). Several lines from both the USDA and the CAES breeding programs served to jump-start TACF's breeding program. Prior to closing the USDA program, Clapper and Diller established two wide-ranging series of test plots (1936-1939 and 1947-1955) of *C. mollissima* and various first- and second-generation hybrids, including material from the CAES program (Berry, 1980; Diller and Clapper, 1969; Diller et al., 1964). A few of the individual backcross chestnut trees survived and grew well in the test plot in southern Illinois (Crab Orchard Wildlife Refuge) with the best tree being cloned by grafting and eventually named 'Clapper' (Clapper, 1963; Little and Diller, 1964) (ancestry *C. mollissima* seedling M16 selected at Glenn Dale, MD from PI 34517, Tianjin, China and *C. dentata* FP.555 used as grandparent and parent). Additional important named *C. mollissima* selections included 'Crane', 'Kuling', 'Meiling', 'Nanking', and 'Orrin' (Berry, 1978). Similar to the USDA program's 'Clapper' tree, CAES produced and identified a highly desirable BC1 named 'Graves' (ancestry *C. mollissima* seedling 'Mahogany' selected by A. H. Graves at Hamden, CT, from PI 70315, northeastern China, *C. dentata* FP.551, pollen received from Bell, MD and a *C. dentata* tree from Clinton Corners, New York used a grandparent and parent, respectively). A. H. Graves made the M16 x FP.551 F1 cross and H. Neinstaedt selected the F1 parent tree at Hamden and made the backcross. (see Burnham et al., 1986 for a detailed summary of all crosses made in both CAES and USDA programs and their performances in various tests). Hebard (1994; 2006) describes the maturation of the TACF breeding program (also see www.acf.org/r_r.php), including breeding, planting, growing, and

inoculating techniques as well as discussions about the inheritance of blight resistance, the necessary progeny sizes in backcross and intercross families, and the number of *C. dentata* and *C. mollissima* parents needed to successfully develop genetically diverse, environmentally adapted, blight resistant populations.

There are many important features of the backcross breeding program regarding genetics, plant breeding, and restoration (Burnham et al., 1986). For example, it is important to use many unrelated *C. dentata* trees at each generation to properly sample the native species alleles and parent trees should originate within the region where the progeny trees will be planted to promote local adaptation. Recent evidence for uncertainty regarding cold tolerance of hybrid-backcross chestnut used in breeding programs for reintroduction in the northeastern U.S. (Gurney et al., 2011) illustrates the importance of adaptation for successful reintroduction. In addition, sources of resistance should include parent trees of both *C. mollissima* and *C. crenata*, because it is likely that trees within and among species will carry different resistance genes. These features are especially important when breeding many locally adapted populations to reintroduce and restore a wide-ranging species (Worthen et al., 2010). To achieve these goals, multiple *C. mollissima* genotypes are being used as resistance sources with several being advanced to the BC2 and BC3 stages. On the *C. dentata* side the basic plan of using 20 sets (i.e., recurrent lines or lines) of four unrelated *C. dentata* trees as parents (producing the F1, B1, B2, and B3 generations) with each *C. mollissima* resistance source has proven to have substantial practical limitations, because the *C. dentata* individual serving as a parent typically dies before enough flowers and progeny can be produced. Thus most of the lines contain more than four *C. dentata* parents, providing the potential for additional genetic diversity among the selections.

Leffel (2004b) has provided a thorough discussion of additional breeding techniques and methods to produce blight resistant *C. dentata*. Some can be considered modifications of the basic backcross breeding plan while others appear novel to *C. dentata*. The modifications are aimed at making the backcross breeding more efficient by utilizing naturally selected BC2F2 trees to make the BC3 generation and/or using cytoplasmic male sterility (CMS) to produce the backcross generations. The former modification allows for much smaller backcross families as all BC3 trees should be equally resistant being heterozygous for most if not all resistance genes. Allowing these BC3 trees to intercross provides a BC3F2 generation that can be planted in seed orchards at close enough spacing to allow natural infections to cull the less than fully resistant trees. The selected trees should then be mostly homozygous for resistance, providing resistant planting stock for forest planting. Leffel (2004a) provides information suggesting that male serility is controlled by a cytoplasmic and a nuclear factor and that *C. mollissima* x *C. dentata* F1 trees are male fertile while the reciprocal crosses are male sterile. If this proves correct male sterile hybrids and backcrosses can be selected allowing surrounding *C. dentata* to open-pollinate to provide the next generation of BC seeds. Allowing natural selection for blight resistance further reduces the work load. A third modification specifically recommends using *C. mollissima* and *C. crenata* as sources of resistance and crossing to avoid male sterility. But instead of backcrossing, the program proceeds with F1 selected for blight resistance and open-pollination to produce F2, select for blight resistance in F2 and allow open-pollination to produce F3, plant F3 in seed orchard at close enough spacing to allow for natural selection for most resistant genotypes. These should breed fairly true for resistance and can be used to produce planting stock for forest planting.

Vegetative Propagation

C. dentata is difficult to vegetatively propagate with only limited success achieved with the various techniques tested (Cummins 1970; Keys 1978; Elkins et al. 1980) including softwood and hardwood rooted cuttings, ground- and air-layering, grafted scions on seedling or sapling rootstocks, rooted micropropagules (i.e., microcuttings) (e.g., Keys and Cech 1982; Serres et al. 1990; Xing et al. 1997) and germinated somatic embryos (e.g., Merkle et al. 1991; Vieitez et al. 1995; Xing et al. 1999). Problems with rooting cuttings (both macro- and micro-propagation) can be circumvented by using juvenile source plants instead of non-juvenile plants or stump sprouts instead of shoots from the higher parts of the tree (Sanchez and Vieitez 1991). Serially grafting onto juvenile rootstocks as a means to rejuvenate mature *Castanea* genotypes has been tested,

again with limited and only short-term (i.e., transient) positive effects (Giovvannelli and Giannini 2000). Stooling seedling stock plants (i.e., macrottage) (Solignat 1964) and inarching (Jaynes 1961) are two other rooting techniques that have been used in various situations. Splice grafting seems to work best compared to whip, cleft and side grafts (Nienstadt and Graves 1955) but in all methods matching sizes of rootstocks and scions are important as well as using as closely related as possible rootstocks and scions. One recommended practice is juvenile rootstocks used for mature scions where the rootstocks are progeny of the ramet being propagated (McKay and Jaynes 1969), although successful grafts can be made using scion and rootstocks of unrelated genotypes and even different species (Clapper 1954b). More recently bark grafting has been recommended for propagating *C. dentata* scions onto juvenile *C. dentata* rootstocks with up to 10% and 50% success rates for mature and juvenile scions, respectively (Elkins et al. 1994). Current state-of-the-art methods including micropropagation and somatic organogenesis and embryogeneis are summarized by Viéitez and Merkle (2004) and Maynard et al. (2008) and offer increasing potential for large scale propagation of *C. dentata* especially if starting with juvenile explants.

