NATIONAL ENVIRONMENTAL POLICY ACT DECISION
AND
FINDING OF NO SIGNIFICANT IMPACT

Permit applications 08-011-106rm and 08-014-101rm
received from ArborGen LLC

Field testing of genetically engineered
Eucalyptus grandis* X *Eucalyptus urophylla

United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services

United States Department of Agriculture (USDA) and the Animal and Plant Health Inspection Service (APHIS), have developed a decision document to comply with the requirements of the National Environmental Policy Act of 1969, as amended, the Council of Environmental Quality's (CEQ) regulations implementing NEPA, and the USDA and APHIS' NEPA implementing regulations and procedures. This NEPA decision document is intended to state APHIS' NEPA decision and present the rationale for its selection.

In accordance with APHIS procedures implementing the NEPA Regulations (7 CFR part 372), APHIS has prepared an Environmental Assessment (EA) to evaluate and determine if there are any potentially significant impacts to the human environment in response to confined environmental release permit applications (APHIS Number 08-011-106rm and 08-014-101rm) received from ArborGen LLC (ArborGen) to continue research on genetically engineered (transgenic) *Eucalyptus* trees currently permitted by APHIS, to allow the planting of additional trees, and to allow genetically engineered (GE) *Eucalyptus* trees to flower at confined field site locations. These plants are a clone[1] coded EH1 derived from a hybrid of *Eucalyptus grandis X Eucalyptus urophylla* and have been genetically engineered with different constructs. The purpose of the confined environmental release is for ArborGen to assess the effectiveness of gene constructs which are intended to confer cold tolerance; to test the efficacy of a gene introduced to alter lignin biosynthesis; and to test the efficacy of a gene designed to alter fertility. In addition the trees have been engineered with a selectable marker gene which confers resistance to the antibiotic kanamycin.

The EA assesses alternatives to issuing two confined environmental release permits to allow the planting, field testing and flowering of a GE *Eucalyptus* hybrid clone engineered to express various genes on 28 confined field site locations in the southeastern United States and analyzes the potential environmental and social effects that result from the proposed action and the alternatives. The proposed action of USDA APHIS, Biotechnology Regulatory Services (BRS) is to issue the APHIS confined field release

[1] Clone – as defined in horticulture and forestry means is a *population* of genetically identical plants that has been derived from one individual. Despite popular use of the word, a clone is not an individual.

permits for a hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla* with supplemental permit conditions (Appendix V of the EA) for the requested three-year period in accordance with 7 CFR part 340. Comments from the public involvement process were reviewed for substantive issues which were considered in developing this NEPA decision.

In 1986, the Federal Government's Office of Science and Technology Policy (OSTP) published a policy document known as the Coordinated Framework for the Regulation of Biotechnology. This document specifies three Federal agencies that are responsible for regulating biotechnology in the United States: USDA-APHIS, the U.S. Department of Health and Human Services' Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). Products are regulated according to their intended use and some products are regulated by more than one agency. USDA-APHIS, FDA, and EPA enforce agency-specific regulations on products of biotechnology that are based on the specific nature of each GE organism. Together, these agencies ensure that the products of modern biotechnology are safe to grow, safe to eat, and safe for the environment.

APHIS regulates GE organisms under the Plant Protection Act of 2000. USDA APHIS-BRS' mission is to protect America's agriculture and environment using a dynamic and science-based regulatory framework that allows for the safe development and use of GE organisms. APHIS regulations at 7 CFR part 340, which were promulgated pursuant to authority granted by the Plant Protection Act, as amended (7 United States Code (U.S.C.) 7701–7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is considered a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation (7 CFR § 340.2) and is also considered a plant pest. A GE organism is also regulated under part 340 when APHIS has reason to believe that the GE organism may be a plant pest or APHIS does not have sufficient information to determine if the GE organism is unlikely to pose a plant pest risk.

When APHIS receives an application for a permit for environmental release, the application is evaluated by APHIS-BRS to determine whether the environmental release, with appropriate conditions imposed, can be carried out while preventing the dissemination and establishment of plant pests. The receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

> *Administrative action on applications*. After receipt and review by APHIS of the application and the data submitted pursuant to paragraph (a) of this section, including any additional information requested by APHIS, a permit shall be granted or denied (7 CFR 340.4(e)).

The applicant has provided the required information associated with this request in the permit application. This information has been reviewed by APHIS-BRS and is analyzed in the EA.

FDA regulates under the authority of the Federal Food, Drug, and Cosmetic Act. The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992 (57 FR 22984-23005). Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. The EPA regulates plant-incorporated protectants under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and certain biological control organisms under the Toxic Substances Control Act (TSCA). Because this GE *Eucalyptus* hybrid is not used of food or feed purposes and does not contain any genetically engineered pesticides or tolerance to herbicides, FDA and EPA consultation is not required.

Public Involvement

In a notice published in the *Federal Register* (74 FR 26648-26649, Docket No. APHIS-2008-0059), APHIS announced the availability of an Environmental Assessment (EA) for public review and comment for a proposed controlled field release of a genetically engineered clone of a *Eucalyptus* hybrid. Comments on the environmental assessment were required to be received on or before July 6, 2009. Commenters noted that one of the documents cited in the environmental assessment, a U.S. Forest Service assessment of hydrological impacts from *Eucalyptus*, was not available for review. Subsequently, APHIS published a notice in the *Federal Register* (75 FR 2845, Docket No. APHIS-2008-0059) on January 19, 2010 announcing the availability of an amended EA, which included the U.S. Forest Service document, and extended the comment period for the environmental assessment an additional 30 days. APHIS also accepted comments received in the interim between the two *Federal Register* notices. There were 45 respondents that supported issuance of the permit; and 12,462 respondents who were opposed. All comments were analyzed to identify new issues, alternatives, or information. Responses to the substantive comments are included as an attachment to this Finding of No Significant Impact.

Major Issues Addressed in the EA

The EA describes the alternatives considered and evaluated using the identified issues. Issues considered in the EA were developed based on APHIS' determination to issue two confined environmental release permits to allow the planting, field testing and flowering of a GE *Eucalyptus* hybrid clone engineered to express various genes on 28 confined field site locations in the southeastern United States. The following issues were identified as important to the scope of the analysis (40 CFR 1508.25):

- Alteration in Susceptibility to Disease or Insects – Potential of the *Eucalyptus* to Harbor Plant Pests
- Expression of the Gene Products, New Enzymes, or Changes to Plant Metabolism - Risk of the Gene Products on the Environment

- Alteration in Weediness characteristics – Potential of the Engineered *Eucalyptus* to be Invasive
- Possibility of Gene Flow Within the Field Test
- Possibility of Gene Flow Outside of the Field Test
- Possibility of Vegetative Propagation / Persistence Outside of the Field Test
- Potential of the *Eucalyptus* in the Field Tests to Become an Invasive Species that Threatens Native Plant and Animal Communities
- Impact on Existing Agricultural Practices
- Potential Impacts to Wildlife
- Potential Impacts by Fire
- Potential Impacts to Human Health
- Transfer of Genetic Information to Organisms with which it Cannot Interbreed - Horizontal Gene Transfer to Other Organisms
- Potential Effects of Growing *Eucalyptus* on Soil Hydrology
- Potential Allelopathic Effects of *Eucalyptus*
- Risks to Threatened and Endangered Species

Affected Environment:
The confined field tests would take place on land controlled by ArborGen or through contracts for field testing. The exact locations are claimed as Confidential Business Information (CBI) and have been submitted as part of the APHIS permit application. Under the two permits, there are 28 research study sites indentified throughout Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Texas where trees have been planted or will be planted, and on 27 of these sites the trees will be allowed to flower (see EA for specific information on each of these 28 sites).

All the confined field test sites listed in the permit application are either on privately owned managed plantation forests and agricultural farm lands or experimental research stations managed by academic institutions and industry. The standard agricultural and silvicultural practices for land preparation, planting, irrigation, and harvesting of plants have been routinely used on these sites. Sites that include managed pastures have had intense activity including the use of heavy machinery for general upkeep, irrigation, fertilization, controlled grazing and management of grasses. Standard silvicultural practices will be used at these sites for the duration of the field tests. Surveys conducted by the applicant at each of these locations indicate that there are not any old growth forests or undisturbed natural areas in the immediate surroundings of the test sites. Depending on the location, the trees will be planted on sites ranging from 0.5 up to 20 acres in size at a planting density of 300 - 600 trees per acre[2].

Alternatives that were fully analyzed:

[2] Planting density typically refers to the number of trees per acre. Planting densities can vary greatly depending upon the tree species and the environment, but densities of short rotation hardwood trees in the southeastern US are typically in the range of 300–800 trees per acre. Therefore sites ranging from 10 to 20 acres can have from 3000 to 16,000 total trees planted in the ground. Twenty acres, as defined by forest plantation standards in the southeast, is considered a small planting.

The EA analyzes the potential environmental consequences of a proposal for APHIS to issue two confined environmental release permits to ArborGen to allow the planting, field testing and flowering of a GE *Eucalyptus* hybrid clone engineered to express various genes on 28 confined field site locations in the southeastern United States. Based upon the permit application submitted by ArborGen, two alternatives are considered and analyzed in the EA: (1) deny the permit and (2) issue the APHIS permit.

Alternative A: No Action – Deny the Permit

Under APHIS–BRS regulations, the Administrator must either grant or deny permits properly submitted under 7 CFR part 340. For the purposes of this EA, the No Action alternative would be the denial of permit applications 08-011-106rm and 08-014-101rm.

Transgenic *Eucalyptus* trees (hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla*) have been previously approved for planting under APHIS authorized notifications, and permits. ArborGen has been allowed to plant trees on 28 sites under permit 08-039-102rm that are requested under the two new permit applications to allow flowering; and two field test sites totaling 7.6 acres have been authorized by APHIS to flower under permits 06-325-111r and 08-151-101r. All three permits are still active. Under the No Action Alternative, the transgenic *Eucalyptus* plants (hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla*) currently approved by APHIS for confined field release under permit 08-039-102rm will continue to not be allowed to flower. The trees could remain in the field, but the applicant will be required to either remove developing flowers or remove the trees from the field test if removing flowers becomes too difficult. Under this alternative, the applicant would not be allowed to gather data on performance of the transgenic trees over a multiyear period and the efficacy of the genes in a wide variety of environments. (Permits 06-325-111r (as amended) and 08-151-101r , both of which already allow flowering, will remain in effect until they expire (unless they are renewed under a new permit). These two permits are not affected by the issuance of permits 08-014-101rm and 08-011-106rm.)

Alternative B: Preferred Alternative – Issue the APHIS Permit

The APHIS-preferred alternative is to issue the APHIS confined field release permits for a hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla* with supplemental permit conditions (Appendix V) for the requested three-year period. The permits will need to be renewed and subsequently approved by APHIS to allow the transgenic plants to remain in the ground beyond the 3 year time period. Under this alternative, APHIS would issue two confined environmental release permits to ArborGen to allow the planting, field testing and flowering of a GE *Eucalyptus* hybrid clone engineered to express various genes on 28 confined field site locations in the southeastern United States. Specifically, these two permits would authorize ArborGen to continue research on GE *Eucalyptus* hybrid trees originally planted under APHIS authorized notifications, to plant additional trees, and to allow all the trees except the trees on one of the sites in South Carolina to flower. Under this alternative, GE *Eucalyptus* hybrid trees would be allowed to grow and flower where the applicant can gather data on performance of the transgenic trees over a multiyear period and the efficacy of the genes in a wide variety of environments. This

alternative would allow the safe development and use of GE organisms under the mission of BRS.

Environmental Consequences of APHIS' Selected Action

The EA contains a full analysis of the alternatives to which we refer the reader for specific details. The following table briefly summarizes the results for each of the issues fully analyzed in the Environmental Consequences section of the EA.

Table 1.

Issue	***Alternative A No Action Deny the Permit***	***Alternative B Preferred Alternative Issue APHIS Permit***
Alteration in Susceptibility to Disease or Insects – Potential of the *Eucalyptus* to Harbor Plant Pests	No increased risk	No increased risk
Expression of the Gene Products, New Enzymes, or Changes to Plant Metabolism - Risk of the Gene Products on the Environment	No expected risk	No expected risk
Alteration in Weediness characteristics – Potential of the Engineered *Eucalyptus* to be Invasive	None	Not likely
Possibility of Gene Flow within the Field Test	None	Minimal
Possibility of Gene Flow Outside of the Field Test	None	Minimal
Possibility of Vegetative Propagation / Persistence Outside of the Field Test	Not likely	Not likely
Potential of the *Eucalyptus* in the Field Tests to Become an Invasive Species that Threatens Native Plant and Animal Communities	None	Not likely
Impact on Existing Agricultural Practices	No change	No change
Potential Impacts to Wildlife	No adverse impacts	No adverse impacts
Potential Impacts by Fire	Minimal risk	Minimal risk
Potential Impacts to Human Health	Negligible	Negligible
Transfer of Genetic Information to Organisms with which it Cannot Interbreed - Horizontal Gene Transfer to Other Organisms	No risk	No risk
Potential Effects of Growing *Eucalyptus* on Soil Hydrology	No negative impacts	No negative impacts
Potential Allelopathic Effects of *Eucalyptus*	Minimal	Minimal
Risks to Threatened and Endangered	No effect	No effect

Species		

Finding of No Significant Impact

The analysis in the EA indicates that there will not be a significant impact, individually or cumulatively, on the quality of the human environment as a result of this proposed action. I agree with this conclusion and therefore find that an EIS need not be prepared. This NEPA determination is based on the following context and intensity factors (40 CFR 1508.27):

Context – The term "context" recognizes potentially affected resources, as well as the location and setting in which the environmental impact would occur. This action would be limited to 28 well dispersed confined field site locations in the southeastern United States and has limited potential to affect resources outside of the confined field test sites. Supplemental permit conditions (*see* Appendix V of the EA) established for these permits will effectively limit the reproductive capabilities and establishment of this GE *Eucalyptus* outside the confined field trial locations.

Intensity – Intensity is a measure of the degree or severity of an impact based upon the ten factors. The following factors were used as a basis for this decision*:*

1. *Impacts that may be both beneficial and adverse.*
   According to the applicant, genetically engineered cold tolerant *Eucalyptus* would enable the production of this hardwood species for pulping and for biofuel applications in managed plantation forests in the southeastern U.S. APHIS issuance of these confined field release permits would allow ArborGen to conduct research to assess the efficacy of the introduced cold tolerance genes and gene to alter lignin biosynthesis in *Eucalyptus* and to research mechanisms for altered fertility. The confined release of the trees in 28 different areas of the southeast U.S. will allow the applicant to obtain data on performance of the transgenic trees and the efficacy of the inserted genes in a wide variety of environments. The establishment and growth of these small confined field tests will not have any impact on existing agricultural practices because they are solely for research purposes. Current agricultural practices will essentially remain unchanged. As indentified by the applicant, the field sites that are being proposed under these permits have been used as forest tree plantations, pastures, or for forestry and agriculture research and are specifically designed for field testing crop plants or forest trees.
2. *The degree to which the proposed action affects public health or safety.*
   The proposed action to issue the APHIS confined field release permits should not pose an unnecessary risk to human health and therefore would have no significant impacts on human health. This GE *Eucalyptus* hybrid is not used of food or feed purposes and does not contain any genetically engineered pesticides or tolerance to herbicides. As described in Section V of the EA, the risk that these field trials will result in a higher incidence a fungal pathogen in the U.S. and thereby pose a risk to human health is considered to be negligible.

3. *Unique characteristics of the geographic area such as proximity to historic or cultural resources, park lands, prime farmlands, wetlands, wild and scenic rivers, or ecologically critical areas.*

There are no unique characteristics of geographic area such as park lands, prime farm lands, wetlands, wild and scenic areas, or ecologically critical areas that would be significantly affected. All the confined field test sites listed in the permit application are either on privately owned managed plantation forests and agricultural farm lands or experimental research stations managed by academic institutions and industry. The standard agricultural and silvicultural practices for land preparation, planting, irrigation, and harvesting of plants have been routinely used on these sites. Surveys conducted by the applicant at each of these locations indicate that there are not any old growth forests or undisturbed natural areas in the immediate surroundings of the test sites. In addition, supplemental permit conditions (*see* Appendix V of the EA) established for these permits will effectively limit the reproductive capabilities and establishment of this GE *Eucalyptus* outside the confined field trial locations.

4. *The degree to which the effects on the quality of the human environment are likely to be highly controversial.*

The effects on the quality of the human environment are not highly controversial. Although there is some opposition to APHIS issuing these confined field release permits, this action is not highly controversial in terms of size, nature or effect. This action would be limited to 28 well dispersed confined field site locations in the southeastern United States on sites ranging from 0.5 up to 20 acres in size. The public comments did not register any specific factual concerns with the data provided APHIS for this permit application and which were presented in the EA.

5. *The degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risks.*

The effects of the proposed action to issue the APHIS confined field release permits are not highly uncertain and do not involve unique or unknown risks. Based on the analysis documented in the EA, the effects on the human environment would not be significant. APHIS has no evidence for any unknown risks of this GE hybrid plant species when released for confined field testing. This GE *Eucalyptus* hybrid has been previously released for confined field testing under APHIS issued notifications and permits in similar geographic locations without any known adverse affects. As described in Section V of the EA and response to comments, the issuance of these confined field release permits to allow the applicant to plant additional trees and to allow all the trees to flower does not present any unforeseen risks. Adherence to the supplemental permit conditions established for these permits by the applicant will effectively limit any potential adverse impacts to the human environment.

6. *The degree to which the action may establish a precedent for future actions with significant effects or represents a decision in principle about a future consideration.*

The proposed action would not establish a precedent for future actions with significant effects or represent a decision in principle about a future decision. Similar to past permit applications reviewed and approved by APHIS, the

decision on whether or not to issue a permit for confined environmental release will be based upon information provided in the applicant. APHIS regulations at 7 CFR part 340, regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. In accordance with these regulations when APHIS receives an application for a permit for environmental release, the application is evaluated to determine whether the environmental release, with appropriate conditions imposed, can be carried out while preventing the dissemination and establishment of plant pests. The applicant has provided the information associated with this request in the permit application and APHIS now must make a determination to either grant or deny the permits. Each permit application that APHIS receives undergoes this independent review to determine if APHIS should grant or deny the individual permit.

7. *Whether the action is related to other actions with individually insignificant but cumulatively significant impacts.*

No significant cumulative effects were identified through this assessment. As discussed in the cumulative effects analysis presented in Section V of the EA, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create cumulative impacts or reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.) associated with the release sites or the ecosystem in which they are situated. No resources will be significantly impacted due to cumulative impacts resulting from the proposed action.

8. *The degree to which the action may adversely affect districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places or may cause loss or destruction of significant scientific, cultural, or historical resources.*

The issuance of these confined field release permits would have no impact on districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places, nor would they likely cause any loss or destruction of significant scientific, cultural, or historical resources. All the confined field test sites listed in the permit application are either on privately owned managed plantation forests and agricultural farm lands or experimental research stations managed by academic institutions and industry. The standard agricultural and silvicultural practices for land preparation, planting, irrigation, and harvesting of plants have been routinely used on these sites. Similar types of agricultural and silvicultural practices would be used by the applicant for managing trees associated with these permits. In addition, supplemental permit conditions (*see* Appendix V of the EA) established for these permits will effectively limit the reproductive capabilities and establishment of this GE *Eucalyptus* hybrid outside the confined field trial locations.

9. *The degree to which the action may adversely affect an endangered or threatened species or its habitat that has been determined to be critical under the Endangered Species Act of 1973.*

APHIS evaluated the potential for negative effects on federal threatened and endangered species as listed by the U.S. Fish and Wildlife Service from the issuance of these confined field release permits and determined that the confined environmental release of this GE *Eucalyptus* hybrid at the 28 test site locations indentified in the permit application would have no effect on federally listed threatened or endangered species or species proposed for listing, or on designated critical habitat or habitat proposed for designation (*see* section on Threatened and Endangered Species in Section V and Appendix IV of the EA).

10. *Whether the action threatens a violation of Federal, State, or local law or requirements imposed for the protection of the environment.*
The proposed action would be in compliance with all federal, state, and local laws. The proposed action to issue the APHIS confined field release permits would be carried out in accordance with 7 CFR part 340. This GE *Eucalyptus* hybrid is not used of food or feed purposes and does not contain any genetically engineered pesticides or tolerance to herbicides, thus FDA and EPA consultation is not required. There are no other Federal, state, or local permits that are needed prior to the implementation of this action.

NEPA Decision and Rationale

I have carefully reviewed the EA prepared for this NEPA determination and the input from the public involvement process. I believe that the issues identified in the EA are best addressed by selecting Alternative B – Issue the APHIS Permit.

As stated in the CEQ regulations, "the agency's preferred alternative is the alternative which the agency believes would fulfill its statutory mission and responsibilities, giving consideration to economic, environmental, technical and other factors." The preferred alternative has been selected for implementation based on consideration of a number of environmental, regulatory, and social factors. Based upon our evaluation and analysis, Alternative B is selected because (1) it allows APHIS to fulfill its statutory mission to protect America's agriculture and environment using a dynamic and science-based regulatory framework that allows for the safe development and use of genetically engineered organisms; and (2) it allows APHIS to fulfill its regulatory obligations. Therefore, it is my decision to implement the preferred alternative as described in the EA. Based on all of the analysis and reasons above, I have determined that there would be no significant impact to the quality of the human environment from the implementation of the chosen alternative (Alternative B) and therefore, no EIS needs to be prepared.

Michael C. Gregoire — MAY 12 2010

Michael C. Gregoire
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Services
U.S. Department of Agriculture

Date

Attachment
Finding of no significant impact
Response to comments
APHIS Nos. 08-011-106rm & 08-014-101rm

In a notice published in the *Federal Register* (74 FR 26648-26649, Docket No. APHIS-2008-0059), APHIS announced the availability of an Environmental Assessment (EA) for public review and comment for a proposed controlled field release of a genetically engineered clone of a *Eucalyptus* hybrid. Comments on the environmental assessment were required to be received on or before July 6, 2009. Commenters noted that one of the documents cited in the environmental assessment, a U.S. Forest Service assessment of hydrological impacts from *Eucalyptus*, was not available for review. Subsequently, APHIS published a notice in the *Federal Register* (75 FR 2845, Docket No. APHIS-2008-0059) on January 19, 2010 announcing the availability of an amended EA, which included the U.S. Forest Service document, and extended the comment period for the environmental assessment an additional 30 days. APHIS also accepted comments received in the interim between the two *Federal Register* notices. There were 45 respondents that supported issuance of the permit; and 12,462 respondents who were opposed.

Respondents supporting APHIS' issuance of these permits came from foresters, paper and packaging companies, or from related industries, academia, agricultural biotech companies, a State government, and individuals. The majority of these respondents cited meeting biomass needs for energy production in accordance with the Energy Independence and Security Act (2007); this *Eucalyptus* hybrid shows little potential for invasiveness, particularly given the small scale of the respective trial locations; there are no non-transgenic trees within the vicinity of the trial locations with which these *Eucalyptus* hybrids could cross breed; the hydrological impacts of these trials are negligible; the inserted genes do not encode exotic, or otherwise uncharacterized novel proteins; and there is no evidence that otherwise suggests that this permit would have a significant impact on the environment.

Respondents opposing APHIS' issuance of these permits came from 21 non-governmental organizations (NGOs), academia, and individuals. The majority of respondents opposing came from 2 NGOs, one of whom submitted 9,525 nearly identical form letters, and another who submitted 959 nearly identical form letters, and a petition bearing 1584 signatories. The majority of respondents opposed believe that APHIS failed to comprehensively analyze all relevant issues related to these proposed field trials, and that an Environmental Impact Statement should have been prepared; that there is great potential for these *Eucalyptus* hybrids to become invasive or cross breed with non-transgenic *Eucalyptus* trees; *Eucalyptus* could be a host for *Cryptococcus neoformans gatti*, a fungi that could potentially cause mycoses in humans and animals; that *Eucalyptus* plantations have been documented to deplete ground water and cause or exacerbate drought situations; *Eucalyptus* trees are known to be an incendiary risk and tend to burn very hot during forest fires, and are likely to occur in the drought prone southeastern U.S.; and there are naturally cold-tolerant *Eucalyptus* already in the U.S. and

no need for transgenic varieties. APHIS' responses to these and all relevant comments submitted are as follows:

APHIS failed to assess the potential impacts of APHIS' deregulation of GE *Eucalyptus* hybrid trees.

Response: The scope of this EA is to analyze the potential impacts to the human environment associated with two permit applications submitted by ArborGen to APHIS for the confined field release of GE *Eucalyptus* hybrid trees. The purpose of this EA is not to analyze the potential impacts of APHIS' deregulation of GE *Eucalyptus* hybrid trees and the issues and impacts associated with large scale deployment as a consequence of deregulation. Comments associated with the deregulation and large scale deployment GE *Eucalyptus* hybrid trees were determined not to be germane and outside the scope of analysis for this EA and therefore, are not specifically addressed in the EA or this response to comment document. APHIS will address issues associated with granting nonregulated status, if and when, APHIS publishes a risk assessment and associated NEPA document for the potential deregulation and large scale deployment GE *Eucalyptus* hybrid trees.

APHIS has failed to comply with mandates of the 2008 Farm Bill.

Response: APHIS has taken action to address Congressional mandates indentified in the 2008 Farm Bill. Specific action items identified in the 2008 Farm Bill are being addressed in APHIS' proposed revision to 7 CFR part 340. On October 9, 2008, APHIS published in the Federal Register (73FR 60007–60048, Docket No. APHIS–2008–0023) a proposal to revise APHIS regulations in 7 CFR part 340 regarding the importation, interstate movement, and environmental release of certain genetically engineered (GE) organisms. The proposed revisions would bring the regulations into alignment with authorities of the Plant Protection Act (7 U.S.C. 7701 *et seq*.) and update the regulations in response to advances in genetic science and technology and our accumulated experience in implementing the current regulations.

While BRS' current regulations have been effective in ensuring the safe introduction of GE organisms, the program is considering potential revisions to update its existing regulations in light of advances in science and technology and to reflect APHIS' experience by incorporating lessons learned. Revising our biotechnology regulations will better position us to address new challenges, as well as meet current needs in evaluating and addressing the plant pest or noxious weed risks associated with the importation, interstate movement, and field release of certain GE organisms. The proposed changes will also improve regulatory processes so they are more transparent to stakeholders and the public, make more efficient use of agency resources, and eliminate unnecessary regulatory burdens.

Currently, APHIS regulates GE organisms within its authority using a two-tiered system that includes a notification and a permit procedure. APHIS is now proposing to remove the notification procedure and instead rely exclusively on a permit procedure for the

importation, interstate movement, and environmental release of GE organisms subject to APHIS regulations. APHIS had originally developed the current notification procedure as an expedited permitting process for GE plants that it considered to be lower risk and that the agency had had extensive experience regulating in the past. Under the current two-tiered system, APHIS requires permits for regulated activities that are not eligible for notification. As currently used, permits are generally more restrictive than notifications and are used for any GE organism that is not a plant, as well as for GE plants that could pose an elevated risk to plant health, such as plants engineered to produce pharmaceutical or industrial compounds. For environmental releases, APHIS is proposing to establish an expanded, multiple category permitting system. For all permit applications, APHIS is providing greater detail in the proposed rule about the basic information requirements that would need to be addressed. Also under the proposed rule, permit conditions specific practices or requirements that an applicant must follow upon issuance of a permit would be listed in a new, separate section of the regulations. Additionally, the proposed regulations clarify the procedure APHIS would use to amend permit conditions, transfer a permit to a different responsible person, and revoke an existing permit.

In recent years, APHIS has strengthened its biotechnology program in order to improve compliance with the regulations, to augment the approaches used to prevent or remediate potential risks to plant health, and to use appropriate enforcement strategies. The proposed rule clarifies the obligation for compliance with regulatory requirements, as well as the approaches available to APHIS to verify compliance. The proposed rule includes requiring permit holders to establish and maintain records related to their permit, as well as requiring them to allow APHIS to review those records. This would improve APHIS' ability to conduct inspections and audit records related to regulated activities, and would also address specific biotechnology provisions in Section 10204 of Title X of the 2008 Farm Bill. The proposed regulations also outline the possible consequences for not complying with the regulations. These include: denial of future permits; revocation of current permits; destruction, treatment, and removal of GE organisms; and issuance of penalties. Additionally, the proposed regulatory changes outline a means to settle alleged civil violations prior to the issuance of an administrative complaint.

APHIS' current regulations have no explicit provisions for the low-level presence (LLP) of regulated materials when mixed into commercial crops, food, feed, or seed. In 2007, APHIS outlined its current LLP policy. The agency policy is to respond with actions appropriate to the level of risk, determined by a scientific assessment and warranted by the facts in each case. In cases in which the occurrence of GE plant material poses no risk to plant health and the environment, APHIS can decide not to take any remedial action in reference to the unauthorized release. APHIS also outlines possible enforcement actions in the proposed regulations to improve transparency regarding how the program would respond to LLP in most instances.

USDA is not adequately assessing the environmental impacts of their decision to issue confined release permits for GE *Eucalyptus* hybrid plants; an environmental impact assessment is needed.

Response: APHIS carefully considered the possible environmental impacts of the proposed action, and is satisfied that the EA prepared by APHIS for permit applications (APHIS Number 08-011-106rm and 08-014-101rm) received from ArborGen LLC is adequate and sufficient. The EA follows all applicable laws, regulations, and guidelines in analyzing potential impacts of this action, including those established by NEPA. In making an informed decision of potential environmental impacts, APHIS used the best available scientific information, data and expert advice.

APHIS has determined that the analysis in its EA showed no significant impact on the quality of the human environment if APHIS was to issue these APHIS confined field release permits and that APHIS did not have to prepare an environmental impact statement (EIS). The EA took a hard look at the need for action, the issues, alternatives, and environmental consequences.

APHIS also reviewed and carefully considered all comments submitted by respondents to the public involvement efforts. As a result of this analysis, APHIS prepared a final EA, from which came the NEPA decision document and a finding of no signification impact (FONSI) that discussed, under each of the ten Council for Environmental Quality (CEQ) points of significance, why each point was not significant, and why an EIS was not required. The agency followed CEQ NEPA regulations and Agency NEPA implementing procedures.

The EA does not assess the potential environmental impacts associated with the movement of GE *Eucalyptus* trees.

Response: APHIS' regulations in 7 CFR part 340 regulate the importation and interstate movement of certain GE organisms and products. The Plant Protection Act directs the USDA to facilitate imports and interstate commerce in agricultural products in ways that will reduce, to the extent practicable, the risk of dissemination of plant pests. In this specific circumstance, the movement of GE *Eucalyptus* hybrid plants that will be used in the proposed confined field trials has not been included in this EA because these types of movements have either already been approved previously by APHIS under a separate permit or notification or will be approved by APHIS prior to interstate movement or importation occurring in the future. In either case the appropriate level of NEPA documentation has been completed or will be completed prior to the movement action taking place.

In accordance with APHIS NEPA implementing regulations (7 CFR part 372) these types of APHIS regulated movements are categorically excluded from additional NEPA analysis because they have been determined not to individually or cumulatively have a significant effect on the quality of the human environment. Each movement application that APHIS receives is carefully reviewed and analyzed to ensure the appropriate level of NEPA analysis is completed prior to the issuance of the movement notification or permit. If at any time during this review process, APHIS determines that the action may have the potential to significantly affect the environment, APHIS will complete the necessary

NEPA analysis to make an informed decision on whether to issue or deny the specific movement request.

The EA does not provide a reasonable number of alternatives to the proposed action. APHIS should include alternatives that analyze potential environmental impacts on a site specific basis or with additional restrictions on space or time.

Response: APHIS has prepared this EA in response to confined environmental release permit applications (APHIS Number 08-011-106rm and 08-014-101rm) received from ArborGen LLC (ArborGen). These permit applications were submitted in accordance with APHIS' regulations in 7 CFR part 340. In accordance with these regulations when APHIS receives an application for a permit for environmental release, the application is evaluated to determine whether the environmental release, with appropriate conditions imposed, can be carried out while preventing the dissemination and establishment of plant pests. The receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

> *Administrative action on applications*. After receipt and review by APHIS of the application and the data submitted pursuant to paragraph (a) of this section, including any additional information requested by APHIS, a permit shall be granted or denied (7 CFR 340.4(e)).

The applicant has provided the information associated with this request in the permit application and APHIS now must make a determination to either grant or deny the permits. Since APHIS decision is to either grant or deny the permit applications, APHIS analysis of the two alternatives (No Action – Deny the permit and Preferred Alternative – Issue the APHIS Permit) identified in the EA are reasonable. The EA analyzes the potential cumulative effects of the proposed field test locations and has shown that the issuance of the permits will not have a significant impact on the human environment. Further analysis on a site by site basis or over a more restricted space or time will not provide any additional information or analysis that would be necessary to make an informed NEPA decision on the issuance of these permits.

Commenters were concerned about the potential invasiveness of the transgenic hybrid trees and were concerned that the trees would escape the field test and become invasive in the environment.

Response: As indicated in the EA, the local environment at each of the proposed field test sites is not conducive to *Eucalyptus* seed germination. *Eucalyptus* is very difficult to establish by seed (Bell and Williams 1997) (Meskimen and Francis 1990). For the establishment of plantations, if seeds are used, these seeds must be germinated under highly controlled environmental conditions. After seedlings are planted a great deal of human care is required to manage the trees so that they can survive and grow (Meskimen and Francis 1990). For this reason plantings are generally established using rooted stem cuttings since germination of seeds is difficult to achieve. As one commenter points out: "the seed from any *Eucalyptus* tree is tiny and competes very poorly with other plants

(this is why most *Eucalyptus* is grown vegetatively from "cuttings" under carefully managed conditions and is not moved into the field until it has a well established root system). Indeed, even when rooted plants are in the field, weed control is recommended for several months after planting to give the delicate young trees a chance to get established." (Oliver Ratcliffe, Mendel Biotechnology).

Therefore, APHIS concludes that the possible germination of seed and establishment of *Eucalyptus* hybrid seedlings and trees outside the confined field test sites is not likely to occur. If any seedlings were to grow outside these field test sites it would be obvious since at most of the proposed locations there are no other *Eucalyptus* trees in the area. In the few locations where there are other known *Eucalyptus* species plantings nearby, these sites are highly managed tests and commercial plantations. Any seedlings that may grow in these locations would be obvious and unusual because of management practices, seedlings are rare under Eucalyptus trees grown in plantation settings. These would be immediately identified and removed. Furthermore, in accordance with the supplemental permit conditions, monitoring for and removal of volunteers within 100m from the edge of transgenic test plot by the applicant would effectively eliminate any seedlings that may be produced and thereby effectively reduce the possibility that any *Eucalyptus* hybrid trees would become established outside the proposed field test sites.

Commenters indicated that there was a lack of conclusive data about the invasiveness of the E. grandis x E. urophylla's hybrid itself, and that APHIS provides no discussion about how and where the parent species have been tested for invasiveness, or where these species have been grown, and for how long.

Response: As APHIS has indicated in the EA and other commenters have pointed out, there are no documented reports of the *Eucalyptus* hybrid escaping plantations or being invasive where it has been grown in both Brazil and Florida. The *E. grandis* x *E. urophylla* hybrid has been grown for forty years in South America and during this time "there has been no evidence of invasiveness by *Eucalyptus* into natural forest areas which are growing as part of an integrated land management system." (Luis Silva, International Paper Company, Brazil - comment to the docket). There is no reason to believe that the *Eucalyptus* hybrid will perform any different from where it has been grown in other locations over many years. Regardless of what models of invasiveness may predict about one of the parents, the empirical data from years of growing the *Eucalyptus* hybrid in plantations support APHIS's conclusion. Given the monitoring requirements put in place for these permits through supplemental permit conditions, APHIS has determined that these are confined field tests and the likelihood of the *Eucalyptus* hybrid establishing outside of the confined field test sites is minimal to non-existent.

APHIS cites no data on the competitiveness of this hybrid with native flora in Alabama, nor any references in the scientific literature that supports its contention that these hybrids would not be competitive or invasive in Alabama if they were to survive the cold.

Response: In making an informed decision, APHIS uses the best available information in its assessment. The best available data are from commercial *Eucalyptus* hybrid plantings that have been conducted in Florida and South America and these data indicate that the hybrid and one of its parents (*E. grandis*) have not been found to be invasive in Florida (D.L, Rockwood, University of Florida, comment to the docket; L. Pearson, ArborGen pers. comm.) or Brazil (Luis Silva, International Paper Company, Brazil, comment to the docket). Indeed, the purpose of the proposed field tests is to determine if the cold tolerant *Eucalyptus* hybrid trees will produce viable seeds and if these trees are competitive in the local environment. To date, based upon field test data and permit reports provided by the applicant for the APHIS approved field tests that have allowed GE *Eucalyptus* hybrid trees to flower, there are no indications that viable seeds are forming, germinating and producing seedlings that are spreading outside the confined field test sites.

One commenter recommend against allowing flowering of both transgenic and non-transgenic hybrids where trials are within 1000 m of each other. If seed production has also been seen in sites where only one of these taxa are present, further restrictions on flowering until experimentation on seedling establishment and survival may be necessary. In Florida, flowering should only be permitted in northern counties where *E. grandis* currently is not naturalizing.

Response: The observations made to date by the applicant on APHIS approved field tests that have allowed GE *Eucalyptus* hybrid trees indicate that the flowering of the hybrid does not overlap the flowering of other *Eucalyptus* in Florida including one of the parents of the hybrid, i.e. *E. grandis*. Also given that the transgenic hybrid is not producing any pollen, there is little if any potential for outcrossing of the hybrid to other species. One of the requirements of the proposed permit is that the applicant must report any overlap in flowering if it occurs with other *Eucalyptus* tree species in the area. If any overlap in flowering were to occur, the only seeds that could be formed from crossing would be on trees within the test plots whose female flowers could be susceptible to incoming pollen. Monitoring of any seeds and seedling formation in the permitted plots by the applicant would show if any seedlings were being formed as hybrids from incoming pollen sources. In accordance with the supplemental permit conditions, monitoring for and removal of volunteers within 100m from the edge of transgenic test plot by the applicant would effectively eliminate any seedlings that may be produced and thereby effectively reduce the possibility that any *Eucalyptus* hybrid trees would become established.

As seedlings are unlikely to establish in the shade of the plantation or in a vegetated buffer, we suggest that at least half the width of the required buffer around test sites be maintained without other vegetation so that any establishment would be evident. This approach would reduce the need to monitor for seedling establishment beyond the buffer, though we would suggest that some level of monitoring be pursued if open soil is present in the surrounding area. We appreciate the plan for monitoring to extend out 100 m from the plantation edges and agree that this additional scrutiny is warranted.

Response: APHIS does not see the utility of such an experiment. This would only answer the question as to how far the seed could disperse and germinate when optimal conditions exist and would not represent a real situation under which trees would be grown in a plantation. APHIS has determined that appropriate safeguards have been put in place to monitor for any escape of seedlings from the field test sites and strict reporting requirements in the supplemental permit conditions will alert APHIS to any loss of confinement. Appropriate mitigation methods are in place. If any seedlings are found they must be reported to APHIS and then destroyed.

There were many commenters who indicated that the EA inadequately addressed the potential of the transgenes for escaping from field sites through pollen flow and introgression into established *E. grandis* populations. There was a concern that these trees cannot be guaranteed genetically sterile, and will almost certainly release pollen that will travel over large distances, potentially contaminating our native species. Studies have shown tree pollen can travel up to 1,000 kilometers.

Response: There are no native species of *Eucalyptus* in the United States and there are no established *E. grandis* populations in any location close to these field tests. All plantings of *E. grandis* are in commercial managed plantations that are harvested and re-established as commercial plantations in Florida. It should be pointed out that the studies that have shown that tree pollen can travel up to very long distances were conducted with loblolly pine which is highly adapted for wind dispersal and is well known to travel long distances (Williams 2010). As indicated in the EA, *Eucalyptus* is adapted for insect pollination, with bees being the predominant vector (Pacheco 1987) (Pacheco et al. 1986) (House 1997). Under ideal conditions of humidity and temperature, viable *Eucalyptus* pollen can only be found within approximately 100 meters from the edge of nearest tree stand (Peters et al. 1990, Linacre and Ades 2004). Pacheco (1987) verified that bees (*Apis* spp.) are the most effective pollinators of *Eucalyptus*, with activity increasing up to 100 meters from the beehive, and decreasing after this distance. de Assis (1996) indicated that the minimum distance to prevent undesirable pollen contamination of seed producing areas is approximately 300 meters. One commenter provided a reference that indicated that isolation distances up to 800 meters can still produce an outcrossing rate of 2.8 % in an *E. grandis* seed orchard (Junghans et al. 1998). However, an examination of this publication shows that the study was designed to maximize outcrossing using a highly self-incompatible clone. The study examined the rate of pollen contamination from trees outside of a seed orchard into the seed orchard and not from a seed orchard to nearby native trees. It can be viewed as an extreme case of pollen contamination in a seed orchard from pollen from a native tropical forest because the study was designed to maximize outcrossing. In another publication cited by the commenter, Jones et al. (2008) show that pollen flow in a typical *E. grandis* orchard from nearby native trees drops off significantly and is less than 1% at 200 meters.