Genetic Modification

It has been argued that the first application of genetically modified organisms (GMO) in forest trees will be for restoration of species decimated by invasive pathogens or pests (Adams et al., 2002; Merkle et al., 2007). *C. dentata* certainly falls into that category and much progress has been made in developing the prerequisite technologies for genetic modification (GM). *In vitro* propagation in *Castanea* spp. was studied over decades in Spain with results summarized by Viéitez and Merkle (2004) and Maynard et al. (2008). Key achievements include derivation of somatic embryogenic cultures from seedling leaf explants (Corredoira et al., 2003) and stable gene transformation using *Agrobacterium* co-cultivation with leaf-derived embryogenic cultures and eventual plantlet formation (Corredoira et al., 2004). Work in *C. dentata* has progressed through similar stages under long-running programs at the University of Georgia (UGA) and the State University of New York, College of Environmental Science and Forestry (SUNY-ESF). In the UGA program, somatic embryogenic cultures were matured into cotyledon-stage embryos (Merkle et al., 1991) and stably transformed embryogenic cultures were obtained using bioliostics (Carraway et al., 1994). This was followed by plantlet formation *in vitro* (Carraway and Merkle, 1997), but survival of trees through acclimation and transfer to greenhouse was not achieved for several more years (Robichaud et al., 2004). Later, application of suspension culture and other cultural changes resulted in 100-fold improvement in efficiency of plantlet formation (Andrade and Merkle, 2005). Use of antibiotic selection in suspension cultures following co-cultivation of embryogenic cultures with *Agrobacterium* led to production of transgenic *C. dentata* plants that grew to the male flowering stage (Andrade et al., 2009). In the SUNY-ESF program, plantlets derived from somatic embryogenic cultures were successfully produced and transferred to the nursery (Xing et al., 1999) and stably transformed cultures and plantlets were produced using *Agrobacterium*-mediated transformation of an antifungal gene (Polin et al., 2006; Rothrock et al., 2007).

Obtaining blight resistant *C. dentata* plants through GM, followed by crossing those plants to a wide array of *C. dentata* trees to produce a blight-resistant, genetically-variable population for reforestation is the goal of the program at SUNY-ESF (W.A. Powell, personal communication). Substantial progress has been made in designing and selecting small proteins, with anti-microbial activity against *C. parasitica* and other necrotrophic pathogens, while showing little or no toxicity to *Castanea, Malus*, or *Salix* pollen (Powell et al., 1995; Powell et al., 2000; Powell and Maynard, 1997; Powell et al., 2006). This suggests a potential path forward in engineering pathogen resistance for plants as demonstrated in transgenic poplar with enhanced resistance to the necrotrophic pathogen *Septoria musiva* (Liang et al., 2002). Another promising lead for chestnut blight resistance is the oxalate oxidase gene (*OxO*) (Polin et al., 2006; Welch et al., 2007). Oxalate production has been shown to be a significant *virulen*ce factor in the blight fungus, *Cryphonectria parasitica* (Chen et al., 2010; Havir and Anagnostakis, 1986). The *OxO* gene, when transformed into in poplar, provides increased tissue tolerance to oxalate and enhanced resistance to *Septoria musiva* (Liang et al., 2001). Co-transformation of two or three genes is a

strategy that may prove useful where post-transformation breeding is required. In *C. dentata* this is routinely accomplished with three genes-- a visual selectable marker (such *GFP*), antibiotic resistance (such as *npt*II) for selection in culture, and the candidate resistance gene (Newhouse et al., 2010; W. A. Powell, personal communication). Because the marker and selection genes are not linked to the resistance gene, they can be removed from the segregating breeding population while progeny inheriting only the candidate resistance genes are maintained. Although potentially useful, co-transformation has limitations such as high variation in gene expression and gene silencing (see Halpin et al., 2001). One way around these limitations is the co-expression of multiple genes in a single open reading frame (i.e., Liang et al., 2005).

Another important consideration for GM trees is the source and tissue specificity of resistance genes and their promoters and regulators. Researchers are identifying and isolating candidate resistance genes from the relatively resistant *C. mollissima* (Forest Health Initiative, FHI, unpublished data) and efforts to clone promoters from *C. dentata* have been successful (Connors et al., 2002). Within the FHI, candidate genes are identified by their presence in genomic regions identified as QTLs for resistance, their up-regulation in inoculated vs. non-inoculated stems in *C. mollissima*, and their presence or absence in suppressive subtraction hybridization (SSH) libraries (Baier and Powell, personal communication) and transcriptomic screens (Barakat et al., 2009). One such gene of interest is a laccase gene that is highly expressed in *C. mollissima* stem tissues and very lowly expressed in *C. dentata*. It also appears that this gene maps to a blight resistance QTL and as such is considered a candidate resistance gene. Utilizing genes from a closely related species in GM, so called intragenics (including cisgenics), has similarities to interspecies backcross breeding (Schouten and Jacobsen, 2008) and may offer new opportunities for restoring species on the verge of extirpation. An example is *Tsuga canadensis* where the exotic hemlock woolly adlegid (*Adelges tsugae*) is decimating the species and no crossable species with resistance exists. However, non-crossable congenic species with co-evolved resistance do exist, e.g., *T. chinensis* (see Montgomery et al., 2009) and offer hope for a form of intragenic technology to intervene on behalf of *T. canadensis*. A recent intragenesis example in poplar, the model forest tree for biotechnology, utilized genomic copies (i.e*.,* cisgenes) of five protein-encoding genes (involved in gibberellin metabolism or signaling) to demonstrate increased genetic variation in growth and wood anatomy traits, including variants that showed either faster growth with no change in wood fiber quality or higher fiber quality with no change in growth rate (Han et al., 2010).

Molecular Marker Applications

Molecular markers have improved our understanding of *C. dentata* genetics by delineating patterns of genetic diversity and dissecting quantitative trait variation (e.g., Casasoli et al., 2006; Dane et al., 2003; Huang et al., 1999; Kubisiak et al., 1997; Kubisiak and Roberds, 2006; Pigliucci et al., 1990). In a similar manner, molecular markers revealed detailed information on the chestnut blight fungus focusing on genetic diversity and mating system mechanics (Marra and Milgroom, 1999; Marra and Milgroom, 2001; Milgroom et al., 1992a; Milgroom et al., 1992b). In the near future marker genotyping a mapping population (i.e*.,* a single cross of 100 progeny) of the fungus scored for canker development in a sample of *C. molissima* x *C. dentata* host trees may provide QTL for *virulen*ce (F. Hebard, personal communication, unpublished data). Early in the DNA marker era (Bernatzky and Mulcahy, 1992; Ellingboe, 1994) suggested using a large number of restriction fragment length polymorphisms (RFLP) markers to map resistance genes in *C. mollissima* and use the markers to facilitate their introgression into *C. dentata* through backcross breeding. Conceptually this is an excellent idea that was proven in numerous systems (Collard and MacKill 2008; Moose and Mumm 2008), however only a few RFLP markers were developed for *Castanea* spp. Random amplified polymorphic DNA (RAPD) markers proved more cost effective for producing larger numbers of markers and they, along with the few RFLPs and the previously developed isozyme markers, were used to map the *C. dentata* genome as well as identify quantitative trait loci (QTL) (Kubisiak et al., 1997). However RAPD and the later developed amplified fragment length polymorphisms (AFLP) markers (see Sisco et al., 2005 for AFLP application in *Castanea* mapping), both being dominant and difficult to track among different families were non-optimal, nor cost effective, for operational use in large breeding programs (F. Hebard, personal communication).

The recent development of large sets of short sequence repeat (SSR or microsatellite) and single nucleotide polymorphism (SNP) markers (Kubisiak et al., *in review*) are likely to provide the practical application envisioned early on by Bernatzky, Mulcahy, Ellingboe and others (e.g., Nance et al. 1992). These markers being codominant and much higher in sequence specificity (providing data on the same loci across families) overcome the two major problems encountered with RAPD and AFLP. However cost effectiveness could still be an issue at least in the near-term. Fortunately newly funded research (i.e., FHI mentioned above) is providing resources to fully test these markers in backcross breeding as well as in assisting with higher-density and higher-resolution mapping for candidate gene discovery. The candidate genes will be isolated from *C. mollissima* and used to transform *C. dentata* to directly test their effectiveness in providing blight resistance. And the highly informative maps will be used to track introgressed *C. mollissima* genes in early and later generation backcross families. In addition these maps will provide estimates of the remaining *C. mollissima* genome at various backcross generations, facilitating the dual selection of resistance provided by *C. mollissima* genes and *C. dentata* silvical traits provided by the *C. dentata* genome. The use of markers in selection for recurrent (*C. dentata*) type (i.e*.,* genomic regions) provides up to a 3X improvement in recovery of recurrent type (Tanksley and Rick 1980; Soller and Beckmann 1986). Traditionally many backcross programs used six backcross generations as a standard (Allard, 1960), however with informative, well-spaced markers only two generations provides similar results ((e.g., Visscher et al., 1996). Given that the TACF backcross program was planned for three backcross generations (Burnham et al., 1986) markers may reduce this to one, allowing for additional sources of resistance sources to be introgressed with a similar level of effort.

Other potentially useful applications for highly polymorphic DNA markers include fingerprinting, paternity analysis, and species classification. Fingerprinting has been used to identify mis-labeled individuals in breeding populations and research crosses (Kubisiak, personal communication; Sisco et al., 2005) and to unravel clonal identities in germplasm banks (Romero-Severson et al. 2009). A form of paternity analysis was used to identify trees with non-*C. dentata* cytoplasm in a large set of trees sampled from across the *C. dentata* range (Kubisiak and Roberds, 2006). This test relied on a single marker difference for the chloroplast genome, differentiating *C. dentata* from all other *Castanea* spp. Highly informative nuclear genome SSR marker sets can be developed for routine fingerprinting and paternity analysis (Jeanne Romero-Severson, personal communication). When fully developed, these tools will open new breeding opportunities such as pedigree-controlled breeding without control-pollination ((El-Kassaby and Lindgren, 2007; El-Kassaby and Lstiburek, 2009; Lambeth et al., 2001) and efficient tracking of clonal lines in tissue culture and genetic modification programs. Species classification relies on a large number of markers distributed across the genome where data are collected on representative trees of each species and on samples of unclassified trees. Computer algorithms (Falush et al., 2003; Pritchard et al., 2000) are then used to classify the individuals based on their genetic marker composition. This has been successfully utilized in loblolly pine (*Pinus taeda* L.) and shortleaf pine (*P. echinata* Mill.) to determine past and current rates of natural hybridization and introgression (Stewart et al., 2010; Stewart et al., *in press*).

Genetics of Blight Resistance

The inheritance of chestnut blight resistance has been studied extensively, especially in interspecies first- (F1) and second-generation (F2 and BC1) crosses (Clapper, 1952; Graves, 1942; Graves, 1950). Burnham et al. (1986) analyzed and summarized the existing knowledge confirming that a two-gene pair model of resistance seemed reasonable as first suggested by (Clapper, 1952) with the resistant *C. mollissima* or *C. crenata* parents providing partially dominant alleles for resistance. Graves (1942) had actually proposed a one-gene model, quickly discounting it due to the intermediate nature of the resistance reaction. Stem canker data from controlled inoculation trials of several segregating (i.e., F2) families (as outlined in Ellingboe, 1994) provided support for the two gene model and indicated that the Asian parents appear homozygous for their resistance genes. In addition, the two Asian species may have different gene pairs (i.e., loci) for resistance suggesting that combining parents from each species in

interspecies breeding may lead to enhanced resistance. This variation is more likely due to allelic differences (resistant vs. susceptible alleles) at the resistance gene loci. Thus, a few major gene loci for resistance may exist that differ among species and are completely lacking or defective in *C. dentata*. There may also be allelic variation at various loci for factors that further affect resistance expression. Although it appears that homozygosity for resistance alleles at two major gene loci is sufficient for survivability to the blight, additional genes will likely improve survivability and increase resistance diversity against a potentially changing (i.e., mutating) pathogen (Ellingboe, 1994).

The most definitive research on blight resistance genetics further supports a two or possibly three gene model as detected by QTL mapping in a *C. mollissima* x *C. dentata* F2 cross (Kubisiak et al., 1997). The resistance genes from *C. mollissima* are partially dominant and obtaining individuals homozygous for two genes provided resistance reactions (i.e., stem canker area at about 8 weeks post-inoculation) on par with *C. mollissima*. The three-locus model accounted for about 70% of the genetic variation, further indicating a combination of major and minor genes contributing to resistance. This F2 cross included one *C. mollissima* grandparent (cv. 'Mahogany') and two related *C. dentata* parents thus they were investigating a rather narrow sample of the potential genetic variation in resistance, but still instructive for revealing the genetic architecture of blight resistance (Hebard, 2006). New work funded by NSF (www.fagaceae.org) and a partnership developing and utilizing biotechnology in forest health (www.foresthealthinitiative.org) are expanding genomic tools for more precisely and comprehensively mapping resistance genes (Kremer et al., 2010). New higher density maps using SSR and SNP markers developed from large-scale expressed gene sequencing (Barakat et al., 2009) has confirmed and slightly refined the genomic locations of the blight QTLs originating in cv. 'Mahogany' (Kubisiak et al., unpublished data). Collaboration with TACF's breeding program will provide much larger population sizes in BC3 and BC3-F2 generations as well as including additional sources of resistance. These features combined with the higher-density genetic maps will allow increased precision in locating blight resistance loci, greater sensitivity in finding smaller effect loci, and the possibility of determining whether different *C. mollissma* trees contribute different resistance loci.

Part 3: Ecology and Restoration

Environmental Controls on Growth

Current knowledge implicates *C. dentata* as an intermediate shade tolerant to shade tolerant species (Joesting et al., 2007; Joesting et al., 2009; McCament and McCarthy, 2005; Wang et al., 2006). Shading produces either a neutral (Rhoades et al., 2009; Wang et al., 2006) or positive (Anagnostakis, 2007; McCament and McCarthy, 2005) effect on germination and/or survival of young *C. dentata*. Seedlings and saplings may persist for years under low light conditions beneath a forest canopy (McEwan et al., 2006; Paillet and Rutter, 1989), exhibiting plasticity by increasing leaf mass per area with greater light availability (Joesting et al., 2009; King, 2003; Wang et al., 2006). *Castanea dentata* seedlings, saplings, and mature trees in a forest in southwestern Wisconsin exhibited light compensation points, quantum efficiency, leaf mass per area, and percent nitrogen content similar to those of shade tolerant species (Joesting et al., 2009). Interestingly, however, understory trees measured in this same study had high maximum rates of photosynthesis, similar to that of fast growing, shade intolerant species such as yellow-poplar (*Liriodendron tulipifera* L.) and eastern cottonwood (*Populus deltoides* Bartram ex Marsh.) (Joesting et al., 2009). Nevertheless, *C. dentata* shows greater growth and photosynthesis with increasing light availability (Joesting et al., 2007; McCament and McCarthy, 2005; Wang et al., 2006) and growth rates of *C. dentata* under high light availability may exceed or equal that of other species exhibiting strong positive responses to light (Boring et al., 1981; Griffin, 1989; King, 2003; Latham, 1992). These ecological attributes distinguish American chestnut from oaks and other co-occurring species (Paillet, 2002).