None of the field test sites are within 1000 meters of any *Eucalyptus* which is a greater distance than the 800 meters cited in the extreme example. And the only sites that have any possibility of the presence of any sexually compatible species are those in Florida

where *E. grandis* is grown. However as pointed out earlier, these *E. grandis* trees do not overlap in flowering with the hybrid. Therefore given that the *Eucalyptus* hybrid trees produce no pollen, there are no sexually compatible trees within 1000 meters of the test site, and there is no overlap in flowering, there is little likelihood that any progeny between this species and the hybrid will be formed. Importantly the only progeny that could be produced from outcrossing with nearby *E. grandis* would be via pollination of incoming *E. grandis* pollen on female parts of the flowers in the field test trees. Any viable seeds that might form would be produced within the planting itself. And in accordance with the supplemental permit conditions any seedlings that might be produced would be found and destroyed through the required monitoring program.

APHIS failed to evaluate these severe storm events in the EA. The southern US, where establishment of commercial GE *Eucalyptus* biofuel feedstock plantations is now being considered, is known to be subject to strong storms, including tornadoes and hurricanes, which have the potential to distribute *Eucalyptus* seeds over very large areas from tens to hundreds of kilometers.

Response: The particular *Eucalyptus* hybrid that is being grown in these proposed field tests produces mature capsules in February and seed fall is shortly after this. Therefore seed release is in early spring and well outside out of the normal hurricane season which occurs between June and November (http://www.nhc.noaa.gov/). The probability that other storm events might distribute seeds that could survive is extremely low. There would have to be a number of events that would have to combine to have any consequence: seed happens to be shedding at the time a severe storm rolls through a field test site, the seed happens to land on a suitable seed bed of bare soil with no weeds, the site is then not disturbed either by plowing or herbicide treatment, and moisture conditions are suitable for a germinating seedling to survive and grow. Research and experience have shown that long distance dispersal of *Eucalyptus* seeds and seedling establishment is very rare. Forsyth et al (2004) point out that "…in most parts of the world where *Eucalyptus* have invaded, they seldom spread considerable distances from planting sites, and their regeneration is frequently sporadic." Richardson (1998) indicates "Eucalypts are also represented on many national or regional weed lists from other parts of the world. Despite this, they have not been nearly as successful in invading alien environments as other widely planted trees such as pines and legumes. Many eucalypts produce large quantities of seeds, so their lack of success as invaders is rather puzzling." This could likely be due to the fact that *Eucalyptus* seeds are very small, have very limited reserves and are intolerant of shade or weedy competition. In order to successfully germinate and establish, *Eucalyptus* seed needs contact with bare mineral soil and lack of competition either as a result of human intervention or naturally following a fire event (Meskimen and Francis 1990, Bell and Williams 1997). In Brazil where *E. grandis, E. urophylla* and their hybrids have been grown since the 1960's and are now planted on several thousand hectares, there is no evidence that wind borne seeds are spreading the trees beyond managed plantations. Over 70,000 hectares of the hybrid has been planted extensively by International Paper, who developed EH1, with no evidence of invasiveness (Luis Silva, International Paper Company, Brazil – comment to the docket). In these environments *Eucalyptus* obviously does not behave like other

windblown seeds of grasses, for example, which can be pioneering species. Therefore the likelihood of significant escape and successful establishment of seeds from the field test sites due to storms is negligible. APHIS has determined that the field test will be confined and storms are not expected to compromise this confinement.

Concerns were expressed that the cold-tolerance genetic construct could have other unanalyzed effects on the environment besides invasiveness, as the genes conferring cold tolerance in this field trial are known to affect the expression of many other genes including genes for drought tolerance. And there was a concern that the hybrid could become invasive if it could survive the cold. All that is known is that one barrier to possible invasiveness or establishment would be removed by the addition of the cold-tolerance genes, i.e., the current inability to survive the cold.

Response: As noted above, these trees will be grown under APHIS permits as confined field trials and would be confined via specific supplemental permit conditions. In the field tests where the trees have been allowed to flower under APHIS confined field release permits, the cold tolerance gene has had no unintended effects on the trees that would indicate that the proposed field test would not remain confined. Under APHIS regulations and permit conditions permittees are required to report any unintended effects or unusual occurrence while trees are grown under permit and none have been reported to APHIS. Therefore, it is reasonable to assume that the *Eucalyptus* hybrid that would be grown under the proposed permits will not result in a weed or invasive plant species.

Several comments noted the potential impacts of *Eucalyptus* on hydrology. The comments centered on concerns that tree plantations have been documented to deplete ground water and cause or exacerbate drought situations. *Eucalyptus* has been shown to threaten water quality, riparian habitat quality, and aquatic species by lowering the water table, affect groundwater recharge, and reduce stream flow.

Response: The impacts by *Eucalyptus* on hydrology relate to the scale of the plantings. These proposed field trial plantings are very small in terms of scale in forestry practices and watersheds. The largest field test plantings under the two proposed permits would be no more than 20 acres. Individual forest plantings in the southern U.S. range in size but can typically be up to 120 acres at a single site in a given year (SFI standards: http://www.sfiprogram.org/files/pdf/sfi_requirements_2010-2014.pdf). A 20 acre planting is considered small in these terms.

The issue of hydrology was addressed in the EA. As noted in the section under Potential Effects of Growing *Eucalyptus* on Soil Hydrology in the EA, the Forest Service has pointed out that the significance of the impact on groundwater and stream flow will depend greatly on the area extent, size, and spatial distribution of the plantations. For example, a few small (less than 10 hectares, i.e. approximately 25 acres) and well-dispersed plantations may only have very localized impacts and negligible impacts at the watershed scale.

The field test sites requested under the two permits are well dispersed and as indicated above are limited in size (none are greater than 20 acres). ArborGen supplied data indicating the maximum size of each of the plantings at each site, the individual watersheds where the plantings occur, the area of the watershed, how much of the watershed will be occupied by the field tests, the location of the closest primary and secondary streams, and the location of any critical habitat for Federally listed threatened and endangered species within the watershed. The data provided by ArborGen show that none of the sites occupy more than 0.03% of any given watershed.

A number of professional foresters provided comments that support APHIS's conclusion: "The proposed research plantations are far too small to have any chance of substantially affecting watershed-scale hydrology. Water-use issues for widespread, large plantations of *Eucalyptus* would be important to quantify, just as they would be important to quantify for widespread plantations of native pine (and all other land uses)…..Dan Binkley, Professor of Forest Ecology, Colorado State Univ. "The field trials represent a negligible fraction of the land area in the States in which these trials are located and will not have any significant impact on hydrology"…..Jim Rakestraw, International Paper Company.

Based on the very small footprint of these proposed confined field tests and the weight of evidence, APHIS concludes that the impacts of these field trials on hydrology will be negligible.

Another commenter indicated that the 29 field sites should be assessed collectively and that the proposal seeks to permit field testing on some 330 acres. A plot of that size should be considered a large-scale plantation.

Response: As described in the EA, the 28 field test sites are widely dispersed across the southeastern US. The sites are 900 miles apart from the farthermost east and west plantings. They average approximately 50 miles apart. The closest plantings have least 3 miles separation between them. No individual planting is greater than 20 acres. There is no reason to assume that there will be any interaction between these distant sites and assessing these plantings as if they were a single block planting of 330 acres is not logical.

While previous field trials of ArborGen trees have been quite small (0.2-7 acres), the widely separated proposed trials will be only slightly larger (0.5-20 acres). The aggregate of the proposed trials is 330 acres distributed across 28 locations in 7 southeastern states. By comparison, 1.8 million acres of loblolly and slash pine trees are planted yearly on plantations in the US southern states (McKeand et al. 2007). The *Eucalyptus* trials would represent .00184 % of all yearly pine plantings or 1.54×10^{-4} % of all forest acres in southern forests. While commercial acreage of *Eucalyptus* is not officially monitored, it is apparently small. In southern Florida, *Eucalyptus* is produced mostly for mulchwood and for energy use on about 15,000 acres, and another several hundred acres is planted throughout the Southern US states (D.L. Rockwood, University of Florida, pers. comm, 2010). *Eucalyptus* acreage is low in the US South because the species that were tested were not well-adapted to the climate (Escalante Fernandez et al.

2002). Southern forests comprise a total of 214 million acres, with the largest forest types composed of loblolly and shortleaf pine, but with large acreage also in longleaf/slash pine, oak/gum cypress and oak/pine species (Sheffield 2009). Therefore 330 acres compared to these numbers is extremely small.

A cumulative impacts analysis in provided in the EA. Based upon this analysis, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create cumulative impacts or reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.) associated with the release sites or the ecosystem in which they are situated. No resources will be significantly impacted due to cumulative impacts resulting from the proposed action.

There was concern expressed about the presence of the barnase gene which has been engineered to alter the fertility of the trees. It was commented that when used, the barnase gene remains present in the cells and tissues of the plant in which it was introduced. Barnase has been found through experiments, even in trace amounts, to be toxic to rat kidneys and human cell lines. Many insects including bees will eat the pollen and it its impacts on wildlife were not assessed.

A related comment stated that a patent application submitted by ArborGen in 2009 stated: "Accordingly, there exists a need for a reproductive ablation system having reduced barnase induced toxicity and minimal leaky expression in a plant's vegetative tissues." The leakiness and toxicity of barnase was not mentioned in the APHIS assessment.

Response: The transgenic *Eucalyptus* hybrid utilizes barnase to produce male sterile plants, reducing the potential for gene flow into the environment. Male sterility is achieved through the localized production of barnase in pollen producing cells. Barnase is a ribonuclease, an enzyme that degrades RNA, thereby regulating protein synthesis. Ribonucleases are highly ubiquitous molecules found in all living cells (Worrall and Luisi 2007).

In patent application 10/946,622 submitted by ArborGen 2004 they maintained "there exists a need for a reproductive ablation system having reduced barnase induced toxicity and minimal leaky expression in a plant's vegetative tissues" (Rottmann et al. 2008). In submitted comments this statement was misconstrued as being indicative of a potential problem with the utilization of barnase. In reality this statement by ArborGen was intended to justify the relevance of their patent application, and to point out inadequacies in other sterility systems, not their own. Transgenic *Eucalyptus* trees covered in ArborGen's patent application, and cited in permit #08-111-106rm, #08-014-101rm, were engineered using barnase gene mutants with reduced toxicity to minimize tissue damage (Rottmann et al. 2008). In order to mitigate "leaky expression" barnase production is controlled by a tissue specific promoter. As with all genes, the gene encoding barnase is present in every cell of the transgenic plant, however, the promoter acts like an "on" switch that controls when and how strongly the gene is expressed. The PrMC2 promoter

used by ArborGen, originally identified in pine (*Pinus radiata*), restricts barnase production to the tapetum, a small layer of cells within the male floral organ, or anther (Walden et al. 1999, Hofig et al. 2003, Rottmann et al. 2008). Within the anther, the tapetum surrounds developing pollen grains. Mariani et al. (1990) developed transgenic tobacco plants using the barnase gene also controlled by a tapetum-specific promoter (Tap29). They observed that in transgenic plants tapetal cells senesced early in their development, preventing pollen formation. All other floral organs, including the anthers, formed normally proving tapetal cells could be eliminated without affecting flower development. Similar results were observed using PrMC2 in *Eucalyptus* trees (Rottmann et al. 2008). After several years of research, no pollen has been observed in transgenic *Eucalyptus* trees (Rottmann et al. 2008). As with other ribonucleases, barnase degrades quickly after the destruction of tapetal cells, and does not accumulate within the plant tissues (Mariani et al. 1990); http://www.fda.gov/Food/Biotechnology/Submissions/ucm161162.htm).

Despite its limited presence in transgenic *Eucalyptus* several commenters express concern that barnase will have unintended effects on human health. Research by Ilinskaya and Vamvakas (1997), Prior et al. (1996), and Balandin (2009) was cited as areas of concern. However, closer evaluation of these studies reveals a more favorable view of barnase with regard to mammalian toxicity.

Ilinskaya and Vamvakas (1997) investigated the effect of barnase on rat kidneys to determine the potential for organ toxicity. The kidneys of male rats were surgically removed and submerged in a low (15 μg/ml) and high (150 μg/ml) dose of purified barnase. At high doses, rat kidneys showed elevated levels of protein excretion, indicating toxicity. There were no harmful effects of barnase at the low level of exposure. Although noteworthy, the relevance of these findings to transgenic *Eucalyptus* is limited. This study focused on extremely high levels of exposure. The concentrations evaluated, both low and high, far exceed the amount of barnase produced by transgenic *Eucalyptus* trees. Barnase is only produced for a short period of time during floral development, is limited to a discrete cell layer within the anthers of the flower, and is rapidly degraded (Mariani et al. 1990), (Rottmann et al. 2008), (Hofig et al. 2003). In previous studies using transgenic radicchio containing the barnase gene researchers were unable to detect accumulation of barnase within floral tissue (http://www.fda.gov/Food/Biotechnology/Submissions/ucm161162.htm). Therefore it is highly unlikely that consumption of, or exposure to, transgenic *Eucalyptus* could contain enough barnase to cause mammalian toxicity. Direct exposure of organ tissue, is also highly unlikely to occur in nature. In addition the FDA has previously reported that consumption of barnase would likely degrade quickly during digestion further reducing the risk of barnase exposure (http://www.fda.gov/Food/Biotechnology/Submissions/ucm161162.htm).

Prior et al. (1996) also investigated the toxicity of barnase. In this study barnase was genetically combined with a bacterial toxin, *Pseudomonas* endotoxin A (PE), creating a new toxin not found in nature, referred to as PE-Bar. Previous studies have shown this type of combination resulted in a more powerful toxin than the natural form of the toxin

(Prior et al. 1996). As expected, PE-bar was found to be toxic to human cells grown *in vitro*; artificially grown on Petri dishes. However when introduced intravenously into live rats PE-Bar was found to be non-toxic. Researchers speculated low toxicity stemmed from rapid degradation of barnase in living tissue. They concluded "furthermore there was no apparent toxicity when the toxin (200μg, 3.6 nmol) was injected intravenously" (Prior et al. 1996). Similarly Balandin et al. (2009) evaluated the toxicity of a modified form of barnase, in this case barnase genetically combined with a human antibody (ScFv 4D5) which targeted cancer cells; referred to as ScFv 4D5-barnase. They found that although this modified form of barnase was toxic to cancer cells it was not toxic against embryonic kidney cells when tested *in vitro* using Petri dishes, or *in vivo* injected into live mice. Unlike Prior et al. (1996) and Balandin et al. (2009), Ilinskaya and Vamvakas (1997) did not conduct intravenous studies, but relied solely on severed kidney tissue for their results.

With regard to toxicity of barnase to bees and other pollinating insects, there is no clear evidence indicating pollinators would be adversely affected by ingestion of barnase. Combined with the fact that barnase is only produced during the short period of tapetum formation, it is quickly degraded, and the lack of pollen produced by the *Eucalyptus* hybrids, it can be concluded the tissue specific production of barnase is unlikely to adversely affect pollinators or other insects. The barnase gene has been deregulated previously in three plant species, corn (petitions 95-288-01p, 97-342-01p, 98-349-01p), rapeseed (petitions 98-278-01p, 01-206-01p) and chicory (petition 97-148-01p) since 1995. APHIS is unaware of any reported cases of mammalian or insect toxicity resulting from barnase consumption or exposure occurring within the past 15 years.

Based on these findings no hazard or exposure associated with the use of barnase has been identified, therefore APHIS concludes that there is no foreseeable risk associated with the use of barnase in transgenic *Eucalyptus* trees in the proposed field tests.

Several comments discussed reports that the incidence of *Cryptococcus gattii* infection is increasing and suggest that field testing of cold tolerant *Eucalyptus* trees developed by ArborGen could further increase the incidence of the disease.

Response: While some reports have linked the incidence of *C. gattii* to the presence of *Eucalyptus* trees (*Eucalyptus camaldulensis)*, several other citations suggest that the fungus occurs in the absence of *Eucalyptus* trees (Ellis and Pfeiffer 1990, Kidd et al. 2004, Upton et al. 2007, Datta et al. 2009).

Cryptococcus gattii is a fungus (yeast) that can infect the pulmonary and central nervous system of humans and animals (Datta et al. 2009). Although the fungus is not considered to be common or ubiquitous, it can be found worldwide (Upton et al. 2007, Datta et al. 2009). It is not clear just how long the fungus has been present in North America. The fungus was first identified as a problem for humans and animals in 1999 in the Pacific Northwest (Datta et al. 2009). Since that time, more than 200 cases of *C. gattii* have been reported in humans, and many more in animals (Datta et al. 2009). Despite its identification as a disease causal agent in this area in 1999, data from genetic studies

suggest that the organism may have been present in that area for more than 30 years (Datta et al. 2009).

Published scientific literature suggests that while the organism remains uncommon, changes in climate, land use and host susceptibility have been responsible for recent spread of the organism and its likely further spread in the future (Upton et al. 2007, Datta et al. 2009). This same literature indicates that the organism has adapted and now can survive in a wide variety of climatic conditions. One citation estimates that the annual incidence of *C. gattii* in the Pacific Northwest is 6.5 cases per million people in British Columbia and 27.9 cases per million on Vancouver Island (Datta et al. 2009). The incidence rate in Vancouver is the highest rate reported worldwide (Datta et al. 2009). This same citation reports that the fatality rate from *C. gattii* disease was about 4.5% (Datta et al. 2009). Beyond the cases in Canada, as of 2009, there were approximately 20 cases of the disease in Washington and Oregon (Datta et al. 2009). *C. gattii* has also been isolated in Washington.

While it is clear that *C. gattii* is an emerging pathogen that can have significant effects on those infected with the fungus, it is much less clear that there is an association between *C. gattii* and *Eucalyptus* trees. Ellis and Pfeiffer (1990) suggest a correlation between the incidence of *C. gatti* and *Eucalyptus camaldulensis* in Australia. At the time of their publication, they suggested that there was no other source of *C. gattii*. However, other publications have not suggested that *C. gatti* is only associated with *Eucalyptus* trees. Kidd et al. (2004) collected more than 700 samples from various locations on Vancouver Island thought to be probable locations of *C. gattii*. The fungus was isolated from 5 tree species, none of which were *Eucalyptus*. Upton et al. (2007) indicate that environmental sampling has shown *C. gattii* to be associated with soil, debris and air around trees. However, there is no indication that there is a correlation between *C. gattii* and only *Eucalyptus* trees. Datta et al. (2009) discuss previous reports of a correlation between *C. gattii* and *Eucalyptus* trees, but also discuss the isolation of *C. gattii* from non-*Eucalyptus* trees and debris.

While several comments suggest that environmental release of *Eucalyptus* trees under APHIS permit could increase the incidence of exposure to and infection by *C. gattii*, based upon available information, it appears that any increase in the incidence of *C. gattii* would be negligible for several reasons. First, available data and information show that the *C. gattii*, while uncommon, is present in many parts of the World. While there are data to support an association between *Eucalyptus* trees and C. gattii, the fungus is also associated with other types of trees. Therefore, release of these *Eucalyptus* trees should not significantly increase the amount of *C. gattii* in the environment. Second, even if the fungus is found to be associated with the trees grown under this proposed permit, because of the location of these field trials, it is unlikely that there will be significant human exposure to the trees, beyond those responsible for conducting the field trial. Finally, the species of *Eucalyptus* from which the fungus has been isolation are different from the hybrid being grown under these proposed permits.

There were comments that were concerned about the risks associated with altered-lignin trees. The assumption is that trees would be weakened structurally, be more vulnerable to storms and more susceptible insects and diseases. It is assumed that low-lignin trees will rot more readily, with potential impacts on soil structure and ecology.

Response: There is no evidence in any of the field tests conducted by the applicant to date that the trees with altered lignin are more susceptible to insects and disease pests as reported in their permit application and annual field test reports supplied to APHIS. Trees covered under the proposed permits would be harvested and grown in a typical forestry field test situation where wood is removed. Therefore rotting of felled trees is not an issue. If there were to be an increase incidence of insects and diseases associated with these proposed field trials, the potential impacts would be localized within the field test itself which is confined. Any trees found to be affected would be immediately rouged. As in any tree or crop genetic improvement program, there may be a certain number of individuals which are found to be more or less susceptible to pests. That is one of the purposes of a field test; to eliminate inferior individuals from the tree improvement program. These are removed by rouging and would be eliminated from the program if they are shown to be more susceptible to pests and diseases.

Several comments noted the high flammability of *Eucalyptus* which allows *Eucalyptus* plantations to be susceptible to initiation or rapid spread of wildfire.

Response: It is not clear that *Eucalyptus* plantations present a greater fire hazard than do the pine plantations which are common in the Southeastern USA. To assess the risk of wildfire in live and dead material, Núñez Regueira et al. (2002a), (2002b) in northern Spain used calorimetry analysis combined with multidimensional assessment of climatic and physical characteristics in plantations of a *Pinus* species (*P. pinaster*) and of a *Eucalyptus* species (*E. globulus*). For pine, the fire risk index for live biomass varied from extremely high in July to little risk in some winter and spring months. Pine residues, however, varied between no risk and extremely high. For *Eucalyptus*, fire risk index for live biomass varied from a middle risk in July to little risk in fall and winter. Dead matter of *Eucalyptus* varied between a middle risk to extremely high in August. Ganteaume et al. (2009) undertook a variety of assays and comparisons of fuel bed flammability and firebrand capacity (the material that can ignite the fuel bed). *Pinus* litter flammability was higher than that of *Eucalyptus*, which in turn was higher than that of a hardwood species (*Ulex*). While differences between southeastern pines and the proposed *Eucalyptus* species and these analyses may be important, basic similarities within the genera are likely relevant. For example, studies of different *Eucalyptus* species have shown that despite the differing climactic specificities of *E. globulus* and *E. nitens* and differences of volatile content and solid mass, the total caloric content of wastes after pulping are similar (Perez et al. 2006).

Some of the comments have cited two large wildfires in arid areas of Australia and California involving *Eucalyptus*. These fires were mentioned as examples of deleterious consequences of *Eucalyptus* plantings.

Response: These fires occurred in areas of extensive plantings of *Eucalyptus*, but it is likely that many factors are important for the frequency and season of occurrence of fires, not just the species of trees within a burned area. One post-fire report on the Oakland, California fire noted that extended drought conditions and freeze-killed *Eucalyptus* contributed large amounts of residue for the fire. However, a wide variety of grasses, brush, trees, and houses combined to provide fuel that sustained this fire, and the fire hadn't initiated in the *Eucalyptus* stands (FEMA 1991). As noted, one of the most important risk factors for *Eucalyptus* production is the presence of accumulating fuels within a stand (Núñez Regueira et al. 2002a). The duration of these field trials will be limited (no more than seven to nine years). ArborGen has seen little accumulation in field trials extending through five years (Les Pearson, ArborGen, personal communication)) and consequently little to no accumulating fuel is expected to arise within the proposed confined field trials.

Other comments said that because of the potential for spread of fire, the area of consideration should include not only acreage of the dispersed field tests but also adjacent lands to which fires could spread.

Response: Based upon the information provided in the previous response on the high flammability of *Eucalyptus,* adding the acreage of surrounding fields to the analysis of potential fire impacts of the test plots would not change the overall impact analysis of either alternative. Since the flammability of the transgenic *Eucalyptus* are not likely to be greater than that of the surrounding pine and the test sites would be located on sites currently used for forest tree production or agriculture/forestry research, it is reasonable to assume the overall risk of fire at any specific location would be the same with or without the proposed confined field trial taking place.

One comment claimed that APHIS did not consider the risks of growing *Eucalyptus* in areas of recent drought stress. *Eucalyptus* production in such conditions would enhance the likelihood of fire, or, *Eucalyptus* itself with its high soil water demand would increase the risk of drought and subsequent fire.

Response: The relationship between drought and increased risk of fire is generally accepted (Keeton et al. 2007, Huntington et al. 2009). Should APHIS expect that drought will have a greater impact on *Eucalyptus* trials than on other types of trees? In assessing risk of forest fires among *Eucalyptus globulus* on the basis of biological and environmental observations and considerations, Nuñez Regueira et al. (2002a) concludes that "*E. globulus* Labill. can be considered as a low risk tree regarding to forest fires. However, residues originating from *Eucalyptus* forestry can become very dangerous in initiating forest fires during seasons with high temperature and low environmental moisture mainly if they are abandoned on the forest without control." The same authors published a fire risk table for seven specific months for both pine (Núñez Regueira et al. 2002b) and *Eucalyptus* (Núñez Regueira et al. 2002a), and pine was generally rated higher risk than *Eucalyptus*. Pine also had the highest risk rating for a single month (July) (Núñez Regueira et al. 2002a). After studying the impacts of drought on rainfed

versus irrigated *Eucalyptus* (*E. globulus* and *E. nitens*) White et al. (1998) showed that water limitation affected tree growth. The ratio of relative rates of leaf area compared to conducting sapwood is reduced under these conditions. It is not clear that this consequence would have any effect on flammability, although sapwood would be less flammable than leaf and other *Eucalyptus* residues (Núñez Regueira et al. 2002a). In two other eucalypts, *E. marginata* and *Corymbia calophylla*, Pekin et al. (2009) surveyed 16 plots of *Eucalyptus* along an aridity gradient in southwestern Australia and concluded that "increased fire and drought… result in denser stands of smaller trees." They also found that "total stand biomass declines with increasing aridity, but has no association with fire frequency." APHIS concludes that whatever the source of the drought, whether climatic or induced by the increased hydrologic demands of *Eucalyptus* production, fire risk will not likely increase significantly by granting this permit.

Although the flammability of tree biomass in *Eucalyptus* may be less than that of pine (Núñez Regueira et al. 2002a, Núñez Regueira et al. 2002b), the flammability of dead residues found in *Eucalyptus* plantations is a potential hazard. Nuñez Regueira et al. (2002a) conclude that cleanup of the more flammable *Eucalyptus* residues is the key to forest fire control.

The applicant notes that substantial amounts of *Eucalyptus* residue have not accumulated at the test sites through five years of field trials (L. Pearson, ArborGen, personal communication). According to observations by a professor of forestry specializing in *Eucalyptus* research, residues in existing plantations in Florida have not exceeded 1"-2" depths, but the age of the *Eucalyptus* at observation was not determined (D.L. Rockwood, University of Florida, personal communication). In Florida, the age of trees in *Eucalyptus* plantations has attained to at least 10 years in some locations (King and Skolmen 1990).

ArborGen is also developing tree plantation products for Brazil and they have extensively consulted with managers of commercial-scale *Eucalyptus* plantations about the best practices with which to maintain the security and value of these trees. From these plantations of *E. grandis, E. urophylla* and the *grandis* x *urophylla* hybrid in Brazil, ArborGen staff observed that residues do not extensively accumulate over the growth cycle of the trees before the scheduled harvest (Les Pearson, ArborGen, personal communication). Before clear-cut harvest, *Eucalyptus* plantations in Brazil range between five and nine years of age (Diaz-Balteiro and Rodriguez 2006).

The permit period for the proposed trials is three years, and will likely be renewed. According to the permit the expected age at harvest of the trees in the field tests is 7–9 years. Therefore a significant buildup of residues is not expected during this short time period.

After some *Eucalyptus* fires, total consumption of surrounding residue may occur without complete loss of trees (Tam Fire: (National Park Service 2006)). Differences in flammability of residues with respect to different *Eucalyptus* species have been anecdotally noted, although the rigorous empirical demonstration of such observations

generally may be lacking (Gill 1997). Pine needles may build up in adjacent pine plantations and are not readily degraded, and according to Nuñez Regueira et al. a fire risk rating of dry pine residues can be just as high in August as are the residues of *Eucalyptus* in the same month (Núñez Regueira et al. 2002a). Flammability of *Eucalyptus* exceeding that of pine in plantation culture has not been demonstrated.

One comment noted that because APHIS does not adequately consider the catastrophic effects of increased wildfires assignable to *Eucalyptus* production, they have not taken account of effects on sensitive and protected species in the action area.

Response: As described in responses to the previous public comments, APHIS does not expect an increase in the fire hazard from planted trees as a result of the proposed *Eucalyptus* field trial plantings. From ArborGen's observations of field trials through five year periods, few *Eucalyptus* residues build up in the test areas within the Southeast US. The residues would be the most important potential source of flammable material during the permit periods. Consequently, APHIS finds that wildfires arising from *Eucalyptus* trials will not be a source of heightened risk to sensitive and protected species in the field test areas.

Some comments claimed that APHIS did not consider the impacts of future commercial *Eucalyptus* acreage on the permit sites, nor possible additional field test acres, nor any ornamental *Eucalyptus* currently grown or planned to be grown.

Response: The EA summarized the existing acreage of other *Eucalyptus* field trials that are adjacent to the proposed trials, and also provided information about proposed, future *Eucalyptus* plantings by ArborGen. All proposed field trials involve limited acreage, and no large regions of *Eucalyptus* production would be created by allowing the field trials to take place. As described in the EA, ArborGen is not aware of any commercial *Eucalyptus* trees being grown within 1000m of any of the field trial sites, although a limited number of non transgenic or transgenic field trial sites may be within 100m of the field trials.

The precise number of commercial acres of non-transgenic trees in the southern US is not readily available, and it would be speculative to predict total future commercial acreage based on current patterns of growth. As noted in the EA, current *Eucalyptus* acreage is quite limited because *Eucalyptus* is highly sensitive to cold temperature, and trials of typical *Eucalyptus* plantation species outside of Florida have not been successful. The only consequential acreage of *Eucalyptus* is in southern Florida, where 15,000 acres are under production (D.L. Rockwood, University of Florida, personal communication). Consequently, based upon current information that APHIS is aware of, APHIS does not expect that commercial acreage of *Eucalyptus* will increase rapidly in most parts of the Southeastern US within the foreseeable future.

At least eight species of ornamental *Eucalyptus* are cold tolerant and likely to grow in the region of the proposed confined field release sites (see EA, p. 26). One of the most

popular ornamental species of *Eucalyptus* is the silver dollar tree, *Eucalyptus cinerea*, which would likely be found in many of the states proposed for field trials (EA, p. 26). These and other ornamentals are likely to be grown as specimen trees (one or a few grown together), and not part of large scale plantations. As presented and analyzed in the EA, potential impacts to ornamental *Eucalyptus* is expected to be minimal to non-existent based upon the reproductive biology of this *Eucalyptus* hybrid and adherence to the supplement permit conditions assigned to these permits.

Comments were received indicating that APHIS failed to consult with the United States Fish and Wildlife Service (USFWS) and/or the National Marine Fisheries Service (NMFS) and instead used its own assessment that was described as inadequate to marginally adequate. Further, APHIS failed to contact USFWS/NMFS to determine if listed species may be present, did not assess potential impacts on protected species' critical habitat, and did not provide the USFWS with information necessary to begin the consultation process, and that at the very least APHIS should have reached a "may affect" determination. In addition, by withholding the location of the field trials, it is not possible for adequate review of potential impacts to protected species.

Response: The ESA requires Federal agencies to ensure that any action they authorize is not likely to jeopardize the continued existence of any threatened or endangered species or result in destruction or adverse modification of critical habitat. It is the responsibility of the agency to assess the effects of their action and to consult with the United States Fish and Wildlife Service (USFWS) if the action "may affect" listed species or critical habitat. To do this, the agency contacts the USFWS/NMFS to request a list of species that may be found in the "action area." The Services would also indicate the presence of critical habitat.

APHIS analyzed the potential for effects from the preferred alternative of this EA on federally listed threatened and endangered species (TES) and species proposed for listing, as well as designated critical habitat and habitat proposed for designation, as required under Section 7 of the Endangered Species Act. APHIS worked with the USFWS to obtain species lists and information and critical habitat information for the proposed field site locations. After analyzing the potential for any effect, APHIS has reached a determination that the proposed environmental release will have no effect on federally listed threatened or endangered species or species proposed for listing, and no effect on designated critical habitat or habitat proposed for designation. Consequently, in accordance with Section 7 of the ESA consultation with the USFWS is not required for the action described in the preferred alternative of this EA. Appendix IV of the EA includes the BRS analysis of threatened and endangered species in the areas of the field releases.

Comments received stated that APHIS did not assess the potential for cumulative effects, propagation, cross-breeding, invasiveness, effects on hydrology, or fire risk on listed species or native ecosystems.

Response: These potential causes of effects are discussed in the EA. The effect of propagation, cross-breeding, and invasiveness on listed species or native ecosystems was not considered a potential impact because of the supplemental permit conditions that prevents these events from occurring. Fire risk to TES was not considered a potential impact because the small size of the field trial combined with the unlikelihood of TES presence within the proposed test area. Likewise, the effect on hydrology was not considered an impact because of the small size of the field trial combined with the distance from water sources that listed species rely upon. In addition, the small size of the field plots and the distance between test sites spread over thousands of miles indicate no cumulative effects would result from APHIS issuing the proposed field release permits.

Comments were received that APHIS failed to consider the effect on migratory birds in potential violation of the Migratory Bird Treaty Act. Further, Executive Order 13186 "Responsibilities of Federal Agencies to Protect Migratory Birds" requires Federal officials to consider the impacts of planned actions on migratory bird populations and habitats for all planning activities. Specifically, commenters cited articles by Stallcup 1997 and Williams 2002, discussing the problem in California of a sticky gum produced by flowering *Eucalyptus* trees (*Eucalyptus globulus*) that can clog bird's faces, resulting in death by asphyxiation or starvation.

Response: The Migratory Bird Treaty Act (MBTA) (16 U.S.C. §701 et seq.), as amended, implements the United States' commitment to four bilateral treaties, or conventions, for the protection of 1007 species of migratory birds. The MBTA provides that it is unlawful to pursue, hunt, take, capture, kill, possess, sell, purchase, barter, import, export, or transport any migratory bird, or any part, nest, or egg or any such bird, unless authorized under a permit issued by the Secretary of the Interior. As specified in the supplemental permit conditions for these permits, these permits do not eliminate the permittee's legal responsibility to obtain all necessary Federal and State approvals. The proposed APHIS permits do not override the applicant's responsibility to ensure that any action they may take during the field test does not violate the MBTA.

Executive Order 13186 states that each federal agency, taking actions that have, or are likely to have, a measurable negative effect on migratory bird populations, is directed to develop and implement, a MOU with the USFWS that shall promote the conservation of migratory bird populations. APHIS has developed a draft MOU with the USFWS as required by this Order and is currently waiting for USFWS approval. APHIS will abide by the MOU once it is finalized and signed by both parties.

The 1997 article "Deadly Eucalyptus" by Rich Stallcup was published in a Point Reyes Bird Observatory periodical (Stallcup 1997). The 2002 article "America's Largest Weed" by Ted Williams was published in *Audubon Magazine* and refers to the 1997 article and includes anecdotes from Stallcup and Keith Hansen, the illustrator (Williams 2002). Neither articles are scientific papers that have been peer reviewed or published in scientific journals. Although it has been over twelve years since the first article was published, a search by APHIS for similar information failed to provide anything

supporting the theory that gum produced by the blue gum tree (*Eucalyptus globulus*) results in avian mortality. Even if the theory were correct, the species grown in these proposed field trials are a hybrid of *Eucalyptus grandis* and *Eucalyptus urophylla* not *Eucalyptus globulus.* There have been no published reports of avian mortality reported in commercial production of these hybrids. As specified in the supplemental permit conditions, the applicant is required to monitor and report any unusual occurrences to APHIS. To date there have been no harm to wildlife reported to APHIS from field trials of *Eucalyptus*. Further, even if the trees did produce a hazardous gum, there would be no effect on listed TES birds that are known to be in the counties of the proposed field tests. According to USFWS field offices contacted by APHIS, there are ten TES birds that are considered to be within the counties where these proposed field trials would occur. As described in Appendix IV of the EA, none of the TES birds would be expected to feed on or be attracted to the *Eucalyptus* flowers. Two are shorebirds (piping plover and least tern) and would not be found anywhere near the test sites. The wood stork and whooping crane are associated with wetlands, the former feeding primarily on fish, and the later feeding on fish, crustaceans, insects, berries, and grains. Audubon's crested caracara is a large raptor that feeds primarily on carrion, live vertebrates, insects, and worms. The Everglades snail kite feeds almost exclusively on freshwater apple snails. The Florida grasshopper sparrow feeds mostly on the surface of the ground, consuming insects, other small invertebrates, grains, and seeds. The Florida scrub-jay prefers open areas of scrub vegetation rarely greater than two meters high, is omnivorous, but feeds primarily on insects and acorns. The ivory billed woodpecker's diet is understandably not well understood, but is believed to be mostly wood boring beetle larvae from dead or dying trees and various nuts. The red cockaded woodpecker inhabits old growth forests, primarily longleaf pine. They forage for insects by prying off pieces of bark, and will also feed on berries.

Comments were received that centered on the use of the land and that a change in forest habitat would be a disaster for forest dwelling species. One said specifically that if pine stands at the sites are converted to *Eucalyptus*, they will not be allowed to mature and be used for red cockaded woodpecker nesting. Further, the conversion of land to *Eucalyptus* denies species the opportunity to use the habitat, resulting in "take".

Response: As described in the EA, the proposed field tests would not take place in unmanaged forest but would be at sites that have either been used in agricultural production or as managed forest with high succession rates. Many of the sites are located at agricultural research facilities where a variety of tree and agricultural crops are grown for short periods of time. The habitats at these sites do not contain the primary constituent elements required by any listed species known to be in any of the counties where the field test sites would be located. Red cockaded woodpeckers preferably nest in old growth longleaf pine, but may nest in other pine species and also bald cypress (NatureServe 4/20/2010). With or without the issuance of these permits, the vegetation at these sites would not be managed to produce mature trees suitable for the red cockaded woodpecker nesting. In addition, the proposed field trials would be of limited size and duration, confined to a specific area, and would not permanently alter the habitat.

A commenter stated that a field survey should have been completed to determine if listed species are using the habitat(s).

Response: As described in the response to an earlier comment questioning the adequacy of
APHIS' ESA analysis, APHIS obtained information from the USFWS about locations and habitat where TES are known to occur. This information was used to eliminate most species from further consideration based on the distance of the field test sites to known populations or suitable habitat (i.e. marine mammals). When there was any doubt, further discussions were held with USFWS biologists, and at their recommendation, surveys of the sites were completed as necessary. As described in Appendix IV of the EA, surveys were completed at some sites for the frosted flatwoods salamander (*Ambystoma cingulatum*), reticulated flatwoods salamander (*Ambystoma bishopi*), gopher tortoise (*Gopherus polyphemus*), bluetail mole skink (*Eumeces egregius lividus*), and the sand skink (*Neoseps reynoldsi*). Based upon these surveys, there was no evidence that these species were present at any of the sites.

One commenter asserted that APHIS failed to assess the close proximity of the proposed field test sites to protected, ecologically sensitive areas and that the field trials represented a risk of biological contamination of natural ecosystems and protected places. That all counties are within a 50-mile radius of ecologically protected areas. The commenter provided a list of protected areas in the following counties: Baldwin County, Alabama; Saint Landry's Parish, Louisiana; Berkeley County, South Carolina; Charleston County, South Carolina; Hardin County, Texas; Jasper County, Texas; Jefferson County, Texas; Newton County, Texas; Columbia County, Florida; Gadsden County, Florida; Highlands County, Florida; Marion County, Florida; and Taylor County, Florida.

Response: ArborGen performed an analysis to determine the distance from the proposed field test locations to each of the listed protected sites. Few of ArborGen's proposed field test locations are in close proximity to any of these sites. In most cases the proposed field test sites are approximately 10 miles or more from any of the listed protected sites, with the majority of the sites being more than 20 miles away. The proposed field test site located in Charleston County, South Carolina is approximately 4 miles from the ACE Basin National Wildlife Refuge. A proposed field test site in Hardin County, Texas is approximately 0.5 miles from the boundary of the Big Thicket National Preserve. As described in the EA, seeds, should any be formed, are expected to disperse only up to 50 meters according to the scientific literature (Cremer 1977, Gill 1997, Linacre and Ades 2004). Therefore, even for the Charleston County and Hardin County sites the closest point is greater than the distance for seed dispersal. The *Eucalyptus* hybrid trees will not produce pollen and there are no other *Eucalyptus* trees in the area with which they could cross pollinate with at these locations. As a result there would be no impact due to pollen flow.

Each of the ecologically sensitive areas listed by the commenter is greater than 1,000 acres in size (some are many hundreds of thousands of acres). Each of the proposed field tests represent an insignificant area relative to the respective closest ecologically sensitive site. As discussed in the EA, the proposed field tests will have only negligible impact on local hydrology and therefore is not expected to impact any of the listed protected sites.

References:

Balandin, T. G., E. Edelweiss, N. V. Andronova, E. M. Treshalina, A. M. Sapozhnikov, and S. M. Deyev. 2009. Antitumor activity and toxicity of anti-HER2 immunoRNase scFv 4D5-dibarnase in mice bearing human breast cancer xenografts. Investigational New Drugs Published on-line: http://www.springerlink.com/content/k2815m053jv80152/?p=f28df7a0b25845a59ff7fb24d4f77fef&pi=0.

Bell, D. T. and J. E. Williams. 1997. Eucalyptus ecophysiology. Pages 168-196 *in* J. E. Williams and J. Woinarski, editors. Eucalyptus Ecology: Individuals to Ecosystems. Cambridge University Press, Cambridge.

Cremer, K. W. 1977. Distance of seed dispersal in Eucalyptus estimated from seed weight. Australian Forest Research 7:225-228.

Datta, K., K. H. Bartlett, and K. A. Marr. 2009. Cryptococcus gattii: Emergence in Western North America: Exploitation of a Novel Ecological Niche. Interdisciplinary Perspectives on Infectious Diseases 2009:1-8.

de Assis, T. F. 1996. Melhoramento genético do eucalipto. Informe Agropecuário, Belo Horizonte (Brazil) 18:32-51.

Diaz-Balteiro, L. and L. C. E. Rodriguez. 2006. Optimal rotations on Eucalyptus plantations including carbon sequestration-A comparison of results in Brazil and Spain. Forest Ecology and Management 229:247-258.

Ellis, D. H. and T. J. Pfeiffer. 1990. Natural Habitat of Cryptococcus neoformans var. gatii. Journal of Clinical Microbiology 28:1642-1644.

Escalante Fernandez, R., S. Monreal-Rangel, J. Stanturf, C. Arseneau, and P. Nantel. 2002. Forest Plantations in North America. Plantations Technical Paper Group. 21st Session of the North American Forest Commission. August 2002. <http://www.fs.fed.us/global/nafc/2002/meeting_info/technical_papers/plantations.doc>

FEMA. 1991. The East Bay Hills Fire, Oakland-Berkeley, California. U.S. Fire Administration/Technical Report Series. USFA-TR-060/October 1991.

Forsyth, G. G., A. D. Richardson, P. J. Brown, and B. W. van Wilgen. 2004. A rapid assessment of the invasive status of Eucalyptus species in two South African provinces. South African Journal of Science 100:75-77.

Ganteaume, A., C. Lampin-Maillet, M. Guijarro, C. Hernando, M. Jappiot, T. Fonturbel, P. Pérez-Gorostiaga, and J. A. Vega. 2009. Spot fires: fuel bed flammability and capability of firebrands to ignite fuel beds. International Journal of Wildland Fire 18:951-969.

Gill, A. M. 1997. Eucalypts and fire-- interdependent or independent? *in* J. E. Williams and J. Woinarski, editors. Eucalypt Ecology: Individuals to Ecosystems. Cambridge University Press, Cambridge.

Hofig, K. P., R. L. Moyle, J. Putterill, and C. Walter. 2003. Expression analysis of four *Pinus radiata* male cone promoters in the heterologous host Arabidopsis. Planta 217:858–867.

House, S. M. 1997. Reproductive biology of eucalypts. Pages 30-56 *in* J. E. Williams and J. Woinarski, editors. Eucalyptus Ecology: Individuals to Ecosystems. Cambridge University Press, Cambridge.

Huntington, T. G., A. D. Richardson, K. J. McGuire, and K. Hayhoe. 2009. Climate and hydrological changes in the northeastern United States: recent trends and implications for forested and aquatic ecosystems. Canadian Journal of Forest Research 39:122-212.

Ilinskaya, O. N. and S. Vamvakas. 1997. Nephrotoxic effects of bacterial ribonucleases in the isolated perfused rat kidney. Toxicology 120:55-63.

Jones, M. E., M. Shepher, R. Henry, and A. Delves. 2008. Pollen flow in Eucalyptus grandis determined by paternity analysis using microsatellite markers. Tree Genetics and Genomes 4:37-47.

Junghans, T. G., I. Peters-Robinson, F. L. Bertolucci, and A. C. Alfenas. 1998. The use of self-incompatibility in the production of hybrid eucalyptus seed by 'Aracruz Celulose' in Brazil. Genetics and Molecular Biology [online] 21:8 pp.

Keeton, W. S., P. W. Mote, and J. F. Franklin. 2007. Chapter 13 Climate variability, climate change and western wildfire with implications for the urban-wildland interface. Advances in the Economics of Environmental Resources 6, 225-253 6.

Kidd, S. E., F. Hagen, R. L. Tscharke, M. Huynh, K. H. Bartlett, M. Fyfe, L. MacDougall, T. Boekhout, K. J. Kwon-Chung, and M. Meyer. 2004. A Rare Genotype of Cryptococcus gattii caused the Cryptococcus outbreak on Vancouver Island (British Columbia, Canada). Proceedings of the National Academy of Science 101:17258-17263.

King, J. R. and R. G. Skolmen. 1990. Robusta Eucalyptus. Myrtaceae -- Myrtle family. In, Russell M. Burns and Barbara H. Honkala, eds, Silvics of Forest Trees of the United States, 1965, revised: December 1990. Volume 2. Agriculture Handbook 654 http://www.na.fs.fed.us/pubs/silvics_manual/volume_2/eucalyptus/robusta.htm.

Linacre, N. A. and P. K. Ades. 2004. Estimating isolation distances for genetically modified trees in plantation forestry. Ecological Modeling 179:247-257.

Mariani, C., M. D. Beuckeleer, J. Truettner, J. Leemans, and R. B. Goldberg. 1990. Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature 347:737-741.

McKeand, S. E., B. J. Zobel, T. D. Byam, and D. A. Huber. 2007. Southern Pine Tree Improvement - A Living Success Story. In, Southern Forest Tree Improvement Conference - 2007. http://www.rngr.net/publications/sftic/2007/southern-pine-tree-improvement-2013-a-living-success-story/?searchterm=McKeand

Meskimen, G. and J. K. Francis. 1990. Rose Gum Eucalyptus. *in* R. M. Burns and B. H. Honkala, editors. Silvics of North America: Volume 2. Hardwoods. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington, DC.

National Park Service, U. S. D. o. t. I., Fire Education Office, Point Reyes Station, CA. 2006. San Francisco Bay Area National Parks 2006. A transcontinental legacy:

Fire management, resource protection and the challenges of Tasmanian blue gum. http://biomass.forestguild.org/casestudies/1001/Eucalyptus.pdf.
Núñez Regueira, L., J. A. Rodrıguez Añón, J. Proupın Castiñeiras, and A. Vilanova Diz. 2002a. Determination of risk indices corresponding to eucalyptus in Galicia using bomb calorimetry. Thermochimica Acta 394:267-278.
Núñez Regueira, L., J. A. Rodrıguez Añón, J. Proupin Castiñeiras, A. Vilanova Diz, and A. Romero Garcia. 2002b. Using bomb calorimetry for determination of risk indices of wildfires originating from pine residues. Thermochimica Acta 394:291-304.
Pacheco, I. A. 1987. Polinização de Eucalyptus saligna Smith (Myrtaceae) por *Apis mellifera* L. 1758 (Hymenoptera, Apidae). Universidade de São Paulo, Piracicaba.
Pacheco, I. A., P. Y. Kageyama, F. M. Wiendl, and E. B. Filho. 1986. Estudo da dispersão de pólen de Eucalyptus saligna Smith por abelhas Apis mellifera L. utilizando-se o radiofósforo. IPEF Piracicaba (Brazil 34:47-52.
Pekin, B. K., M. M. Boer, C. Macfarlane, and P. F. Grierson. 2009. Impacts of increased fire frequency and aridity on eucalypt forest structure, biomass and composition in southwest Australia. Forest Ecology and Management 258:2136-2142.
Perez, S., C. J. Renedo, A. Ortiz, M. Manana, and D. Silio. 2006. Energy evaluation of the *Eucalyptus globulus* and the *Eucalyptus nitens* in the north of Spain (Cantabria). Thermochimica Acta 451:57-64.
Peters, G. B., J. S. Lonie, and G. F. Moran. 1990. The breeding systems, genetic diversity and pollen sterility in *Eucalyptus pulverulenta*, a rare species with small disjunct population. Australian J. Botany 38:559-570.
Prior, T. I., S. Kunwar, and I. Pastan. 1996. Studies on the Activity of Barnase Toxins *in Vitro* and *in Vivo*. Bioconjugate Chemistry 7:23-29.
Richardson, A. D. 1998. Forestry Trees as Invasive Aliens. Conservation Biology 12:18-26.
Rottmann, W. H., K. H. Norris-Caneda, and C. Zhang. 2008. *Reproductive ablation constructs* - Patent number 7,453,025. ArborGen, LLC United States.
Sheffield, R. 2009. Planted forests and plantations. *in* W. B. Smith, P. D. Miles, C. H. Perry, and S. A. Pugh, editors. Forest resources of the United States, 2007. Gen. Tech. Rep. WO-78. Washington, DC: U.S. Department of Agriculture, Forest Service, Washington Office. 67-69.
Stallcup, R. 1997. Deadly eucalyptus. Observer No. 108. Point Reyes Bird Observatory. http://www.prbo.org/OBSERVER/Observer108/Focus108.2.html.
Upton, A., J. A. Fraser, S. E. Kidd, C. Bretz, K. H. Bartlett, J. Heitman, and K. A. Marr. 2007. First Contemporary Case of Human Infection with Cryptococcus gattii in Pugent Sound: Evidence for Spread of the Vancouver Island Outbreak. . Journal of Clinical Microbiology 45:3086-3088.
Walden, A. R., C. Walter, and R. C. Gardner. 1999. Genes expressed in *Pinus radiata* male cones include homologs to anther-specific and pathogenesis response genes. Plant Physiology 121:1103-1116.
White, D., C. Beadle, D. Worledge, J. Honeysett, and M. Cherry. 1998. The influence of drought on the relationship between leaf and conducting sapwood area in *Eucalyptus globulus* and *Eucalyptus nitens*. Trees 12:406-414.

Williams, C. G. 2010. Long-distance pine pollen still germinates after meso-scale dispersal. American Journal of Botany 97:1-11.
Williams, T. 2002. America's largest weed. . Audubon. http://www.audubonmagazine.org/incite/incite0201.html.
Worrall, J. A. and B. F. Luisi. 2007. Information available at cut rates: structure and mechanism of ribonucleases. Current Opinion in Structural Biology 17:128-137.

United States
Department of
Agriculture

Marketing and
Regulatory
Programs

Animal and
Plant Health
Inspection
Service

Permit applications 08-011-106rm and 08-014-101rm received from ArborGen LLC

Field testing of genetically engineered *Eucalyptus grandis* X *Eucalyptus urophylla*

Final Environmental Assessment April 2010

Biotechnology Regulatory Services

Agency Contact

Cynthia Eck, Document Control Officer
USDA, APHIS, BRS
4700 River Road, Unit 147
Riverdale, MD 20737-1237
Phone: (301) 734-0667
Fax: (301) 734-8669
Cynthia.A.Eck@aphis.usda.gov

TABLE OF CONTENTS

I. Summary

United States Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS) has prepared an environmental assessment (EA) in response to confined environmental release permit applications (APHIS Number 08-011-106rm and 08-014-101rm) received from ArborGen LLC (ArborGen) to continue research on genetically engineered (transgenic) *Eucalyptus* trees currently permitted by APHIS, to allow the planting of additional trees, and to allow genetically engineered (GE) *Eucalyptus* trees to flower at confined field site locations. These plants are a clone [1] coded EH1 derived from a hybrid of *Eucalyptus grandis X Eucalyptus urophylla* and have been genetically engineered with different constructs. The purpose of the confined environmental release is for ArborGen to assess the effectiveness of gene constructs which are intended to confer cold tolerance; to test the efficacy of a gene introduced to alter lignin biosynthesis; and to test the efficacy of a gene designed to alter fertility. In addition the trees have been engineered with a selectable marker gene which confers resistance to the antibiotic kanamycin.

ArborGen has been granted several permits for transgenic *Eucalyptus* trees, some of which allow flowering and some of which do not allow flowering. ArborGen previously applied for a permit (06-325-111r) requesting that transgenic *Eucalyptus* trees be allowed to flower at a confined field site in Alabama on 1.1 acres. The permit was granted following the completion of an EA and reaching of a FONSI (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf). This permit was subsequently amended to allow flowering on an additional 5.1 acres at the same location bringing the total acres at this site allowed to flower to 6.2 acres. ArborGen has also been granted permit 08-151-101r to allow flowering of transgenic *Eucalyptus* on 1.4 acres on a confined field trial site in Florida. Under permits 06-325-111r and 08-151-101r, a total of 7.6 acres of trees at 2 sites are authorized by APHIS to flower. These two permits were issued for three years and will expire on June 27, 2010 and June 30, 2011repectively. ArborGen has been allowed to plant trees under a different permit 08-039-102rm, but they cannot flower under this permit which expires April 25, 2011. All multiyear field trials are issued under three-year permits, and if applicants wish to continue the field trials thay can renew these permits for an additional 3 years.

ArborGen has submitted two new permit applications 08-014-101rm and 08-011-106rm, for planting and growing trees on 28 sites and is also requesting that trees on 27 of these sites be allowed to flower (the subject of this EA). The original request was to plant 29 sites and allow 28 to flower, however, one location was removed from permit application 08-014-101rm, which reduced the number to 28 sites, with 27 allowed to flower. The 28 sites include those already authorized for planting under permit 08-039-102rm, As of April 2010, ArborGen is currently growing approximately 37 acres of the transgenic *Eucalyptus* hybrid on 15 sites under APHIS permit 08-039-102rm that would be allowed to flower under the two new permits if approved. These two new permits (08-014-101rm and 08-011-106rm) combined would allow flowering on up to 330 acres across 27

[1] Clone – as defined in horticulture and forestry means is a *population* of genetically identical plants that has been derived from one individual. Despite popular use of the word, a clone is not an individual.

locations. The size of each confined field test site in these new permit applications ranges from 0.5 to 20 acres.

The genes are the same as those in permits 06-325-111r and 08-151-101r, with the addition of a gene aimed at altering lignin biosynthesis that is being tested on some of the sites. The status of the field tests permitted under permits 06-325-111r and 08-151-101r is included in Appendix I.

II. Purpose and Need

A. Proposed Action

The proposed action is for APHIS, Biotechnology Regulatory Services (BRS) to issue two confined environmental release permits to allow the planting, field testing and flowering of a GE *Eucalyptus* hybrid clone engineered to express various genes on 28 confined field site locations in the southeastern United States. The genes are intended to confer increased tolerance to cold temperatures, alter fertility and alter lignin biosynthesis. In addition there is a gene used as a selectable marker that confers no other benefit to the GE *Eucalyptus* trees.

B. Purpose of this Environmental Assessment

The purpose of this EA is to assess potential environmental impacts of field research studies being conducted on 28 research sites in Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Texas. Two permit applications were received by APHIS–BRS in January 2008 from ArborGen to continue research on GE *Eucalyptus* hybrid trees originally planted under APHIS authorized notifications, to plant additional trees, and to allow all the trees except the trees on one of the sites in South Carolina to flower. These are permit application numbers 08-011-106rm and 08-014-101rm.

This EA was conducted pursuant to: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. § 4321 et seq.), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500-1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Generally, issuances of permits for confined field trials of regulated articles are categorically excluded under APHIS NEPA Implementing Procedures. However, if APHIS determines that a confined field release of genetically engineered organisms may have the potential to significantly impact the quality of the human environment, as those terms are defined in 40 CFR 1508.27 and 1508.14, an environmental assessment may be prepared, pursuant to 7 CFR § 372.5(d) to determine if an Environmental Impact Statement is warranted. In this case, an EA has been prepared because the permittee intends to allow GE *Eucalyptus* trees to grow under an APHIS confined environmental release permit for 7 years to allow the trees to reach maturity and flower. The actions described in the permit applications involve the confined field release of transgenic *Eucalyptus grandis* x *Eucalyptus urophylla* into the environment. APHIS has prepared this EA to address concerns that the increased number of locations and size of the releases that would be allowed to flower could potentially result in the unconfined release of pollen and/or seed into the

environment. Other impacts such as hydrology, allelopathy and fire are also addressed in this EA.

C. Need for This Action

USDA-APHIS-BRS mission is to protect America's agriculture and the environment using a dynamic and science-based regulatory framework that allows for the safe development and use of GE organisms. APHIS' regulations in 7 CFR part 340, which were promulgated pursuant to authority granted by the Plant Protection Act, as amended, (7 U.S.C. 7701–7772), regulates the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. The Plant Protection Act directs the USDA to facilitate imports and interstate commerce in agricultural products in ways that will reduce, to the extent practicable, the risk of dissemination of plant pests. Under APHIS regulations, the APHIS Administrator has authority to regulate any organism or product altered or produced through genetic engineering that the Administrator determines is a plant pest or has reason to believe is a plant pest. When APHIS receives an application for a permit for environmental release, the application is evaluated to determine whether the environmental release, with appropriate conditions imposed, can be carried out while preventing the dissemination and establishment of plant pests. The receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

> *Administrative action on applications*. After receipt and review by APHIS of the application and the data submitted pursuant to paragraph (a) of this section, including any additional information requested by APHIS, a permit shall be granted or denied (7 CFR 340.4(e)).

The applicant has provided the information associated with this request in the permit application. This information has been reviewed and analyzed in this EA.

D. Purpose and Description of the Research

The purpose of the ArborGen research is to assess the efficacy of the introduced cold tolerance genes and gene to alter lignin biosynthesis in *Eucalyptus*. According to the applicant, genetically engineered cold tolerant *Eucalyptus* would enable the production of this hardwood species for pulping and for biofuel applications in managed plantation forests in the southeastern U.S. In addition, the applicant is researching mechanisms for altered fertility. The confined release of the trees in different areas of the southeast U.S. will allow the applicant to obtain data on performance of the transgenic trees and the efficacy of the inserted genes in a wide variety of environments.

III. Affected Environment

Biology of *Eucalyptus* and Status in the United States

The genus *Eucalyptus* belongs to family Myrtaceae (subfamily: Leptospermoideae) which includes over 700 species. *Eucalyptus* is native to Australia with the exception of some species that are native to the Timor Islands (Groves 1994, Ladiges 1997). There are no wild relatives of *Eucalyptus* that occur naturally in the United States. An overview of the biology of *Eucalyptus grandis* has been published by the US Forest Service (Meskimen and Francis 1990). *Eucalyptus* has been planted as an ornamental species in the extreme southern United States where mild winters will allow some species to grow. *Eucalyptus* normally propagates in its native range via seeds. It does not spread vegetatively like other trees such as poplar or willow. In the United States it is usually propagated and sold commercially as rooted stem cuttings.

There have been numerous attempts to grow *Eucalyptus* as a commercial forest tree in the southeastern United States, but due to its sensitivity to cold temperatures, these attempts have not met with success. It is only grown in commercial plantations in central and southern Florida, where it normally survives freezing temperatures. *Eucalyptus* is adapted to live in the mild arid and semi-arid climate of Australia. Severe freezing events that can occur in the southern United States have limited its establishment as a commercial forest tree. There are plantations of *Eucalyptus grandis* and *E. amplifolia* currently grown in south central Florida as short rotation energy crops and for mulch production (Stricker et al. 2000, Rockwood et al. 2004). These trees are generally planted in areas where severe freezing events are rare.

The species hybrid *E. grandis* x *E. urophylla* that ArborGen wishes to allow to mature and flower under this permit has not been categorized as invasive. The *E. grandis* x *E. urophylla* hybrid has been grown for forty years in South America and during this time there has been no evidence of invasiveness by into natural forest areas which are growing as part of an integrated land management system (Luis Silva, International Paper Company, Brazil, comment to docket APHIS-2008-0059). One of the parents of the hybrid, *E. grandis* has been shown to be naturalized in the State of Florida (http://www.florida.plantatlas.usf.edu). In South Africa, *E. grandis* has been shown to be an invasive species (Forsyth et al., 2004). It is also naturalized in both New Zealand (New Zealand Plant Conservation Network 2005: http://www.nzpcn.org.nz/exotic_plant_life_and_weeds/weed_list.asp.) and Ecuador (http://i3n.iabin.net/participants/ecuador.html). Daehler (1998) listed *E. grandis* as an invader of natural areas. These and other data were recently used in the University of Florida's IFAS Assessment of Non-native Plants in Florida's Natural Areas. The assessment conclusion for *E. grandis* is that it is now: "Predicted to be invasive: recommend only under specific management practices that have been approved by the IFAS Invasive Plant 2 Working Group" (http://plants.ifas.ufl.edu/assessment/predictive_response_forms.html). Specific management practices for four specific cultivars of *E. grandis* have been approved by the Working Group (http://plants.ifas.ufl.edu/assessment/ -- see Approved, Specified and Limited Uses link). The University of Florida IFAS has not made an assessment of the *E. grandis* and *E. urophylla* hybrid.

Numerous species of *Eucalyptus* were introduced into California during that State's early history (see Santos: http://wwwlibrary.csustan.edu/bsantos/euctoc.htm), and some of these species have become established. Two of these, *E. globulus* (Tasmanian blue gum) and *E. camaldulensis* (Red gum) are now categorized as invasive by the California Invasive Plant Council (http://www.cal-ipc.org/ip/inventory/weedlist.php). Neither of these species is being proposed to be planted at the permitted field site.

Traits Engineered into *Eucalyptus*

ArborGen LLC wishes to field test genetically engineered (transgenic) *Eucalyptus* trees during which time the trees may flower. These plants are a clone coded EH1 derived from a hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla*. These have been genetically engineered with different constructs. The purpose of the field trials is to test the effectiveness of the CBF gene which is intended to confer cold tolerance and to test the efficacy of the Barnase gene designed to alter fertility. In a small set of experiments the CBF and Barnase genes are also being tested in combination with genes introduced to alter lignin biosynthesis (claimed as CBI). In addition the trees have been engineered with a common selectable marker gene (*nptII*) which confers resistance to the antibiotic kanamycin.

Confined Field Test Locations

The confined field tests are taking place on land controlled by ArborGen or through contracts for field testing. The exact locations are claimed as Confidential Business Information (CBI) and have been submitted as part of the APHIS permit application. Under the two permits, there are 28 sites where trees have been planted or will be planted, and on 27 of these sites the trees will be allowed to flower. See below for the States and Counties in which these 28 sites are located. An additional site in South Carolina is a holding area for plants in pots and trees. Trees will be held there for planting and will not be allowed to flower.

All the confined field test sites listed in this permit application are either on privately owned managed plantation forests and agricultural farm lands or experimental research stations managed by academic institutions and industry. The standard agricultural and silvicultural practices for land preparation, planting, irrigation, and harvesting of plants have been routinely used on these sites. Sites that include managed pastures have had intense activity including the use of heavy machinery for general upkeep, irrigation, fertilization, controlled grazing and management of grasses. Standard silvicultural practices will be used at these sites for the duration of the field tests. Surveys conducted by the applicant at each of these locations indicate that there are not any old growth forests or undisturbed natural areas in the immediate surroundings of the test sites. The trees will be planted from 0.5 up to 20 acres, depending on the location. In the case of these tests the planting density will be from 300 - 600 trees per acre[2]. An acre is about the size of a football field.

[2] Planting density typically refers to the number of trees per acre. Planting densities can vary greatly depending upon the tree species and the environment, but densities of short rotation hardwood trees in the southeastern US are typically in the range of 300–800 trees per acre. Therefore sites ranging from 10 to 20

Baldwin County Alabama Site:

This location has been an agricultural research station for more than 20 years. The location has been used for managed production of annual agricultural crops and forest trees. Site preparation involved herbicide application, subsoiling, and planting of trees in flat beds. The surrounding areas of the test site consist of field plantings of agricultural crops, experimental forest trees and an abandoned pecan orchard. Approximately 6.2 acres of existing field trials of genetically modified *Eucalyptus* of the same clone (EH1) are being grown under issued permit # 06-325-111r under which these trees are allowed to flower. The oldest of these trees at this site are now entering their fifth growing season.

Up to 8,000 containerized trees, including non-transgenic trees could be transferred to this field test site and planted in field plots of up to 10 acres (at around 300 - 600 trees per acre) over the next three years. An additional field trial of the same clone (EH1) was established at this site on approximately 0.8 acres now covered under permit 08-039-102rm. This trial would be transferred to permit # 08-011-106rm to allow flowering.

Escambia County Alabama Site:

This location had previously been used as an intensely managed pasture for more than 5 years and was planted with grasses suitable for cattle grazing. Site preparation involved herbicide application to remove existing grasses, subsoiling, preparation for possible irrigation, and planting of the test trees in flat beds. The surrounding areas of the test site consist of experimental forest trees; approximately 30 year-old slash pine and a re-forested area with less than 7 year-old mixed stands of pine and hardwood species.

Up to 8,000 containerized trees, including non-transgenic trees could be transferred to this field test site and planted in field plots of up to 10.3 acres over the next three years. The existing field trials of the same clone (EH1) were planted at this site on approximately 0.5 acres that are now covered under Permit #08-039-102rm. These trials would be transferred to permit # 08-011-106rm to allow flowering.

Evans County Georgia Site:

This location has been a commercial nursery for forest seedling production for over 30 years. Site preparation involved herbicide application, subsoiling and planting of trees in flat beds. The areas surrounding the test site consist of nursery beds of forest tree seedlings, experimental forest trees, agricultural crops and mixed stands of hardwood and pine.

Up to 4,000 containerized trees, including non-transgenic trees, could be transferred to this field test site and planted on field plots of up to 5 acres over the next three years. A field trial of the same clone (EH1) was planted on approximately 0.2 acres at this site that is now covered under BRS permit # 08-039-102rm. This trial would be transferred to permit # 08-011-106rm to allow flowering.

---

acres can have from 3000 to 16,000 total trees planted in the ground. Twenty acres, as defined by forest plantation standards in the southeast, is considered a small planting.

Saint Landry's Parrish Louisiana Site:

This location has been an experimental agricultural farm for more than 25 years, used for conducting research experiments with soybean, cotton and wheat. Site preparation involved herbicide application, subsoiling and planting of trees in flat beds. The areas surrounding the test site consist of agricultural fields of rice, sugarcane and millet.

Up to 8,000 containerized trees, including non-transgenic trees could be transferred to this field test site and planted in field plots of up to 11.7 acres over the next three years. Field trials of the same clone (EH1) were planted at this site on approximately 1.9 acres under Permit #08-039-102rm. All tests except 0.2 acres were subsequently terminated. The remaining 0.2 acres would be transferred to permit # 08-011-106rm to allow flowering.

Marshall County Mississippi Site:

This location has been an agricultural research station for more than 50 years, used for conducting research experiments with agricultural crops and grasses. The test site was previously used for experimental planting of grasses. Site preparation involved herbicide application, subsoiling and planting of trees in flat beds. The areas surrounding the test site consist of experimental forest trees, agricultural fields, and less than 5 year-old pine plantations.

A field test of the same clone (EH1) was established at this site on 0.5 acres under permit 08-039-102rm. This test was subsequently terminated. No additional plantings are currently anticipated at this site. Additional tests, if planted at this site, would be allowed to flower under permit # 08-011-106rm.

Pearl River County Mississippi Site:

This location has been used as an agricultural research station for more than 5 years for conducting research experiments with agricultural crops and grasses. The test site was used for experimental planting of grasses. Site preparation involved herbicide application to remove existing grasses, subsoiling, preparation for irrigation installation, and planting of trees in flat beds. The areas surrounding the test site consist of a grape research farm, mixed stands of hardwoods and pine, and a residential area.

Up to 4,000 containerized trees, including non-transgenic trees could be transferred to this field test site and planted in field plots of up to 7 acres over the next three years. The existing field trials of the same clone (EH1) were established at this site on approximately 3.0 acres that are now covered under permit 08-039-102rm. These trials would be transferred to permit # 08-011-106rm to allow flowering.

Bamberg County South Carolina Site:

This location has been a managed forest plantation for more than 14 years. The location has been specifically used for short-rotation planting of hardwoods and softwood trees for forestry research. Standard silvicultural practices for site preparation, irrigation, fertilization, planting and harvesting have been used at this location. Similar practices will be used for the additional field tests to be established at this site. The areas surrounding the test site consist

of experimental forest trees, young pine plantations, mixed stands of hardwoods and pine, and agricultural fields. There are experimental test plots of non-transgenic cold-hardy *E. macarthurii, E. benthamii, E. viminalis, E. badjensis,* and *E. dorrigoensis* planted at least 1000 meters from the test plot location.

Up to 4,000 containerized trees, including non-transgenic trees could be transferred to this field test site and planted in field plots of up to 8.3 acres over the next three years. Field trials of the same clone (EH1) were established at this site on 3.5 acres under permit 08-039-102rm. All tests except 0.2 acres were subsequently terminated. The remaining 0.2 acres would be transferred to permit # 08-011-106rm to allow flowering.

Berkeley County South Carolina Site 1:

This is an extension of a greenhouse facility that has been used for acclimatization of transgenic and non-transgenic plants for more than 7 years. The 0.5 acre release site is located adjacent to greenhouse facilities and is surrounded by hardwoods and pine plantations. This site is a secure fenced holding area where trees growing in containers are transferred from the greenhouse to the out-of-doors for acclimatization prior to field planting. Trees will not be allowed to flower at this location.

Berkeley County South Carolina Site 2:

This location has been a managed forest plantation for more than 7 years. The location has been specifically used for short rotation planting of cottonwood and *Eucalyptus* Hybrid (EH1) for forestry research. Site preparation involved herbicide application, subsoiling, drip irrigation installation, and planting of trees in flat beds. The test site is located adjacent to greenhouse facilities and is surrounded by pine plantations.

Field trials of the same clone (EH1) were established at this site on 0.5 acres under permit 08-039-102rm. These tests were subsequently terminated. No additional plantings are currently anticipated at this site. Additional tests, if planted at this site, would be allowed to flower under permit # 08-011-106rm.

Charleston County South Carolina Site:

This location has been a managed forest plantation for more than 10 years. The location has been specifically used for short-term planting of hardwoods and softwood trees for forestry research. Standard silvicultural practices for site preparation, irrigation, fertilization, planting and harvesting were used at this location. Similar practices were used for the additional field tests established at this site. The areas adjacent to the field test site include young mixed stands of hardwoods and pines.

Field trials of the same clone (EH1) were established at this site on 3.1 acres under permit 08-039-102rm. All tests were subsequently terminated. No additional trials are currently planned for this site. Additional tests, if planted at this site, would be allowed to flower under permit # 08-011-106rm.

Marlboro County South Carolina Site:

This location has been a commercial nursery for forest seedling production for over 30 years. Site preparation involved herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas consist of field plantings of agricultural crops, nursery beds of forest tree seedlings and less than 30 year-old mixed hardwood and pine plantations.

A field trial of the same clone (EH1) was established at this site on 0.3 acre under permit 08-039-102rm. This test was subsequently terminated. No additional trials are currently planned for this site. Additional tests, if planted at this site, would be allowed to flower under permit # 08-011-106rm.

Hardin County Texas Site:

This location has been a managed forest plantation for more than 30 years. The location consists of mixed hardwood tree plantations planted using standard silvicultural practices and was harvested by the owner in 2004. The test site is within the larger harvested area and was re-bedded by the owner for planting. Site preparation included herbicide application and sub-soiling. The areas surrounding the test site consist of mixed hardwood stands and managed loblolly pine plantations. There are experimental test plots of non-transgenic cold-hardy *Eucalyptus* species, *E. macarthurii*, *E. benthamii*, and *E. viminalis* planted within 100 m of the transgenic test plot location.

Up to 16,000 containerized trees, including non-transgenic trees, could be transferred to this field test site and planted in field test plots of up to 20 acres over three years. A field trial of the same clone (EH1) was established at this site on 19.7 acres under permit 08-039-102rm. This trial would be transferred to permit # 08-011-106rm to allow flowering.

Jasper County Texas Site 1:

This location has been a managed pine plantation for more than 25 years. Previous plantings were cultivated in beds using standard silvicultural practices. The existing pine plantation at this site was harvested by the owner in 2007 and re-bedded for planting. The test site is within the larger harvested and bedded site prepared by the site owner. Further site preparation involved herbicide application, subsoiling and planting of trees in flat beds. The areas surrounding the test site consist of harvest age pine plantations.

Up to 16,000 containerized trees, including non-transgenic trees, could be transferred to this field test site and planted in field test plots of up to 20 acres over three years. A field trial of the same clone (EH1) was planted at this site on approximately 0.7 acres under BRS permit # 08-039-102rm. This trial would be transferred to permit # 08-011-106rm to allow flowering.

Jasper County Texas Site 2:

This location has been in agriculture for more than 30 years. The test site was used for pasture for the past 10 years. Site preparation involved herbicide application to remove existing grasses, subsoiling, preparation for irrigation installation, and flat plantings of trees. The areas surrounding the test site consist of natural stands of mixed pine and hardwoods.

Up to 8,000 containerized trees, including non-transgenic trees, could be transferred to this field test site and planted in field test plots of up to 10 acres over three years. A field trial of the same clone (EH1) was established at this site on 1.0 acre under permit 08-039-102rm. This trial would be transferred to permit # 08-011-106rm to allow flowering.

Jefferson County Texas Site:

This location has been used for managed agricultural production of rice for more than 5 years. Site preparation will involve herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of rice plantations. Up to 8,000 containerized trees, including non-transgenic trees, could be transferred to this field test site and planted in field test plots of up to 10 acres over three years

Newton County Texas Site 1:

This location has been a managed loblolly pine plantation for at least 30 years. The previous plantings were cultivated in beds using standard silvicultural practices and the areas have recently been harvested. Site preparation has or will include herbicide application, plowing, and planting of trees in raised or flat beds. The areas surrounding the test sites consist of managed loblolly pine plantations and/or mixed hardwood stands. Up to 16,000 containerized trees, including non-transgenic trees, could be transferred to this field test site and planted in field test plots of up to 20 acres over three years.

Newton County Texas Site 2:

This location has been a managed loblolly pine plantation for at least 30 years. The previous plantings were cultivated in beds using standard silvicultural practices and the areas have recently been harvested. Site preparation has or will include herbicide application, plowing, and planting of trees in raised or flat beds. The areas surrounding the test sites consist of managed loblolly pine plantations and/or mixed hardwood stands. Up to 16,000 containerized trees, including non-transgenic trees, could be transferred to this field test site and planted in field test plots of up to 20 acres over three years.

Newton County Texas Site 3:

This location has been a managed loblolly pine plantation for at least 30 years. The previous plantings were cultivated in beds using standard silvicultural practices and the areas have recently been harvested. Site preparation has or will include herbicide application, plowing, and planting of trees in raised or flat beds. The areas surrounding the test sites consist of managed loblolly pine plantations and/or mixed hardwood stands. Up to 16,000 containerized trees, including non-transgenic trees, could be transferred to this field test site and planted in field test plots of up to 20 acres over three years.

Newton County Texas Site 4:

This location has been a managed loblolly pine plantation for at least 30 years. The previous plantings were cultivated in beds using standard silvicultural practices and the areas have recently been harvested. Site preparation has or will include herbicide application, plowing, and planting of trees in raised or flat beds. The areas surrounding the test sites consist of

managed loblolly pine plantations and/or mixed hardwood stands. Up to 16,000 containerized trees, including non-transgenic trees, could be transferred to this field test site and planted in field test plots of up to 20 acres over three years.

Bay County Florida Site:

This location was used as an intensely managed pasture for more than 15 years, and was planted with grasses suitable for cattle grazing. Site preparation involved herbicide application to remove existing grasses, subsoiling, preparation for possible irrigation installation, and planting of trees in flat beds. The areas surrounding the test site consist of experimental forest trees, agricultural crops and less than 25 year-old hardwoods and pine.

Up to 4,000 containerized trees, including non-transgenic trees, could be transferred to this site and planted in field test plots of up to 5 acres over the next three years. Field trials of the same clone (EH1) were planted at this site on approximately 1.35 acres of test plots under permit #08-039-102rm. All tests except approximately 0.2 acre were subsequently terminated. The remaining 0.2 acres would be transferred to permit # 08-014-101rm to allow flowering.

Columbia County Florida Site:

This location has been a managed pine plantation for more than 20 years. Previous plantings were cultivated in beds using standard silvicultural practices. This area of the tract was burned in a fire in May 2007. After the fire, the area was raked and bedded by the site owner in preparation for re-planting. The test site is within the larger harvested and bedded area, which is surrounded by existing pine plantations and additional harvested tracts. Up to 16,000 containerized trees, including non-transgenic trees, could be transferred to this site and planted in field test plots of up to 20 acres over the next three years.

Gadsden County Florida Site 1:

This location has been used as an agricultural research station for more than 10 years for conducting research experiments on agricultural crops. Standard silvicultural practices were used for site preparation, including herbicide application, plowing and planting of trees in raised or flat beds. The areas surrounding the test site consist of mixed pine-hardwood forests and pine plantations as well as research plantings of agricultural and horticultural crops. There is an experimental plot of non-transgenic *Eucalyptus* species including *E. grandis, E. amplifolia*, and *E. camaldulensis* at least 1000 meters away from the transgenic test location.

Up to 8,000 containerized trees, including non-transgenic trees, could be transferred to this site and planted in field test plots of up to 10 acres over the next three years. A field trial of the same clone (EH1) was planted at this site on approximately 0.2 acres under permit # 08-039-102rm. This trial would be transferred to permit # 08-014-101rm to allow flowering.

Gadsden County Florida Site 2:

This location has been an agricultural research station for more than 10 years. The field has been fallow for approximately seven years. Standard silvicultural practices will be used for

site preparation, including herbicide application, plowing and planting of trees in raised or flat beds. The areas surrounding the test site consist of mixed pine-hardwood forests and pine plantations, as well as research plantings of agricultural and horticultural crops. Up to 12,000 containerized trees, including non-transgenic trees, could be transferred to this site and planted in field test plots of up to 15 acres over the next three years.

Glades County Florida Site:

Due to changes in experimental plans this site will not be used for field trials of these trees and was removed from the permit application per the applicant's request.

Highlands County Florida Site:

This location was previously used for managed production of citrus for at least 15 years. The planting area at this location had been used for field trials of transgenic *Eucalyptus* for more than 6 years. Site preparation involved herbicide application, plowing, and planting of trees in flat beds. Areas surrounding the test site consist of less than 5 year-old second-growth pine and hardwood with mixed grasses. Field trials of 1.4 acres of some of EH1 translines are being grown under issued permit # 08-151-101r under which these trees are allowed to flower. These trees are now entering their fifth growing season. An additional 2.3 acres of tests of different lines with the same EH1 hybrid are being grown under permits 08-039-102rm (2.0 acres) and 09-070-101rm (0.3 acres).

Up to 8,000 containerized trees, including non-transgenic trees, could be transferred to this site and planted in field test plots of up to 10 acres over the next three years.

Marion County Florida Site:

This location has been used as an agricultural research station for more than 5 years for conducting research experiments on agricultural crops. Site preparation involved herbicide application, plowing, and planting of trees in flat beds. Areas surrounding the test site consist of agricultural fields and plantings of horticultural crops. An experimental test of non-transgenic *E. amplifolia* is planted approximately 200 meters from the test plot location. In addition, a field test of 0.3 acres of different lines with the same EH1 hybrid was planted at this site under permit 9-070-101rm.

Up to 8,000 containerized trees, including non-transgenic trees, could be transferred to this site and planted in field test plots of up to 10 acres over the next three years. A field trial of some of the same lines of EH1 clone was established at this site on 0.9 acres under permit 08-039-102rm. This trial would be transferred to permit # 08-014-101rm to allow flowering.

Taylor County Florida Site 1:

This location has been a managed pine plantation for over 20 years. The previous plantings were cultivated in beds using standard silvicultural practices. The existing pine plantations at these sites were harvested by the owner and prepared for re-planting. Additional site preparation involved herbicide application, plowing to remove stumps, and planting of trees

in raised beds. Areas surrounding these test sites consist of managed stands of pine plantations.

Up to 16,000 containerized trees, including non-transgenic trees, could be transferred to this site and planted in field test plots of up to 20 acres over the next three years. A field trial of the same clone (EH1) was planted at this site on approximately 4.3 acres under permit # 08-039-102rm. This trial would be transferred to permit # 08-014-101rm to allow flowering.

Taylor County Florida Site 2:

This location has been under managed pine plantations for over 20 years. The previous plantings were cultivated in beds using standard silvicultural practices. The existing pine plantations at these sites were harvested by the owner and prepared for re-planting. Additional site preparation involved herbicide application, plowing to remove stumps, and planting of trees in raised beds. Areas surrounding these test sites consist of managed stands of pine plantations.

Up to 16,000 containerized trees, including non-transgenic trees, could be transferred to this site and planted in field test plots of up to 20 acres over the next three years. A field trial of the same clone (EH1) was planted at this site on approximately 3.7 acres under permit # 08-039-102rm. This trial would be transferred to permit # 08-014-101rm to allow flowering.

Taylor County Florida Site 3:

This location has been under managed pine plantations for over 20 years. The previous plantings were cultivated in beds using standard silvicultural practices. The existing pine plantations at these sites were harvested by the owner and prepared for re-planting. Additional site preparation involved herbicide application, plowing to remove stumps, and planting of trees in raised beds. Areas surrounding these test sites consist of managed stands of pine plantations.

Up to 16,000 containerized trees, including non-transgenic trees, could be transferred to this site and planted in field test plots of up to 20 acres over the next three years. A field trial of the same clone (EH1) was planted at this site on approximately 1.3 acres under permit # 08-039-102rm. This trial would be transferred to permit # 08-014-101rm to allow flowering.

IV. Alternatives

This EA analyzes the potential environmental consequences of a proposal for APHIS to issue two confined environmental release permits to ArborGen to allow the planting, field testing and flowering of a GE *Eucalyptus* hybrid clone engineered to express various genes on 28 confined field site locations in the southeastern United States. Specifically, these two permits would authorize ArborGen to continue research on GE *Eucalyptus* hybrid trees allowed to be planted under APHIS authorized notifications and permits on 28 sites, to plant additional trees, and to allow all the trees except the trees on one of the sites in South Carolina to flower. Therefore 27 of 28 sites originally allowed to be planted with the GE *Eucalyptus* would be allowed to flower. Based upon the permit

applications submitted by ArborGen, two alternatives are considered and analyzed in this EA: (1) deny the permits and (2) issue the APHIS permits.

A. No Action – Deny the Permit

Under APHIS–BRS regulations, the Administrator must either grant or deny permits properly submitted under 7 CFR part 340. For the purposes of this EA, the No Action alternative would be the denial of permit applications 08-011-106rm and 08-014-101rm.