Increasing light availability was shown to have a greater influence on *C. dentata* growth than soil parameters (McCament and McCarthy, 2005) or site type, i.e., xeric vs. mesic (Rhoades et al., 2009). This combined evidence reflects the capacity of *C. dentata* to survive for prolonged periods

as stump sprouts or advance regeneration under suppressed conditions, while maintaining the ability to rapidly respond to release following disturbance. For example, *C. dentata* sprouts vigorously following cutting and growth may exceed that of any other hardwood species following clearcutting (Mattoon, 1909; Smith, 1977). Radial growth rates approach 5 mm year-1 in plantation or natural stand settings, with maximum values of 10-12 mm year-1 (Jacobs and Severeid, 2004; McEwan et al., 2006; Paillet and Rutter, 1989; Zeigler, 1920). Productivity of mature *C. dentata* trees in Connecticut was measured to be at least 25% greater than that of oak species (Frothingham, 1912). A productivity of 2.9m ha-1 year-1 was reported for *C. dentata* stands on 60-year rotations in the Blue Ridge Mountains (Buttrick et al., 1925).

The former dominance of *C. dentata* in upland habitats suggests its drought tolerance compared to co-occurring species (Jacobs, 2007). For example, *C. dentata* exhibited higher instantaneous water use efficiency relative to several species of upland oaks (*Quercus* spp.) and dry site red maples (*Acer rubrum* L.) subjected to drought under controlled conditions (Bauerle et al., 2006). Additionally, sprouts of *C. dentata* had higher leaf water potential than several species of upland oaks during an early summer drought in Pennsylvania (Abrams et al., 1990). However, the oaks in this study were newly planted, whereas the *C. dentata* were of sprout origin, suggesting potential bias associated with root system development. *Castanea dentata* resists high pH soils (Russell, 1987), and growth may be negatively correlated with pH (Tindall et al., 2004). Specific responses to varying nutrient availability are less well documented, although *C. dentata* has been shown to increase leaf, shoot, and root biomass with increasing availability of specific nutrients including nitrogen, potassium, and magnesium (Latham (Latham, 1992; McCament and McCarthy, 2005; Rieske et al., 2003).

Competitive Ability

Castanea dentata historically grew with many forest tree species due to its occurrence in a wide variety of mixed forest types.. Under the submesic or subxeric sites on which *C. dentata* dominated, it was primarily associated with upland oaks (*Quercus* spp.), maples (*Acer* spp.), hickories (*Carya* spp.), and other mixed hardwoods depending on region (McEwan et al., 2006; Russell, 1987). Historical and more recent observations have reported on the rapid early growth and competitiveness of *C. dentata* as well as its dominance in pre-blight stands. For example, *C. dentata* trees that were introduced into a site in southwestern Wisconsin rapidly invaded an adjacent woodland and largely outcompeted and replaced associated species, such as oaks and hickories, maintaining themselves over time as the dominant forest canopy trees (McEwan et al., 2006; Paillet and Rutter, 1989). Thus, *C. dentata* exhibits characteristics of both a pioneer (facilitated by aggressive stump sprouting and juvenile competitiveness) and late-successional species (based on its extended stand longevity).

By studying development of American chestnut relative to six co-occurring species across a broad range of light and nutrient levels under controlled conditions, Latham (1992) helped to elucidate mechanisms for American chestnut's competitive ability. *Castanea dentata* outranked all other species in traits associated with competitive ability over the wide range of resource level combinations, implicating *C. dentata* as both a broad generalist and strong competitor (Latham, 1992). Furthermore, there is evidence that leachate from *C. dentata* litter may have allelopathic properties that suppress the development of common competitors (Vandermast and Van Lear, 2002).

Dispersal

Similar to other large hard mast species, nuts of *C. dentata* fall close to the parent tree although blue jays, squirrels, and other rodents have been noted as significant historical consumers and dispersers (Diamond et al., 2000; Russell, 1987; Steele et al., 2005). A blight-free stand of *C. dentata* in southwestern Wisconsin provided unique insight into dynamics of regeneration and migration (and potential competitive dominance) of the species (Paillet and Rutter, 1989). In 70 years, nine original planted *C. dentata* trees supplied sufficient regeneration to spread the species over 1 km; within about 0.5 km from the original source trees, *C. dentata* comprised at least 25% of total canopy basal area and predominated among advanced saplings entering the canopy.

Evidence from this stand suggests that migration of *C. dentata* regeneration involved a multi-step process, including i) establishment of individuals or groups of pioneer trees following seed dissemination in light gaps, ii) development of large pools of advanced regeneration in the understory of these pioneer trees, and iii) persistence of these seedlings and saplings underneath the established canopy until being released by disturbance to assume canopy dominance (Jacobs, 2007; Paillet and Rutter, 1989).

Pathogens and Herbivores

Several pathogens and pests other than chestnut blight pose a threat to *C. dentata*. Principal among these is the introduced soilborne oomycete pathogen, *P. cinnamomi* Ronds, which forms lesions on roots that inhibit water and nutrient uptake (Maurel et al., 2001a; Maurel et al., 2001b) leading to reduced tree vigor and potential mortality (Anagnostakis, 2001; Rhoades et al., 2003). Growth, reproduction, and dissemination of the disease are favored under compacted, saturated soils with poor aeration because this promotes sporangia formation and zoospore release (Rhoades et al., 2003; Wilcox and Mircetich, 1985). The impact of the disease was noted in the southern US prior to introduction of chestnut blight (Anagnostakis, 2001), and current evidence suggests that the pathogen presents another significant obstacle for *C. dentata* reintroduction (Rhoades et al., 2009; Rhoades et al., 2003). Careful site selection, identification of ectomycorrhizae that confer protection to roots, and additional resistance breeding have been suggested as means to help combat the impact of *P. cinnamomi* (Anagnostakis, 2001; Rhoades et al., 2003). In addition, evidence has specifically implicated the oriental gall wasp (*Dryocosmus kuriphilus* Yasumatsu), Gypsy moth (*Lymantria dispar* L.), and ambrosia beetles (*Xylosandrus crassiusulus* Mot. and *Xylosandrus saxeseni* Blandford) as pests that may also negatively affect *C. dentata* following reintroduction (Anagnostakis, 2001; Oliver and Mannion, 2001; Rieske et al., 2003).