Transgenic *Eucalyptus* trees (hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla*) have been previously approved for planting under APHIS authorized notifications, and permits. ArborGen has been allowed to plant trees on 28 sites under permit 08-039-102rm that are requested under the two new permit applications to allow flowering; and two field test sites totaling 7.6 acres have been authorized by APHIS to flower under permits 06-325-111r and 08-151-101r. All three permits are still active. Under the No Action Alternative, the transgenic *Eucalyptus* plants (hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla*) currently approved by APHIS for confined field release under permit 08-039-102rm will continue to not be allowed to flower. The trees could remain in the field, but the applicant will be required to either remove developing flowers or remove the trees from the field test if removing flowers becomes too difficult. Under this alternative, the applicant would not be allowed to gather data on performance of the transgenic trees over a multiyear period and the efficacy of the genes in a wide variety of environments. (Permits 06-325-111r (as amended) and 08-151-101r , both of which already allow flowering, will remain in effect until they expire (unless they are renewed under a new permit). These two permits are not affected by the issuance of permits 08-014-101rm and 08-011-106rm.)

B. Preferred Alternative – Issue the APHIS Permit

The APHIS-preferred alternative is to issue the APHIS confined field release permits for a hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla* with supplemental permit conditions (Appendix V) for the requested three-year period. The permits will need to be renewed and subsequently approved by APHIS to allow the transgenic plants to remain in the ground beyond the 3 year time period. Under this alternative, APHIS would issue two confined environmental release permits to ArborGen to allow the planting, field testing and flowering of a GE *Eucalyptus* hybrid clone engineered to express various genes on 28 confined field site locations in the southeastern United States. Specifically, these two permits would authorize ArborGen to continue research on GE *Eucalyptus* hybrid trees originally planted under APHIS authorized notifications, to plant additional trees, and to allow all the trees except the trees on one of the sites in South Carolina to flower. Under this alternative, GE *Eucalyptus* hybrid trees would be allowed to grow and flower where the applicant can gather data on performance of the transgenic trees over a multiyear period and the efficacy of the genes in a wide variety of environments. This alternative would allow the safe development and use of GE organisms under the mission of BRS.

V. Environmental Consequences

A. No Action

Under the no action alternative, the applicant would not be allowed to let the trees flower on 27 sites currently authorized by APHIS for planting under permit 08-039-102rm. At this time 15 of the 27 sites have been planted with *Eucalyptus* hybrid trees. The applicant could continue to plant trees under permit 08-039-102rm, but would not allow the trees to flower. Preventing flower formation would prove impossible over time because the trees grow too tall and too many flowers are produced to remove manually. Therefore the trees on these sites under 08-039-102rm would likely be cut down prior to maturity. The *Eucalyptus* trees currently planted under APHIS permits would remain in the ground but would likely be cut down within 2 or 3 years to prevent flowering. At each of the field sites the locations could remain planted in short rotation *Eucalyptus* field tests if approved by APHIS under a notification or permit, or the sites could be returned to other forest tree production or agriculture/forestry research. Some sites could be returned to pasture or other agricultural activities. Intense activity including the use of heavy machinery for land preparation, general upkeep, irrigation, and fertilization for the management of tree plantings and grasses would continue. For forest tree plantings, standard silvicultural practices would continue to be used at these sites.

B. Preferred Alternative

Under this alternative, the applicant would be allowed to let GE *Eucalyptus* trees produce flowers and to plant more trees. This means that the trees would remain in the ground for at least 3 years and most likely longer since the applicant has indicated that they are planning to renew the permits. If the renewal permits are approved by APHIS, the trees could be allowed to stay in the ground until maturity or when normally harvested (age 7-9). The standard silvicultural practices for land preparation, planting, irrigation, and harvesting of trees would continue to be routinely used on these sites.

Potential Environmental Impact of the Preferred Alternative

Alteration in Susceptibility to Disease or Insects – Potential of the *Eucalyptus* to Harbor Plant Pests

Overall impacts on disease or insect susceptibility would be similar to the no action alternative.

As presented in the permit application submitted by ArborGen:

- There has been no intentional genetic change in these plants to affect their susceptibility to disease or insect damage.

- All of the genes have been previously tested in *Eucalyptus* in existing field tests and the permittee has observed no changes in the incidence of pests, beneficial insects or pathogens between the transgenic and non-transgenic controls.
- None of the genes being engineered into the *Eucalyptus* plants are expected to alter the susceptibility of the transgenic *Eucalyptus* plants to disease or insect damage.

There might be a concern that altered lignin could lead to an increase in insect or disease susceptibility, but the results so far with this particular gene do not indicate that this is the case (*see* below). As prescribed in the supplemental permit conditions assigned to these permits, periodic monitoring of the field plots will allow the detection of any unexpected infestation by plant disease organisms or animal pests. The permittee is required to report any such unanticipated effects to APHIS under the terms of the permit - see 7 CFR 340.4(f)(10)(ii).

Although the trees originated from New Zealand, the trees were propagated in sterile tissue culture and were free of pests upon importation into the U.S. prior to their introduction. All materials were handled in accordance with the USDA–APHIS requirements for import and quarantine under a USDA–APHIS PPQ Post-entry quarantine permit.

Expression of the Gene Products, New Enzymes, or Changes to Plant Metabolism - Risk of the Gene Products on the Environment

Overall impacts of genes for the selectable marker, cold tolerance, altered flowering and altered lignin would be similar to the no action alternative. These same types of genes are currently being used in GE *Eucalyptus* trees being field tested under current APHIS notifications or permits for confined field release. The same genes are being expressed whether the trees are allowed to flower or not to flower.

Gene used as selectable marker

The kanamycin resistance selectable marker gene (*nptII*) engineered into the trees is generally accepted as being safe (Fuchs et al. 1993) and has been used in thousands of field tests with no evidence that it has led to an increase in plant pest characteristics. This gene does not alter the expression of a gene product or change plant metabolism in such a way that it would be expected to cause risk to the environment. In a number of instances, plants transformed with this gene have been deregulated by APHIS (e.g. corn, petition 01-137-01p; rapeseed, petition 01-206-02p; cotton, petition 95-045-01p; and papaya, petition 96-051-01p).

Genes conferring cold tolerance

The C-Repeat Binding Factor (*CBF)* genes are transcription factors that belong to the AP2/EREBP family of DNA binding proteins (Riechmann and Meyerowitz 1998) and like other transcription factors act as control switches for the coordinated expression of other genes in defined metabolic pathways. *CBF* protein recognizes and binds to a cold-

and drought-responsive DNA regulatory sequence designated as the C-repeat (CRT)/dehydration-responsive element (DRE) (Baker et al. 1994), (Yamaguchi-Shinozaki and Shinozaki 1994), which is found in the promoter regions of many cold-inducible genes (Maruyama et al. 2004).

A common observation across experiments in which *CBF* genes are overexpressed in transgenic plants is that constitutive expression of *CBF* negatively impacts a number of other traits (Hsieh et al. 2002). In potato, for example, constitutive expression of *Arabidopsis CBF* genes using the CaMV35S promoter was associated with smaller leaves, stunted plants, delayed flowering, and reduction or lack of tuber production (Pino et al. 2007). In contrast, *CBF* genes under the control of a cold-induced promoter, rd29A (Yamaguchi-Shinozaki and Shinozaki 1993, Kasuga et al. 1999), (Narusaka et al. 2003), increased freezing tolerance to the same level as constitutive expression (about 2 °C, or ~3 °F) while restoring growth and tuber production to the levels similar to wild-type plants (Pino et al. 2007). In the rd29A controlled *CBF* plants the same level of freezing tolerance as the CaMV35S versions was observed after only a few hours of exposure to low but non-freezing temperatures. These results suggest that using a stress-inducible promoter to direct *CBF* transgene expression could significantly improve freeze tolerance without negatively impacting other agronomically important traits. In the case of these *Eucalyptus* trees, the CBF gene is under the control of a cold inducible promoter which causes the gene to be expressed under cold temperatures, thus mitigating the potential of reduced growth by overexpression. Under this promoter the trees exhibit normal plant growth.

The CBF gene is not expected to produce any toxic substances and is not expected to alter the characteristics of the engineered plants other than imparting tolerance to cold temperatures. These genes do not alter the expression of a gene product or change plant metabolism in such a way that it would be expected to cause risk to the environment. These genes are in a number of previous and existing field tests and have not produced unanticipated phenotypes that would indicate there have been changes to plant metabolism leading to increase plant pest characteristics.

Gene for altered fertility

The barnase gene has been engineered into other crops that have been previously reviewed and addressed in multiple environmental assessments by APHIS. Male sterile corn (USDA APHIS petitions for deregulation 95-288-01p, 97-342-01p and 98-349-01p), rapeseed (petitions 98-278-01p and 01-206-01p) and chicory (petition 97-148-01p) have been reviewed and granted non-regulated status by APHIS. There is no reason to believe that the function and expression of this gene will be any different from the plants in which it has been previously assessed. There were no toxicity or allergenicity issues found with this gene in previous FDA reviews (See BNF Nos. 31, 32, 45, 57 and 66 at: http://www.cfsan.fda.gov/~lrd/biocon.html).). The presence of this gene is likely to reduce the ability of the trees to produce progeny and thus further reduce the likelihood of the release of the regulated article into the environment. In greenhouse tests using tobacco and an early flowering model *Eucalyptus* (*E. occidentalis*), the applicant has found that the barnase gene has demonstrated 100% efficacy in preventing pollen

formation. In developing flower buds from field grown transgenic *Eucalyptus* lines containing this cassette, 90% of lines showed complete pollen ablation. Recent observations from the replicated field study being conducted in Alabama under the approved BRS permit (BRS # 06-325-111r-a1) confirm that cold tolerant trees grown at the site and allowed to flower did not produce any viable pollen (see also Appendix I).

Gene for altered lignin

This gene has been engineered into other crops that have been previously released into the environment under both notifications and permits. The gene engineered into the plants in these field tests has been previously tested in ArborGen field trials for more than two years. There might be a concern that altered lignin could lead to an increase in insect or disease susceptibility since lignin is often associated with resistance to insects and disease organisms (Pederson et al. 2005), but the results of field tests with this particular gene have shown no differences in plant pest susceptibility. Growth measurements have indicated that trees containing this gene had normal to a moderately reduced growth phenotype. The trees were also visually inspected on a monthly basis for the presence of any insect and disease damage and these observations found that there have been no differences in insect or diseases occurrence in the transgenic lines compared to the control trees. However, if during the tests there is evidence of increase disease or insect susceptibility, the applicant is required to report this to APHIS. The permittee is required to report any such unanticipated effects (including excessive mortality or morbidity) to APHIS under the terms of the permit - see 7 CFR 340.4(f)(10)(ii).

Non-coding sequences

The transgenic *Eucalyptus* also contains non-coding regulatory sequences[3] derived from plants and plant pathogens. The non-coding regions of the plant pathogens will not result in the production of an infectious entity or cause plant disease symptoms. None of these sequences are expected to pose a plant pest risk.

Method of transformation

The genes were transferred to *Eucalyptus* via well-characterized laboratory techniques that utilize DNA sequences from *Agrobacterium tumefaciens* to transfer introduced genes into the chromosome of the recipient plant (see reviews by (Zambryski 1988, Klee and Rogers 1989) *A. tumefaciens* is a bacterial plant pathogen that can cause crown gall disease on a wide range of dicotyledonous plant species. Although some of the DNA sequences used in the transformation process were derived from the *A. tumefaciens*, the genes that cause crown gall disease are first removed, and therefore the recipient plant does not have crown gall disease. Following transformation, the bacteria are eliminated

[3] A non-coding sequence is the strand of DNA that does not carry the information necessary to make a protein. In this case the non-coding sequences are strands of DNA such as promoters and terminators that drive the expression of the gene but do not result in the formation of a protein, which is the product of the gene. Therefore promoters and terminators, by themselves, cannot result in the production of a disease-causing entity.

from the transformed plant tissue, and the DNA sequences introduced into the plant are maintained and inherited as any other genes of the plant cell.

Alteration in Weediness characteristics – Potential of the Engineered *Eucalyptus* to be Invasive.

The potential of the engineered *Eucalyptus* to be weedy and become invasive was covered in a previous EA and response to comments for APHIS permit 06-325-111r (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf) and is herein incorporated by reference.

The hybrid *Eucalyptus* EH1 used to produce the transgenic trees has not been shown to be weedy or invasive in the U.S (L. Pearson, ArborGen pers. comm.), but no formal assessment has been conducted on its weediness or invasiveness potential. None of the genes introduced into *Eucalyptus* code for traits that would be expected to make the GE hybrids more weedy or invasive. The genes introduced to affect cold tolerance could make the engineered *Eucalyptus* more adapted to cold temperatures in the southern United States, but this trait alone would not impart invasive or weediness characteristics (Kolar and Lodge, 2001) to the engineered plants. The trees would be considered weedy or invasive if they were to produce many seedlings that were readily spread away from the field test sites. Where the non-engineered hybrid *Eucalyptus* (EH1) has been grown in Brazil, on an estimated 400,000 acres planted over 15 years, there has been no indication that large numbers of seedlings are being produced and are becoming invasive from the commercial plantations (L. Pearson, ArborGen pers. comm. and Luis Silva, International Paper Company, Brazil, comment to docket APHIS-2008-0059).

Eucalyptus generally has difficulty establishing without human intervention, even in warmer climates. *Eucalyptus* is intolerant of shade or weedy competition. In order to successfully germinate and establish, *Eucalyptus* seed need contact with bare mineral soil and the removal of competing plants, either as a result of human intervention or naturally following a fire event (Meskimen and Francis 1990, Bell and Williams 1997). The areas surrounding the field release sites would not be readily conducive to the establishment of seedlings because they are managed or unmanaged areas where other plant species are growing. So any seeds that attempted to germinate would face competition and would likely be unsuccessful. The addition of the cold-tolerance genes are not expected to affect the reproductive biology such as seed production or vegetative reproduction capabilities. The gene introduced to alter lignin biosynthesis would also not be expected to affect seed production or vegetative reproduction capabilities. The selectable marker gene, when used previously, did not contribute to weediness or invasive properties of the genetically engineered plants. The gene for altered fertility should not contribute to weediness or invasive properties and should reduce the ability of the trees to produce progeny. None of the traits introduced into the transgenic *Eucalyptus* will compromise the ability to control these plants as weeds.

In addition, the supplemental permit conditions assigned to these permits will limit the reproductive capabilities of this GE *Eucalyptus* outside the confined field trial locations.

Possibility of Gene Flow Within the Field Test

All of the trees in the test plots, including control non-transgenic trees, have the same parental genotype EH1. The high level of self incompatibility in *Eucalyptus* (Campinhos et al. 1998, Pound et al. 2002) is expected to significantly reduce the potential for crossing[4] (gene flow) within the test plots. Seed set from any self pollination is expected to be poor, and the vigor of any selfed progeny is also expected to be greatly reduced. In experiments conducted in Brazil and Alabama, the control self-pollinated seed obtained from this genotype had abnormal morphology and failed to germinate (ArborGen, unpublished results). In recent field releases allowed to flower in Alabama and Florida, ArborGen has observed a low level of seed production. However, no seedlings have been found established beneath the trees or in the surrounding areas (Appendix I). Even if seed are produced in the test, several factors in the biology of *Eucalyptus* would limit the potential for seed dissemination. Although *Eucalyptus* seed is very light and small, it is not adapted to wind dispersal and consequently the dispersal of seed is very limited, generally being confined within a radius of twice the tree or canopy height (approximately 50 meters for a 25 meter tall tree at harvest age)(Cremer 1977, Gill 1997, Linacre and Ades 2004). Another consequence of the very small size of *Eucalyptus* seeds is that they have very limited reserves and are intolerant of shade or weedy competition. In order to successfully germinate and establish, *Eucalyptus* seed needs contact with bare mineral soil and lack of competition either as a result of human intervention or naturally following a fire event (Meskimen and Francis 1990, Bell and Williams 1997). *Eucalyptus* plantations are typically established using rooted plantlets because of poor establishment using direct seeding methods. Even for the rooted plants, competition control is recommended for several months after planting to ensure good survival (Meskimen and Francis 1990). Therefore there is limited possibility that volunteer seedlings could become established in any unmanaged areas that may be close to the site. However, if they were to appear, the supplemental permit conditions for these two permits will require that all volunteers be reported to APHIS, found and destroyed to prevent any spread of trees from the field release site.

Eucalyptus seeds do not have any dormancy barriers to prevent germination of volunteer seeds (Grose 1960, Wellington 1989, Gill 1997) and seed viability and storage of *Eucalyptus* seeds in soil are less than one year (Gill 1997). The *Eucalyptus* species that have become invasive in California are particularly adapted to a Mediterranean climate subject to summer fog, which is conducive to seed germination in those species (http://ucce.ucdavis.edu/datastore/detailreport.cfm?usernumber=48&surveynumber=182). This type of climate does not exist in the Southeastern U.S. In the event that any viable seeds are produced and are deposited on in an area conducive to germination from this field trial, these seeds would be expected to germinate within 7 – 14 days (Meskimen and Francis 1990). In accordance with the supplemental permit conditions for these permits,

[4] When plants or trees "cross" the male pollen from one tree can pollinate (fertilize) the female ovule (or egg) on the same tree or on another tree. Unlike animals, some plants can fertilize themselves when the pollen and ovule are produced on the same tree. In this case all the trees are genetically identical (i.e. the same clone)(see footnote 1). Eucalyptus has a built-in mechanism that will inhibit self-fertilization. So these GE trees are likely to exhibit reduced fertility and reduced numbers of viable seed compared to fully sexually compatible Eucalyptus trees since they are genetically identical individuals.

the bordering fields within 100 meters from the edge of the trials will be monitored every six months for germinating seedlings by the applicant. This distance is twice the 50 meter distance that seeds would be expected to be dispersed from a tree at harvest age (Cremer 1977, Gill 1997, Linacre and Ades 2004). If transgenic seedlings are observed they will be destroyed by the applicant either by uprooting or by spraying with EPA approved herbicides (e.g., glyphosate or other herbicides to which these trees are susceptible) and APHIS will be notified of their occurrence.

Possibility of Gene Flow Outside of the Field Test

Eucalyptus is adapted for insect pollination, with bees being the predominant vector (Pacheco et al. 1986, Pacheco 1987, House 1997). Under ideal conditions of humidity and temperature, viable *Eucalyptus* pollen can only be found within approximately 100 meters from the edge of nearest tree stand (Peters et al. 1990, Linacre and Ades 2004). Pacheco (1987) verified that bees (*Apis* spp.) are the most effective pollinators of *Eucalyptus*, with activity increasing up to 100 meters from the beehive, and decreasing after this distance. de Assis (1996) indicated that the minimum distance to prevent undesirable pollen contamination of seed producing areas is approximately 300 meters. Even if bees were to transport pollen farther distances from the field test sites, there are no sexually compatible species nearby with which they could cross and produce offspring (see description of the field test sites below).

There could be two possible routes of gene flow outside of the confined field test to other *Eucalyptus* species trials. One could be with nearby transgenic *Eucalyptus* field test trees and the other could be with other nearby non-transgenic *Eucalyptus* species trials.

- Transgenic trials being conducted by the applicant under approved under current BRS notifications and permits, of the same hybrid *Eucalyptus* variety EH1, are planted adjacent to or within the proposed field test plot locations at the sites in Escambia and Baldwin Counties in Alabama; Evans County in Georgia; Saint Landry's Parish, Louisiana; Pearl River County in Mississippi; Jasper County in Texas; and Bay, Highlands, Taylor and Gadsden (Site 1) Counties in Florida. The applicant has indicated that they are not aware of any commercial plantings of compatible *Eucalyptus* species within 1000 meters of the proposed test plot location at any of these sites. Therefore, based upon the limited distance that viable pollen is likely to occur outside a tree stand, it is highly unlikely that gene flow would occur outside of the confined field test sites at these locations. The two field trials in Alabama and Florida that have allowed these hybrids to flower under an APHIS permit are producing low numbers of viable seeds (see Appendix I). Based upon monitoring conducted from the applicant, no volunteer seedlings have been observed at these two field trials to date.

- At all test sites in Berkeley, Charleston, and Marlboro Counties in SC; Marshall County in MS; Jefferson and Newton Counties in Texas; Columbia and Gadsden (except Site 1) Counties in Florida, there are no known existing transgenic or non-transgenic Eucalyptus field trials. The applicant is not aware of any commercial plantings of compatible *Eucalyptus* species within 1000 meters of the proposed

test plot location at these sites. Therefore, based upon the limited distance that viable pollen (if it were to be produced) is likely to occur outside a tree stand, it is highly unlikely that gene flow would occur outside of the confined field test sites at these locations.

- At the Bamberg County South Carolina site, there are transgenic trials, covered under permit # 08-039-102rm, of the same hybrid Eucalyptus variety EH1 planted adjacent to the proposed field test plot location at this site. There are also experimental test plots of non-transgenic cold-hardy *Eucalyptus* species (*E. macarthurii*, *E. benthamii*, *E. viminalis*, *E. badjensis*, and *E. dorrigoensis*) planted by the applicant within 1000 meters of the proposed test plot location. These cold hardy *Eucalyptus* species are highly unlikely to be reproductively compatible with the hybrid *Eucalyptus* variety because of distant phylogenetic relationship and asynchronous flowering. *E. grandis* and *E. urophylla*, for which hybrids have been generated in directed breeding programs, are in the Salignae and Resiniferae series, respectively, of Section Transversaria (http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/eucclass.pl?gn=*Eucalyptus*). In contrast, *E. macarthurii*, *E. benthamii*, *E. viminalis*, *E. badjensis*, and *E. dorrigoensis* are far removed genetically from the genotype used in this field trial on the evolutionary scale and reside within different Series and in the Section Maidenaria of genus *Eucalyptus* (see http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/eucclass.pl?gn=*Eucalyptus* for details on sections and series in *Eucalyptus*). The published literature supports the fact that natural hybridization among distantly related species within genus *Eucalyptus* is rare and hybrid inviability increases with increasing taxonomic distance between parents (Potts and Dungey 2004). In accordance with the supplemental permit conditions, the applicant will monitor flower development in both the transgenic trial and the non-transgenic trial to determine if there is any overlap in the occurrence of mature flowers. If overlaps are observed the applicant is required to report their findings to APHIS. In the unlikely event that viable pollen from the non transgenic *Eucalyptus* trees fertilizes the transgenic trees, any seed dispersal is expected to be within 100 m of the test plot. Should any hybridization and viable seed production occur, in accordance with supplemental permit conditions, the monitoring for and removal of volunteers within 100 m from the edge of transgenic test plot by the applicant would effectively eliminate any seedlings that may be produced.

- At the Hardin County Texas site, there are transgenic trials, covered under permit # 08-039-102rm, of the same hybrid Eucalyptus variety EH1 planted adjacent to the proposed field test plot location at this site. Experimental test plots of non-transgenic *E. macarthurii, E. benthamii and E. viminalis* are planted within 100 meters of the proposed test plot location. These cold-hardy *Eucalyptus* species are unlikely to be reproductively compatible with the hybrid *Eucalyptus* variety EH1 because of distant phylogenetic relationship and asynchronous flowering. *E. grandis* and *E. urophylla*, for which hybrids have been generated in directed breeding programs, are in the Salignae and Resiniferae series, respectively, of Section Transversaria (http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/eucclass.pl?gn=*Eucalyptus*). In contrast, *E. macarthurii, E. benthamii and E.*

viminalis are far removed genetically from the genotype used in this field trial on the evolutionary scale and reside within a different Series and in the Section Maidenaria of genus *Eucalyptus* (see http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/eucclass.pl?gn=*Eucalyptus* for details on sections and series in *Eucalyptus*). The published literature supports the fact that natural hybridization among distantly related species within genus *Eucalyptus* is rare and hybrid inviability increases with increasing taxonomic distance between parents (Potts and Dungey 2004). In accordance with the supplemental permit conditions, the applicant will monitor flower development in both the transgenic trial and the non-transgenic trial to determine if there is any overlap in the occurrence of mature flowers. If overlaps are observed the applicant is required to report their findings to APHIS. In the unlikely event that viable pollen from the non transgenic *Eucalyptus* trees fertilizes the transgenic trees, any seed dispersal is expected to be within 100 m of the test plot. Should any hybridization and viable seed production occur, in accordance with supplemental permit conditions, the monitoring for and removal of volunteers within 100 m from the edge of transgenic test plot by the applicant would effectively eliminate any seedlings that may be produced.

- At the Marion County Florida site, a transgenic trial, covered under Permit # 08-039-102rm, of the same hybrid Eucalyptus variety EH1 is planted at the proposed field test plot location. An experimental test of non-transgenic *E. amplifolia* is planted approximately 200 meters from the proposed test plot location. This *Eucalyptus* species is unlikely to be reproductively compatible with the hybrid *Eucalyptus* variety EH1 because of its distant phylogenetic relationship and asynchronous flowering. Phenology of the *Eucalyptus* hybrid used in the transgenic trial (mature flowers in mid to late summer) and *E. amplifolia* (mature flowers in the spring) indicates that there would not be any overlap in flowering times. As noted above, viable pollen dispersal is generally limited to within 100 m of the tree stand with the minimum distance to prevent undesirable pollen contamination of seed producing areas set at approximately 300 m. All trees to be planted in this proposed field trial have been shown in previous trials to exhibit the reduced fertility trait (see Appendix I). Results from these studies have shown that the reduced fertility trait is highly effective in preventing the formation of mature viable pollen. Based upon this information, the hybrid *Eucalyptus* variety EH1 are not expected to successfully fertilize the non transgenic *E. amplifolia* trees. Observations will be made by the applicant on developing flowers in the test site to verify that these trees do have reduced fertility. The applicant will monitor flower development in both the transgenic trial and the non-transgenic trial to determine if there is any overlap in the occurrence of mature flowers. In the unlikely event that viable pollen from the non transgenic *E. amplifolia* trees could fertilize the transgenic trees, any seed dispersal is expected to be within 100 m of the test plot. Should any hybridization and viable seed production occur, in accordance with supplemental permit conditions, the monitoring for and removal of volunteers within 100m from the edge of transgenic test plot by the applicant would effectively eliminate any seedlings that may be produced.

- At the Gadsden County Florida Site 1, a transgenic trial, covered under Permit # 08-039-102rm, of the same hybrid Eucalyptus variety EH1 is planted at the proposed field test plot location. There are experimental plantings of non-transgenic *Eucalyptus* species including *E. grandis*, *E. amplifolia*, and *E. camaldulensis* which are at least 1000 meters away from the transgenic test location. Therefore, based upon the limited distance that viable pollen (if it were to be produced) is likely to occur outside a tree stand, it is highly unlikely that gene flow would occur outside of the confined field test sites at these locations. In addition, even though the hybrid genotype used in these studies for transformation may be compatible with *E. grandis* trees grown in Florida, *E. grandis* produces mature flowers in the fall whereas the hybrid genotype used in these tests produces mature flowers in mid to late summer. Therefore, because of asynchronous flowering, hybridization of transgenic trees with the E. grandis at this site would be virtually negligible.

There are other species of cold-hardy *Eucalyptus* that can possibly be grown in the Southeast U.S. These species include *E. neglecta, E. niphophila, E. pauciflora, E. camphora, E. nova-anglica, E. macarthurii, E. gunnii* and *E. cinerea*. These could occur in the same states as the proposed field trials . Among these species, *E. cinerea*, also known as the silver dollar tree or Argyle Apple, is the most popular species grown for its ornamental foliage (http://www.australiaplants.com/Eucalyptus_cinerea.htm).

The transgenic *Eucalyptus* hybrids that will be grown in the proposed field trials are not likely to be sexually compatible with any of the cold hardy species listed above. For example, *E. grandis* and *E. urophylla*, for which hybrids have been generated in directed breeding programs, are in the Salignae and Resiniferae series, respectively, of section Transversaria (http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/eucclass.pl?gn=*Eucalyptus*). In contrast, *E. cinerea*, and other cold hardy species mentioned above are far removed genetically from the genotype used in this field trial on the evolutionary scale and reside within different Series and Sections of genus *Eucalyptus* (see http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/eucclass.pl?gn=*Eucalyptus* for details on sections and series in *Eucalyptus*). Even among the closely related species of *Eucalyptus*, hybridization rates are generally very low (Volker 1995). The published literature supports the fact that natural hybridization among distantly related species within genus *Eucalyptus* is rare and hybrid inviability increases with increasing taxonomic distance between parents (Potts and Dungey 2004). Where hybridization is possible, it often requires significant human intervention in directed breeding/crossing efforts. (Potts and Dungey 2004) make reference to the high degree of inviability in F_1 hybrids (offspring). Inviability of these offspring may be expressed at germination, in the nursery and even after planting in the field. Slower germination of hybrid seed often occurs, along with reduced survival of germinants in the nursery, and many seedlings have abnormal phenotypes. Griffin et al. (1988) surveyed natural and manipulated hybrids in the genus *Eucalyptus* and discussed the challenges of developing even human-made hybrids from such wide crosses (in this case *E. grandis* and *E. globulus* in sections Transversaria and Maidenaria respectively), with only 4.4% of seed germinating and only 3.2% of these producing trees that were worthy of further evaluation. To achieve the development of viable hybrids sometimes hundreds of hand pollinations must be made to find a viable

hybrid that will grow normally. An example of the procedures required to make these wide-cross hybrids is given in (Barbour and Spencer 2000).

A further barrier to potential crossing between the transgenic trees with ornamental *E. cinerea* and other species is the expected differences in flowering times between species (Gore and Potts 1995, Potts et al. 2003). For example, *E. cinerea* flowers in spring, while the transgenic hybrid genotype used in this test initiates flowers in early summer with expected maturation in mid to late summer. In the United States, ArborGen data indicate that flowering of the clone being tested occurs in the summer.

Based on the above information, there is little if any significant risk for outcrossing to or from other *Eucalyptus* species because: 1) to date the trees that have been allowed to flower have shown no mature pollen formation; 2) other species that are or could be grown in the area are unlikely to be compatible; 3) it is unlikely that flowering time in other species will overlap with the hybrid used in this test and; 4) hybrids, in the event that they could form, would be expected to be of very poor vigor.

Possibility of Vegetative Propagation / Persistence Outside of the Field Test

Overall impacts on the possibility of vegetative propagation and persistence outside the field test location would be similar to the no action alternative.

Unlike some other hardwood forest trees, *Eucalyptus* does not spread in the environment via natural abscissions of branches, or cladoptosis. The asexual propagation of shoots via rooted cuttings requires specific environmental conditions such as a greenhouse or a high humidity environment (Hartney 1980), so it is highly unlikely that any shoots that fall or that are removed from the trees would propagate themselves in the wild.

Suckering (production of shoots from subterranean roots) does not occur in this *Eucalyptus* hybrid. Regrowth of shoots from stumps of felled trees is common and this practice, known as coppicing, is used to regrow trees in a plantation after harvest (Meskimen and Francis 1990). In accordance with supplemental permit conditions this regrowth will be managed in this confined field test at termination by devitalizing any sprouts that form from the stumps of harvested trees using EPA registered herbicide treatments.

Potential of the *Eucalyptus* in the Field Tests to Become an Invasive Species that Threatens Native Plant and Animal Communities.

There could be a concern that adding the cold tolerance trait would make the engineered *Eucalyptus* more adaptive and invasive in the southeastern U.S. It has been hypothesized that engineered traits such as cold tolerance could significantly affect the engineered variety's ability to propagate, survive, and impact native ecosystems.

There is no evidence to date that the untransformed clone of the *Eucalyptus* hybrid in these permits is weedy or invasive in the U.S. (L. Pearson, ArborGen pers. comm.) None of the genes introduced into *Eucalyptus* code for traits that would be expected to make

the plants more weedy or invasive. The genes introduced to affect cold tolerance could make the engineered *Eucalyptus* more adapted to cold temperatures in the southern United States, but this trait in and of itself would not impart invasive or weediness characteristics (Kolar and Lodge 2001) to the engineered plants.

As discussed in previous sections of this EA, there are multiple mechanisms in place that would prevent these *Eucalyptus* hybrids from establishing themselves in the wild. Since only one clone is being planted, viable seed set is likely to be limited due to self incompatibility. In accordance with supplemental permit conditions monitoring of seed set and seedling viability will be required by the applicant during these confined field tests so that the extent of seed production and seedling establishment can be determined. In addition altered fertility leading to the lack of viable pollen development has been engineered into the trees. The addition of the cold-tolerance genes are not expected to affect the reproductive biology such as seed production or vegetative reproduction capabilities. The gene introduced to alter lignin biosynthesis would also not be expected to affect seed production or vegetative reproduction capabilities. The selectable marker gene, when used previously, did not contribute to weediness or invasive properties of the genetically engineered plants. The gene for altered fertility should not contribute to weediness or invasive properties and should reduce the ability of the tree to produce progeny.

In unlikely event that seeds are formed and seedlings are produced, none of the traits introduced into the transgenic *Eucalyptus* will compromise the ability to control these plants as weeds so spread of seedlings and trees from the field test sites is highly unlikely. Volunteers can be readily identified and controlled. Should any hybridization and viable seed production occur, in accordance with supplemental permit conditions, the monitoring for and removal of volunteers within 100m from the edge of transgenic test plot by the applicant would effectively eliminate any seedlings that may be produced. If transgenic seedlings are observed they will be destroyed by the applicant either by uprooting or by spraying with EPA approved herbicides (e.g., glyphosate or other herbicides to which these trees are susceptible) and APHIS will be notified of their occurrence.

Impact on Existing Agricultural Practices

Overall impacts on existing agricultural practices would be similar to the no action alternative.

The establishment and growth of these small confined field tests will not have any impact on existing agricultural practices because they are solely for research purposes. Current agricultural practices will essentially remain unchanged. As indentified by the applicant, the field sites that are being proposed under these permits have been used as forest tree plantations, pastures, or for forestry and agriculture research and are specifically designed for field testing crop plants or forest trees.

Potential Impacts to Wildlife

Native floral communities

The field sites in the permit applications are located in Bamberg, Berkeley, Charleston, and Marlboro counties, South Carolina; Evans county, Georgia; Baldwin and Escambia counties, Alabama; St. Landry's Parish, Louisiana; Marshall and Pearl River counties, Mississippi; Hardin Jasper, Jefferson and Newton counties, Texas; and Bay, Columbia, Gadsden, Highlands, Marion, and Taylor counties, Florida. These sites are a mixture of pasture, crop lands and forested areas. In the unlikely event that viable seeds are formed, these areas are unsuitable for the establishment of the *Eucalyptus* hybrid clone. As discussed in previous sections of this EA, *Eucalyptus* is intolerant of shade or weedy competition. In order to successfully germinate and establish, *Eucalyptus* seed need contact with bare mineral soil and the removal of competing plants, either as a result of human intervention or naturally following a fire event. With the exception of the field test area, the agricultural areas surrounding the field sites are not conducive to the establishment of *Eucalyptus*. The surrounding agricultural and tree crops would provide a shady canopy and competition for light and other resources that would impede seedling establishment of *Eucalyptus*. The inhospitable conditions for seed germination, in combination with the supplemental permit conditions established for these permits, will make the establishment of *Eucalyptus* in the surrounding area highly unlikely. Should any hybridization and viable seed production occur, in accordance with supplemental permit conditions, the monitoring for and removal of volunteers within 100m from the edge of transgenic test plot by the applicant would effectively eliminate any seedlings that may be produced. If transgenic seedlings are observed they will be destroyed by the applicant either by uprooting or by spraying with EPA approved herbicides (e.g., glyphosate or other herbicides to which these trees are susceptible) and APHIS will be notified of their occurrence.

Terrestrial animals

The most likely animals to encounter the transgenic *Eucalyptus* trees in this confined field trial would be browsing mammals (e.g., deer), burrowing animals (such as rodents), and leaf consuming insects (considered plant pests). In the event of consumption of plant material or seeds by other animals, the gene products produced by the selectable marker gene and genes of interest do not produce any toxin or have any similarity to known toxins (*see* Section above on - Risk of the Gene products on the Environment). Therefore APHIS concludes that the *Eucalyptus* hybrid would have no adverse impacts on any native vertebrate or invertebrate animal species.

Aquatic organisms

Eucalyptus germinates in areas of bare mineral soils and would not be expected to establish in aquatic or riparian environments. There is no expectation of toxicological effects on any aquatic organism due to the ingestion of the transgenic plant material in this confined field trial (*see* Section above on - Risk of the Gene products on the Environment). Therefore, APHIS concludes that the *Eucalyptus* hybrid would have no adverse impacts on any aquatic species.

Potential Impacts by Fire

Overall impacts of fire would be similar to the no action alternative.

Most *Eucalyptus* communities in Australia have evolved in the presence of periodic fire, and fires are an integral part of the *Eucalyptus* ecosystem (Ashton 1981) (Gill 1997). Many *Eucalyptus* species are known to be highly flammable and depending upon the species, location and age, they can be very resistant or susceptible to fire damage (Gill 1997). *Eucalyptus* fires can be very hot and move rapidly. The bark catches fire readily, and deciduous bark streamers tend to carry fire into the canopy and to disseminate fire ahead of the main front (Ashton 1981) (Skolmen and Ledig 1990) (Esser 1993). Other features of *Eucalyptus* that promote fire spread include heavy litter fall, flammable oils in the foliage, and open crowns bearing pendulous branches, which encourages maximum updraft (Esser 1993, Gill 1997). In the U.S., there have been reports of significant fires in California and many have been blamed on the widespread planting of *Eucalyptus*. Fuel buildup occurs very rapidly in unmanaged bluegum *Eucalyptus* stands in California which has lead to significant forest fires. The buildup of litter and dead grass are primary responsible for the spread of these fires (see Santos: http://wwwlibrary.csustan.edu/bsantos/euctoc.htm).). The Forest Service indicates that fuel reduction programs and the establishment of firebreaks in *Eucalyptus* plantings can reduce wildfire hazard. (Esser 1993).

There is a historical risk of forest fire in the southeastern U.S., however, the probability that these confined field trials will increase the risk and severity of forest fires in their respective locations is very small. These plantings are small (none greater than 20 acres) and they will be managed by the applicant to prevent litter buildup. These sites are also physically isolated from nearby plantations. If they were to catch fire, the fires would likely be readily contained. The sites are managed to reduce the risk of fire spreading to or from the study areas by maintaining a firebreak between the test plots and adjacent forested areas. Depending on local conditions at each site the firebreak may be a road, a cultivated strip or a plowed fire line.

Potential Impacts to Human Health

Overall impacts on human health would be similar to the no action alternative.

During the comment period for the EA prepared for permit 06-325-111r, there were concerns expressed that *Eucalyptus* field tests could be a source of *Cryptococcus neoformans gattii*. APHIS conducted a thorough review of *C. neoformans gattii* and the possibility that the field tests could pose a risk to human health (APHIS 2004) EA and response to comments for permit 06-325-111r (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf) and concluded that the field trial would not lead to a higher incidence of *C. gattii* in the U.S. and therefore should not pose an unnecessary risk to human or animal health.

C. neoformans gattii is a fungal pathogen that is hosted on a variety of species of *Eucalyptus* as well as other tree species (Upton et al. 2007). It causes systemic fungal

infections in humans, leading to fungal meningitis and death (Datta et al., 2009). *C. neoformans gattii* has been found on a number of *Eucalyptus* hosts, some of which are being grown in commercial plantations and imported and exported for ornamental use. People have contracted and died from cryptococcosis in India, Africa, Taiwan, South America and California (Datta e al, 2009). *C. neoformans* infections are found particularly in AIDS patients due to their weak immune systems (Chaturvedi et al, 2005),. Infections with this fungus are rare in those with fully functioning immune systems. For this reason, *C. neoformans* is sometimes referred to as an opportunistic fungus. There was an outbreak of cryptococcal disease on the eastern portion of Vancouver Island, British Columbia in 1999 (Datta et al., 2009). The disease was previously only known to occur in tropical or semi-tropical climates.

It is unlikely that the trees that are the subject of the proposed field release can be a source that might introduce the pathogen into the U.S because the trees were derived from sterile tissue culture lines. The transgenic *Eucalyptus* started as a hybrid developed in Brazil. In Brazil, small pieces of the tissue derived from the hybrid were put into sterile tissue culture and sent to New Zealand for transformation. The transformed lines were sent to the U.S as sterile tissue culture lines that were inspected by APHIS Plant Protection and Quarantine inspectors prior to entry into the U.S. *C. gattii* spores readily germinate in culture. If *C. gattii* spores were present in the tissue culture, contamination, would be evident and the affected lines would be discarded prior to regeneration of trees for introduction into the environment. Another reason it is unlikely that spores could be or were ever present in the hybrid lines used in the proposed confined field trial is that in the *Eucalyptus* species where *C. gattii* is associated, the pathogen is primarily found colonizing the bark or decaying wood in hollows of older trees (Kidd et al, 2007). The tissue culture used for the *Eucalyptus* hybrid was not derived from woody tissue nor was woody tissue generated during tissue culture. Because the trees were derived from tissues that are not known to be a source of the spores and were derived from sterile tissue culture lines that by all appearances were free from any fungal contamination, there is a negligible risk that the hybrid trees used in the field trial could be or have been contaminated with *C. gattii*.

The risk that these field trials will result in a higher incidence of the fungus in the U.S. and thereby pose a risk to human health is considered to be negligible for the following reasons. First, there is not a clear association between *E. grandis* or *E. urophylla* and *C. gattii*. Second, there is no reason to believe that the genetic modification of the hybrids will alter the association of the trees with *C. gattii*. Third, the scale of the field tests is miniscule compared to the vast expanses of native trees that could potentially harbor the pathogen. Based on the above considerations we have concluded that an increase of additional acreage planted to *Eucalyptus* would not impact the likelihood that these field trials should lead to a higher incidence of *C. gattii* in the U.S. and therefore should not pose an unnecessary risk to human health.

Transfer of Genetic Information to Organisms with which it Cannot Interbreed - Horizontal Gene Transfer to Other Organisms

Overall impacts on horizontal gene transfer would be similar to the no action alternative.

Horizontal gene transfer (HGT) is any process in which an organism incorporates genetic material from another organism without being the offspring of that organism. HGT is a common phenomenon among bacteria but is not common between higher organisms (Keese 2008). HGT and expression of DNA from these *Eucalyptus* hybrid plant species to bacteria is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko et al. 2000, Wood et al. 2001, Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al. 2001, Brown 2003). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic (i.e., bacterial) expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, many common transgenes used in plant biotechnology are derived from bacteria commonly found in the environment. The FDA has evaluated horizontal gene transfer from the use of selectable marker genes and concluded that the likelihood of transfer of such genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Biotechnology/ucm096135.htm. Therefore APHIS concludes that horizontal gene transfer poses no environmental risk.

C. Cumulative Effects

The field test sites in this permit application have been in agricultural or forest research, or in agricultural production or forest tree plantations for 5 to 50 years. Therefore the land has been in continuous agricultural or forest tree production at all the proposed field sites for at least 5 years prior to these proposed releases and it is reasonably foreseeable that if the permit were not issued that the sites would continue to be maintained under similar agriculture or forestry production. It is also reasonably foreseeable that the applicant may request to further extend the permit for this environmental release for additional years beyond the 3 years indicated in the pending permit to observe the growth of these trees to maturity. Moreover, APHIS has received a petition for the deregulation of these transgenic *Eucalyptus* trees, however, the environmental effects of that petition will be analyzed in a separate NEPA document. The temporary change from agricultural crops to a tree crop may result in a temporary change in resident animal and plant species, but after harvest and termination of the proposed permits, it is reasonably foreseeable that the land will return to agriculture or be replanted to tree production or research. At the end of the field test, transgenic plant material will be removed from the test site and/or destroyed in accordance with supplemental permit conditions established for these permits. Therefore the only past, present, and reasonably foreseeable actions associated with the locations for the proposed releases under permit are those related to agricultural or forest tree production. APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create cumulative impacts or reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.)

associated with the release sites or the ecosystem in which they are situated. No resources will be significantly impacted due to cumulative impacts resulting from the proposed action.

D. The Degree to Which the Possible Effects are Highly Uncertain or Involve Unique or Unknown Risks

Potential Effects of Growing *Eucalyptus* on Soil Hydrology

Eucalyptus is recognized as having impacts on hydrology and large widespread plantings could have potential impacts on hydrology in the southeastern United States (Farley et al. 2005a). Since large plantings of *Eucalyptus* have not been grown in many parts of the southeastern US (other than southern Florida) the potential impacts of such plantings on hydrology are unknown. APHIS requested additional information on potential impacts of hydrology from ArborGen and also consulted with the USDA Forest Service to assess the potential impacts of planting *Eucalyptus* on hydrology. The additional information supplied by ArborGen and the Forest Service are included as Appendices II and III. The document supplied by the Forest Service represents only their opinion on the potential impacts of these field tests on hydrology and does not represent the position of the USDA on the pros and cons of deploying *Eucalyptus* as a biofuel, bioenergy or fiber crop.

The Forest Service indicates that planting large-scale *Eucalyptus* plantations may potentially lower the water table, and affect groundwater recharge and stream flow dynamics. *Eucalyptus* is very efficient at using water. It can produce more biomass per unit water consumed than native southeastern pines; however, their extremely rapid biomass production has proportionally higher transpirational costs and therefore greater water use. The Forest Service has estimated that a mature *Eucalyptus* plantation growing in southwest Georgia could potentially transpire 882 mm per year, exceeding all other forest types on average by a factor of 2.5. *Eucalyptus* transpiration could exceed that of pine plantations by a factor of 1.6, and previous pasture land by a factor of 3.5. The comparison with agricultural crops is more variable where *Eucalyptus* transpiration may be greater or lesser than that of crop plants depending on the crop, the growing season, and the management practices.

Eucalyptus has a dimorphic rooting pattern which means that it has surface roots that draw water from the surface as well as deep roots which draw water from deep within the soil. The mean maximum rooting depth for *Eucalyptus* is 15 meters, which is a characteristic of a dimorphic rooting pattern. In contrast, mean maximum rooting depths of pine plantation (*P. taeda* and *P. elliottii*) and grass species are 3 meters and 2.6 meters, respectively (Canadell et al. 1996a). According to the Forest Service, conversion to *Eucalyptus* on sites where the water tables are less than 10 meters will likely lower down-slope water tables via direct means (i.e., direct use of ground water by deep roots), affect groundwater-aquifer dynamics, and result in evapotranspiration rates that exceed precipitation input, as have been reported for this species in other locations (Calder et al. 1997a).

Recent research suggests that *Eucalyptus* plantations would reduce stream flow more than pine plantations, and could potentially eliminate low flows. In a review of more than 20 catchment[5] conversion studies, Farley and others (Farley et al. 2005a) showed that converting existing vegetation to *Eucalyptus* plantations reduced stream flow by 20% more than converting it to a pine plantation. This review also showed that the loss of low flows were more complete for *Eucalyptus* plantations compared to pine plantations (100% vs. ~80% reduction of low flows). Elimination of low stream flows could have important ramifications for threatened and endangered aquatic species, such as the gulf strain striped bass, and species of endemic freshwater mussels (Golladay et al. 2004a, Couch and McDowell 2006b).

Due to a lack of available data in the southeastern U.S. on planting *Eucalyptus*, it is difficult to determine the significance of the effects on hydrology if large acreage of *Eucalyptus* were to be planted. The Forest Service has indicated that collection of data and modeling will be useful to determine the long-term impacts of planting large acreages of the genus. The Forest Service has also pointed out that the significance of the impact on groundwater and stream flow will depend greatly on the area extent, size, and spatial distribution of the plantations. For example, a few small (less than 10 hectares, i.e. approximately 25 acres) and well-dispersed plantations may only have very localized impacts and negligible impacts at the watershed scale.

The field test sites requested under the two permits are well dispersed and are limited in size (none are greater than 20 acres) and it is anticipated that they are not likely to have significant impacts on hydrology. At the request of APHIS, ArborGen has supplied data indicating the maximum size of each of the plantings at each site, the individual watersheds where the plantings occur, the area of the watershed, how much of the watershed will be occupied by the field tests, the location of the closest primary and secondary streams, and the location of any critical habitat for Federally listed threatened and endangered species within the watershed.

Using the 8 digit HUC (Hydrologic Unit Code) as the Watershed to be analyzed, the data provided by ArborGen show that none of the sites occupy more than 0.03% of any given watershed. The closest critical habitat for an aquatic species (such as a fish and mussel) is 6 kilometers at one location and ranges from 6 to 90 km for any of the sites having any proximity to habitats that could be impacted. There are no nearby threatened or endangered plant species that could be impacted by hydrological effects (see also Appendix IV). Any effects would be very localized on existing nearby agricultural and forestry plantings. Therefore APHIS concludes that while the effects on hydrology, including the watershed and aquifers, are unknown and uncertain for very large plantings of *Eucalyptus*, these small-scale field tests are unlikely to have any significant negative impacts on hydrology and on native flora and fauna.

Potential Allelopathic Effects of *Eucalyptus*

[5] A catchment or drainage basin is an extent of land where water from rain or snow-melt drains downhill into a body of water, such as a river, lake, reservoir, estuary, wetland, sea or ocean. The drainage basin includes both the streams and rivers that convey the water as well as the land surfaces from which water drains into those channels, and is separated from adjacent basins by a drainage divide.

Allelopathy refers to "any process involving secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems" (See: International Allelopathy Foundation - http://www.allelopathy-journal.com/allelopathy.aspx). Allelochemicals from plants are released into the environment by exudation from roots, leaching from stems and leaves, or decomposition of plant material. Allelopathy can have both negative and positive impacts on the environment (Eljarrat and Barceló 2001, Xuan et al. 2005, Kohli et al. 2006). There has been increased research activity in this area, one of which is taking advantage of plants that produce allelopathic compounds in developing agroforestry and sustainable agriculture systems (Kohli et al. 2006, Narwal 2006). Allelopathy has been demonstrated in many commercially important tree species including *Acacia, Ailanthus Eucalyptus, Juglans, Quercus, Leucaena, Pinus, Picea, Aibes, Populus* and *Acer*; and has been demonstrated in agronomic crops such as rye, wheat and alfalfa (Nandal et al. 1994, Ferguson and Rathinasabapathi 2003, Reigosa and Gonzáles 2006, Mallik 2008).

There have been extensive studies conducted on allelopathy in *Eucalyptus* and there are several comprehensive reports and review articles on this genus (Ong 1993, Sunder 1995) (Nandal et al. 1994, Davidson 1995, White 1995). *Eucalyptus* species are known to produce chemical compounds that are required by the plant for defense against herbivores and pathogens. There are several studies in the literature that demonstrate the negative, positive and neutral allelopathic interaction of *Eucalyptus* species and their hybrids with other crop plants (Sanginga and Swift 1992) (Khan et al. 2004) (Espinosa-Garcia et al. 2008). These interactions vary greatly depending upon the crop species and conditions under which they are grown. There is inconclusive data as to whether these compounds produced by *Eucalyptus* are exclusively responsible for allelopathic influence on understory vegetation in *Eucalyptus* plantations. Most allelopathic studies in *Eucalyptus* species have involved laboratory experiments with extracts obtained from different plant parts or leaf litter to investigate allelopathic effects on seed germination and growth in potted plants. These laboratory bioassays and pot culture studies may or may not be applicable to field conditions. The perceived allelopathic effects observed in the field on growth of understory or adjacent intercropped food crops could also result from competition for water, nutrients and light.

Allelopathy tends to be an inexact science and many studies in allelopathy are inconclusive and difficult to interpret due to potential interactions with other aspects of the environment. For example in a recent study, (Nandal and Dhillon 2005) tested the allelopathic effects of poplar (*Populus deltoides*) leaf extracts on germination and growth of ten wheat varieties under laboratory conditions. They reported that lower concentration of leaf extracts from poplar had stimulatory effects on root length in all wheat varieties whereas higher concentrations adversely affected germination and seedling growth of some of the wheat varieties tested. In a field experiment, the performance of all ten wheat varieties was also evaluated under four different poplar spacings in an agri-silviculture system. Although the grain yield of wheat varieties was significantly lower under all spacings of poplar compared to controls, yields increased significantly with increased spacing of poplar, possibly due to reduced competition for light and nutrients.

However, no correlation was found between the laboratory bioassay using leaf extracts and the field studies.

In a recent study, the allelopathic interaction of *Eucalyptus grandis*, *E. urophylla* and *E. grandis x urophylla* on the germination and early growth of four annual crops (maize, bean, watermelon and squash) was investigated (Espinosa-Garcia et al. 2008). Soil samples were collected from different soil horizons and at varying distances from *Eucalyptus* trees growing at the plantation edge and used for growth studies in pots. The dried soil samples used for growth studies were also analyzed for total soluble phenolics present in the soil. The study showed that soil samples from different plantations had differential effects ranging from no effect, to slightly inhibitory, to a stimulatory effect on germination and radicle[6] growth of test crops. Among the three *Eucalyptus* species tested, the soil samples from *E. grandis x urophylla* plantations had an inhibitory effect on germination of maize, bean and watermelon but had a stimulatory effect on squash. The soil from *E. grandis* plantations had an inhibitory effect on squash. The total soluble phenolics varied in different soil samples but did not explain the differential effects on the test crops. The authors concluded that soil samples collected from plantations of *Eucalyptus* species contained allelochemicals that affected germination and early growth of some annual crops but such effects could be avoided by planting crops at a distance of 15 meters away from the edge of plantations.

Even though the *Eucalyptus* under this permit could demonstrate allelopathic properties, the presence of any allelochemicals is not going to make the *Eucalyptus* planted under these permits more invasive or present a plant pest risk. Since all these field tests are confined and limited in size, any allelopathic effects should be small. As a standard silvicultural practice, herbicides will also be used within the field test sites and any of their effects on understory vegetation will be as severe or more severe than any allelopathic effects. In the future, should any negative allelopathic or other competitive interactions be observed under field conditions outside of the immediate field tests sites, these could be mitigated by adjusting the tree spacing, irrigation and fertilization practices or by planting the field tests at least 15 meters away from any agronomic crops or sensitive areas. Any unusual observations at the field test sites are to be reported to APHIS under the supplemental permit conditions of the permit; including any indications of allelopathic effects.

E. Risks to Threatened and Endangered Species

APHIS analyzed the potential for effects from the preferred alternative of this EA on federally listed threatened and endangered species (TES) and species proposed for listing, as well as designated critical habitat and habitat proposed for designation, as required under Section 7 of the Endangered Species Act. APHIS worked with the United States Fish and Wildlife Service to obtain species lists and information and critical habitat information for the proposed field site locations. After analyzing the potential for any effect, APHIS has reached a determination that the proposed environmental release will

[6] The radicle is the first part of a seedling (a growing plant embryo) to emerge from the seed during the process of germination. It is an embryonic root.

have no effect on federally listed threatened or endangered species or species proposed for listing, and no effect on designated critical habitat or habitat proposed for designation. Consequently, consultation with the United States Fish and Wildlife Service is not required for the action described in the preferred alternative of this EA. Appendix IV includes the BRS analysis of threatened and endangered species in the areas of the field releases.

F. Other Considerations

Consideration of Executive Orders, Standards and Treaties Relating to Environmental Impacts.

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children.

Each alternative was analyzed with respect to the above EO 12898 and 13045. The human health and environmental impacts of the action alternatives are presented in Section V of this EA. No human health or environmental effects were identified for any of the action alternatives that would have a disproportionate adverse effect or that would exclude a particular group of persons or populations, including minority and low-income populations, or children, from expected benefits.

EO 13112, "Invasive Species", states that federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. As presented in Section V of this EA, the hybrid species of *Eucalyptus* being grown is not considered an invasive species and does not establish itself without human intervention. Based on historical experience with the *Eucalyptus* in these field tests, the engineered plant is not expected to have an increased invasive potential.

Executive Order 12114, "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories and possessions that result from actions being taken. APHIS has given this due consideration and does not expect an environmental impact

outside the United States should APHIS choose any of the two alternatives. These confined field tests are being conducted in the continental U.S. and would not be expected on have environmental effects outside of the U.S.

Migratory Bird Treaty Act, 1918 as amended and Executive Order 13186. Migratory birds include all native wild birds found in the United States except the house sparrow, starling, feral pigeon, and resident game birds such as pheasant, grouse, quail, and wild turkeys. A reference list of migratory game birds is found in Title 50, Code of Federal Regulations, Part 10. The Migratory Bird Treaty Act makes it unlawful for anyone to kill, capture, collect, possess, buy, sell, trade, ship, import, or export any migratory bird, including feathers, parts, nests, or eggs. Executive Order 13186 "Responsibilities of Federal Agencies to Protect Migratory Birds" requires Federal officials to consider the impacts of planned actions on migratory bird populations and habitats for all planning activities. APHIS has determined that it is reasonable to assume that the activities at the field test sites such as planting, collecting samples and eventual harvest of the trees should have no adverse impact on migratory birds since they would not be expected to nest or permanently inhabit these types of field test sites.

Consistency of Proposal with other Environmental Requirements:

The proposal is believed to be consistent with other environmental requirements. This environmental assessment was prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C 4321 *et seq*.); (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500-1508); (3) USDA regulations implementing NEPA (7 CFR part 1b); and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372).

G. Conclusion

As outlined under the Purpose and Need sections of this document, this EA was prepared because it was necessary for APHIS to evaluate the potential environmental impacts resulting from the increased number of locations and size of the releases of flowering *Eucalyptus*, which could potentially lead to a lack of confinement of the field tests and other impacts to the environment. APHIS has evaluated the permit applications to determine whether the environmental release, with appropriate conditions imposed, can be carried out while preventing the dissemination and establishment of plant pests. After preparing this draft EA, APHIS has concluded that even though there is an increase in the number of sites where trees will be allowed to reach maturity and flower, over those already allowed to flower under permits 06-325-111r and 08-151-101r, there is no substantially greater risk of loss of confinement and risk to the environment. APHIS concludes that the releases will remain as confined field tests and that the genetically engineered trees will not pose a significant plant pest risk. In addition, APHIS concludes that granting permits will not significantly affect the quality of the human environment. No threatened and endangered species or critical habitat should be impacted by letting the trees reach maturity and flower at the increased number of locations.

VI. Listing of Agencies and Persons Consulted

James M. Vose - USDA-Forest Service Coweeta Hydrologic Laboratory, Otto, NC
Chelcy R. Ford - USDA-Forest Service Coweeta Hydrologic Laboratory, Otto, NC
Jody Smithen – US Fish and Wildlife Service – Daphne, Alabama Field Office
Kathy Chapman - US Fish and Wildlife Service - Coastal Georgia Field Office
James Harris – US Fish and Wildlife Service – Lacombe, Louisiana Field Office
Laura Zimmerman - US Fish and Wildlife Service – Charleston, South Carolina Field Office
Caroline Stahaller - US Fish and Wildlife Service - Panama City, Florida Field Office
Brad Rick - US Fish and Wildlife Service - Vero Beach, Florida Field Office
Candice Martino - US Fish and Wildlife Service - Jacksonville, Florida Field Office

VII. References

APHIS. 2004. The emergence and colonization of *Cryptococcus gattii* in British Columbia. Emerging disease notice. December 22, 2004. Available online at: http://www.aphis.usda.gov/vs/ceah/cei/taf/emergingdiseasenotice_files/cryptococcal_disease_britishcolumbia.htm.

Ashton, D. H. 1981. Fire in tall open-forests (wet sclerophyll forests). Pages 339-366 *in* A. M. Gill, R. H. Groves, and I. R. Noble, editors. Fire and the Australian biota. The Australian Academy of Science, Canberra City, ACT.

Baker, S. S., K. S. Wilhelm, and M. F. Thomashow. 1994. The 5'-region of *Arabidopsis thaliana cor15a* has cis-acting elements that confer cold-, drought- and ABA-regulated gene
expression. Plant Mol Biol. 24:701-713.

Barbour, E. L. and N. Spencer. 2000. The potential of a crossing technique for interspecific hybridization between *E. globulus* and *E. dunnii*. Pages 390-394 *in* Hybrid Breeding and Genetics of Forest trees" Proceedings of QFRI/CRC_SPF Symposium, 9-14 April 2000

Bell, D. T. and J. E. Williams. 1997. Eucalyptus ecophysiology. Pages 168-196 *in* J. E. Williams and J. Woinarski, editors. Eucalyptus Ecology: Individuals to Ecosystems. Cambridge University Press, Cambridge.

Brown, J. R. 2003. Ancient horizontal gene transfer. Genetics 4:121-132.

Calder, I. R., P. T. W. Rosier, K. T. Prasanna, and S. Parameswarappa. 1997. Eucalyptus water use greater than rainfall input-a possible explanation from southern India. Hydrology and Earth System Sciences 1:249-256.

Campinhos, E. N., I. Peters-Robinson, F. L. Bertolucci, and A. C. Privets. 1998. Interspecific hybridization and inbreeding effect in seed from *Eucalyptus grandis* x *E. urophylla* clonal orchard in Brazil. Genet. Mol. Biol. [online], 21, No. 3. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1415-47571998000300014 &lng=en&nrm=iso>.

Canadell, J., R. B. Jackson, J. R. Ehleringer, H. A. Mooney, O. E. Sala, and E. D. Schulze. 1996. Maximum rooting depth of vegetation types at the global scale. Oecologia 108:583-595.

Chaturvedi, S., M. Dyavaiah, R. A. Larsen, and V. Chaturvedi. 2005. Cryptococcus gattii in AIDS patients, southern California. Emerging Infectious Diseases 11:1686-1692.

Couch, C. A. and R. J. McDowell. 2006. Flint River Basin Regional Water Development and Conservation Plan. Georgia Dept. of Natural Resources, Environmental Protection Division. Page 242.

Cremer, K. W. 1977. Distance of seed dispersal in Eucalyptus estimated from seed weight. Australian Forest Research 7:225-228.

Datta, K., K. H. Bartlett, and K. A. Marr. 2009. *Cryptococcus gattii*: Emergence in Western North America: Exploitation of a Novel Ecological Niche. Interdisciplinary Perspectives on Infectious Diseases 2009:1-8.

Daehler, C. C. 1988. The taxonomic distribution of invasive angiosperm plants: ecological insights and comparison to agricultural weeds. Biological Conservation 84: 167-180.

Davidson, J. 1995. Ecological aspects of *Eucalyptus* plantations.*in* Proceedings of the Regional Expert Consultation on *Eucalyptus*. FAO Regional Office for Asia and the Pacific, Bangkok.

de Assis, T. F. 1996. Melhoramento genético do eucalipto. Informe Agropecuário, Belo Horizonte (Brazil) 18:32-51.

Eljarrat, E. and D. Barceló. 2001. Sample handling and analysis of allelochemical compounds in plants. Trends in Analytical Chemistry 20:584-590.

Espinosa-Garcia, F. J., E. Martinex-Herandez, and A. Quiroz-Flores. 2008. Allelopathic potential of *Eucalyptus* spp plantations on germination and early growth of annual crops. . Allelopathy Journal 21:25-37.

Esser, L. L. 1993. Eucalyptus globulus. In: Fire Effects Information System. U.S. Department of Agriculture, Forest Service - Rocky Mountain Research Station, Fire Sciences Laboratory (Producer) - Available: http://www.fs.fed.us/database/feis/.

Farley, K., J. E., and J. R.B. 2005. Effects of afforestation on water yield: a global synthesis with implications for policy. Global Change Biology 11:1565-1576.

Ferguson, J. J. and B. Rathinasabapathi. 2003. Allelopathy: How plants suppress other plants. University of Florida HS944 http://edis.ifas.ufl.edu/.

Forsyth, G. G., D. M. Richardson, et al. (2004). A rapid assessment of the invasive status of Eucalyptus species in two South African provinces. South African Journal of Science 100(1): 75-77.

Fox, A. M., D. R. Gordon, J. A. Dusky, L. Tyson, and R. K. Stokcker. 2008. IFAS Assessment of the Status of Non-Native Plants in Florida's Natural Areas.

Fuchs, R. L., J. E. Ream, B. G. Hammond, M. W. Naylor, R. M. Leimgruber, and S. A. Berberich. 1993. Safety assessment of neomycin phosphotransferase II (NPTII) protein. Nature Biotechnology 11:1543-1547.

Gill, A. M. 1997. Eucalyptus and Fires: Interdependent or Independent. Pages 151-167 *in* J. E. Williams and J. Woinarski, editors. Eucalyptus Ecology: Individuals to Ecosystems. Cambridge University Press, Cambridge.

Golladay, S. W., P. Gagnon, M. Kearns, J. M. Battle, and D. W. Hicks. 2004. Response of freshwater mussel assemblages (Bivalvia:Unionidae) to a record drought in the Gulf Coastal Plain of southwestern Georgia J. N. Am. Benthol. Soc. 23:494-506.

Gore, P. and B. M. Potts. 1995. The genetic control of flowering time in *Eucalyptus globulus, E. nitens* and their F1 hybrids. Pages 241-242 *in* Eucalyptus Plantations: Improving Fiber Yield and Quality, Proc CRCTHF - IUFRO Conference. CRC for Temperate Hardwood Forestry, Hobart. Australia, 19 -24 Feb.

Griffin, A. R., I. P. Burgess, and L. Wolf. 1988. Patterns of Natural and Manipulated Hybridisation in the Genus Eucalyptus L'Herit - A review. Australian J. Botany 36:41-66.

Grose, R. J. 1960. Effective seed supply for natural regeneration of *Eucalyptus delegatensis*. Journal of Australian Pulp and Paper Industry Association 13:131-147.

Groves, R. H., editor. 1994. Australian vegetation. Cambridge University Press, Cambridge.

Hartney, V. J. 1980. Vegetative propagation of the eucalypts. Australian Forest Research 10:191-211.

House, S. M. 1997. Reproductive biology of eucalypts. Pages 30-56 *in* J. E. Williams and J. Woinarski, editors. Eucalyptus Ecology: Individuals to Ecosystems. Cambridge University Press, Cambridge.

Hsieh, T. H., J. T. Lee, P. T. Yang, L. H. Chiu, Y. Y. Charng, Y. C. Wang, and M. T. Chan. 2002. Heterology expression of the *Arabidopsis* C-repeat/dehydration response element binding factor 1 gene confers elevated tolerance to chilling and oxidative stresses in
transgenic tomato. Plant Physiol. 129:1086-1094.

Kaneko, T., Y. Nakamura, S. Sato, E. Asamizu, T. Kato, S. Sasamoto, A. Watanabe, K. Idesawa, A. Ishikawa, K. Kawashima, T. Kimura, Y. Kishida, C. Kiyokawa, M. Kohara, M. Matsumoto, A. Matsuno, Y. Mochizuki, S. Nakayama, N. Nakazaki, S. Shimpo, M. Sugimoto, C. Takeuchi, M. Yamada, and S. Tabata. 2000. Complete Genome Structure of the Nitrogen-fixing Symbiotic Bacterium Mesorhizobium loti. DNA Research **7**:331-338.

Kaneko, T., Y. Nakamura, S. Sato, K. Minamisawa, T. Uchiumi, S. Sasamoto, A. Watanabe, K. Idesawa, M. Iriguchi, K. Kawashima, M. Kohara, M. Matsumoto, S. Shimpo, H. Tsuruoka, T. Wada, M. Yamada, and S. Tabata. 2002. Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* DNA Research 9:189-197.

Kasuga, M., Q. Liu, S. Miura, K. Yamaguchi-Shinozaki, and K. Shinozaki. 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stressinducible
transcription factor. Nature Biotechnology 17:287-291.

Keese, P. 2008. Review Article: Risks from GMOs due to horizontal gene transfer. Environ. Biosafety Res. 7: 123-149.

Khan, E. A., M. A. Khan, H. K. Ahmad, and K. F. U. 2004. Allelopathic effects of *Eucalyptus* leaf extracts on germination and growth of cotton (*Gossypiumhirsutum*). Pakistan J. Weed Sci. Res. 10:145-150.

Kidd, S. E., Y. Yat Chow, S. Mak, P. J. Bach, H. Huiming Chen, A. O. Hingston, J. W. Kronstad, K. H., and K. H. Bartlett. 2007. Characterization of Environmental Sources of the Human and Animal Pathogen Cryptococcus gattii in British Columbia, Canada, and the Pacific Northwest of the United States. . Applied and Environmental Microbiology 73:1433-1443.

Klee, H. J. and S. G. Rogers. 1989. Plant gene vectors and genetic transformation: plant transformation systems based on the use of Agrobacterium tumefaciens. Pages 1-23 *in* I. K. Vasil, editor. Cell Culture and Somatic Cell Genetics of Plants. Academic Press, Orlando, FL.

Kohli, R. K., D. R. Batish, and H. P. Singh. 2006. Allelopathic interactions in agroecosystems. Pages 465-493 *in* R. Reigosa, N. Pedrol, and L. Gonzáles, editors. Allelopathy: A Physiological Process with Ecological Implications. Springer.

Kolar, C.S. and D. M. Lodge. 2001. Progress in invasion biology: predicting invaders. Trends in Ecology and Evolution 16:199-204.

Koonin, E. V., K. S. Makarova, and L. Aravind. 2001. Horizontal gene transfer in prokaryotes: Quantification and classification. Annual Review of Microbiology 55:709-742.

Ladiges, P. Y. 1997. Phylogenetic history and classification of Eucalyptus. Pages 16-29 *in* J. E. Williams and J. Woinarski, editors. Eucalyptus Ecology: Individuals to Ecosystems. Cambridge University Press, Cambridge.

Linacre, N. A. and P. K. Ades. 2004. Estimating isolation distances for genetically modified trees in plantation forestry. Ecological Modeling 179:247-257.

Mallik, A. U. 2008. Allelopathy in forested ecosystems.*in* R. S. Zeng, A. U. Mallik, and S. M. Lou, editors. Allelopathy in sustainable agriculture and forestry. Springer.

Maruyama, K., Y. Sakuma, M. Kasuga, I. Y., M. Seki, H. Goda, Y. Shimada, S. Yoshida, K. Shinozaki, and K. Yamaguchi-Shinozaki. 2004. Identification of cold-inducible downstream genes of the *Arabidopsis* DREB1A/CBF3 transcriptional factor using two
microarray systems. Plant Journal 38:982-993.

Meskimen, G. and J. K. Francis. 1990. Rose Gum Eucalyptus.*in* R. M. Burns and B. H. Honkala, editors. Silvics of North America: Volume 2. Hardwoods. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington, DC.

Nandal, D. P. S., S. S. Bisla, S. S. Narwal, and J. C. Kaushik. 1994. Allelopathic Interactions in Agroforestry Systems Pages 93-130. *in* S. S. Narwal and P. Tauro, editors. Allelopathy in Agriculture and Forestry. Scientific Publishers, Jodhpur, India.

Nandal, D. P. S. and A. Dhillon. 2005. Allelopathic effects of poplar (*Populus deltoides* Bartr Ex Marsh): an assessment on the response of wheat varieties under laboratory and field conditions. Fourth World Congress in Allelopathy,, Wagga Wagga, NSW Australia.

Narusaka, Y., K. Nakashima, Z. K. Shinwari, Y. Sakuma, T. Furihata, H. Abe, M. Narusaka, K. Shinozaki, and K. Yamaguchi-Shinozaka. 2003. Interaction between two cis-acting elements, ABRE and DRE, in ABA-dependent expression of *Arabidopsis rd29A* gene in
response to dehydration and high-salinity stresses. Plant J. 34:137-148.

Narwal, S. S. 2006. Allelopathy in ecological sustainable agriculture.*in* M. J. Reigosa, N. Pedrol, and L. Gonzáles, editors. Allelopathy: A Physiological Process with Ecological Implications. Springer.

Ong, C. K. 1993. On the difference between competition and allelopathy. Agroforestry Today 5:12-14.

Pacheco, I. A. 1987. Polinização de Eucalyptus saligna Smith (Myrtaceae) por *Apis mellifera* L. 1758 (Hymenoptera, Apidae). Universidade de São Paulo, Piracicaba.

Pacheco, I. A., P. Y. Kageyama, F. M. Wiendl, and E. B. Filho. 1986. Estudo da dispersão de pólen de Eucalyptus saligna Smith por abelhas Apis mellifera L. utilizando-se o radiofósforo. IPEF Piracicaba (Brazil 34:47-52.

Pederson, J. F., K. P. Vogel, and D. L. Funnell. 2005. Impact of reduced lignin on plant fitness. Crop Science 45:812-819.

Peters, G. B., J. S. Lonie, and G. F. Moran. 1990. The breeding systems, genetic diversity and pollen sterility in *Eucalyptus pulverulenta*, a rare species with small disjunct population. Australian J. Botany 38:559-570.

Pino, M. T., J. S. Skinner, E. J. Park, Z. Jeknić, H. P.M., M. F. Thomashow, and T. H. Chen. 2007. CBF3/DREB1A expression and plays a central role in stress tolerance in
Arabidopsis. Use of a stress inducible promoter to drive ectopic AtCBF expression improves potato freezing tolerance while minimizing negative effects on tuber yield. Plant Biotechnol J. 5:591-604.

Potts, B. M., R. C. Barbour, A. B. Hingston, and V. R.E. 2003. Turner Review: the risk of genetic pollution of native Eucalyptus gene pools. Australian J. Botany 51:1-25.

Potts, B. M. and H. S. Dungey. 2004. Interspecific hybridization of Eucalyptus: Key issues for breeders and geneticists. New Forest 27:115-138.

Pound, L. M., M. A. B. Wallwork, B. M. Potts, and M. Sedgley. 2002. Self-incompatibility in *Eucalyptus globulus* ssp. globulus (Myrtaceae). Australian Journal of Botany 50:365-372.

Reigosa, M. J. and L. Gonzáles. 2006. Forest Ecosystems and Allelopathy. Pages 451-463 *in* R. Reigosa, N. Pedrol, and L. Gonzáles, editors. Allelopathy: A Physiological Process with Ecological Implications. Springer.

Riechmann, J. L. and E. M. Meyerowitz. 1998. The AP2/EREBP family of plant transcription
factors. . Biol Chem 379:633-646.

Rockwood, D. L., D. R. Carter, and J. A. Stricker. 2004. Commercial Tree Crops for Phosphate Mine Lands, Fourth Year Cumulative Progress Report - Prepared for Florida Institute of Phosphate Research FIPR Project Number 99-03-141R. University of Florida.

Sanginga, N. and M. J. Swift. 1992. Nutritional effects of *Eucalyptus* litter on the growth of maize (*Zea mays*). Agriculture, Ecosystems and Environment 41:55-65.

Skolmen, R. G. and F. T. Ledig. 1990. Bluegum Eucalyptus.*in* R. M. Burns and B. H. Honkala, editors. Silvics of North America: Volume 2. Hardwoods. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington, DC. .

Stricker, J., D. L. Rockwood, S. A. Segrest, G. R. Alker, R. M. Prine, and D. R. Carter. 2000. Short rotation woody crops for Florida. Pages 15-23 *in* Proc. 3rd Biennial Short Rotation Woody Crops Operations Working Group Conference, October 10-13, 2000, Syracuse, NY.

Sunder, S. 1995. The Ecological, Economic and Social Effects of *Eucalyptus*. *in* Proceedings of the Regional Expert Consultation on *Eucalyptus*. FAO Regional Office for Asia and the Pacific, Bangkok.

Upton, A., J. A. Fraser, S. E. Kidd, C. Bretz, K. H. Bartlett, J. Heitman, and K. A. Marr. 2007. First Contemporary Case of Human Infection with Cryptococcus gattii in Pugent Sound: Evidence for Spread of the Vancouver Island Outbreak. . Journal of Clinical Microbiology 45:3086-3088.

Volker, P. W. 1995. Evaluation of *Eucalyptus nitens* x *globulus* for commercial forestry. Pages 222-225 *in* Eucalyptus Plantations: Improving Fiber Yield and Quality, Proc CRCTHF - IUFRO Conference. CRC for Temperate Hardwood Forestry, Hobart. Australia. 19-24 Feb

Wellington, A. B. 1989. Seedling regeneration and population dynamics of eucalyptus. Pages 155-167 *in* J. C. Noble and R. A. Bradstock, editors. Mediterranean Landscapes in Australia: Mallee Ecosystems and their Management. CSIRO, Melbourne.

White, K. J. 1995. Silviculture of *Eucalyptus* Plantings – Learning from the Region (Australia).*in* Proceedings of the Regional Expert Consultation on *Eucalyptus*. FAO Regional Office for Asia and the Pacific, Bangkok.

Wood, D., J. Setubal, R. Kaul, D. Monks, J. Kitajima, V. Okura, Y. Zhou, L. Chen, G. Wood, A. J. N., L. Woo, Y. Chen, I. Paulsen, J. Eisen, P. Karp, S. Bovee, D., P. Chapman, J. Clendenning, G. Deatherage, W. Gillet, C. Grant, T. Kutyavin, R. Levy, M. Li, E. McClelland, C. Saenphimmachak, Z. Wu, P. Romero, D. Gordon, S. Zhang, H. Yoo, Y. Tao, P. Biddle, M. Jung, W. Krespan, M. Perry, B. Gordon-Kamm, L. Liao, S. Kim, C. Hendrick, Z. Zhao, M. Dolan, F. Chumley, S. Tingey, J. Tomb, M. Gordon, M. Olson, and E. Nester. 2001. The Genome of the Natural Genetic Engineer *Agrobacterium tumefaciens* C58. Science 294:2317-2323.

Xuan, T. D., S. Tawata, T. D. Khanh, and I. M. Chung. 2005. Decomposition of Allelopathic Plants in Soil. J. Agronomy and Crop Science 191:162-171.

Yamaguchi-Shinozaki, K. and K. Shinozaki. 1993. Characterization of the expression of a desiccation-responsive *rd29* gene of *Arabidopsis thaliana* and analysis of its promoter in
transgenic plants. Mol. Gen. Genet. 23:331-340.

Yamaguchi-Shinozaki, K. and K. Shinozaki. 1994. A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt
stress. Plant Cell 6:251-264.

Zambryski, P. 1988. Basic processes underlying Agrobacterium mediated DNA transfer to plant cells. Annual Review of Genetics 22:1-30.

APPENDIX I: Status of Existing Field Tests Allowed to Flower

The applicant has been allowed to let transgenic *Eucalyptus* trees flower under APHIS permits 06-325-111r-a2 and 08-151-101r. These trees have the same genetic constructs as those *Eucalyptus* hybrid trees under APHIS consideration in the two permit applications submitted by ArborGen. The following is a summary of information that has been collected by the applicant and provided to APHIS as part of their annual reports under the existing permits that allow flowering.

All transgenic and non-transgenic trees in these field tests, spanning 6.2 acres in Alabama and 1.4 acres in Florida, produced mature flowers in late August-early September of 2007 and/or 2008. On the 25-30 foot tall trees in these tests there were estimated to be several thousand flowers on each tree. No differences were noted in flower formation in transgenic trees compared to non-transgenic controls (the same parental genotype).

In January 2008, mature (but not yet opened) capsules were collected from non-transgenic and transgenic trees under permit 06-325-111r. Replicate samples were collected from transgenic trees and non-transgenic controls in two separate blocks. Capsules from a subset of transgenic trees plus non-transgenic controls were dried in the laboratory and allowed to open to evaluate the presence of seed or seed like structures in the capsules. Approximately 100 capsules for each of the two replicate samples were analyzed. Microscopic examination of the material inside the capsules did not show any seed or seed like structures in capsules of either non-transgenic or transgenic lines. Controlled germination studies of the material extracted from the capsules did not produce any germinating seeds. Observations from the replicated field study being conducted in Alabama under permit No. 06-325-111r confirm that cold tolerant lines grown in this field test also did not produce any pollen. The results to date have shown that the barnase gene that has been engineered into these trees is effective at preventing pollen formation.

After the initial draft EA was published, the applicant submitted annual reports for permits (06-325-111r-a2 and 08-151-101r) as required under the supplemental conditions. The transgenic and non-transgenic trees planted under these permits in Baldwin County, Alabama and Highlands County, Florida were previously allowed to flower. The following is a summary of this report.

In Alabama, the transgenic trees produced several hundred to a few thousand flowers per tree whereas the non-transgenic controls had much fewer flowers as a result of significant cold damage. In Florida, there was no difference in the number of flowers produced by both transgenic and non-transgenic trees. Mature seed capsules, prior to opening, were sampled from select trees in three field tests in Baldwin County, Alabama and trees from the single field test in Highlands County, Florida in early March 2009. Samples consisted of approximately 70 to 100 capsules collected from different positions in the crown. The capsules were returned to ArborGen's greenhouse facility where they were dried and contents of the capsules were extracted and stored at 4°C. A controlled germination test was conducted using approximately 0.1 g of the extracted contents of

each sample spread on moist filter paper in a standard Petri dish. Open pollinated seed of EH1 obtained from Brazil were germinated as control seedlings for comparison.

Of the samples collected and analyzed from trees in Baldwin County, AL, approximately 4% of the seeds showed a low level of germination while approximately 83% of the samples collected from trees in the field trial in Highlands County, FL, showed germination, including samples from both transgenic and non-transgenic control trees. For both transgenic and non-transgenic tree samples analyzed, the applicant observed 2-8 seedlings for each 0.1g sample plated for germination. In the literature, for the same sample size tested for *E. grandis* 31 to 65 seedlings have been reported. The applicant indicates that, as expected from limited self pollination that may have occurred in these trees, the number of seedlings observed in these tests is much lower than would be expected for open pollinated seeds.

The controlled germination tests from samples collected in spring 2009 from field trials at two sites indicate that a very low level of seedlings can be produced from these trees. The number of seedlings produced is significantly less than what would be expected for an open-pollinating mixed stand of *Eucalyptus*. So far the data are consistent with the hypothesis that limited self pollination can occur from viable pollen produced by the non-transformed control trees. The applicant has also not observed any volunteer seedlings in or around the test sites. Volunteer monitoring and reporting the presence of volunteers is required in the supplemental permit conditions.

Monthly field test monitoring observations have not identified any differences in diseases and insects or other non-target organisms between the transgenic and non transgenic trees in the field test.

APPENDIX II: Hydrology considerations for planted *Eucalyptus* submitted by ArborGen LLC

Submitted by ArborGen. LLC to USDA APHIS BRS in support of consideration for approval of permits for field trials of *Eucalyptus* at multiple sites.

August 12, 2008

Introduction

The relevant scientific literature and conclusions drawn by experts in the field of hydrology, ecology, and plantation management on the hydrology of *Eucalyptus* plantations are discussed in this document. Extensive research on hydrology has been conducted in countries where large plantations of *Eucalyptus* have been established for many years, including India, China, South Africa and Brazil, as well as in its native Australia. The FAO, in response to the criticisms and concerns expressed about *Eucalyptus* plantations, has developed several expert reviews on the ecological impacts of *Eucalyptus* (discussed below).