Compared to blight resistance, less is known about the genetics of resistance in *Castanea* spp. to *Phytophthora* root rot and Oriental gall wasp. For root rot resistance both two and one gene models have been proposed (Bowles, 2006; Guedes-Lafargue and Salesses, 1999). An informative early (first-year seedlings) screening system is being deployed to screen (i.e., progeny test) interspecies backcross parents produced in the TACF breeding program (Jeffers et al., 2009; Sisco, 2009). Segregation for resistance is often noted (P. Sisco, personal communication) providing a rich source of genotypic and phenotypic material for genetically mapping the resistance factors. The genetic situation for gall wasp resistance is beginning to emerge (Anagnostakis et al., 2009) with the chestnuts (*C. mollissima, C. crenata,* and *C. dentata*) apparently being more susceptible relative to the chinkapins (*C. pumila*, *C. pumila* var. *ozarkensis* and *C. henryi*). Strong differences among trees within interspecies backcrosses were noted in a field test in North Carolina where gall wasp pressure was high. Segregation for resistance (i.e., no or few galls per tree vs. many galls) was noted in both crosses suggestive of a single, dominant gene controlling resistance (Anagnostakis et al., 2009). Additional crosses will need to be evaluated under high gall wasp pressure to further evaluate the inheritance of gall wasp resistance. In all three cases—blight, root rot, and gall wasp— resistance is available in the Asian chestnuts for the first two and the chinkapins for the third. Resistance to all pests appears be at least partially dominant and much of the variation appears controlled by one or two genes. Whether these genes are the same in different species or even genotypes within species remains to be seen but application of emerging genomic technologies (Wheeler and Sederoff, 2009) should help to resolve the situation and provide tools for precisely tracking the genes in breeding programs, enabling introgression of genes for resistance to root rot, gall wasp, and blight together.

Optimal Restoration Habitats

Restoration of *C. dentata* to its native range may be initiated through reforestation and afforestation plantings of blight-resistant seedlings. Recent evidence has demonstrated excellent growth and competitiveness of *C. dentata* over a wide range of sites in natural stands (McCament and McCarthy, 2005; McEwan et al., 2006; Rhoades et al., 2009). In addition, mine reclamation sites and marginal agricultural lands would provide abundant planting sites for afforestation of *C.*

dentata (Jacobs, 2007; Jacobs and Severeid, 2004). Despite the characteristic competitiveness of juvenile *C. dentata*, effective silvicultural management may be necessary to ensure vigorous establishment of high-value blight-resistant seedlings following planting (McCament and McCarthy, 2005; McNab, 2003; Rhoades et al., 2009). Specific recommendations for underplanting (Wang et al., 2006) or thinning and burning (McCament and McCarthy, 2005) have been proposed to promote competitiveness of *C. dentata* in natural stands. Similarly, recommendations are available for herbicide application to control competing vegetation and promote *C. dentata* development in field plantations (Robertson and Davis, 2011; Selig et al., 2005).

Several specific considerations may prove important in selecting optimal sites for restoration. The absence of *C. dentata* in high pH, limestone derived soils (Russell, 1987) suggests that these site types should be discriminated against for restoration plantings. Additionally, the susceptibility of *C. dentata* to *P. cinnamomi* indicates that careful site selection may be needed to strategically locate restoration plantings on very well drained sites (Rhoades et al., 2003). Furthermore, public opinion regarding harvesting, fire, and other forms of forest disturbance may restrict the capacity of land managers to employ silvicultural treatments that have been demonstrated to promote *C. dentata* development, particularly on public lands (Jacobs, 2007; McEwan et al., 2006). This implies that target sites for *C. dentata* restoration may shift toward reforestation and afforestation of private lands (Jacobs, 2007). Much of the large-scale hardwood afforestation plantings in the US for carbon sequestration, conservation, wildlife, and timber occur in the Midwest, which encompasses a limited portion of the original *C. dentata* range. This presents a new challenge, as targeting *C. dentata* plantings in this region is incongruent with the fundamental mission to restore *C. dentata* to the original species range (Jacobs, 2007). Additionally, *C. dentata* has demonstrated its ability to thrive when introduced outside of its native range (Jacobs and Severeid, 2004; McEwan et al., 2006; Paillet and Rutter, 1989), raising ecological considerations regarding its potential to suppress indigenous vegetation (Jacobs, 2007).

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Component #5 – Regulatory Landscape Review

Navigating Existing US regulations on Forest Biotechnology Research

May 2011 by Mr. Tom Redick

In agriculture, biotech crops were rapidly adopted in the US and to a lesser extent worldwide (over 800 million hectares in just over a decade[11]). This brought substantial environmental or human health benefits and improved various agricultural systems while increasing yield in some instances[12;13;14]. Biotech trees released into forests have the potential to promote ecosystem sustainability, and bring life cycle benefits for greenhouse gas mitigation[15]. This can occur via traits that speed growth or increase nut yields, reduce runoff in forest management, reduce pest damage, and improve stress tolerances so trees can be grown with less water, fertilizer, or crop protection inputs. To reap the benefits of biotech trees in U.S. forests, innovators in tree breeding must first navigate the research and development pathway. This includes regulatory and marketplace approval, including stakeholders who value the forest for aesthetic, recreational and ecological interests. A recent survey of forest scientists about how regulations affect the development of transgenic forest biotechnology in the USA cited research in "containment options" as the number one research priority[16]. However, the development and field verification of containment technology performance is itself made extremely difficult by today's process-based regulations, including by a ban on field trials with GURTs (genetic use restriction technologies) that has been recommended by parties to the Cartagena Protocol on Biosafety[17].

While the US has only one federal law specific to GE crops (the 2000 Plant Protection Act) this law was tinkered with the 1986 "Coordinated Framework"[18] and again in the 2008 Farm Bill, arguably expanding USDA's authority to regulate biotech crops. This existing regulatory authority to regulate biotech organisms is distributed through three agencies:

1. The United States Department of Agriculture, Animal and Plant Health Inspection Service (APHIS), for all crops under their authority to regulate introductions of agricultural pests and noxious weeds;
2. The Environmental Protection Agency (EPA) for plant-incorporated protectants (PIPs), including fungicides, against various plant pests; and
3. The Food and Drug Administration (FDA). These cover virtually all recombinant DNA plant breeding, while comparable traits (e.g., herbicide-resistance, pest tolerance) obtained through conventional breeding continues to be unregulated.

In 2009, the USDA Forest Service, in cooperation with a power company (Duke Energy) and a foundation (the U.S. Endowment for Forestry and Communities), formed the Forest Health Initiative (FHI). See www.foresthealthinitiative.org (last visited Mar. 23, 2010). FHI is a "collaborative effort to advance the country's understanding and role of biotechnology to address some of today's most pressing forest health challenges." FHI plans to build on the extensive

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[11] International Service for the Acquisition of Agri-biotech Applications. 2007. Global Status of Commercialized Biotech/GM crops. ISAAA. 16 July 2010; www.isaaa.org/Resources/Publications/ briefs/37/executivesummary/default.html

[12] Brookes G, Barfoot P. 2005. GM crops: The global economic and envi- ronmental impact: The first nine years 1996–2004. AgBioForum 8: 187–196.

[13] Fernandez-Cornejo J, Caswell M. 2006. The First Decade of Genetically Engineered Crops in the United States. US Department of Agriculture Economic Information Bulletin 11. (25 June 2010; www.ers.usda.gov/ publications/eib11/eib11.pdf)

[14] Kleter GA, Bhula R, Bodnaruk K. 2007. Altered pesticide use on transgenic crops and the associated general impact from an environmental per- spective. Pest Management Science 63: 1107–1115.

[15] Sheehan JJ. 2009. Biofuels and the conundrum of sustainability. Current Opinion in Biotechnology 20: Table 1: 318–324.

[16] Strauss SH, Schmitt M, Sedjo R. 2009. Forest scientist views of obstacles to research and commercial development of transgenic forest biotechnology. Journal of Forestry 107: 350–357.

[17] Strauss SH, et al. 2009. Strangled at birth? Forest biotech and the Conven- tion on Biological Diversity. Nature Biotechnology 27: Box 1: 519–527.