The main hydrological concerns voiced against *Eucalyptus* plantations are that they deplete water supplies. The authors of an early FAO report (Poore and Fries, 1985) noted that these same criticisms would apply equally to any other plantation tree species, and that society tends to judge more harshly forestry crops relative to agricultural crops. As stated in Poore and Fries, 1985, "… most crops in many parts of the world are of foreign origin (wheat, maize, rice, potatoes, manioc, rubber, oil palm, coconut and many others. No one is surprised either that the soil under agricultural crops becomes depleted if these are continuously cropped without adding fertiliser. But both of these features are considered grounds for criticism in forestry." Ironically, *Eucalyptus* evolved to be water efficient as the Australian continent itself became more dry (Davidson, 1995). In fact *Eucalyptus* uses less water per unit weight of biomass produced than do other kinds of trees (Chaturvedi, 1987) and many agricultural crops (Davidson, 1995).

It is also important to take into account the breadth of the genus *Eucalyptus*, often referred to broadly as eucalypts, where different species have different characteristics which may prove detrimental or beneficial under different situations. There are several different ecological situations globally in which eucalypts may be planted: in place of existing closed forest; in place of other natural vegetation such as savannah, scrub or grassland; on degraded or waste land either as a potential crop or to assist in the control of erosion or salinity; within agricultural land as shelter belts or as components of agroforestry systems, or as intensively managed crops for wood production. It is important to understand the particular application in order to evaluate the potential ecological impact of *Eucalyptus* in a scientific manner. For example, the effect on soil moisture content and the water table can only satisfactorily be judged with reference to the pre-existing conditions before the establishment of the planting of *Eucalyptus*. In the

case of field trials of *Eucalyptus* requested in ArborGen's permit applications, all sites are on land previously managed for forestry, agricultural production or maintained as pasture land.

The Water Cycle

In considering the potential hydrological impacts of *Eucalyptus* it is important to put these in the context of the water cycle. Poore and Fries (1985) provide a good overview of the water cycle (see Figure 1 in Poore and Fries, 1985, or see Figure 1-1 in NRC, 2008). One key variable is the amount of rain that is intercepted by the canopy and is then evaporated back into the atmosphere. As a result, such intercepted water does not contribute to water in the soil. Of the water that does reach the soil some of this is absorbed while some fraction runs off the surface or is evaporated. A certain amount of water is maintained in the soil layer against the forces of gravity (called the 'fieldcapacity' and dependant on soil texture and organic content) while any excess drains to the water table, the level at which the soil is permanently saturated. Depending on the depth of the water table it may be accessed by deep rooting plants such as trees. Even without roots that reach to the water table, plants may be able to access water from deeper, wetter soil layers through capillary action, depending on the soil type, where there can actually be an upward movement of water.

Through normal transpiration plant roots take up available water which is transported through the stem to the leaves, the majority of which is lost to the atmosphere. Evapotranspiration is the total water returned to the atmosphere through transpiration and evaporation from the ground, bodies of water, plus intercepted water in the canopy. The relative rates of evapotranspiration and precipitation are often compared in assessments of hydrological systems. Where precipitation exceeds evapotranspiration then there is a net water gain to groundwater or downstream systems. Where evapotranspiration exceeds precipitation then the available water resources may be depleted. It is important to understand, as pointed out in Poore and Fries (1985), that water loss is a "price that plants must pay for growth". When stomata in plant leaves close then photosynthesis and growth both cease. In general terms, the rate of growth or biomass production in a plant is proportional to its water use. Consequently fast growing trees, of any species, use large quantities of water. As described below, *Eucalyptus* is actually more efficient in terms of water used per gram of biomass produced than many other tree species.

Hydrology of *Eucalyptus* – Key Literature Reviews

There has been extensive literature published on the hydrology of *Eucalyptus*. Google Scholar for example lists over 60,000 hits for the keywords '*Eucalyptus*' and 'water'. It is therefore not possible to provide summaries of the entire breadth of the literature. Several review articles are available including reviews sponsored by FAO that assessed the ecological impacts of *Eucalyptus* plantations, including analysis of the impact of *Eucalyptus* on hydrology. We provide here a summary of these reviews together with data from some specific reports (see below) where the hydrology of *Eucalyptus* has been studied in detail. FAO has also released two annotated bibliographies (FAO, 2002a, 2002b) that collate and summarize publications on environmental, social and economic

impacts of Eucalypts, and which include many references to water use. While there are specific examples and geographic regions where *Eucalyptus* (and other trees) can negatively impact hydrology, in general, the literature indicates that *Eucalyptus* can be grown in a sustainable manner and that its associated water use is not a major ecological threat. Most of the reviews indicate that soil and water characteristics of the site should be taken into consideration when establishing and maintaining a *Eucalyptus* plantation, in the same way that would be appropriate for plantings of agricultural crops.

The first FAO review was published in 1985 by Poore and Fries. At that time the authors suggested that there were relatively few existing studies in several important areas including hydrology (Lima, 1984; Poore and Fries, 1985). Where comparative studies showed that for dry alpine conditions the water regime for *Eucalyptus* did not differ from adjacent grasslands (see Lima, 1984), this was attributed to *Eucalyptus*' ability to control the rate of transpiration, an evolutionary adaptation for survival of drought stress which is often typical of the rainfall regimens of their native habitats. For deep soils and higher rainfall *Eucalyptus* plantations might be expected to reduce streamflow or groundwater recharge but that this is comparable to these same effects in pine plantations. In contrast, the water intercepted and re-evaporated by the foliage, and therefore not available to the soil, is less for *Eucalyptus* when compared to pines, due in part to the near vertical orientation of leaves in *Eucalyptus* (Whitehead and Beadle, 2004). It was concluded that the conditions of a particular site need to be taken into account as well as balancing local demands for forest products and water.

By 1993, at an FAO sponsored regional Expert Consultation on *Eucalyptus* (White et al, 1995) more information was available about hydrology and *Eucalyptus* plantations. These experts presented their experiences with *Eucalyptus* plantations from Asia. The report recognized the potential benefits of *Eucalyptus* and noted that many of the criticisms of the species were based on inappropriate government policies on afforestation or social concerns rather than the biology of the trees themselves (see also Casson, 1997). Calder et al (2004) highlights that many early policies were based on public misconceptions about the impacts of forests on water. With regard to hydrological effects on intercropping with other species, the experts in the FAO report concluded that while *Eucalyptus* can have negative effects in drier climates, in regions where rainfall is above 1,200 mm/year this is not expected to be a problem. The report suggests that for *Eucalyptus* plantings in those regions where water is scarce or demanded by other sectors, biomass production could be adjusted to match the amount of water available, for example by planting fewer trees per unit area or by thinning existing plantations.

As part of this expert consultation Sunder (1995) reported that the overall use of water by *Eucalyptus* is limited to the total rainfall of the area, in the absence of access of the tree to the water table. He concluded that there is an equilibrium between rainfall and evapotranspiration in *Eucalyptus* and that this does not differ significantly from other trees. As an example, monthly evapotranspiration of an *E. globulus* plantation in Portugal was the same as that of a natural open stand of cork oak (*Quercus suber*) with a developing understory of shrubs (de Almeida and Riekerk, 1990). Patil (1995) reported data on water consumption at sites in India, which although high in *Eucalyptus*, was the most efficient in terms of water consumed per gram of biomass produced (see also Silva

et al 2004). In fact, water use efficiency in *Eucalyptus* actually increases with greater water availability (Stape et al, 2004a, 2004b). Patil (1995) also noted that there were no hydrological impacts of *Eucalyptus* on adjacent crops at these sites. White (1995) stated that large plantings of *Eucalyptus* may reduce water yield and lower water tables but this varies from one situation to another and most importantly can be mitigated through management practices such as changes in tree stocking regimes. The environmental considerations of *Eucalyptus* are the same as those for agricultural crops. Davidson (1995) noted that drawing water from shallow or deep wells to supply high water demanding crops such as rice or cotton can have a greater impact on drawing down water tables than fast growing tree plantations. He also concluded that many potential adverse effects are reversible, as noted earlier by Poore and Fries (1985).

A review of the environmental issues of *Eucalyptus* plantations in Brazil was published by Oak Ridge National Laboratory (Couto and Betters, 1995). This report summarized that the hydrology of *Eucalyptus* plantations was comparable to other tree plantations or natural forest cover and that any effects would largely depend on management practices. Numerous studies demonstrate that forest cover and any changes in this alter water yield: reducing forest cover typically increases water yield and vice versa (Bosch and Hewlett, 1982; Sahin and Hall, 1996).

More recent reviews support the points made above. Binkley and Stape (2004) contend that very large tree plantations must address similar issues of sustainability as seen in agriculture. They refer to the many hundreds of trials that have been conducted in Brazil, with particular reference to a very large watershed project conducted in collaboration with Aracruz Cellulose Company (reported by Almeida et al 2007, described in more detail below). Binkley and Stape conclude that in semi-arid environments afforestation with any species of trees may increase water use, lower ground water levels and reduce streamflow. Given appropriate silvicultural management however, wood production should face no barriers to sustainability.

Whitehead and Beadle (2004) provided a comprehensive review of the physiological regulation of water use in *Eucalyptus*. These species have evolved several mechanisms to allow them to cope with drought conditions in their native habitats. These include dynamic changes in leaf area index (LAI), arrangement of leaves, high stomatal sensitivity to air saturation deficit, osmotic manipulation to maintain turgor in leaves, as well as an ability to form deep roots. Maximum potential rates of photosynthesis are high in *Eucalyptus* compared to other broad-leaved trees, but actual rates are often much less because of water limitations. Some examples are noted where *Eucalyptus* plantings have led to reductions in yields of water catchments. Conversely, the high water usage by *Eucalyptus* may be valuable in purposefully lowering water tables to reduced potential salinity problems. It is therefore important to assess productivity and water use in relation to climate variables, nutrient supply and options for silvicultural management, and careful matching of species to sites where available water may be limited. One of the physiological responses of *Eucalyptus* to limited water noted by Whitehead and Beadle is to reduce LAI, thus although *Eucalyptus* are evergreen species there can be large seasonal changes in LAI in response to dry seasons. Similar observations were made in reduced LAI along a gradient of water availability by Ares and Fownes (2000). The root systems

of Eucalypts are dimorphic, with widely spreading lateral root systems below the surface plus a deep tap root system. In a plantation of 7 year old *Eucalyptus* trees in Brazil the tap root extended to a depth of about 2.5m (Almeida and Soares, 2003) consistent with other observations (see Srivastava et al, 2003). When artificially stressed, by using plastic sheets on the soil surface to prevent rain entering the soil, young *Eucalyptus* developed roots 8 m or greater in depth. Under other conditions water was utilized from soil below the root zone by upward movement from wetter levels. In considering these physiological adaptations Whitehead and Beadle conclude that in the case of South Africa, where planted *Eucalyptus* replaced native grasslands, the decreased water yields resulted from increased transpiration in the evergreen and deep rooted *Eucalyptus* during the dry season compared to the seasonally dormant grasses. It is well established that forests have greater evapotranspiration than grasslands (Zhang et al, 1999).

Specific Hydrology Issues for Planted *Eucalyptus*:

***Eucalyptus* Afforestation and Hydrology.**

In those cases where *Eucalyptus* has been shown to have negative impacts on hydrology this has been associated with afforestation, most notably of lands where trees were previously absent. Typically, these are areas of low rainfall that are normally dominated by grasses. Under these conditions afforestation with different species of trees, including *Eucalyptus*, has lead to changes in the water balance including lowering of water tables and restricting stream flows. Calder and colleagues have published several reports on afforestation efforts in India including examples where deep-rooted *Eucalyptus* were able to tap into water resources not previously utilized by short-rooted species (Calder et al , 1997), but also describes cases where water use by *Eucalyptus* was comparable to indigenous forests at some sites (Calder, 1994). Similar studies of native grasslands have documented negative impacts of *Eucalyptus* on the water balance in South Africa (Lesch and Scott, 1997; Scott and Lesch, 1997; Scott et al, 1998) and Argentina (Jobbagy and Jackson, 2004; Engel et al, 2005; Nosetto, 2005). In many of these cases other introduced trees including pines had similar impacts and particularly in South Africa impacts on water balance result from a wide variety of introduced species (Le Maitre et al, 2000; 2002).

These examples contrast with the experience in Brazil where there has been extensive reforestation with *Eucalyptus* over many decades. Much of this literature is in Portuguese but often abstracts are published in English. Lima and colleagues have published a number of reports that analyzed potential impacts of both *Eucalyptus* and pine plantations on the *cerrado* grasslands in Brazil. Lima et al (1990) showed that in the region there was adequate rainfall to meet the evapotranspiration demands of *Eucalyptus*. A comparison of 6-year old *Eucalyptus* and pine plantings showed comparable levels of evapotranspiration during the dry season (May through September) as herbaceous vegetation (Lima and Freire, 1976). In these trials *Eucalyptus* actually showed greater interception than pine (Lima, 1976) and contrasts with references above, but likely reflected the greater average height of the *Eucalyptus* at 13.4 m compared to an average of 6 m for pine.

Similarly, an examination of the water balance of *Eucalyptus* plantations in China were not considered to be deleterious for water supplies (Lane et al 2004). While evapotranspiration exceeded precipitation in the dry season, water storages were replenished during the wet season.

Comparison of Water Use by *Eucalyptus* with other Tree Species.

In addition to the reports cited above many authors have concluded that the hydrological impacts of *Eucalyptus* are comparable to and should be viewed in the context of other tree species (see for example Myers et al, 1995; Wullschleger et al, 1998).

One of the largest studies comparing *Eucalyptus* and native trees conducted to date has been a catchment area in Brazil of over 280 hectares (owned by Aracruz Cellulose S.A.) consisting of 190 ha of planted hybrid *Eucalyptus* and almost 90 ha of native Atlantic rainforest, that was analyzed over a period of six years. Average precipitation at this site was 1147 mm, which is similar or less than the sites listed in this permit (range from ~1160 mm in Glades County Florida to almost 1750 mm in Escambia County, Alabama). Mean high temperature at the Aracruz site was 32.6 C (~91 F) for February (the summer season in the southern hemisphere), again, comparable to mean high temperatures in the summer for the sites in this permit. Data from the studies of this catchment area in Brazil indicated that evapotranspiration was strongly influenced by precipitation (Almeida et al, 2007). In an unusually dry year evapotranspiration was about half that compared to when water was readily available. In this dry year evapotranspiration exceeded precipitation but conversely in wetter years evapotranspiration was much less than precipitation. Over the length of the study evapotranspiration was ~95% of precipitation. This adjustment in response to varying conditions and water availability was indicative that these hybrid trees exert strong stomata control and utilize water according to its availability. In a series of studies in this same area conducted over a period of 8 years Almeida and Soares (2003, text in Portuguese with abstract and figure legends in English) examined a number of other hydrological parameters. Stomatal conductance was steady over several months with adequate water and then dropped significantly as available water dropped and the predawn leaf water potential (Ψ) increased, again demonstrating strong stomatal control. Rainfall interception by the Eucalypts averaged ~11% compared to ~24% in the native forest and water availability (at a depth of 2.5m) is almost identical in the native forests and the *Eucalyptus* plantations during the wet summer months but is less in the area with *Eucalyptus* during the drier winter. The authors attribute this to the deeper roots systems (>5m) of the native trees accessing water at deeper levels, while the *Eucalyptus* (with roots only to ~2.5m) are limited to the available water in the shallower levels. Finally, the authors compared the ratio of evapotranspiration and precipitation (ET/P) of the planted *Eucalyptus* with the native forest. In years with normal precipitation ET/P was comparable for both the *Eucalyptus* and native forest. In years with less than normal precipitation the native forest had higher ET/P (that is, evapotranspiration was much greater than precipitation) compared to the *Eucalyptus*. Based on their data, the authors suggest that the native forest has a greater consumption of water relative to the growth/harvest cycle of *Eucalyptus*, since in the first few years after planting transpiration in the plantation is much less than the native forest.

Competition for Resources between *Eucalyptus* and Adjacent Crops

There has been speculation that water use by *Eucalyptus* could have a negative impact on water resources available for adjacent vegetation or agricultural crops. Such issues have been extensively researched in relation to widespread agroforestry systems (reviewed by Nuberg 1998, and Schroth, 1999). There are important tradeoffs between the positive effects from windbreaks and shelter belts versus potential competition for light, nutrients and water resources. Such effects typically occur within 1 to 2 tree-heights (50 to 100 feet for a 50 foot tall tree, Nuberg, 1998) and can be attributed to direct competition by roots for available soil moisture. Often this can be managed by root pruning to reduce the area occupied by the tree roots. Impacts attributed to *Eucalyptus* depend on specific site conditions, and as with other concerns, there are examples where no negative impact on adjacent agricultural crops were observed (e.g. Patil, 1995). At some sites this could be attributed to deeper rooted trees versus shallow rooting crops utilizing water from different soil profiles. Finally, tree planting has been proposed as a mitigation strategy where rising water levels increase salinity and reduce crop yields (Hatton and George, 2001).

Summary and Conclusions:

Many studies report that water use in *Eucalyptus* is comparable to other tree species. There are some cases where afforestation with *Eucalyptus* (or other tree species) has lead to reduced water run-off and supply of streams or changes in water table levels, especially in regions with limited rainfall. However, in many well documented cases *Eucalyptus* plantations do not have any significant negative impacts on hydrology. Where there have been purported negative impacts, these often reflect more complex issues such as socioeconomic and land ownership disputes rather than the physiology of *Eucalyptus* itself. A key finding of many experiments has been that *Eucalyptus* is highly effective in regulating its water consumption relative to available supplies and regulates its growth accordingly. Based on numerous comparisons that have been made between the potential hydrological impacts of *Eucalyptus* and other tree species, we do not expect that the *Eucalyptus* trials planted under these permits would be any more impactful on local hydrology than planting other fast growing trees species.

References:

Almeida, A.C. and Soares, J.V. (2003). Comparison of water use in *Eucalyptus grandis* plantations and Atlantic rainforest in eastern coast of Brazil. Revista Arvore 27(2): 159-170.

Almeida, A.C., Soares, J.V., Landsberg, J.J. and Rezende, G.D. (2007). Growth and water balance of *Eucalyptus grandis* hybrid plantations in Brazil during a rotation for pulp production. Forest Ecology and Management, 251:10-21

Ares, A. and Fownes, J.H. (2000) Productivity, nutrient and water-use efficiency of *Eucalyptus saligna* and *Toona ciliata* in Hawaii. Forest Ecology and Management 139:227-236

Binkley,D. and Stape, J. L. (2004). Sustainable Management of *Eucalyptus* Plantations in a Changing World. In: Borrelho, N., et al., *Eucalyptus* in a Changing World Proc. IUFRO Conference, Aviero, 11-15, October 2004.

Bosch, J.M. and Hewlett, J.D. (1982). A review of catchment experiments to determine the effect of vegetation changes on water yield and evapotranspiration. Journal of Hydrology, 55:3-23.

Calder I.R., Rosier, P.T.W., Prasanna, K.T. and Parameswarappa, S. (1997). *Eucalyptus* water use greater than rainfall input-a possible explanation from southern India. Hydrology and Earth System Sciences, 1:244-256.

Calder, I., Amezaga, J., Aylward, B., Bosch, J., Fuller, L., Gallop, K., Gosain, A., Hope, R., Jewitt, G., Miranda, M., Porras, I. and Wilson, V. (2004). Forest and Water Policies. The need to reconcile public and science perceptions. Geologica Acta, 2:157-166

Calder, I.R. (1994). *Eucalyptus*, Water and Sustainability: A Summary Report. ODA Forestry Series No. 6, United Kingdom Office of Development Assistance, London.

Casson, A. (1997). The controversy surrounding eucalypts in social forestry programs of Asia. National Center for development Studies. Working Paper 97/1

Chaturvedi, A.N. (1987). Silvicultural requirements of *Eucalyptus* for small farms. In Proc. International Workshop Multipurpose tree species for small farms. Winrock International Institute for Agricultural Development USA, International Development Research Centre of Canada.

Couto, L. and D. R. Betters. (1995). Short-rotation Eucalypt Plantations in Brazil: Social and Environmental Issues, prepared for the Biofuels Feedstock Development Program, ORNL/TM-12846, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Available on-line at: http://bioenergy.ornl.gov/reports/euc-braz/toc.html

Davidson, J., (1995). Ecological aspects of *Eucalyptus* plantations. In: Proceedings of the Regional Expert Consultation on *Eucalyptus*, 4-8 October, 1993, Vol. 1, FAO Regional Office for Asia and the Pacific, Bangkok. Available on-line at: http://www.fao.org/docrep/005/ac777e/ac777e00.htm#Contents
de Almeida, P.A. and Riekerk, H. (1990). Water balance of *Eucalyptus globulus* and *Quercus suber* forest stands in south Portugal. For. Ecol. Manage., 38: 55-64.

Engel V., Jobbagy, E.G., Stieglitz, M., Williams, M. and Jackson, R.B. (2005). Hydrological consequences of *Eucalyptus* afforestation in the Argentine Pampas. Water Resour. Res., 41, W10409

FAO (2002a). Annotated bibliography on environmental, social and economic impacts of Eucalypts (Spanish Version), Forest Plantations Working Papers, Working

Paper 17 (S). Palmberg, C., Forest Resources Development Service, Forest Resources Division. FAO, Rome (unpublished).

FAO (2002b). Annotated bibliography on environmental, social and economic impacts of Eucalypts (Spanish Version), Forest Plantations Working Papers, Working Paper 16. Forest Resources Development Service, Forest Resources Division. FAO, Rome (unpublished).

Hatton,T. and George, R. (2001). The Role of Afforestation in Managing Dryland Salinity. In Plantations, Farm Forestry and water: Workshop Proceedings RIRDC Publication No. 01/20. Namibar, E.K.S. and Brown, A.G. (eds).

Jobbagy, E.G. and Jackson, R.B. (2004). Groundwater use and salinization with grassland afforestation. Global Change Biology 10:1299–1312

Lane, P.N.J., Morris, J., Zhang, N., Guangyi, Z., Guangyi, Z., Guoyi, Z. and Daping, X. (2004). Water balance of *Eucalyptus* plantations in southeastern China. Agricultural and Forest Meteorology, 124: 253-267

Le Maitre, D.C., Versfeld, D.B. and Chapman, R.A. (2000). The impact of invading alien plants on surface water resources in South Africa: A preliminary assessment. Water SA 26:397-408

Le Maitre, D.C., van Wilgen, B.W., Gelderblom, C.M., Bailey, C., Chapman, R.A. and Nel, J.A. (2002). Invasive alien trees and water resources in South Africa: case studies of the costs and benefits of management. Forest Ecology and Management 160:143–159

Lesch. W., and Scott, D.F. (1997). The response in water yield to the thinning of *Pinus radiata*, *Pinus patula* and *Eucalyptus grandis* plantations. Forest Ecology and Management 99:295-307

Lima, W. de P. (1976). Interceptação da Chuva em Povoamentos de Eucalipto e de Pinheiro. IPEF, n.13, p.75-90. (Portuguese with English abstract)
Lima, W.P. (1984). The Hydrology of Eucalypts Forests in Australia - A Review. IPEF n.28, p.11-32

Lima, W. de P. and Freire, O. (1976). Evapotranspiração em Plantações de Eucalipto e de Pinheiro, e em Vegetação Natural. IPEF, n.12, p.103-117. (Potuguese with English abstract)

Lima, W.D. Zakia, M.J.B. Libardi, P.L. and Filho, A. (1990). Comparative evapotranspiration of *Eucalyptus*, pine and natural vegetation measured by the soil water balance method. IPEF International Piracicaba. 1:5-11.

Myers, B.J. Theiveyanathan, S. O'Brien, N.D. and Bond, W.J. (1995). Growth and water use of *Eucalyptus grandis* and *Pinus radiata* plantations irrigated with effluent.

Tree Physiology, 16:211-219.

Nosetto, M.D., Jobbagy, E.G. and Paruelo, J.M. (2005). Land-use change and water losses: the case of grassland afforestation across a soil textural gradient in central Argentina. Global Change Biology (2005) 11, 1101–1117

NRC (2008). Hydrologic Effects of a Changing Forest Landscape (Prepublication copy available on-line at http://www.nap.edu/catalog/12223.html)

Nuberg, I.K. (1998). Effect of shelter on temperate crops: a review to define research for Australian conditions. Agroforestry Systems 41:3–34.

Patil, V. (1995). Local Communities and *Eucalyptus* - An Experience in India. In: Proceedings of the Regional Expert Consultation on *Eucalyptus*, 4-8 October, 1993, Vol. 1, FAO Regional Office for Asia and the Pacific, Bangkok. Available on-line at: http://www.fao.org/docrep/005/ac777e/ac777e00.htm#Contents

Poore, M.E.D. and Fries, C. (1985). The Ecological Effects of *Eucalyptus*. FAO Forestry Paper No. 59

Sahin, V. and Hall, M.J. (1996). The effects of afforestation and deforestation on water yield. Journal of Hydrology 178:293-309

Schroth , G. (1999). A review of belowground interactions in agroforestry, focusing on mechanisms and management options. Agroforestry Systems 43:5–34.

Scott, D.F. and Lesch, W. (1997). Streamflow responses to afforestation with *Eucalyptus* grandis and *Pinus patula* and to felling in the Mokobulaan experimental catchments, South Africa. Journal of Hydrology 199:360-377

Scott, D.F., Le Maitre, D.C. and Fairbanks D.H.K. (1998). Forestry and streamflow reductions in South Africa: A reference system for assessing extent and distribution. Water SA 24:187-200

Silva, W., Sediyama, T., Silva, A., and Cardoso, A. (2004). Consumption and Water Use Efficiency Index by *E. citriodora* and *E. grandis* plants cultivated in pots with three different water contents in the soil jointly with *Brachiaria brisantha* populations. Foresta, 32 (3): 325-335. (Potuguese with English abstract)

Srivastava, R,J., Kumar, A. and Prasad, K. (2003). Studies on Soil Moisture Variations Under *Eucalyptus* Plantations. XII World Forestry Congress, 2003, Quebec City, Canada. Available on-line at: http://www.fao.org/DOCREP/ARTICLE/WFC/XII/0500-B2.HTM

Stape, J.L., Binkley, D. and Ryan, M.G. (2004a). *Eucalyptus* production and the supply, use and efficiency of use of water, light and nitrogen across a geographic gradient in Brazil. Forest Ecology and Management 193:17–31

Stape, J.L. Binkley, D. Ryan, M.G. and Gomes, A.N. (2004b). Water use, water limitation, and water use efficiency in a *Eucalyptus* plantation. Bosque 25:35–41.
Sunder, S. (1993). The Ecological, Economic and Social Effects of *Eucalyptus*. In: Proceedings of the Regional Expert Consultation on *Eucalyptus*, 4-8 October, 1993, Vol. 1, FAO Regional Office for Asia and the Pacific, Bangkok

White, K.J. (1995). Silviculture of *Eucalyptus* Plantings – Learning from the Region (Australia). In: Proceedings of the Regional Expert Consultation on *Eucalyptus*, 4-8 October, 1993, Vol. 1, FAO Regional Office for Asia and the Pacific, Bangkok

White, K., Ball, J. and Kashio, M. (eds) (1995). Proceedings of the Regional Expert Consultation on *Eucalyptus*, 4-8 October, 1993, Vol. 1, FAO Regional Office for Asia and the Pacific, Bangkok

Whitehead, D. and Beadle, C.L. (2004). Physiological regulation of productivity and water use in *Eucalyptus*: A review. Forest Ecology and Management. 193:113-140

Wullschleger, S. Meinzer, F. and Vertessy, R. (1998). A Review of Whole Plant Water Use Studies in Trees. Tree Physiology, 18: 499-512.

Zhang, L., Dawes, W.R. and Walker, G.R (1999). Predicting the effect of vegetation changes on catchment average water balance. Cooperative Research Center for Catchment Hydrology Technical report 99/12

APPENDIX III: USDA Forest Service assessment of impacts on hydrology

This document was prepared by C. R. Ford and J. M. Vose in response to the document titled "Hydrology considerations for planted *Eucalyptus*" submitted by ArborGen LLC to USDA APHIS BRS in support of consideration for approval of permits for field trials of *Eucalyptus* at multiple sites

Executive Summary

We reviewed the materials provided by ArborGen and synthesized the literature on water use by *Eucalyptus* and other vegetation in the southeastern US. Based on these materials and our best professional judgment, we provide the following assessments:

1. ***Water use efficiency (WUE) is not a good metric to evaluate impacts on hydrology***
   From a hydrologic standpoint, total water use (transpiration + interception) is a more appropriate metric to assess hydrologic impacts. A species may have high WUE (defined by ArborGen as volume of wood produced per amount of water required), but still transpire and intercept a significant amount of water.

2. ***Annual E_t losses by* Eucalyptus *hybrid plantations planted in the southeast US will greatly exceed E_t by other native southeastern forest types***
   Our review of the literature and estimate of *Eucalyptus* transpiration suggests that water use is at least 2-fold greater than most other native forests in the southeastern US.

3. ***If* Eucalyptus *invades native forests, forest water use will increase***
   Due to a combination of physiological and structural characteristics, *Eucalyptus* will use more water than most native species regardless of whether it is planted or invades native forests.

4. ***Afforestation from existing vegetation into* Eucalyptus *plantations reduces stream flow more so than afforestation to pine plantations***
   Our review of the literature suggests that stream flow will be about 20% lower in *Eucalyptus* plantations vs. pine plantations.

5. ***Planting* Eucalyptus *hybrid plantations will lower the water table, and affect groundwater recharge and stream flow dynamics***
   The combination of shallow and deep roots typical of *Eucalyptus* species has the potential to impact **both** surface and groundwater hydrology.

6. ***It is unlikely that lower stocking levels will be an acceptable management practice to reduce hydrologic impacts of* Eucalyptus *plantations.***

High biomass production requires fully stocked stands. Reducing stocking to minimize hydrologic impacts is likely to counter the benefits of planting fast growing *Eucalyptus*.

Possible impacts of *Eucalyptus* hybrid plantations on southeastern US hydrology

Water use efficiency (WUE) is not a good metric to evaluate impacts on hydrology

From a physiological standpoint, water use efficiency is defined as the ratio of the moles of carbon fixed to the moles of water lost. WUE is a leaf-level metric. The Hydrology document prepared by ArborGen provided a ratio of liters of water consumed to grams of biomass produced. While these ratios provide good information regarding the transpirational cost of biomass production, they do not incorporate information on the *magnitude* of evapotranspirational losses, nor do they integrate stand management effects (e.g., planting density, rotation age). Hence, WUE is a poor metric to evaluate the effects of *Eucalyptus* on water resources. For example, *Eucalyptus* can produce more biomass per unit water consumed than native southeastern pines; however, their extremely rapid biomass production has proportionally higher transpirational costs and hence greater water use. Better metrics of evaluating the impacts of *Eucalyptus* hybrid plantations on hydrology exist. In order of scale, these are evapotranspiration (ET, mm H_2O yr^{-1}), transpiration (E_t, mm H_2O yr^{-1}), and whole-tree water use (Q, kg H_2O day^{-1}). Evapotranspiration (ET) integrates water loss by E_t, interception (E_i), and soil evaporation (E_s), and is often estimated at the landscape scale using precipitation input minus stream flow output on paired-catchments (P-R_o). The net effects of greater evapotranspiration losses are reduced soil moisture, reduced groundwater depth and recharge, and reduced stream flow. These parameters can also be used to evaluate impacts on hydrology.

Annual E_t losses by *Eucalyptus* hybrid plantations planted in the southeast US will greatly exceed E_t by other southeastern forest types

Previous studies have quantified annual E_t from various southeastern US forested and crop lands (Table 1). Native pine plantations consume nearly twice the water consumed by longleaf pine savannas, but only marginally more than mature upland hardwood forests. In contrast, a mature *Eucalyptus* plantation (age 5, 1111 trees ha^{-1}, LAI of 6 m^2 m^{-2}) growing in southwest GA could potentially transpire 882 mm yr^{-1}, exceeding all other forest types on average by a factor of 2.5. The Hydrology document prepared by ArborGen states that the proposed sites are on land previously managed for forestry, agricultural production or maintained as pasture land. In these cases, we may expect *Eucalyptus* E_t to exceed that of previous pine plantations by a factor of 1.6, and previous pasture land by a factor of 3.5. The comparison with agricultural crops is more variable; *Eucalyptus* E_t may be greater or lesser than crop E_t, depending on the crop, the growing season, and the management practices.

Table 1

Vegetation type	*Mean transpiration (mm yr^{-1})*	*Reference*
Longleaf pine savanna	244	(Ford et al. 2008)
Old field	250	(Stoy et al. 2006)
Oak-pine-hickory forest	278	(Oren and Pataki 2001)
Upland oak forest	313	(Wullschleger et al. 2001)
Mixed pine hardwood	355	(Phillips and Oren 2001)
Mixed pine hardwood	442	(Stoy et al. 2006)
Planted loblolly pine	490	(Stoy et al. 2006)
Mixed pine hardwood	523	Schafer and others 2002
Slash pine flatwoods	563	(Powell et al. 2005)
Eucalyptus hybrid plantation	882	Estimated for SW GA in average climate and rainfall year from model published in (Mielke et al. 1999)
Cotton (non-irrigated, annual)	392	(Howell et al. 2004)
Strawberries (irrigated, 7-month crop, 5-month fallow)	1397	(Clark 1994, Allen et al. 1998)
Watermelon (irrigated, 3-month crop, 9-month fallow)	237	(Allen et al. 1998, Shukla et al. 2007)

Eucalyptus has much higher stomatal conductance (g_s) in humid environments compared to native species

The Hydrology document prepared by ArborGen states that *Eucalyptus* has evolved several mechanisms that allows it to cope with drought conditions in their native habitats, including high stomatal sensitivity to air vapor pressure deficit (VPD). Across many taxa of plants, two main g_s responses to VPD exist (Figure 1). Both strategies regulate g_s (and thus transpiration) according to allowable variation in leaf water potential. The benefit of having a high δ, is having a high conductance in humid environments ($g_s$ref). The southeastern US is a relatively humid environment, with average daily VPD values around 1.5 kPa (Ford et al. 2004). In this humid environment, we can expect that *Eucalyptus* hybrid plantations will have stomatal conductance rates that are roughly double the conductance rates of native southeastern pine species. This is one mechanism that confers a greater transpiration rate of the former compared to the latter.

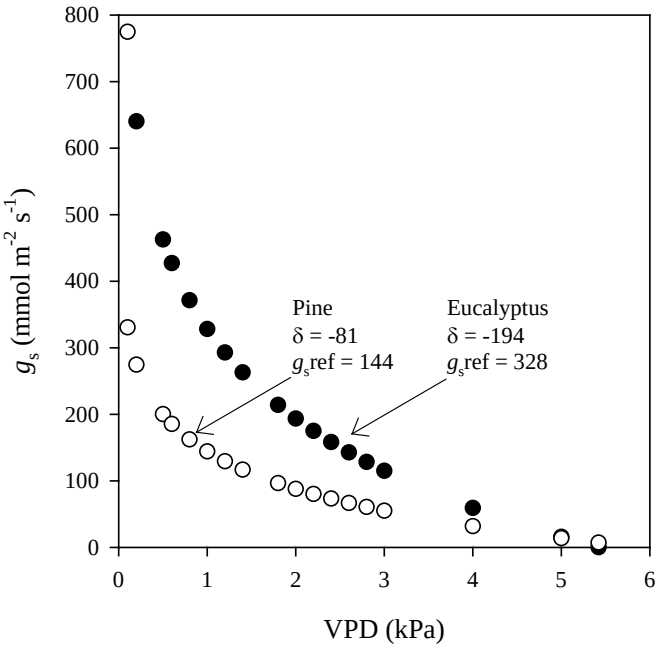

Figure 1: Stomatal conductance response to VPD for pine and *Eucalyptus* (Oren et al. 1999)

If *Eucalyptus* invades native forests, forest water use will increase

In general, species that evolved in arid climates have a more sensitive g_s response to VPD than those in more mesic environments (McDowell et al. 2008). When comparing water use of native species to plant species that have invaded a system (invasives), a recent meta-analysis across all biomes shows that stomatal conductance and photosynthesis are significantly greater in the invasive species compared to any of the native species in the system (Cavaleri and Sack 2008). Specifically, for systems that had been invaded by tree life-forms, stand level transpiration was significantly greater compared to un-invaded systems (Cavaleri and Sack 2008).

Afforestation from existing vegetation into *Eucalyptus* plantations reduces stream flow more so than afforestation to pine plantations

The Hydrology document prepared by ArborGen states that afforestation with any tree species may reduce stream flow; and that while *Eucalyptus* plantations might reduce stream flow, the reduction would be comparable to the reduction by pine plantations. Recent research suggests that *Eucalyptus* plantations would reduce stream flow more than pine plantations, and more importantly, *Eucalyptus* plantations could eliminate low flows. In a review of more than 20 catchment conversion studies, Farley and others (2005b) showed that converting existing vegetation to *Eucalyptus* plantations reduced stream flow by 20% more than converting it to a pine plantation. This review also

showed that the loss of low flows were more complete for *Eucalyptus* plantations compared to pine plantations (100% vs. ~80% reduction of low flows).

In perennial streams throughout the southeast which have base flows sustained by subsurface flow from the water table (or unconfined aquifers), elimination of low flows may have important ramifications for threatened and endangered aquatic species, such as the gulf strain striped bass, and thee species of endemic freshwater mussels (Golladay et al. 2004b, Couch and McDowell 2006a).

Planting *Eucalyptus* hybrid plantations will lower the water table, and affect groundwater recharge and stream flow dynamics

The Hydrology document prepared by ArborGen states that afforestation with any tree species may lower ground water levels. This is highly dependent on subsurface flow patterns, local hillslope hydrology, and species-specific rooting patterns. For example, in sites where the water table can be recharged laterally, if roots extend to the water table, then stomatal conductance and transpiration can be maintained even when water in the upper soil layers is insufficient to maintain transpiration. If *Eucalyptus* hybrid plantations mine water from the saturated zone (i.e., water table), groundwater recharge could be reduced. The southeastern Coastal Plain is characterized in many places by karst geology in which groundwater from the semi-confined Upper Floridan Aquifer (UFA) is hydraulically connected to the water table (surface water) (Opsahl et al. 2007). Mean water table depths typically range 3–8 m (Ford et al. 2008). The mean maximum rooting depth for *Eucalyptus* is 15 m, characteristic of its dimorphic rooting pattern; in contrast, mean maximum rooting depths of pine plantation (*P. taeda* and *P. elliottii*) and grass species are 3 m and 2.6 m, respectively (Canadell et al. 1996b). The average age of groundwater in the UFA is ~20 years (Happella et al. 2006) and groundwater is regularly recharged by surface water in this region (Opsahl et al. 2007). Conversion to *Eucalyptus* on sites with water tables <10 m will likely lower down-slope water tables via direct means (i.e., direct use of ground water by deep roots), affect groundwater-aquifer dynamics, and result in ET rates that exceed precipitation input, as have been reported for this species in other locations (Calder et al. 1997b).

The Hydrology document prepared by ArborGen states that the high water usage by *Eucalyptus* may be valuable in purposefully lowering water tables to reduced potential salinity problems. *Eucalyptus* has been used to afforest areas and lower the saline groundwater in highly weathered landscapes (e.g., AUS). This application is not relevant to the southeastern US, as soils are not saline. Furthermore, receding groundwater levels in the UFA are being replaced in costal areas by saltwater (i.e., saltwater intrusion) (Andersen et al. 2006). Thus, lowering the water table, and the groundwater levels in the UFA would not reduce salinity problems (as stated in the Hydrology document), and may actually exacerbate them.

Key Point: The significance of the impact on groundwater and stream flow will depend greatly on the area extent, size, and spatial distribution of the plantations. For example, a few small (i.e., < 10 ha) and well dispersed plantations may only have very localized impacts and negligible impacts at the watershed scale.

Management of *Eucalyptus* as coppice stands will affect water use of future rotations
Management practices may create a perennial root stock in *Eucalyptus* plantations. If *Eucalyptus* plantations are managed as coppice stands, the remaining mature, deeply-penetrating root stock may be able to supply the second rotation stems with more water resources for use than similar sized stems in their first rotation (Swift and Swank 1981).

It is unlikely that lower stocking levels will be an acceptable management practice to reduce hydrologic impacts of *Eucalyptus* plantations
Some of the reports cited in the ArborGen document discuss the potential for altering management practices to minimize the impacts of intensively managed *Eucalyptus* plantations on hydrology. The most viable option for reducing hydrologic impacts is to manage stocking ("stocking" is a term to describe the how much of the site is occupied by the species of interest; stand basal area expressed in m^2 stem area $hectare^{-1}$ is often used as a measure of stocking). Water use is highly regulated by stand leaf area and reducing basal area will result in lower stand leaf area. Empirical research (Douglass and Swank 1972) at Coweeta has shown that stand basal area needs to be reduced by at least 15 % before any impact on stream flow is obtained. Large and sustained increases in stream flow typically require significant reductions in stocking because trees growing in more open conditions will increase transpiration rates in response to changes in micrometeorological conditions in the tree crown.

From a practical standpoint, *it is unlikely that lower stocking levels will be an acceptable management practice for Eucalyptus plantations* because one of the primary objectives of growing *Eucalyptus* is to maximize biomass production -- this requires fully stocked stands.

Monitoring Impacts on Hydrology
Several options are available to monitor the impacts of *Eucalyptus* plantations on hydrology. Options include direct or indirect measurements of impacts, and vary in complexity and cost. The ArborGen document provided basic information on the hydrologic cycle and its components so these will not be repeated here.