[18] Office of Science and Technology Policy. 1986. Coordinated framework for regulation of biotechnology. Federal Register 51: 23302–23309.

research already accomplished on the American chestnut by the American Chestnut Foundation and others as a model system for how biotechnology can potentially protect trees. This approach is being accomplished in conjunction with coordinated efforts directed to social/environmental issues and regulatory requirements.

A first step will be to safely and effectively develop an American chestnut that resists chestnut blight and root rot. Researchers have biotech versions of the American chestnut in sapling form, too young to determine their viability against the fungus. This could restore the original American chestnut – fast growing to 100 feet tall – back to its former glory in the forests of the Eastern U.S.

The Food and Drug Administration (FDA) would expect, under its "voluntary consultation" process, to hear about plans to develop a GE American Chestnut to introduce to North American forests[19] and which produces nuts that are used for food by humans, as well as wildlife (the latter is only an FDA concern insofar as they conduct cursory assessments of environmental impacts). This tree can also be an energy crop due to its very fast growth on poor soils and its calorie-rich wood, and parallel to the biotech breeding effort, traditional breeding methods are producing 15/16 Chinese-American hybrid chestnuts. Before tree breeders succeed in restoring the American Chestnut to all or part of its natural range, they must develop and test a number of varieties to find those that are adequately resistant to the invasive exotic chestnut blight fungus that devastated this tree in the first half of the 20th Century. Chestnuts might encounter other serious pest threats like the *Phytophthora* blight that is attacking oaks in the US. Full and timely restoration is likely to require a combination of conventional hybridization/backcross and biotech tree breeding methods.

The EPA regulates plants with genes that provide protection against any form of pest, but only if breeding employs GE methods (as this is considered most likely to lead to new types of toxicological exposures)[20]. If a fungicidal trait were bred into a Chestnut tree using biotech methods, this would be a regulated PIP. There are new methods of genetic manipulation ("Cisgenics") that move DNA around within closely related plant species, and which might not trigger the same regulatory response (but there is no guarantee that the regulatory review might expand to address this, as Canada's regulatory oversight expanded to cover non-GMO herbicide-resistance and other traits produced using mutagenesis forms of plant breeding).

APHIS has generally been reviewing and approving biotech crops under its "plant pest" authority as "regulated articles" that require field trials, with EPA approval required for PIPs. For field trials over 10 acres, EPA needs an "Experimental Use Permit". Evaluations of a crop with a new GE trait would normally proceed thorough four general stages:

1. Lab/greenhouse studies to observe, under controlled conditions, the existence of a desirable trait imparted by a gene in a model species and a model crop variety;
2. Limited field trials in one or a few model varieties and environments to see if the trait persists in the field to a useful degree, or has adverse consequences for other traits. Most genes that pass stage 1 fail at this second stage.
3. Testing of several different forms of the gene that might have different promoters to vary expression pattern and level, and includes a large number of different insertion events to identify those with favorable expression patterns. This stage also normally includes an initial analysis of agronomic properties, though in a limited sample of commercial varieties and environments. During this process, there are usually hundreds to thousands of other genotypes under evaluation at the same locations for general plant breeding goals that must be kept free of any possible comingling. Thus, the tracking of all of the inserts, and accounting for the containment of each, presents a logistical problem, even without flowering.

[19] Merkle SA, et al. 2007. Restoration of threatened species: A noble cause for transgenic trees. Tree Genetics and Genomes 3: 111–118.
[20] Environmental Protection Agency. 2001. Regulations under the Federal Insecticide, Fungicide, and Rodenticide Act for Plant-incorporated Protectants (Formerly Plant-Pesticides). Federal Register 66: 37771–37817.

4. Movement of the gene into a variety of different commercial genotypes and testing in a wide variety of environments for the new trait and agronomic properties. These tests are essentially normal breeding trials, except for the required regulatory approvals, monitoring, use of buffer zones, and other steps required to assure segregation from actual commercial varieties and products. As evidenced by the many cases of adventitious presence of unapproved GE varieties that have entered the food supply at a low level, this is perhaps the most risky step in crop development when using transgenes. For example, a chestnut grove growing food chestnuts for export to overseas buyer would have to avoid pollen flow from a flowering biotech American Chestnut.

Effective confinement of propagules therefore generally means the complete prevention of flowering, via GURTs or manual bagging over all flowers on every experimental plant. Manual bagging is extremely difficult and costly for large-scale plant breeding in any crop, and may be too risky given the legal consequences of comingling discussed above, especially for public sector breeders or small companies[21]. Due to most trees' large size, it is virtually impossible to remove or bag all flowers on large trees such as poplars once they are beyond the small scale field trial stage and into larger scale field evaluation and variety development (stages 3 and 4 above).

Potential Impact of Emerging US regulations on Biotechnology Research

As noted above, USDA is considering a number of changes in its regulations about transgenic plants, publishing a draft environmental impact statement in 2007 (APHIS 2007a, APHIS 2007c), and draft rules for GE crops in 2008 (APHIS 2008c).

In the 2007 and 2008 GE regulatory proposals, APHIS has suggested a preferred alternative to regulate all GE organisms as noxious weeds, ensuring that all GE crops are subject to regulation. At present, APHIS only has authority to regulate GE crops with sequences derived from plant pests, or that are truly plant pests (e.g., a parasitic GE plant). In practice, however, all commercialized GE crops appear to have gone through USDA for approval. Although APHIS seems unlikely to treat every GE plant as equivalent to a noxious weed, it remains unclear what a change in regulatory coverage may mean for researchers, developers, markets, and public perception.

The 2000 Plant Protection Act (PPA) expanded APHIS authority over "noxious weeds" but APHIS has not yet determined what "other effects" it might assert responsibility to manage. Secretary Vilsack has promised to finalize regulations and issue them for public comment soon, since USDA did not meet the 18 month deadline set by Congress. Coexistence issues are at the top of USDA's agenda, given litigation stopping approval of two Roundup Ready® crops – sugar beets and alfalfa (Monsanto's Roundup Ready "RR" Alfalfa reached the US Supreme Court). This litigation could force USDA to consider, for any biotech chestnut tree, various indirect commingling "injury" or economic damage to alternative agriculture (organic or non-GMO) chestnut. This economic risk should not make a "noxious weed" of a biotech plant that has US Approval, but APHIS may soon have new regulatory authority to look beyond plant pest risks and agronomic impacts to "other effects" under the PPA. USDA is considering a number of changes in its regulations about transgenic plants, publishing a draft environmental impact statement in 2007[22;23] and draft rules for GE crops in 2008[24].

USDA's controversial decision to grant nationwide approval for RR Alfalfa in February 2011 was due to its perceived "limited authority" legally restricting its review to "plant pest" risks. Other pending litigation by CFS against GE eucalyptus trees challenges USDA to use the 2008 Farm Bill (which still has not been fully implemented by USDA) to expand its regulatory oversight to include

[21] Vinluan F. 2009. Genetically modified rice leads to ruling against Bayer CropScience. Triangle Business Journal. 7 December. (16 July 2010; http://greenbio.checkbiotech.org/news/genetically_modified_rice_leads_ ruling_against_bayer_cropscience)
[22] APHIS 2007. Programmatic Environmental Impact Statement. USDA APHIS. (25 June 2010; www.aphis.usda.gov/publications/biotechnology/content/printable_version/fs_programmatic_eis.pdf)
[23] AHPIS 2007. Introduction of organisms and products altered or produce through genetic engineering. Federal Register 72: 39021–39025.
[24] APHIS 2008. Proposed rules for the importation, interstate movement, and release into the environment of certain genetically engineered organisms. Federal Register 73: 60008–60048.

"other effects" of "noxious weeds" to put herbicide-resistant crops into tighter containment. This litigation is pressuring the USDA to issue new rules under the 2008 Farm Bill, which could expand its authority over "noxious weeds" to make approvals more cumbersome for many biotech crops.

USDA Secretary Vilsack spoke in early April to the Organic Trade Association policy conference, noting that the now-overdue Plant Protection Act regulations (which Congress wanted by 2010) would address hot-button issues as noxious weeds and economic harm from biotech crops commingling with organic or non-GMO crops. He assured the producers that USDA is in the process of finalizing the needed regulations in order to "properly evaluate biotech crops" and their potential impacts. USDA will not dictate "co-existence" rules but instead to get the "good, hard-working, fundamentally sound folks" on both sides of the biotech issue to agree on reasonable solutions among themselves. Moreover, he still supports a compensation fund for organic and NonGMO contamination claims as a possible option. The bottom line – plant pest-based regulatory limits are soon to be a thing of the past. This will mean more extensive review of economic impacts and perhaps some USDA victories on NEPA claims in the courts, with even more need for the third party review that USDA is seeking to test out.

The threat posed by NEPA litigation and nationwide injunctions stopping sale (which occurred to Roundup Ready ("RR") Alfalfa in 2007) is among the biggest potential barriers to entry for the pipeline of biotech American chestnuts and other biotech trees. In response to this threat, the biotech tree industry has begun to discuss with USDA the concept of using industry-funded third party consultants to conduct the USDA-required environmental assessments or more detailed "environmental impact statements" ("EIS"). USDA does not have the resources to conduct these EA's and court-ordered EIS reviews (as occurred with RR Alfalfa and RR Beets under court orders now on appeal, one to the Supreme Court). With third-party review, USDA may open its bottleneck in regulatory approval while simultaneously addressing the threat of NEPA injunctions. In another effort to stem the tide of NEPA litigation that is extending US biotech approvals into multi-year messes, the APHIS biotech unit has asked for volunteers for a NEPA pilot project that will attempt to improve EIS handling, increasing improve the quality, timeliness, and cost effectiveness of NEPA environmental impact reviews. See, Solicitation of Letters of Interest To Participate in National Environmental Policy Act Pilot Project**,** 76 Federal Register 19309-19310, April 7, 2011 at www.edocket.access.gpo.gov/2011/2011-8329.htm. APHIS has asked for volunteers for a NEPA pilot project involving non-USDA consultants to conduct regulatory environmental reviews, including any court-ordered Environmental Impact Statement ("EIS") that will lead to faster approvals. While larger companies can afford to pay this for faster approval, smaller companies consider it a cost that they can ill afford. As a result, this pilot may not lead to changes in USDA policy.

In the case of nationwide approval for planting biotech trees, the USDA may need policy support from the regulatory framework that already exists to conduct third party review, mainly occurring to date under the USDA Forest Service, which has been subject to EIS requirements under NEPA for decades on a localized project basis. The industry approach is likely to seek a "pilot" or trial period to determine the viability of this process – biotech trees could be in such a pilot. This proposed approach invites comparison to the Food and Drug Administration's use of third-party reviewers for medical devices, which succeeded and broke a logjam in approvals in the 1990's (with some notable rollbacks that merit study to avoid similar problems with a biotech tree pilot program).

Potential Impact of Existing and Near-Term Case Law on Biotechnology Research

There are two types of cases pending that will determine how biotech seed companies need to manage the risks of causing economic loss to non-biotech growers. The first is a series of federal lawsuits, starting with *Geertson v. USDA,* under the National Environmental Policy Act (NEPA), with anti-biotech activists and organic growers suing to challenge USDA policy of conducting relatively quick environmental assessments (with a finding of no significant impact) rather than the multi-year environmental impact statements that California federal courts have ordered. The second involves the common law liability of biotech seed companies, in a jury trial underway in

St. Louis federal court (*In re LL601 Rice Contamination),* for experimental rice that commingled, prior to US approval, with the foundation seed used in rice production throughout the US, causing loss of the European Union market for export-oriented growers. The LLRice 601 case is costly civil liability arising from unauthorized releases from biotech rice field trials that led to comingling of research genes with the commercial seed supply, and this billion-dollar liability is likely to cause reexamination of the the risks of many cooperative breeding programs between biotechnology companies and research universities.

A. Under Geertson, Federal Law Mandates Segregation of Biotech Crops

In the first case addressing agricultural biotechnology's environmental impacts, the Supreme Court in *Geertson v. USDA*[25] ruled 7-1 on June 22, 2010 to reverse a three-year-old injunction against planting Monsanto's Roundup Ready™ alfalfa ("RR Alfalfa "). This ban was granted in 2007 on a preliminary injunction motion by California District Court Judge Charles Breyer (the brother of the environmental regulation scholar, Justice Breyer, who recused himself) effectively halting further planting of Monsanto's Roundup Ready™ alfalfa.

First, the good news for the biotech seed industry: USDA is freed from the nationwide injunction. Second, the Supreme Court found USDA's granting nationwide approval to RR Alfalfa may have been overly broad, given impacts to organic and "non-GMO" crops. While RR Alfalfa is unchained, USDA has to find a way to change the nationwide launch of biotech crops that it approves (wherever there is a legally recognized risk of an economic impact to organic, non-GMO or export-oriented crops.) A Monsanto representative hailed the decision as "exceptionally good news" that would allow farmers to plant the crop in the coming season, which would presumably include late-season planting in 2010. The Center for Food Safety, which is filing NEPA cases (including the pending RR Beets and Eucalyptus cases noted below) warn that the ruling still makes it illegal for farmers to use the seed until the USDA EIS is out. USDA just closed comment on the EIS which will be out in early 2011.

Second, Justice Samuel Alito wrote that while the court went too far in issuing a nationwide ban on the seeds, the court correctly ruled in sending the deregulation of the crop back to the Agriculture Department to conduct an environmental impact study. While "contamination" of other crops must be avoided, the choice of remedy was too drastic given USDA's proposed judgment.

The future of USDA containment of contamination may lie in its proposed "partial deregulation" that would have kept RR Alfalfa contained using the following mandates:

1. Isolation distances between RRA and other alfalfa to avoid gene flow;
2. Harvesting conditions;
3. Steam-cleaned planting and harvesting equipment after RRA and before further use with other alfalfa;
4. Identification and handling (i.e., traceability) for RRA seed;
5. RRA grower contracts requiring compliance with all other limitations set out in the proposed judgment.

A partial release pending EIS review would have prevented the injury to organic and non-GMO farmers, and the Supreme Court found that this fact was conceded by plaintiffs; hence the District Court should have remanded the matter "to the [USDA] so that it could determine whether to pursue a partial deregulation during the pendency of the EIS process." *Geertson* at 4. If USDA had been allowed to establish a regional approach, it could have found the middle path and removed the threat of harm that the plaintiff-respondents feared. (If USDA's approach failed to address the risk, it can expect another lawsuit challenging that partial deregulation decision).