Indirect Measurements:

Transpiration & Interception
Instrumentation required = rain gauges, throughfall collectors, sap flow sensors
PROS: direct measure of change in water use component on hydrologic cycle
CONS: does not directly measure impacts on stream flow or groundwater; expensive, high maintenance

Soil Moisture
Instrumentation = TDR probes and data loggers (automated); soil probe for gravimetric (manual)
PROS: easy to implement, relatively inexpensive
CONS: does not directly measure impacts on stream flow or groundwater

Direct Measurements:

Groundwater Depth

Instrumentation = access wells & pressure transducers (automated); access wells (manual)

PROS: direct measure of impacts; reliable

CONS: expensive

Stream flow

Instrumentation = flumes & data loggers (automated); pressure transducer, rating curve, stream survey

PROS: direct measure of impacts; reliable

CONS: expensive, requires stream, expertise

Regardless of the monitoring approach chosen, the monitoring design will require a suitable control for comparison. Ideally, the plantation site(s) and the control site(s) would be measured for at least 1 to 2 years prior to be being planted, and then both sites would be measured for the duration of the monitoring period. We recommend that the monitoring period begin at plantation establishment and continue through canopy closure (approximately 5 years).

Additional analyses on groundwater dynamics and linkages with aquatic ecosystems are required

We recommend that APHIS solicit input from experts on groundwater hydrology (e.g., from USGS) to assess the potential impacts on groundwater recharge and associated dynamics. In addition, our analysis suggests that stream flow will be reduced by at least 20% relative to pine plantations and perhaps even greater reductions will be observed relative to native ecosystems. We recommend that APHIS solicit input from aquatic ecologists to assess the potential impacts on aquatic ecosystems and associated species.

References

Allen RG, Pereira LS, Raes D, Smith M. 1998. Crop evapotranspiration - Guidelines for computing crop water requirements. FAO - Food and Agriculture Organization of the United Nations. Paper 56.

Andersen PF, Mercer JW, White Jr. HO. 2006. Numerical modeling of salt-water intrusion at Hallandale, Florida. Ground Water 26: 619–630.

Calder IR, Rosier PTW, Prasanna KT, Parameswarappa S. 1997. Eucalyptus water use greater than rainfall input-a possible explanation from southern India. Hydrology and Earth System Sciences 1: 249–256.

Canadell J, Jackson RB, Ehleringer JR, Mooney HA, Sala OE, Schulze ED. 1996. Maximum rooting depth of vegetation types at the global scale. Oecologia 108: 583-595. <Go to ISI>://A1996VX11000001

Cavaleri M, Sack L. 2008. Native vs. invasive plant water use at multiple scales: A global meta-analysis. *Presented at the* Ecological Society of America Annual Meeting *in* Milwaukee, WI. August 3–8.

Clark GA. 1994. Water requirements for drip irrigated strawberries in south central Florida. Agricultural and Biological Engineering Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. AE253.

Couch CA, McDowell RJ. 2006. Flint River Basin Regional Water Development and Conservation Plan. Georgia Dept. of Natural Resources, Environmental Protection Division. p. 242.

Douglass JE, Swank WT. 1972. Streamflow modification through management of eastern forests. USDA Forest Service, Southeastern Forest Experiment Station. Research Paper SE-94.

Farley K, Jobbagy E, Jackson RB. 2005. Effects of afforestation on water yiel: a global synthesis with implications for policy. Global Change Biology 11: 1565–1576.

Ford CR, Goranson CE, Mitchell RJ, Will RE, Teskey RO. 2004. Diurnal and seasonal variability in the radial distribution of sap flow: predicting total stem flow in *Pinus taeda* trees. Tree Physiol. 24: 951-960.

Ford CR, Mitchell RJ, Teskey RO. 2008. Water table depth affects productivity, water use, and the response to nitrogen addition in a savanna system. Can. J. For. Res. 38: 2118–2127.

Golladay SW, Gagnon P, Kearns M, Battle JM, Hicks DW. 2004. Response of freshwater mussel assemblages (Bivalvia:Unionidae) to a record drought in the Gulf Coastal Plain of southwestern Georgia. Journal of the North American Benthological Society 23: 494-506.

Happella JD, Opsahl S, Top Z, Chanton JP. 2006. Apparent CFC and $^{3}H/^{3}He$ age differences in water from Floridan Aquifer springs. J. Hydrol. 319: 410–426.

Howell TA, Evett SR, Tolk JA, Schneider AD. 2004. Evapotranspiration of full-, deficitirrigated, and dryland cotton on the northern Texas high plains. Journal of Irrigation an Drainage Engineering 130: 277–285. doi:10.1061/(ASCE)0733-9437(2004)130:4(277)

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA. 2008. Tansley review: Mechanisms of plant

survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol. 178: 719–739.
Mielke MS, Oliva MA, deBarros NF, Penchel RM, Martinez CA, Almeida ACd. 1999. Stomatal control of transpiration in the canopy of a clonal *Eucalyptus grandis* plantation. Trees- Structure and Function 13: 152-160.
Opsahl SP, Chapal SE, Hicks DW, Wheeler CK. 2007. Evaluation of ground-water and surface-water exchanges using streamflow difference analyses. Journal of the American Water Resources Association 43: 1132–1141.
Oren R, Pataki DE. 2001. Transpiration in response to variation in microclimate and soil moisture in southeastern deciduous forests. Oecologia 127: 549-559.
Oren R, Sperry JS, Katul GG, Pataki DE, Ewers BE, Phillips N, Schafer KVR. 1999. Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to Vapour pressure deficit. Plant Cell Environ. 22: 1515-1526.
Phillips N, Oren R. 2001. Intra- and inter-annual variation in transpiration of a pine forest. Ecol. Appl. 11: 385–396.
Powell TL, Starr G, Clark KL, Martin TA, Gholz HL. 2005. Ecosystem and understory water and energy exchange for a mature, naturally regenerated pine flatwoods forest in north Florida. Can. J. For. Res. 35: 1568-1580.
Shukla S, Jaber F, Srivastava S, Knowles J. 2007. Water use and crop coefficient for watermelon in southwest Florida. Institute of Food and Agricultural Sciences (IFAS), University of Florida. Report No. WRP-LY-0009 submitted to Southwest Florida Water Management District
Stoy P, Katul G, Siqueira M, Juang J, Novick K, McCarthy HR, Oishi AC, Umbelherr J, Kim H, Oren R. 2006. Separating the effects of climate and vegetation on evapotranspiration along a successional chronosequence in the southeastern US. Global Change Biology 12: 2115–2135.
Swift LW, Swank WT. 1981. Long term responses of streamflow following clearcutting and regrowth. Hydrological Sciences Bulletin 26: 245–256.
Wullschleger SD, Hanson PJ, Todd DE. 2001. Transpiration from a multi-species deciduous forest as estimated by xylem sap flow techniques. For. Ecol. Manage. 143: 205-213.

APPENDIX IV: Threatened and Endangered Species Analysis

The threatened and endangered species analysis was accomplished by considering the possibility of effects on all listed species including species proposed for listing and designated critical habitat including habitat proposed for designation within the county where the test site is located. Normally consideration would be limited to only those species and critical habitat identified by the United States Fish and Wildlife Service and/or National Marine Fisheries Service to be likely found in the area of the field tests. However, because the locations are declared by the applicant to be confidential business information, APHIS instead obtained species lists and critical habitat information for the entire county where the field tests are to occur. Although it is very clear that there could be no effect on some species (e. g. humpback whale) all species on the list and all critical habitat in each county are included in the discussion below.

The analysis was originally completed in early 2008, and updated in February 2009 to reflect changes to species' regulatory status. After receiving comments in response to the draft EA, it was decided to review the entire analysis to ensure that it was up to date and satisfies the requirements of Section 7 (a) (2) of the Endangered Species Act. This was completed in April 2010. Species lists and critical habitat for each county were verified with USFWS field offices to ensure accuracy. The review resulted in the addition of some new species to the county lists, and the removal of others from consideration. The Glades County, Florida site was not included in the revised analysis because the applicant removed this location from the permit applications. There are no significant changes to the potential for species to be affected by the action, or the likelihood of any species to be found in the test sites.

The following resources were used in the analysis:
US Fish and Wildlife Service Endangered Species Homepage: http://www.fws.gov/endangered/
US Fish and Wildlife Service ECOS system: http://ecos.fws.gov/ecos_public/index.do
NatureServe Explorer: http://www.natureserve.org/explorer/index.htm
Google Earth with critical habitat metadata supplied by US Fish and Wildlife Service
County species lists from FWS field offices in Alabama, Georgia, Louisiana, Mississippi, South Carolina, Texas and Florida.
South Carolina Heritage Trust Database
Telephone contacts with USFWS Field Office personnel
Discussions with property owners of field test sites

Baldwin County, AL (AL-BAL-01)

This location has been an agricultural research station for more than 20 years. The location has been used for managed production of annual agricultural crops and forest trees. Approximately 6.2 acres of field trials of genetically modified Eucalyptus Hybrid (EH1) trees of some of the lines in the permit request are being grown under issued permit # 06-325-111r under which these trees are allowed to flower. The oldest of these trees at this site are now entering their fifth growing season. An additional 0.75 acres of tests with some of these lines are being grown under permit 08-039-102rm. Site preparation involved herbicide application, subsoiling, and planting of trees in flat

beds. The surrounding areas of the test site consist of field plantings of agricultural crops, experimental forest trees and an abandoned pecan orchard.

Fourteen TES animals and one TES plant are listed in Baldwin County. The TES Animals include loggerhead sea turtle (*Caretta caretta*), green sea turtle, (*Chelonia mydas*), Alabama red-belly turtle (*Pseudemys alabamensis*), Alabama beach deermouse (*Peromyscus polionotus ammobates*), Perdido Key beach deermouse (*Peromyscus polionotus trissyllepsis*), piping plover (*Charadrius melodus*), least tern (*Sternula antillarum*), West Indian manatee (*Trichechus manatus*), wood stork (*Mycteria americana*), Kemp's ridley sea turtle (*Lepidochelys kempii*), gulf sturgeon (*Acipenser oxyrinchus desotoi*), Alabama sturgeon (*Scaphirhynchus suttkusi*), heavy pigtoe mussel (*Pleurobema taitianum*), and inflated heelsplitter mussel (*Potamilus inflatus*). The listed plant is American chaffseed (*Schwalbea americana*).

The American chaffseed occurs on sandy peat, sandy loam, acidic, seasonally moist to dry soils. It is generally found in habitats described as open, moist pine flatwoods, fire-maintained savannas, ecotonal areas between peaty wetlands and xeric sandy soils, and other open grass-sedge systems. According to Jody Smithen (contacted February 20, 2008) of the Daphne Field Office USFWS, the only location this plant is known to be in the county is in the northeast corner, far from the release site. The plant has no designated critical habitat.

The four turtle species (loggerhead sea turtle, green sea turtle, Alabama redbelly turtle and Kemp's ridley sea turtle), the gulf and Alabama sturgeons, and the West Indian manatee occur in aquatic habitats, and their habitat systems (bays, lagoons, salt marshes, creeks, ship channels, and other saltwater and freshwater environments) do not overlap with the trial site. The wood stork primarily inhabits wetland systems notably cypress or mangrove swamps and would not use the field test site. The two mouse species listed above (Alabama beach mouse and Perdido Key Beach deermouse) are found only in coastal dune areas and Perdido Key Beach, respectively, where they feed on sea oats, bluestems, and a variety of insects. Both habitats are located over 60+ miles from the proposed field trial. The piping plover uses sparsely vegetated dunes and coastal beaches in southern Baldwin County, also far away from the field site (about 60 miles). The Least Tern breeds on seacoasts, beaches, bays, estuaries, lagoons, lakes, and rivers and rests on sandy beaches, mudflats, and salt-pond dikes which are far away from the field test site.

Three species have been removed from the county list since the original TES effects analysis was completed in 2008. The FWS has determined that the species are so unlikely to be found in the county that it would be unreasonable for federal agencies to include them in TES analysis for projects within the county. These are the red-cockaded woodpecker (*Picoides borealis*), the reticulated flatwoods salamander (*Ambystoma bishopi*), and the Eastern indigo snake (*Drymarchon corais couperi*). However, we are retaining the information and findings from the earlier analysis as follows: The red-cockaded woodpecker could potentially visit the field tests but this species prefers mature pine stands as habitat for nesting. The reticulated flatwoods salamander uses the wet pine flat-woods associated with ephemeral wetlands. They typically breed in the low wetlands where they lay their eggs. After hatching, they spend 11 to 18 weeks as larvae before metamorphosing into adults and leaving the wetland for higher ground to burrow into the soil. It is important that the area through which the species moves is vegetated with grasses. Reticulated flatwood salamanders may move as far as 450 meters from their breeding sites. The area

surrounding the release site out to beyond this distance has been intensively managed for over 20 years as an agricultural research station, making it unlikely that the species would find this habitat useable. The Eastern indigo snake is known to inhabit a wide range of habitats (agriculture fields, pine flat-woods, wet depressions, stream bottom thickets and margins of swamps). It appears to be very rare in Baldwin County where a case has been reported in an unknown location (US Forest Service). According to Jody Smithen (contacted February 20, 2008) of the Daphne Field Office USFWS, the species has not been documented in the county for many years, but there are occasionally unsubstantiated reports. They do not feel this field trial poses concern for this species. Although it is highly unlikely that the species would be found at the site, the applicant will provide all workers with identifying characteristics of the snake and instructions on what to do if the species is encountered. These measures are a variation of standard protective measures the USFWS uses when they have reached a "may affect" determination for construction sites.

Critical Habitat: Most of the TES animals within the county area use inshore or wetland systems most of which are concentrated essentially in the southern and southeastern coastal beaches of Baldwin County. There is critical habitat listed for the Perdido Key beach deermouse and the piping plover. Notice of designation of critical habitat for the reticulated flatwood salamander (final rule) was published in the *Federal Register* on February 10, 2009 (http://edocket.access.gpo.gov/2009/pdf/E9-2403.pdf). There is no designated critical habitat for this species in Alabama. The closest critical habitat (for the Perdido Key Beach deer mouse and the piping plover) is about 65 miles away. The gulf sturgeon has critical habitat in Alabama but this does not occur in Baldwin County.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Escambia County, AL (AL-ESC-01)

This location had previously been used as an intensely managed pasture for more than 5 years and was planted with grasses suitable for cattle grazing. Site preparation involved herbicide application to remove existing grasses, subsoiling, preparation for possible irrigation, and planting of the test trees in flat beds. Field tests with some of the lines in these permit requests were established in 2007 (0.3 acres) and 2008 (0.2 acres) and are currently covered under permit 08-039-102rm. The surrounding areas of the test site consist of experimental forest trees, ~ 30 year-old slash pine and a re-forested area with less than 7 year-old mixed stands of pine and hardwood species.

Three TES animals are listed for Escambia County. The animals are gulf sturgeon, (*Acipenser Oxyrinchus desotoi*), red-cockaded woodpecker (*Picoides borealis*) and wood stork (*Mycteria americana*). The gulf sturgeon occurs in the Gulf of Mexico and spawns in freshwater rivers. It will not be affected by the field test since the closest river is over 3.5 miles away. The red cockaded woodpecker inhabits old growth forests, primarily longleaf pine. It could visit the field

test site but would not nest there. The wood stork primarily inhabits wetland systems notably cypress or mangrove swamps and would not find the field test site hospitable.

Critical Habitat: The designated critical habitat for the gulf sturgeon includes the Escambia River System in Santa Rosa and Escambia counties, Florida and Escambia, Conecuh, and Covington counties, Alabama. The establishment of the field test site would not impact this habitat. It is about 3.5 miles away from the Conecuh River.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Evans County, GA (GA-EVA-01)

This location has been a commercial nursery for forest seedling production for over 30 years. Site preparation involved herbicide application, subsoiling and planting of trees in flat beds. A field test of 0.2 acres of some of the lines included in these permit requests were established under permit 08-039-102rm in 2008. The areas surrounding the test site consist of nursery beds of forest tree seedlings, experimental forest trees, agricultural crops and mixed stands of hardwood and pine.

There are four TES animals listed for Evans County. There are no listed plants. The animals are the Eastern indigo snake (*Drymarchon corais couperi*), wood stork (*Mycteria americana*), red-cockaded woodpecker (*Picoides borealis*), and frosted flatwoods salamander (*Ambystoma cingulatum*).

The listed birds (red-cockaded woodpecker and wood stork) could potentially visit the field test site but would not nest there. The red-cockaded woodpecker nests in old growth longleaf pine. The wood stork nests in marshes, floodplain lakes, and swamps. The flatwoods salamander (*Ambystoma cingulatum*) was a species that required further analysis because it typically inhabits longleaf or slash pine forests lying between drier land upslope and wetlands and seasonally inhabits wet pine flat-woods with vernal pools. Originally it was associated with a unique community of longleaf pine/wire grass, but much of this habitat is now replaced by slash pine plantations. According to Kathy Chapman (contacted February 22 and 26, 2008) of the Coastal Georgia Field Office, there should not be an effect on the species because there would be no change to the habitat. However, she suggested verifying that there is no suitable breeding habitat near the release site. She provided information on life history and suitable habitat for the species. Viewing the site using Google Earth does not readily identify any suitable breeding habitat. To ensure that there is no suitable habitat in the area, BRS provided the species information to the applicant and in early March 2008, the applicant conducted a breeding habitat survey of the area within 450 meters (1476 feet) of the release site. No suitable habitat was found. For the eastern indigo snake (*Drymarchon couperi*) according to Kathy Chapman (contacted February 22 and 26, 2008) of the Coastal Georgia Field Office, the species is associated with the gopher tortoise in sandhill areas with wetlands. However,

land use is a determining factor on the likelihood of either species being present. The site has been used as a commercial nursery for forest seedling production for over 30 years. She indicated that she was not concerned with the site because there would be no change to the habitat. Although it is highly unlikely that the species would be found at the site, the applicant will provide all workers with identifying characteristics of the snake and instructions on what to do if the species is encountered. These measures are a variation of standard protective measures the USFWS uses when they have reached a "may affect" determination for construction sites.

Critical Habitat: There is no designated critical habitat or habitat proposed for designation in the county.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

St. Landry's Parish, LA (LA-SLP-01)

This location has been an experimental agricultural farm for more than 25 years, used for conducting research experiments with soybean, cotton and wheat. Site preparation will involved herbicide application, subsoiling and planting of trees in flat beds. Field tests of some of the lines included in these permit requests were established during 2006, 2007 and 2008 on 1.9 acres total, now covered under permit 08-039-102rm. Approximately 1.7 acres were subsequently terminated. The areas surrounding the test site consist of agricultural fields of rice, sugarcane and millet.

There are two TES animals listed for St. Landry's Parish; the pallid sturgeon (*Scaphirhynchus albus*) and the Louisiana black bear (*Urus americanus luteolus*). There are no listed plants.

The pallid sturgeon occurs in larger channels of the Mississippi-Missouri river system. The field test site is approximately 24 miles away from the Mississippi river, so would not be impacted by the field test. The Louisiana black bear (*Ursus a. luteolus*) prefers bottomland forests with diverse food resources, including a variety of hard-mast-producing species. Its habitat includes remote areas with little or no human activity so it would not likely be found at the site.

Critical Habitat: There is no designated critical habitat or habitat proposed for designation in the parish. The Louisiana black bear does have critical habitat in adjoining Pointe Coupee parish, but this is over 10 miles from the release site.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for

designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Marshall County, MS (MS-MAR-01)

This location has been an agricultural research station for more than 50 years, used for conducting research experiments with agricultural crops and grasses. The test site was previously used for experimental planting of grasses. Site preparation involved herbicide application, subsoiling and planting of trees in flat beds. A field test of 0.5 acres of some of the lines included in these permit requests were established in 2007, now under permit 08-039-102rm. This test was subsequently terminated. The areas surrounding the test site consist of experimental forest trees, agricultural fields, and less than 5 year-old pine plantations.

There are no listings for TES animals or plants in the county.

Critical Habitat: There is no designated critical habitat or habitat proposed for designation in the county.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Pearl River County, MS (MS-PRC-01)

This location has been used as an agricultural research station for more than 5 years for conducting research experiments with agricultural crops and grasses. The test site was used for experimental planting of grasses. Site preparation involved herbicide application to remove existing grasses, subsoiling, preparation for irrigation installation, and planting of trees in flat beds. Field tests of some of the lines included in these permit requests were established in 2007 (2.0 acres) and 2008 (0.98 acres), now covered under permit 08-039-102rm. The areas surrounding the test site consist of a grape research farm, mixed stands of hardwoods and pine, and a residential area.

There are five TES animals listed in this county and one TES plant. The animals are the ringed map turtle (*Graptemys oculifera*), gopher tortoise (*Gopherus polyphemus*), Louisiana black bear (*Ursus a. luteolus*), gulf sturgeon (*Acipenser oxyrhynchus desotoi*) and inflated heelsplitter (*Potamilus inflatus*). The listed plant is Louisiana quillwort (*Isoetes louisianensis*).

The ringed map turtle (*Graptemys oculifera*) inhabits wide rivers with strong currents, adjacent white sand beaches, and an abundance of basking sites in the form of brush, logs, and debris. The field test will not impact this aquatic species which occurs in the Pearl River system – approximately 11 miles from the field test site. The gopher tortoise (*Gopherus polyphemus*) inhabits dry sand ridges dominated by pine and areas maintained by fire. It is common in longleaf pine forests, but its numbers have decreased with the replacement of longleaf pine forests with

loblolly pine forests. This field test is located in an agricultural research station that would be an inhospitable environment for the gopher tortoise. According to James Harris (contacted February 6 and 7, 2008), Supervisory Wildlife Biologist with the USFWS in Lacombe, LA, the species is found in a large geographic area that includes the release site. However, the species is not found everywhere within this geographic area. The species is not likely to be on the site because of its location on a facility used for many years as an agricultural research station. The research facility was contacted to determine if gopher tortoises have been observed at the facility. The farm manager has not observed the species at the facility, and no sightings have been reported to him during his nine years as manager. He is familiar with the species and has seen them at another location about 7-8 miles from the release site. Another employee contacted has worked at the site for over 35 years and has never seen a gopher tortoise at the facility but did observe one approximately ten years ago about ¼ mile from the facility. The applicant surveyed the site for the presence of gopher tortoise burrows on January 29, 2008 and none were found. Considering the use of the facility, testimony of the facility employees, and the negative result of the survey, it can be concluded that the species is not present now and would be unlikely to use the site while it operates as an agricultural research station. The Louisiana black bear (*Ursus a. luteolus*) prefers bottomland forests with diverse food resources, including a variety of hard-mast-producing species. Its habitat includes remote areas with little or no human activity so it would not likely be found at the site. The gulf sturgeon, the inflated hellsplitter mussel, and the Louisiana quillwort occur in aquatic environments so would not be affected by the field test.

Critical habitat: The gulf Sturgeon has designated critical habitat in this county in the Pearl River system. The Pearl River is about 11 miles from the field test site. Tributaries of the Pearl River system are approximately 3.5 and 7.7 miles from the test site.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Bamberg County, SC (SC-BAM-01)

This location has been a managed forest plantation for more than 14 years. The location has been specifically used for short-rotation planting of hardwoods and softwood trees for forestry research. Standard silvicultural practices for site preparation, irrigation, fertilization, planting and harvesting have been used at this location. Similar practices will be used for the additional field tests to be established at this site. Several field tests of some of the lines included in these permit requests (3.5 acres total) were established from 2006 to 2008. These tests were covered under permit 08-039-102rm, and all but 0.2 acres were subsequently terminated. The areas surrounding the test site consist of experimental forest trees, young pine plantations, mixed stands of hardwoods and pine, and agricultural fields.

Three TES are listed in Bamberg County. The TES species listed are a plant, Canby's dropwort

(*Oxypolis canbyi*), and two animals, the wood stork (*Mycteria americana)* and the red cockaded woodpecker (*Picoides borealis*).

Canby's dropwort is an herbaceous perennial whose existing populations are maintained mainly through asexual reproduction. This species is strongly clonal, reproducing vegetatively by means of stoloniferous rhizomes. It has been found in a variety of habitats, including cypress ponds, grass-sedge dominated Carolina bays, wet pine savannahs, shallow pineland ponds and cypress-pine swamps or sloughs. The largest and most vigorous populations reported occur in open bays or ponds which are flooded throughout most of the year and which have little or no canopy cover. It grows in soils with a medium to high organic content, high water table, that are deep, poorly drained, and acidic. The Lisa Matthews Memorial Bay is a 52 acre site in Bamberg County which was given to the South Carolina Native Plants Society by the Nature Conservancy for the purpose of preserving Canby's dropwort (*Oxypolis canbyi*). This depression wetland is apparently a remnant Carolina Bay which is being restored and expanded to protect this endangered species. The location of the field test under this permit is a managed forest research area and does not provide the proper habitat for the species.

The wood stork inhabits riparian areas with lagoons and shallow water. It lives in freshwater situations: marshes, swamps, lagoons, ponds, flooded fields and brackish wetlands. It nests mostly in the upper parts of cypress trees, mangroves, or dead hardwoods over water or on islands along streams or adjacent to shallow lakes and feeds in freshwater marshes, swamps, lagoons, ponds, flooded pastures and flooded ditches, and in depressions in marshes. The field trial location is unsuitable habitat for the wood stork so it will be very unlikely to occur in this location. The red cockaded woodpecker inhabits old growth forests, primarily longleaf pine and might visit the field test site but would not nest there.

Critical Habitat: There is no designated critical habitat or habitat proposed for designation in the county.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Berkeley County, SC (SC-BER-01 and SC-BER-02)

There are two release site locations in Berkeley County. One site is an extension of a greenhouse facility that has been used for acclimatization of transgenic and non-transgenic plants for more than 7 years. The release site is located adjacent to greenhouse facilities and is surrounded by hardwoods and pine plantations. The trees are held here on a temporary basis until field planting. The other location has been a managed forest plantation for more than 7 years. The location has been specifically used for short rotation planting of cottonwood and Eucalyptus for forestry research. Site preparation involved herbicide application, subsoiling, drip irrigation installation,

and planting of trees in flat beds. The test site is located adjacent to greenhouse facilities and is surrounded by pine plantations. Field tests of some of the lines included in these permit requests were established in 2006 (0.3 acres) and 2007 (0.2 acres) that were covered under permit 08-039-102rm but subsequently terminated.

Five threatened or endangered species (TES) animals and three plants are listed in Berkeley County. The TES animal species are shortnose sturgeon (*Acipenser brevirostrum*), frosted flatwoods salamander (*Ambystoma cingulatum*), red-cockaded woodpecker (*Picoides borealis*), West Indian manatee (*Trichechus manatus*), and wood stork (*Mycteria americana*). The TES plants are pondberry (*Lindera melissifolia*), Canby's dropwort *(Oxypolis canbyi*) and American chaffseed (*Schwalbea americana*).

The shortnose sturgeon (*Acipenser brevirostrum*) occurs in rivers and estuaries. The West Indian manatee (Trichechus manatus) occurs in shallow coastal waters, rivers, bays and lakes; none of which are close to any of these release locations. The red cockaded woodpecker (*Picoides borealis*) inhabits old growth forests, primarily longleaf pine and might visit the field test site but would not nest there. The wood stork (*Mycteria americana*) primarily inhabits wetland systems notably cypress or mangrove swamps and would not find the field test site hospitable. The frosted flatwoods salamander (*Ambystoma cingulatum*) inhabits longleaf or slash pine forests lying between drier land upslope and wetlands and seasonally inhabits wet pine flat-woods with vernal pools. Discussions with Laura Zimmerman (contacted February 14 and 20, 2008) of the Charleston Field Office of the USFWS indicate that the species is not known to be in the area of the release. Known populations in the county are in the Francis Marion National Forest, far from the release site. A check of the SC Heritage Trust Database did not identify any occurrences in the area of the release. One site in Berkeley County is a fenced research plot/holding area so it is highly unlikely that these animal species would occur at this location. The other site is a managed forest plantation for short rotation planting of cottonwood for forestry research. The bird species might visit the location but would not nest there.

Canby's dropwort *(Oxypolis canbyi*) is an herbaceous perennial whose existing populations are maintained mainly through asexual reproduction. This species is strongly clonal, reproducing vegetatively by means of stoloniferous rhizomes. It has been found in a variety of habitats, including cypress ponds, grass-sedge dominated Carolina bays, wet pine savannahs, shallow pineland ponds and cypress-pine swamps or sloughs. The largest and most vigorous populations reported occur in open bays or ponds which are flooded throughout most of the year and which have little or no canopy cover. It grows in soils with a medium to high organic content, high water table, that are deep, poorly drained, and acidic. The pondberry (*Lindera melissifolia*) occurs in similar locations, in wetland habitats such as bottomland and hardwoods in the interior areas, and the margins of sinks, ponds and other depressions in the more coastal sites. The plants generally grow in shaded areas but may also be found in full sun. The chaffseed (*Schwalbea americana*) occurs on sandy peat, sandy loam, acidic, seasonally moist to dry soils. It is generally found in habitats described as open, moist pine flatwoods, fire-maintained savannas, ecotonal areas between peaty wetlands and xeric sandy soils, and other open grass-sedge systems. The site in Berkeley County is a fenced research plot/holding area which would be very inhospitable to these species. Laura Zimmerman (contacted February 14 and 20, 2008) of the Charleston Field Office of the USFWS states that she does not believe the species would be likely to be in the area. According to

the species' recovery plan, most known occurrences are on US Forest Service land and the only two occurrences on private land are not near the release site. A check of the SC Heritage Trust Database did not identify any occurrences in the area of the release.

Critical Habitat: Notice of designation of critical habitat for the frosted flatwood salamander (final rule) was published in the *Federal Register* on February 10, 2009 (http://edocket.access.gpo.gov/2009/pdf/E9-2403.pdf). Unit FFS-6 is within Berkeley County. This is located in the Francis Marion National Forest which is about 19-20 miles away from the release location.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at these release sites. If they were to enter the sites, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The sites are not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Charleston County, SC (SC-CHA-01)

This location has been a managed forest plantation for more than 10 years. The location has been specifically used for short-term planting of hardwoods and softwood trees for forestry research. Standard silvicultural practices for site preparation, irrigation, fertilization, planting and harvesting were used at this location. Similar practices were used for the additional field tests established at this site. Field tests of 3.1 acres of some of the lines included in the permit request were established during 2006 and 2007. These tests were covered under permit 08-039-102rm, and were all subsequently terminated. The test plots adjacent to the field test site include young mixed stands of hardwoods and pines. No additional trials are currently planned for this site.

Thirteen TES animals and 4 TES plants are listed for Charleston County. The animal species are: shortnose sturgeon (*Acipenser brevirostrum*), West Indian manatee (*Trichechus manatus*), Kemp's ridley sea turtle (*Lepidochelys kempii*), leatherback sea turtle (*Dermochelys coriacea*) and green sea turtle, (*Chelonia mydas*), loggerhead sea turtle (*Caretta caretta*), wood stork (*Mycteria americana*), red-cockaded woodpecker (*Picoides borealis*), piping plover (*Charadrius melodus*), frosted flatwoods salamander (*Ambystoma cingulatum*), finback whale (*Balaenoptera physalis*), humpback whale (*Megaptera novaengliae*), and right whale (*Balaena glacialis*). The plant species are: seabeach amaranth (*Amaranthus pumilus*), Canby's dropwort (*Oxypolis canbyi*), pondberry (*Lindera melissifolia*) and American chaffseed (*Schwalbea americana*).

The shortnose Sturgeon, West Indian manatee, Kemp's ridley sea turtle, leatherback sea turtle, green sea turtle, loggerhead sea turtle, West Indian manatee, finback whale, humpback whale, and right whale are all aquatic animals and would not be affected by the field test. The birds (piping plover, red-cockaded woodpecker and wood stork) could all potentially visit the field test site but would not nest there. The piping plover nest along the coast. The red-cockaded woodpecker nests in old growth longleaf pine. The wood stork nests in marshes, floodplain lakes, and swamps. The frosted flatwoods salamander inhabits longleaf or slash pine forests lying between drier land

upslope and wetlands and seasonally inhabits wet pine flat-woods with vernal pools. Discussions with Laura Zimmerman (contacted February 14 and 20, 2008) of the Charleston Field Office of the USFWS indicate that the species is not known to be in the area of the release. Known populations in the county are far from the release site in the Santee Coastal Reserve. A check of the SC Heritage Trust Database did not identify any occurrences in the area of the release.

For the plants, the seabeach amaranth occurs in areas just above the high tide line on accreting shorelines; those where the beach is building up or expanding. Canby's dropwort occurs in a variety of coastal plain communities, including pond cypress savannahs, the shallows and edges of cypress and pond pine ponds, sloughs, and wet pine savannas. Pondberry is found in swamp and pond margins, sandy sinks, swampy depressions or wet flats that are subject to drying but the roots are submerged at times. American chaffseed is found in various sandy soil areas on the coastal plain; plants are usually found on margins of savannas and cypress ponds that are seasonally wet; best managed by prescribed fire. The location of the field test, which has been a managed forest plantation for more than 10 years, would not be hospitable habitat for any of these species.

Critical Habitat: There is designated critical habitat for the piping plover in Charleston County but it is along the coast and over 15 miles from the field test. Notice of designation of critical habitat for the frosted flatwood salamander (final rule) was published in the *Federal Register* on February 10, 2009 (http://edocket.access.gpo.gov/2009/pdf/E9-2403.pdf). Unit FFS-7 is within Charleston County. This is located in the Santee Coastal Reserve which is over 50 miles away from the Charleston County site.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not adversely modify designated critical habitat or habitat proposed for designation.

Marlboro County, SC (SC-MAR-01)

This location has been a commercial nursery for forest seedling production for over 30 years. Site preparation involved herbicide application, subsoiling and planting of trees in flat beds. A 0.3 acre field test of some of the lines included in these permit requests were established in 2007, now covered under permit 08-039-102rm but subsequently terminated. The surrounding areas consist of field plantings of agricultural crops, nursery beds of forest tree seedlings and less than 30 year-old mixed hardwood and pine plantations. No additional trials are currently planned for this site.

There are two TES animals listed for Marlboro County; the red-cockaded woodpecker (*Picoides borealis*) and the shortnose sturgeon (*Acipenser brevirostrum*). The listed plant is Canby's Dropwort (*Oxypolis canbyi*).

The red-cockaded woodpecker nests in old growth longleaf pine not found at the location of the field test. The shortnose sturgeon occurs in rivers and estuaries. Little is known about the status of any of the populations in South Carolina. The closest body of water where the sturgeon could possibly live is about 5 miles away. Canby's dropwort occurs in a variety of coastal plain communities, including pond cypress savannahs, the shallows and edges of cypress and pond pine ponds, sloughs, and wet pine savannas. The location of the field test, which has been a managed nursery for more than 30 years, would not be hospitable habitat for any of these species.

Critical Habitat: There is no designated critical habitat or habitat proposed for designation in the county.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Hardin County, TX (TX-HAR-01)

This location has been a managed forest plantation for more than 30 years. The location consists of mixed hardwood tree plantations planted using standard silvicultural practices and was harvested by the owner in 2004. The test site is within the larger harvested area and was re-bedded by the owner for planting. Site preparation included herbicide application and sub-soiling. A field test of 19.7 acres of some of the lines included in these permit requests were established under permit 08-039-102rm in 2009. The areas surrounding the test site consist of mixed hardwood stands and managed loblolly pine plantations.

One TES animal and one TES plant are listed for Hardin County. The animal is the red-cockaded woodpecker (*Picoides borealis*), and the plant is the Texas trailing phlox (*Phlox nivalis* ssp. texensis).

The red-cockaded woodpecker might visit the field test site but would not nest there since it prefers old growth pine forests, particularly longleaf pine. The Texas trailing phlox is endemic to the Pineywoods of the west gulf coastal plain of east Texas. It occurs in deep sandy soils in fire-maintained openings in upland longleaf pine savannas or post oak-bluejack oak woodlands. Since this site has been under managed forest plantations for more than 30 years this species would not find the field test site a suitable habitat.

Critical Habitat: There is no designated critical habitat or proposed critical habitat listed for this county.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the

species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Jasper County, TX (TX-JAS-01 and TX-JAS-02)

There are two locations in Jasper County Texas. The first site, TX-JAS-01, has been a managed pine plantation for more than 25 years. Previous plantings were cultivated in beds using standard silvicultural practices. The existing pine plantation at this site was harvested by the owner in 2007 and re-bedded for planting. The test site is within the larger harvested and bedded site prepared by the site owner. Further site preparation involved herbicide application, subsoiling and planting of trees in flat beds. A field test of 0.7 acre of some of the lines included in these permit requests were established under permit 08-039-102rm in 2008. The areas surrounding the test site consist of harvest age pine plantations.

The second site, TX-JAS-02, has been in agriculture for more than 30 years. The test site was used for pasture for the past 10 years. Site preparation involved herbicide application to remove existing grasses, subsoiling, preparation for irrigation installation, and flat plantings of trees. A field test of 1.0 acre of some of the lines included in these permit requests were established under permit 08-039-102rm in 2008. The areas surrounding the test site consist of natural stands of mixed pine and hardwoods.

Two threatened or endangered species (TES) animals and one plant are listed in Jasper County. The animals are the red-cockaded woodpecker (*Picoides borealis*) and the Louisiana black bear (*Ursus americanus luteolus*). The plant is Navasota ladies'-tresses (*Spiranthes parksii*).

The red-cockaded woodpecker (*Picoides borealis*) inhabits old growth forests, primarily longleaf pine and might visit the field test sites but would not nest there. The Louisiana black bear (*Ursus americanus luteolus*) depends on diverse, productive bottomland forest with diverse food resources, including a variety of hard-mast-producing species. High quality habitat includes remote areas with little or no human activity so it would not likely occur at either site. Navasota ladies'-tresses (*Spiranthes parksii*) occurs at the margins of post oak (*Quercus stellata*) woodlands in sandy loams along intermittent tributaries of rivers. The field test sites have none of these characteristics.

Critical Habitat: There is no designated critical habitat or habitat proposed for designation in the county.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at these release sites. If they were to enter the sites, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The sites are not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Jefferson County, TX (TA-JEF-01)

This location has been used for managed agricultural production of rice for more than 5 years. Site preparation will involve herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of rice plantations.

There are six TES animals listed for Jefferson County. There are no plants listed. The animals are green turtle (*Chelonia mydas*), hawksbill turtle (*Eretmochelys imbricata imbricate*), Kemp's ridley sea turtle (*Lepidochelys kempii*), leatherback turtle (*Dermochelys coriacea*), loggerhead sea turtle (*Caretta caretta*) and piping plover (*Charadrius melodus*).

All of the turtles occur in the open waters of the Gulf of Mexico or in shallow coastal and estuarine waters. The field test site is about 30 miles away from the Gulf. The piping plover uses sparsely vegetated dunes and coastal beaches in southern Jefferson County, which is also at least 30 miles from the field test site. None of these species would be impacted by the field test.

Critical Habitat: There is critical habitat listed for the piping plover but it does not occur in this county. The closest critical habitat is in neighboring Galveston County, approximately 40 miles from the field test site.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Newton County, TX (TX-NEW-01, TX-NEW-02, TX-NEW-03, TX-NEW-04)

There are four locations in Newton County. All locations have been under managed loblolly pine plantations for at least 30 years. The previous plantings were cultivated in beds using standard silvicultural practices and the areas have recently been harvested. Site preparation has been or will include herbicide application, plowing, and planting of trees in raised or flat beds. The surrounding areas of the test site consist of managed loblolly pine plantations and/or mixed hardwood stands.

Two TES animals are listed for Newton County. The animals are the red-cockaded woodpecker (*Picoides borealis*), and Louisiana black bear (*Ursus americanus luteolus*). There are no plants listed for this county.

The red-cockaded woodpecker might visit the field test site but would not nest there since it prefers old growth pine forests, particularly longleaf pine. The Louisiana black bear (*Ursus a. luteolus*) prefers bottomland forests with diverse food resources, including a variety of hard-mast-producing species. According to the Black Bear Recovery Plan (http://www.bbcc.org/web/images/stories/information/pdf/FinalRestorationPlanwithFigures.pdf) in

east Texas, potentially occupied habitat may occur in at least four Counties: Cass, Shelby, Panola and Angelina Counties. All of these locations are from fifty to over a hundred miles away from these test sites. Louisiana black bear habitat includes remote areas with little or no human activity so it is unlikely to be found at the site.

Critical Habitat: The proposed critical habitat is located in Avoyelles, East Carroll, Catahoula, Concordia, Franklin, Iberia, Iberville, Madison, Pointe Coupee, Richland, St. Martin, St. Mary, Tensas, West Carroll, and West Feliciana Parishes, Louisiana. All of these locations are over a hundred miles away from these test sites.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at these release sites. If they were to enter the sites, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The sites are not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Bay County, FL (FL-BAY-01)

This location was used as an intensely managed pasture for more than 15 years, and was planted with grasses suitable for cattle grazing. Site preparation involved herbicide application to remove existing grasses, subsoiling, preparation for possible irrigation installation, and planting of trees in flat beds. Field tests of some of the lines included in these permit requests were established in 2007 (1.0 acre) and 2008 (0.35 acres), now covered under permit 08-039-102rm. All tests except 0.18 acre were subsequently terminated. The areas surrounding the test site consist of experimental forest trees, agricultural crops and less than 25 year-old hardwoods and pine.