[25] http://en.wikipedia.org/wiki/Monsanto_Co._v._Geertson_Seed_Farms

The Supreme Court sent a message to both USDA and biotech seed companies, however, when it rejected Monsanto and other petitioners argument[26] that protection against the risk of commercial harm was not "an interest that NEPA was enacted to address." The Supreme Court held that the uncontested fact that there was a "risk that the RRA gene conferring glyphosate resistance will infect conventional and organic alfalfa" was a "significant" impact worth protecting from harm. Moreover, Monsanto's hope to plant RR alfalfa in Fall 2010 might run afoul of the order that no RR alfalfa "can be grown or sold until such time as a new deregulation decision is in place."*Geertson* at 22.

In sum, this historic Supreme Court ruling shunned the blunt-object nationwide approaches that both the District Court and USDA took to complex agricultural management and coexistence questions. USDA will have to manage the interrelated economic and environmental impacts of biotech crops better; the federal courts may be called upon to determine if a partial deregulation is adequate.

Geertson will influence two NEPA cases pending against "GMOs". First, in a hearing to be held in August 2010, USDA and Monsanto will seek to prevent another nationwide injunction (against RR Beets, after a summary judgment requiring an EIS). The judge denied a previous preliminary injunction motion due to excessive delay in bringing the motion, since over 90% of US acres are planted in RR Beets. Briefing filed on July 9 by plaintiffs suggested that they would like to see a partial deregulation from USDA that protected the interests they represent – but they reserve the legal right to challenge such decisions.

The second NEPA case was filed promptly on July 1, 2010 in Florida U.S. District Court (by the same activist groups who delayed with RR beets) suing USDA for its approval of Arborgen's biotech eucalyptus in *Center for Biological Diversity v. U.S. Department of Agriculture*, U.S. District Court, Southern District of Florida (West Palm Beach)[27].

Both judges should follow the Geertson lead and the case to USDA for partial deregulation pending completion of the EIS. With more limited launches and legal challenges to EIS findings ahead, however, innovation in biotech crops may suffer over the coming decade. For biotech trees, the increased level of regulatory oversight – now confirmed by the US Supreme Court – will provide innovators with a clearer path to market. If USDA takes its new mandate seriously, its partial deregulations will include plans for peaceful coexistence without litigation over economic impacts. This could lead to a friendlier legal environment for biotech trees.

B. Bayer Mass Tort Trials Add up to Billions in Liability Risk

In the second landmark case, *In re LL601 Rice Contamination*, Bayer Crop Sciences is defending nuisance and negligence claims for an illegal release of experimental herbicide-resistant Liberty Link® rice that commingled in export-bound rice in 2006-2007, causing rice prices to drop. The jury trials of test plaintiffs (from a growing pool of 6,000) started in December 2009, continuing through this year. Plaintiffs prevailed in four trials, with juries finding Bayer Cropsciences negligent in allowing its Louisiana-based field trials of herbicide-resistant Liberty Link rice to be too close to the foundation seed used in US rice production. While the planting distances that the Louisiana State researcher used were adequate, commingling occurred during post-harvest handling, according to plaintiffs' experts on identity preservation.

Bayer has lost a series of trials, and the statute of limitations of five years has not yet run, so more cases may be filed. Bayer is facing compensatory damage awards estimated at over $4 billion, if 6,000 farmers recover similar amounts (verdicts are averaging over $550,000 in compensatory damages). Most notably, an Arkansas jury awarded Arkansas farmers $48 million in punitive damages on April 14, 2010 in the fourth trial. Bayer may feel compelled to appeal this case to higher courts – potentially making legal precedent that will influence future cases.

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[26] *Bennett v. Spear*, 520 U. S. 154, 162–163 (1997)

[27] http://www.biologicaldiversity.org/news/center/articles/2010/businessweek-07-01-2010.html

Like the Supreme Court, these jurors are seeing economic damage to other crops as a problem that biotech seed companies should have paid more attention to in years past. The future of biotech crops and the companies that sell them will depend upon continuous improvement in stewardship strategies that protect export, non-GMO and organic interests from undue economic impact.

C. Will State Common Law Nuisance Evolve in Response to Geertson?

US courts have yet to rule that the sale of USDA-approved biotech crops that lack approval in major markets overseas is common law nuisance or negligence, where there is a prevailing standard of care that requires biotech seed companies to avoid export impact. For example, the American Soybean Association has long required, as a matter of "due care" in stewardship, that biotech seed companies obtain export approvals in all major overseas markets before commercial launch. Any biotech soybean lacking this approval has to be produced in closed-loop identity preservation (similar to the USDA proposed "partial deregulation").

Moreover The Supreme Court's finding of "contamination" under NEPA could influence common law rulings. For example, this finding could also influence some courts to require that biotech crops be "fenced-in" in regions that depend on exports or non-GMO/organic markets, as has occurred with livestock in the East. See, A. Bryan Endres, **Coexistence Strategies, the Common Law of Biotechnology and Economic Liability Risks**, 13 Drake J. Agric. L. 115-148 (Spring 2008).

The preemptive or presumptive power of US approval under new partial deregulation decisions remains to be determined. Will evidence of compliance with federally-mandated identity preservation provide a defense for biotech seed companies? For example, if USDA implements a partial deregulation approach for a controversial new biotech corn from Syngenta that contains amylase, this Environmental Assessment Finding of No Significant Impact (EA-FONSI) might survive legal challenges. (See, Environmental Assessment for Syngenta Event 3272, available at www.aphis.usda.gov/brs/aphisdocs/05_28001p_ea.pdf *).* This may also be sufficiently protective of economic impacts to avoid common law nuisance liability.

D. Conclusion

Going forward, both USDA and biotech seed companies will need to monitor and prevent economic impacts, even after regulatory approval. USDA assessments of environmental impacts must include relevant economic interests, and maintain peaceful coexistence between and among biotech, non-GMO and organics. In avoiding new commingling episodes, they will prevent nuisance liability for the seed companies selling those biotech crops. As biotech trees, including the American Chestnut, enter the marketplace and environment in the coming decade, their economic impacts will be assessed and they will find their proper place.

Acronyms and Definitions

EA An acronym for 'Environmental Assessment' that is a NEPA compliance document used to determine if an action would have a significant effect on the human environment. If not, a finding of no significant impact (FONSI) is written. If so, an environmental impact statement (EIS) is written. Note that an EA usually requires less time and resources than completing an Environmental Impact Statement (EIS).[28]

EIS An acronym for 'Environmental Impact Statement' that is A NEPA compliance document used to evaluate a range of alternatives when solving the problem would have a significant effect on the human environment. The EIS is more than a document, it is a formal analysis process which mandates public comment periods. An EIS covers purpose and need, alternatives, existing conditions, environmental consequences, and consultation and coordination. Note that an EIS usually requires more time and resources than completing an Environmental Assessment (EA).[29]

NEPA An acronym for 'National Environmental Policy Act' of 1969 (42 USC § 4331). The NEPA process begins when an agency proposes to take an action (this can include proposals to adopt: rules and regulations; formal plans that direct future actions; program; and specific projects - see 40 C.F.R. § 1508.18). Once the proposal is conceptualized and any reasonable alternatives have been developed, the agency must determine if the action has the potential to affect the quality of the human environment. This process results in one of three levels of NEPA analysis. Agencies may:

a. apply a Categorical Exclusion;
b. prepare an Environmental Assessment (EA); or
c. prepare an Environmental Impact Statement (EIS).[30]

[28] http://www.usbr.gov/library/glossary
[29] http://www.usbr.gov/library/glossary
[30] http://www.nepa.gov

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