Sixteen TES animals and 6 TES plants are listed for Bay County. The animals are gulf moccasinshell (*Medionidus penicillatus*), oval pigtoe (*Pleurobema pyriforme*), gulf sturgeon (*Acipenser oxyrinchus*) loggerhead turtle (*Caretta caretta*), eastern indigo snake (*Drymarchon corais couperi*), piping plover (*Charadrius melodus*), red-cockaded woodpecker (*Picoides borealis*), West Indian manatee (*Trichechus manatus*), Choctawhatchee beach mouse (*Peromyscus polionotus allophrys*), St. Andrews beach mouse (*Peromyscus polionotus peninsularis*), reticulated flatwoods salamander (*Ambystoma bishopi*), green turtle (*Chelonia mydas*), leatherback turtle (*Dermochelys coriacea*), hawksbill turtle (*Eretmochelys imbricata imbricate*), Kemp's ridley turtle (*Lepidochelys kempii*), and wood stork, (*Mycteria americana*). The plants are telephus spurge (*Euphorbia telephioides*), white birds-in-a-nest (*Macbridea alba*), Godfrey's (violet) butterwort (*Pinguicula ionantha*), Florida skullcap (*Scutellaria floridana*), Harper's beauty, (*Harperocallis flava*) and Crystal Lake nailwort, (*Paronychia chartacea minima*).

The gulf moccasinshell, oval pigtoe, gulf sturgeon, West Indian manatee, and the turtles (loggerhead, green, leatherback, hawksbill and Kemp's ridley) are all aquatic animals and would not be affected by the field test. The birds (piping plover, red-cockaded woodpecker and wood stork) could all potentially visit the field test site but would not nest there. The piping plover nests along the coast which is about 20 miles away from the release site. The red-cockaded woodpecker

nests in old growth longleaf pine. The wood stork nests in marshes, floodplain lakes, and swamps. The two mouse species live along the coast in beach areas so would not be impacted by the field release. For the eastern indigo snake, according to Caroline Stahaller (contacted February 20, 2008) of the Panama City Field Office USFWS, the species has not been positively identified in the county or anywhere else on the Florida panhandle in 15-20 years. Considering the area is currently managed as a pasture, a habitat where the species is unlikely to occur, there is no concern for the species at this site. Although it is highly unlikely that the species would be found at the site, the applicant will provide all workers with identifying characteristics of the snake and instructions on what to do if the species is encountered. These measures are a variation of standard protective measures the USFWS uses when they have reached a "may affect" determination for construction sites. The reticulated flatwoods salamander inhabits longleaf or slash pine forests lying between drier land upslope and wetlands and seasonally inhabits wet pine flat-woods with ephemeral wetlands. They typically breed in the low wetlands where they lay their eggs. After hatching, they spend 11 to 18 weeks as larvae before metamorphosing into adults and leaving the wetland for higher ground to burrow into the soil. It is important that the area through which the species moves is vegetated with grasses. Management of the area with controlled burning is generally needed to provide quality flatwoods salamander habitat. Reticulated flatwoods salamanders may move as far as 450 meters from their breeding sites. Although the release site is not an area with known populations, and is at least 10 miles from the critical habitat, Ms. Stahaller of USFWS suggested a closer look at the site to determine if the habitat would be suitable for the species. The property owner was forwarded survey protocol information provided by USFWS. In late March 2008, the owner, (who is 64 and has lived at the site his entire life) walked the site within 450 meters of the release. The owner was contacted on April 7, 2008 to discuss his observations. The owner looked for adult salamanders but did not find any, and does not recall ever seeing them at the site. There were four naturally wet areas that were found. One was in the pasture itself. It was a small site of about 8 ft. by 10 ft. and is surrounded by an area that has been in continuous agricultural use for at least 60 years. The other two sites were heavily wooded areas surrounded by pasture and remain constantly wet in most years. They are approximately 250 yards from the release site. The fourth site is approximately 400 to 500 yards from the release site and is a 5 acre wetland that shares the same elevation with an adjacent creek and remains constantly wet, but does dry around the perimeter. Vegetation in the site is longleaf pine with a wiregrass groundcover – habitat the species finds most suitable. Vegetation between this potential breeding site and the release site is pasture. It is unlikely that the flatwoods salamander would be using the release site for a number of reasons. First, the only possible breeding sites within 450 meters of the release remain constantly wet during most years, a condition that would not serve as breeding habitat. The five acre site where the perimeter dries could perhaps allow for breeding in some years but it is about at the farthest possible distance from the release site that the adults are known to travel from their breeding area. Second, the sites are either surrounded by land that has been used for agricultural purposes for at least 60 years or is separated from the release site by such land. Activities associated with agricultural production, disking, plowing, application of pesticides etc. would make the habitat unsuitable for the species. In addition, none of this area is managed by controlled burning, a management practice that is generally used in areas where the species has viable populations. The lack of suitable breeding habitat, combined with the ongoing and historic agricultural activities at the site, point to a conclusion that the reticulated flatwood salamander will not be present in the area of the release, nor will they be likely to enter the site during the field trial. None of the six listed plant species would be expected to grow in the existing pasture. They grow in wiregrass

dominated longleaf pine savannahs, in the mesic flatwoods, on low sand ridges of pine-scrub oak near the Gulf of Mexico or in wet prairies and seepage bogs.

This location is being converted from a pasture to a forest tree research plot. Based on the above evaluation, APHIS has determined that disturbance of the field site for the release (e.g. plowing, removing vegetation, burning etc.) will not directly or indirectly affect a federally listed threatened or endangered species or species proposed for listing, or affect the habitat for these species.

Critical Habitat: There is designated critical habitat for the Gulf sturgeon along the entire coast of Bay County. Critical habitat for the Choctawhatchee beach mouse, St. Andrew's beach mouse, and the piping plover is along the coast in the Panama City area. The field test site is approximately 18 miles away from the coast so would not impact these critical habitats. Notice of designation of critical habitat for the reticulated flatwood salamander (final rule) was published in the *Federal Register* on February 10, 2009 (http://edocket.access.gpo.gov/2009/pdf/E9-2403.pdf). Unit RFS-6, subunit B is just beyond the northern border of Bay County in neighboring Washington County. The field test site is approximately 28 miles away from this location. Unit RFS-9 subunit A is located in neighboring Calhoun County approximately 10 miles from the site. There is proposed critical habitat listed for the West Indian manatee but it does not occur in this county.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Columbia County, FL (FL-COL-01)

This location has been under managed pine plantations for more than 20 years. Previous plantings were cultivated in beds using standard silvicultural practices. This area of the tract was burned in a fire in May 2007. After the fire, the area was raked and bedded by the site owner in preparation for re-planting. The test site is within the larger harvested and bedded area, which is surrounded by existing pine plantations and additional harvested tracts.

Five TES animals are listed for Columbia County. The animals are oval pigtoe (*Pleurobema pyriforme*), gulf sturgeon (*Acipenser oxyrinchus*), eastern indigo snake (*Drymarchon corais couperi*), red-cockaded woodpecker (*Picoides borealis*), and wood stork (*Mycteria americana*). There are no plants listed for this county.

The oval pigtoe and gulf sturgeon are aquatic animals and would not be affected by the field test. The birds (red-cockaded woodpecker and wood stork) could all potentially visit the field test site but would not nest there. The red-cockaded woodpecker nests in old growth longleaf pine. The wood stork nests in marshes, floodplain lakes, and swamps. For the eastern indigo snake, according to Caroline Stahaller (contacted February 20, 2008) of the Panama City Field Office USFWS, the species has not been positively identified in the Florida panhandle in 15-20 years.

Considering the area has been managed as a pine plantation for many years, it is unlikely to be found at this site. Although it is highly unlikely that the species would be found at the site, the applicant will provide all workers with identifying characteristics of the snake and instructions on what to do if the species is encountered. These measures are a variation of standard protective measures the USFWS uses when they have reached a "may affect" determination for construction sites.

Critical Habitat: There is designated critical habitat for the Gulf sturgeon but it is not listed in this county. There is no proposed critical habitat listed for this county.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Gadsden County, FL (FL-GAD-01, FL-GAD-02)

There are two planting sites at this location. One location, FL-GAD-01, has been an agricultural research station for more than 10 years and has been used for conducting research experiments on agricultural crops. Standard silvicultural practices were used for site preparation, including herbicide application, plowing and planting of trees in raised or flat beds. A field test of 0.2 acre of some of the lines included in these permit requests were established under permit 08-039-102rm in 2008. The areas surrounding the test site consist of mixed pine-hardwood forests and pine plantations as well as research plantings of agricultural and horticultural crops.

The second location, FL-GAD-02, has been an agricultural research station for more than 10 years. The field has been fallow for approximately seven years. Standard silvicultural practices will be used for site preparation, including herbicide application, plowing and planting of trees in raised or flat beds. The areas surrounding the test site consist of mixed pine-hardwood forests and pine plantations, as well as research plantings of agricultural and horticultural crops.

Ten TES animals and six TES plants are listed for Gadsden County. The animals are: fat threeridge mussel (*Amblema neislerii*), purple bankclimber mussel (*Elliptoideus sloatianus*), shinyrayed pocketbook (*Hamiota subangulata*), ochlockonee moccasinshell (*Medionidus simpsonianus*), oval pigtoe (*Pleurobema pyriforme*), chipola slabshell (*Elliptio chipolaensis),* gulf sturgeon (*Acipenser oxyrinchus desotoi*), wood stork (*Mycteria americana*), red-cockaded woodpecker, (*Picoides borealis*), Eastern indigo snake (*Drymarchon corais couperi*). The plants are: Florida torreya (*Torreya taxifolia*), Chapman's rhododendron (*Rhododendron chapmanii*), American chaffseed (*Schwalbea americana*), fringed campion (*Silene polypetala*), Miccosukee gooseberry (*Ribes echinellum*) and gentian pinkroot (*Spigelia gentianoides*).

The six mussel species (fat threeridge mussel, purple bankclimber mussel, shinyrayed pocketbook, Ochlockonee moccasinshell, oval pigtoe and chipola slabshell), and the gulf sturgeon (*Acipenser*

oxyrinchus desotoi) are all aquatic animals and would not be affected by the field test. (See CH info below on the gulf sturgeon). The red cockaded woodpecker (*Picoides borealis*) inhabits old growth forests, primarily longleaf pine. It could visit the field test site but would not nest there. The wood stork (*Mycteria americana*) primarily inhabits wetland systems notably cypress or mangrove swamps and would not find the field test site hospitable. For the Eastern indigo snake (*Drymarchon corais couperi*), according to Caroline Stahaller, (contacted February 20, 2008) of the Panama City Field Office USFWS, the species has not been seen anywhere on the Florida panhandle in 15-20 years. She had no concern that the action could affect the species and agreed the action would have "no effect." Although it is highly unlikely that the species would be found at the site, the applicant will provide all workers with identifying characteristics of the snake and instructions on what to do if the species is encountered. These measures are a variation of standard protective measures the USFWS uses when they have reached a "may affect" determination for construction sites.

None of the six listed plant species would be expected to grow at the field site/research station. For American chaffseed (*Schwalbea Americana*), according to the species recovery plan, the only known extant population in the county is on private land. A site visit in 1994 found the area changed to a residential development. It is assumed that the population may be extirpated. For Chapman's rhododendron (*Rhododendron chapanii*), according to the Federal Register notice listing the species, there is only one population in Gadsden County. The population is on land owned by a paper company near the Gadsden – Liberty county line, and is many miles from the release site. The Florida torreya (*Torreya taxifolia*) occurs on hardwood hammock slopes, ravines, and bluffs of the Apalachicola River region, usually in steephead ravines (deep cuts made by erosion into coastal plain sediments). The ravines are much cooler and more moist than the land surface above and harbor remnants of the more temperate flora that existed in the region during the Tertiary ice ages. The fringed campion (*Silene polypetala*) prefers well-drained, sandy-loam soils of deciduous woods, usually hillsides. The Miccosukee gooseberry (*Ribes echinellum*) is only known to exist on sites along east bank of Lake Miccosukee which is about 36 miles away from the field test site. The gentian pinkroot (*Spigelia gentianoides*) is also listed in Gadsden County and specimens were once collected in the county, but presently the only population currently known to exist is at the Three Rivers State Recreation Area, Lake Seminole, Jackson County, FL. (http://www.centerforplantconservation.org/). None of these species would find the field test site as suitable habitat.

Critical Habitat: The designated critical habitat for the gulf sturgeon includes the Apalachicola River mainstream, beginning from the Jim Woodruff Lock and Dam, Gadsden and Jackson Counties, Florida, downstream to its discharge at East Bay or Apalachicola Bay, Franklin County, Florida. The establishment of the field test site would not impact this habitat. The field site is about 19 miles away from the Apalachicola river mainstream below the Jim Woodruff Lock and Dam. The mussel species have designated critical habitat, but the nearest to the release site is in the Ochlokonee River over 10 miles away.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to found at these release sites. If they were to enter the sites, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species

proposed for listing. The sites are not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Highlands County, FL (FL-HIG-01)

This location was previously used for managed production of citrus for at least 15 years. The planting area at this location had been used for field trials of transgenic *Eucalyptus* for more than 6 years. Site preparation involved herbicide application, plowing, and planting of trees in flat beds. Field trials of 1.4 acres of some of the lines included in these permit requests are being grown under issued permit # 08-151-101r under which these trees are allowed to flower. The oldest of these trees at this site are now entering their fifth growing season. An additional 2.3 acres of tests of different lines with the same EH1 hybrid are being grown under permits 08-039-102rm and 09-070-101rm. Areas surrounding the test site consist of less than 5 year-old second-growth pine and hardwood with mixed grasses.

Fifteen TES animals, nineteen TES plants, and one TES lichen are listed for Highlands County. The animals are: American alligator (*Alligator mississippiensis*), wood stork (*Mycteria americana*), Audubon's crested caracara (*Caracara cheriway*), red-cockaded woodpecker (*Picoides borealis*), Florida scrub-jay (*Aphelocoma coerulescens*), Florida grasshopper sparrow (*Ammodramus savannarum floridanus*), Everglade snail kite (*Rostrhamus sociabilis plumbeus*), ivory-billed woodpecker (*Campephilus principalis*), Whooping crane (*Grus americana*) (non-essential experimental population), Florida panther (*Puma concolor coryi*), Puma (*Puma concolor* – similarity of appearance), West Indian manatee (*Trichechus manatus*), Eastern indigo snake (*Drymarchon corais couperi*), bluetail mole skink (*Eumeces egregius lividus*) and sand skink (*Neoseps reynoldsi*).

The plants are: Florida bonamia (*Bonamia grandiflora*), pygmy fringetree (*Chionanthus pygmaeus*), pigeon wings (*Clitoria fragrans*), short-leaved rosemary (*Conradina brevifolia*), Avon Park hare-bells (*Crotalaria avonensis*), Garrett's mint (*Dicerandra christmanii*), scrub mint (*Dicerandra frutescens*), scrub buckwheat (*Eriogonum longifolium var. gnaphalifolium*), snakeroot (*Eryngium cuneifolium*), highlands scrub hypericum (*Hypericum cumulicola*), scrub blazingstar (*Liatris ohlingerae*), Britton's bear-grass (*Nolina brittoniana*), papery whitlow-wort (*Paronychia chartacea ssp. Chartacea*), Lewton's polygala (*Polygala lewtonii*), wireweed (*Polygonella basiramia*), sandlace (*Polygonella myriophylla*), scrub plum (*Prunus geniculata*), Carter's mustard (*Warea carteri*), and Florida ziziphus (*Ziziphus celata*).

The lichen is: Florida perforate cladonia (*Cladonia perforate*).

The American alligator (*Alligator mississippiensis*) is no longer biologically endangered or threatened; however, it is listed by USFWS as Threatened throughout its entire range due to similarity of appearance to other endangered or threatened crocodilians. The wood stork (*Mycteria Americana*) primarily inhabits wetland systems notably cypress or mangrove swamps and would not find the field test site hospitable. The crested caracara (*Caracara cheriway*) is associated with open country; dry prairie with scattered cabbage palms, wetter prairies, and to some extent also improved pastures and sometimes wooded areas having associated limited areas of open grassland.

The center of range is the Kissimmee Prairie, an area of shallow ponds and sloughs with scattered hummocks of live oaks and cabbage palms. The red cockaded woodpecker (*Picoides borealis*) inhabits old growth forests, primarily longleaf pine. The Florida scrub-jay (*Aphelocoma coerulescens*) prefers oak scrub on white, drained sand, in open areas without a dense canopy associated with Palmetto, sand pine and rosemary. This includes scrub with no canopy, sandpine scrub, scrubby flatwoods, and coastal scrub. The Florida grasshopper sparrow (*Ammodramus savannarum floridanus*) prefers dry prairie with stunted saw palmetto and dwarf oaks, bluestems and wiregrass and unimproved cattle pastures. Its habitat is maintained by periodic fires. The snail kite (*Rostrhamus sociabilis plumbeus*) prefers large, open freshwater marshes and lakes with shallow open waters. The ivory-billed woodpecker (*Campephilus principalis*) formerly occurred in the southeastern United States and Cuba and has declined to extinction or near extinction. It once occupied swampy forests, especially large bottomland river swamps of coastal plain and Mississippi Delta and cypress swamps of Florida, in areas with many dead and dying trees. It would not occur in an agricultural environment such as the field test site. Whooping crane (*Grus americana*) prefers freshwater marshes and wet prairies. It nests in dense emergent vegetation (sedge, bulrush) in shallow ponds, freshwater marshes, wet prairies, or along lake margins, within large expanses of undisturbed wilderness. The cranes listed in this county are an experimental reintroduction of whooping cranes in Florida initiated in 1993 to establish a non-migratory population. This is an experimental, non-essential population. Any of the bird species could potentially visit the field test site but would not nest there. The Florida panther (*Puma concolor coryi*) generally occurs in heavily forested areas in lowlands and swamps, also upland forests in some parts of range; areas with adequate deer or wild hog population. Habitats include tropical hammocks, pine flatwoods, cabbage palm forests, mixed swamp, cypress swamp, live oak hammocks, sawgrass marshes, and Brazilian pepper thickets. It depends on large contiguous blocks of wooded habitat, though interspersed fields and early successional habitats may be beneficial through their positive effect on prey populations. Its day-use sites typically are dense patches of saw palmetto surrounded by swamp, pine flatwoods, or hammock. It would not occur in the trial area due to the openness and continued presence of humans in the area. The West Indian manatee (*Trichechus manatus*) occurs in shallow coastal waters, rivers, bays and lakes; none of which are close to any of this release location. For the Eastern indigo snake (*Drymarchon cosair couperi*) – according to Candice Martino (904-232-2580 ext. 129) Section 7 Endangered Species biologist from the Jacksonville, FL Field Office, (contacted February 25, 2008) the species is seldom seen but could be anywhere. However, the habitat at the release site would not be suitable. The historic and continuous use of the release site and the surrounding area as a citrus grove and *Eucalyptus* field trials makes it extremely unlikely that the species would be found in the area. Therefore, the appropriate determination would be “no effect.” Although it is highly unlikely that the species would be found at the site, the applicant will provide all workers with identifying characteristics of the snake and instructions on what to do if the species is encountered. These measures are a variation of standard protective measures the USFWS uses when they have reached a “may affect” determination for construction sites.

The bluetail mole skink (*Eumeces egregius lividus*) inhabits sand pine-rosemary scrub or, less frequently, longleaf pine-turkey oak association sandhills. It occupies localized pockets of sufficient leaf litter and moisture to provide abundant food and nesting sites. The sand skink (*Neoseps reynoldsi*) occurs only on Florida's central ridges, at elevations of 27 m or more. It inhabits loose sands of sand pine-rosemary scrub, less often longleaf pine-turkey oak sandhills or

turkey oak barrens adjacent to scrub, especially high pine-scrub ecotones. It was determined that the release site is within a geographic area where these two skink species are found. According to Brad Rick, (contacted February 27 and 28, 2008) of the Vero Beach Field Office USFWS, sand skinks and bluetail mole skinks are found in scrub habitat with areas of open sand. The literature indicates that skinks are sometimes found in active and abandoned citrus groves and the applicant confirmed that sandy soils are predominant in the area. It was decided to have the applicant conduct a survey of the species using USFWS protocols to determine if the species is present. The protocols were provided by the Vero Beach Field Office. The USFWS protocol recommends that surveys be conducted between March 1 and May 15 as this is an ideal time to observe evidence of the skinks. A coverboard survey was conducted over a one month period from March 18 to April 15. The coverboards were checked weekly on March 25, April 1, April 8 and April 15. No evidence indicating the presence of sand skinks or bluetail mole skinks was observed and they are presumed absent. Therefore, the appropriate determination would be "no effect."

For the plants, the Florida bonamia (*Bonamia grandiflora*) grows in natural clearings of bare ground and invades disturbed areas of open sand. Although not common, it is often locally abundant where there is little or no shade from trees or shrubs. It is locally abundant on deep, white, dry sands of ancient dunes and sandy ridges in clearings or openings of scrub habitat on the Central Ridge of Florida. Pygmy fringetree (*Chionanthus pygmaeus*) is generally found in xeric, coarse white sand of scrub/oak scrub areas found at the southern end of the Central Florida Ridge. It is also found occasionally in longleaf pine-turkey oak vegetation, high pineland, dry hammocks, and transitional habitats. Pigeon wings (*Clitoria fragrans*) is widely scattered in undisturbed clearings of xeric sandhill and scrub communities on well-drained upland soils. It is typically found in undisturbed clearings in scrub areas but also occurs in very open scrub as well. Short-leaved rosemary (*Conradina brevifolia*), is found in white sands of sand pine-oak scrub with scattered overstory of sand pine and scrub oak. Avon Park hare-bells (*Crotalaria avonensis*) occurs in upland habitats (scrub and sandhill), often along trails. It grows in full sun or partial shade provided by characteristic scrub shrubs or sand pine. Garrett's mint (*Dicerandra christmanii*) occurs in openings in sand pine-oak scrub on yellow soils of the Central Florida Ridge. Scrub mint (*Dicerandra frutescens*) occurs in well-drained soils of scrub or sandhill vegetation. It is locally abundant in and around the sand pine-evergreen oak scrub, where it may occur in the low shrub layer or in open stands, clearings, or adjacent sandy places. It is not found in areas cleared for pasture, or areas in which wholesale site preparation has taken place. Scrub buckwheat (*Eriogonum longifolium var. gnaphalifolium*) is long-lived, slow growing and flowers and reproduces primarily after fires or other disturbances (e.g. logging, mowing) that increase light availability. It prefers dry pinelands, sandhills, and scrub (longleaf pine-turkey oak, scrub oaks) and is more commonly found in transition habitats between scrub and high pine and in turkey oak barrens than in either dense scrub or open high pine. Snakeroot (*Eryngium cuneifolium*) is generally found in areas of open sand, including blowouts and other highly disturbed soil surfaces, such as road shoulders. It occurs in exposed sunny openings; areas in scrub, especially rosemary scrub. Highlands scrub hypericum (*Hypericum cumulicola*) occurs in patches of open, nutrient-poor sand within oak and rosemary scrub. It is often associated with reindeer lichen (*Cladonia* spp.) and snakeroot (*Eryngium cuneifolium*). Scrub blazingstar (*Liatris ohlingerae*) occurs in openings in oak-rosemary scrub and sand pine scrub. Britton's bear-grass (*Nolina brittoniana*) occurs in deep, fine-textured, well-drained sands of sand pine-evergreen oak scrub or longleaf pine-turkey oak sandhills. *Nolina* is entirely dependent on fire or some other mechanism to maintain an

open successional stage in scrub or sandhills. Papery whitlow-wort (*Paronychia chartacea ssp. Chartacea*) is a sand scrub that occurs on ancient dunes in the lake region, in white sand clearings or blowouts. Lewton's polygala (*Polygala lewtonii*) occurs in sandhills characterized by longleaf pine and low scrub oaks, including low turkey oak woods, and transitional sandhill/scrub habitats. This species occasionally inhabits powerline clearings or new roadsides. Wireweed (*Polygonella basiramia*) is restricted to bare patches within sand pine-evergreen oak scrub vegetation. It grows on areas of bare sand within sand pine (*Pinus clausa*) and Florida rosemary (*Ceratiola ericoides*). Sandlace (*Polygonella myriophylla*) occurs in areas of sand pine scrub and ancient sand dunes. Scrub plum (*Prunus geniculata*) has a very narrow range and small widely scattered populations. It frequently forms small colonies of several plants but may grow as solitary individuals. It grows in deep, yellow sands of longleaf pine-turkey oak sandhill and white, excessively leached, wind-deposited soils of evergreen scrub oak-sand pine scrub. Carter's mustard (*Warea carteri*) occurs in sandy clearings in sand scrub and sandhills; scattered overstory of sand; longleaf or slash pine and scrub oaks. Florida ziziphus (*Ziziphus celata*) is a scrub that occurs on gently rolling hills with vegetation dominated by *Carya floridana* and *Quercus* species. It prefers open, sunny areas. The Perforate Reindeer Lichen (*Cladonia perforate*) occurs in sandy openings in stabilized sand dunes with Florida scrub vegetation. It is often associated with *Ceratiola*. None of the plants and the lichen listed above would find the field test site as suitable habitat and would not be present given the historic and continuous use of the release site and the surrounding area as a citrus grove and research area used for growing *Eucalyptus*.

Critical Habitat: There is proposed critical habitat listed for the West Indian manatee but none of the proposed habitat occurs in this county. The whooping crane population in this county is a non-essential experimental population and does not have critical habitat.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Marion County, FL (FL-MAR-01)

This location has been used as an agricultural research station for more than 5 years for conducting research experiments on agricultural crops. Site preparation involved herbicide application, plowing, and planting of trees in flat beds. A field test of 0.9 acre of some of the lines included in these permit requests were established under permit 08-039-102rm in 2008. In addition, a field test of 0.3 acres of different lines with the same EH1 hybrid was planted in 2009 under permit 9-070-101rm. Areas surrounding the test site consist of agricultural fields and plantings of horticultural crops.

Seven TES animals and four TES plants are listed for Marion County. The animals are: sand skink (*Neoseps reynoldsi*), Eastern indigo snake (*Drymarchon corais couperi*), wood stork (*Mycteria americana*), Everglades snail kite (*Rostrhamus sociabilis plumbeus*), red-cockaded woodpecker

(*Picoides borealis*), Florida scrub-jay (*Aphelocoma coerulescens*), and West Indian manatee (*Trichechus manatus*). The plants are: Florida bonamia (*Bonamia grandiflora*), longspurred mint (*Dicerandra cornutissima*), scrub buckwheat (*Eriogonum longifolium var. gnaphalifolium*), and Lewton's polygala (*Polygala lewtonii*).

The Everglades snail kite (*Rostrhamus sociabilis plumbeus*) prefers large, open freshwater marshes and lakes with shallow open waters. The red cockaded woodpecker (*Picoides borealis*) inhabits old growth forests, primarily longleaf pine. The Florida scrub-jay (*Aphelocoma coerulescens*) prefers oak scrub on white, drained sand, in open areas without a dense canopy associated with Palmetto, sand pine and rosemary. This includes scrub with no canopy, sandpine scrub, scrubby flatwoods, and coastal scrub. The wood stork (*Mycteria americana*) primarily inhabits wetland systems notably cypress or mangrove swamps and would not find the field test site hospitable. Any of the four bird species could potentially visit the field test site but would not nest there. The West Indian manatee occurs in shallow coastal waters, rivers, bays and lakes; none of which are close to any of this release location. For the Eastern indigo snake (*Drymarchon corais couperi*) – according to Candice Martino, Section 7 Endangered Species biologist from the Jacksonville, FL Field Office, (contacted February 25, 2008) the species is seldom seen but could be anywhere. However, the habitat at the release site would not be suitable. The historic and continuous use of the release site and the surrounding area as an agricultural research station makes it extremely unlikely that the species would be found in the area. Therefore, the appropriate determination would be “no effect”. Although it is highly unlikely that the species would be found at the site, the applicant will provide all workers with identifying characteristics of the snake and instructions on what to do if the species is encountered. These measures are a variation of standard protective measures the USFWS uses when they have reached a “may affect” determination for construction sites. Also for the sand skink (*Neoseps renoldsi*) – according to Candice Martino (contacted February 25, 2008), although the release site may be within a greater geographic area where the species may be found, the habitat of the release site would not be suitable for the species. The historic and continuous use of the release site and the surrounding area as an agricultural research station makes it extremely unlikely that the species would be found in the area. Therefore, the appropriate determination would be “no effect”.

For the plants, the Florida bonamia (*Bonamia grandiflora*) grows in natural clearings of bare ground and invades disturbed areas of open sand. Although not common, it is often locally abundant where there is little or no shade from trees or shrubs. It is locally abundant on deep, white, dry sands of ancient dunes and sandy ridges in clearings or openings of scrub habitat on the Central Ridge of Florida. The longspurred mint (*Dicerandra cornutissima*) is scattered in openings (natural or artificial) in longleaf pine-turkey oak scrub/sandhill or on low rises in slash pine-palmetto scrub. The scrub buckwheat (*Eriogonum longifolium var. gnaphalifolium*) is an herbaceous perennial that occurs in dry pinelands, sandhills, and scrub (longleaf pine-turkey oak, scrub oaks). It is more commonly found in transition habitats between scrub and high pine and in turkey oak barrens than in either dense scrub or open high pine. Lewton's polygala (*Polygala lewtonii*), occurs in sandhills characterized by longleaf pine and low scrub oaks, including low turkey oak woods, and transitional sandhill/scrub habitats. This species occasionally inhabits powerline clearings or new roadsides. None of the four plant species listed above would find an agricultural research area as suitable habitat and would not be present given the historic and continuous use of the release site and the surrounding area as an agricultural research station.

Critical Habitat: There is no designated critical habitat or habitat proposed for designation in the county. There is proposed critical habitat listed for the West Indian manatee but it is not in this county.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to found at the release site. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The site is not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Taylor County, FL (FL-TAY-01, FL-TAY-02 and FL-TAY-03)

There are three release site locations in Taylor County.

These locations have been under managed pine plantations for over 20 years. The previous plantings were cultivated in beds using standard silvicultural practices. The existing pine plantations at these sites were harvested by the owner and prepared for re-planting. Additional site preparation involved herbicide application, plowing to remove stumps, and planting of trees in raised beds. Approximately 9.3 acres of field tests of some of the lines included in these permits requests were established across these sites under permit 08-039-102rm in 2008 and 2009. Areas surrounding these test sites consist of managed stands of pine plantations.

There are ten TES animals listed for Taylor County. There are no listed plants. The animals are: Eastern indigo snake (*Drymarchon corais couperi*), wood stork (*Mycteria americana*), piping plover (*Charadrius melodus*), red-cockaded woodpecker (*Picoides borealis*), West Indian manatee (*Trichechus manatus*), Gulf sturgeon (*Acipenser oxyrinchus desotoi*), green turtle (*Chelonia mydas*), leatherback turtle (*Dermochelys coriacea*), Kemp's ridley sea turtle (*Lepidochelys kempii*) and loggerhead sea turtle (*Caretta caretta*).

According to Caroline Stahaller, (contacted February 20, 2008) of the Panama City Field Office USFWS, the Eastern indigo snake (*Drymarchon couperi*) has not been seen anywhere on the Florida panhandle in 15-20 years. She had no concern that the action could affect the species. Although it is highly unlikely that the species would be found at the site, the applicant will provide all workers with identifying characteristics of the snake and instructions on what to do if the species is encountered. These measures are a variation of standard protective measures the USFWS uses when they have reached a “may affect” determination for construction sites. The birds (piping plover, red-cockaded woodpecker and wood stork) could all potentially visit the field test site but would not nest there. The piping plover nests along the coast which is seven or more miles away from the field test sites. The red-cockaded woodpecker nests in old growth longleaf pine. The wood stork nests in marshes, floodplain lakes, and swamps. The remaining species: West Indian manatee (*Trichechus manatus*), Gulf sturgeon (*Acipenser oxyrinchus desotoi*), green turtle (*Chelonia mydas*), leatherback turtle (*Dermochelys coriacea*), Kemp's ridley sea turtle (*Lepidochelys kempii*) and loggerhead sea turtle (*Caretta caretta*) are all aquatic species and would

not occur anywhere close to the field test site. The closest body of water is the Econfina river which is about 2.5 miles away from one of the test sites.

Critical Habitat: There is designated critical habitat listed for the piping plover in Taylor County. It is located along the coast in the southwest part of the county. It is approximately 15, 27 and 32 miles away from the three test sites. There is proposed critical habitat listed for the West Indian manatee but it does not occur in this county.

Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to be found at these release sites. If they were to enter the sites, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. The sites are not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Overall Conclusions

The field test sites in these permit applications have been in agricultural or forest research, or in agricultural production or forest tree plantations for from 5 to 50 years. No federally listed threatened or endangered species or species proposed for listing are likely to be found at any of the release sites. If they were to enter the site, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to habitat used by any listed species or species proposed for listing. The sites are not within or near designated critical habitat or habitat proposed for designation. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation. Because of this no effect determination, consultation and/or the concurrence of the USFWS and/or the NMFS are not required.

APPENDIX V: Supplemental Permit Conditions

For Release of *Eucalyptus grandis* x *Eucalyptus urop*hylla under permits 08-011-106rm and 08-014-101rm

(1) The test sites and adjacent land within 100 meters shall be monitored for any volunteer *Eucalyptus* plants every 6 months during the field test (as indicated in the permit) and for one year after completion of the field test, during which time any volunteer plants will be destroyed before they flower. During the monitoring period following completion of the field test, the site will not be planted with *Eucalyptus*, so that any volunteer seedlings that emerge can be easily identified. If volunteers or stump sprouts are still emerging at the end of the first year, a second year will be added to the monitoring period to ensure that no shoots are continuing to be produced.

(2) Please note that transportation of all test and plant materials to and from the field test location must be done in accordance with APHIS/USDA regulations outlined in "Container requirements for the movement of regulated articles", 7CFR 340.8(b) unless a shipping container variance has been approved by APHIS-BRS.

(3) BRS should be notified in writing of any proposed changes to the permit application (or approved permit) including for example confinement protocols, transgenic lines or constructs, release locations, acreage, etc. Changes usually require amendments to the permit and must be pre-approved by BRS. Requests should be directed to Regulatory Permit Specialist, USDA APHIS BRS, Biotechnology Permit Services, 4700 River Road, Unit 91, Riverdale, Maryland 20737.

(4) Any regulated article introduced not in compliance with the requirements of 7 CFR part 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. The responsible party may be subject to fines or penalties as authorized by the Plant Protection Act (7 U.S.C. 7701-7772).

(5) This Permit does not eliminate the permittee's legal responsibility to obtain all necessary Federal and State approvals, including for the use of: (1) any non-genetically engineered plant pests or pathogens as challenge inoculum; (2) plants, plant parts or seeds which are under existing Federal or State quarantine or restricted use; (3) experimental use of unregistered chemical; and (4) food or feed use of genetically engineered crops harvested from the field experiment.

(6) APHIS/BRS and/or an APHIS/PPQ personnel may conduct inspections of the test location, facilities, and/or records at any time.

(7) Harvested plant material may not be used for food or animal feed unless it is first devitalized and approved for such use by the U.S. Food and Drug Administration; and for plant-incorporated protectants, a tolerance for the pesticide must first be established by the U.S. Environmental Protection Agency.

(8) The permittee shall provide all workers with identifying characteristics of the threatened Eastern indigo snake and instructions on what to do if the species is encountered. This shall be done for release sites within the known range of the snake which include the following counties: Baldwin AL, Evans GA, Bay FL, Highlands FL, Marion FL, Gadsden FL, Columbia, FL and Taylor FL.

(9) Reporting an Unauthorized or Accidental Release
1. According to the regulation in 7 CFR 340.4(f)(10), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

- For immediate verbal notification, contact APHIS BRS Compliance Staff at (301) 734-5690 and ask to speak to a Compliance and Inspection staff member. Leave a verbal report on voicemail if the phone is not answered by a Compliance Officer.
- In addition, in the event of an emergency in which you need to speak immediately to APHIS personnel regarding the situation, you may call:

The APHIS/BRS Regional Biotechnologist assigned in the region where the field test occurs:
For Western Region, contact the Western Region Biotechnologist at (970) 494-7513
or e-mail: BRSWRBT@aphis.usda.gov
For Eastern Region, contact the Eastern Region Biotechnologist at (919) 855-7622 or e-mail: BRSERBT@aphis.usda.gov
Or
The APHIS State Plant Health Director for the state where the unauthorized release occurred. The list of APHIS State Plant Health Directors is available at:
http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml.
or http://pest.ceris.purdue.edu/stateselect.html

2. Written notification should be sent by one of the following means:
By e-mail:
BRSCompliance@aphis.usda.gov
By mail:
Biotechnology Regulatory Services (BRS)
Compliance and Inspection Branch
USDA/APHIS
4700 River Rd. Unit 91
Riverdale, MD 20737

3. Additional instructions for reporting compliance incidents may be found at
http://www.aphis.usda.gov/biotechnology/compliance_incident.shtml

(10) Reporting Unintended Effects:
According to the regulation in 7 CFR 340.4(f)(10)(ii), APHIS shall be notified in writing as soon as possible but within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the permit application or suffers any

unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

Written notification should be sent by one of the following means:
By e-mail:
BRSCompliance@aphis.usda.gov
By mail:
Biotechnology Regulatory Services (BRS)
Compliance and Inspection Branch
USDA/APHIS
4700 River Rd. Unit 147
Riverdale, MD 20737

(11) Reports and Notices:
Send notices and all reports (CBI and CBI-deleted or non-CBI copies) to BRS by e-mail, mail, or fax.
BRS E-mail:
BRSCompliance@aphis.usda.gov
BRS Mail:
Animal and Plant Health Inspection Service (APHIS)
Biotechnology Regulatory Services (BRS)
Compliance and Inspection Branch
4700 River Rd. Unit 91
Riverdale, MD 20737
BRS Fax:
Compliance and Inspection Branch
(301) 734-8669

a. Planting Report
Within 28 calendar days after planting, submit a report, in paper format or electronically, that includes the following information for each field test location:
i. Permit number;
ii. Regulated article;
iii. Release location [provide state, county, internal identification number (if available), and either a single GPS coordinate as a reference point (center of plot or specify corner) or specific address];
iv. List of all constructs and specific transformed lines (event) planted;
v. Total acreage of regulated article planted;
vi. Total acreage of any border rows planted;
vii. The actual planting date(s)
If multiple plantings occur that are separated in time by more than a month, then a planting report is required within 28 days of each planting.

b. Annual Report
Within 30 days after the anniversary date (one year increments from the effective date) an Annual Report must be submitted to APHIS. FAILURE TO SUBMIT ANNUAL REPORTS MAY RESULT IN REVOCATION OF THE PERMIT. The Annual Report shall reflect the current status

and observations to date for each location. It shall include the information submitted in the Planting Report, plus the following:
i. An accounting of the acreage or number of plants per line (event) for each construct that remain in the ground;
ii. A detailed map of the plantings;
iii. Total remaining acreage (include acreage of border rows if appropriate);
iv. The methods of observation;
v. The resulting data and analysis regarding all deleterious effects on plants, non-target organisms, or the environment. This should include, but not be limited to, data on insect damage, disease susceptibility, gross morphology and any indications of weediness;
vi. If any material was harvested, removed, or terminated or otherwise destroyed, a disposition table with the following information for each line (event) released should be provided:
date(s) of harvest, removal, and/or termination; a formal record of how the regulated material was removed from the environment; what material and how much was harvested or removed and where it was transported, stored and further processed up to the time it is or was to be taken to a contained facility; and what was done to devitalize residual and/or harvested material at the location.

In this report also provide data documenting which trees produced flowers and which if any produced viable seeds. Also document seedling volunteer monitoring, including any volunteers found and the method of devitalization.

c. Field Test Report
Within 6 months after the expiration date of the permit, the permittee is required to submit a Field Test Report.
NOTE: If a new application is approved to continue the field test past its scheduled expiration date, an annual report should continue to be submitted until the final expiration date, at which point the Field Test Report will be due after 6 months. Field Test Reports provide the final status and observations at each location and shall include:
i. List of all constructs and specific transformed lines (event) planted;
ii. Planting date(s), and harvest dates if any material was harvested;
iii. Total acreage of regulated article planted;
iv. Total acreage of any border rows planted;
vi. The methods of observation;
vii. The resulting data and analysis regarding all deleterious effects on plants, non-target organisms, or the environment. This should include, but not be limited to, data on insect damage, disease susceptibility, gross morphology and any indications of weediness.
viii. A disposition table with the following information:
Site name (or GPS), crop, harvest date(s), and disposition of harvested material. Date(s) of harvest, removal, and/or termination; a formal record of how the regulated material was removed from the environment; what material and how much was harvested or removed and where it was transported, stored and further processed up to the time it was taken to a contained facility; and what was done to devitalize residual and/or harvested material at the location.

We encourage the inclusion of other types of data if the applicant anticipates submission of a petition for determination of non-regulated status for their regulated article. APHIS considers these data reports as critical to our assessment of plant pest risk and development of regulatory policies

based on the best scientific evidence. Failure by an applicant to provide data reports in a timely manner for a field trial may result in the withholding of permission by APHIS for future field trials.

d. Flowering monitoring report
In the locations where there are other species of eucalyptus within 1000 meters of the test plots, if there is any overlap in flowering between the transgenic trial and the non-transgenic trees, this must be reported to APHIS.

e. Monitoring Report
The final monitoring report is due no later than 2 months from the end of the volunteer monitoring period.
The report must include:
i. Dates when the field location and perimeter fallow zone were inspected for volunteer plants;
ii. Number of volunteers observed;
iii. Any actions taken to remove or destroy volunteers.