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*

Revised Draft Environmental Assessment December 17, 2009

Biotechnology Regulatory Services

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I. Summary

USDA/APHIS has prepared a draft environmental assessment in response to permit applications (APHIS Number 08-011-106rm and 08-014-101rm) received from ArborGen LLC (ArborGen), to continue research on transgenic *Eucalyptus* trees currently permitted, to be issued permits to plant additional trees, and to be issued permits for the environmental release of genetically engineered (transgenic) *Eucalyptus* trees which will be allowed to flower. These plants are a clone[1] coded EH1 derived from a hybrid of *Eucalyptus grandis X Eucalyptus urophylla.* These have been genetically engineered with different constructs. The purpose of the environmental release is 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.

ArborGen previously applied for a permit (06-325-111r) requesting that *Eucalyptus* trees be allowed to flower in the field in Alabama. 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). The original permit allowed flowering on 1.1 acres. It was subsequently amended to allow flowering on 5.1 acres at the same location. In addition, the company was granted permit 08-151-101r to allow flowering on 1.4 acres at another site in Florida. As of the date of this EA, ArborGen had approximately 50 acres of the transgenic *Eucalyptus* hybrid planted and has permits that allow 7.6 acres of the trees to flower. ArborGen has now submitted two new permit applications for planting and growing trees on 29 sites and requesting that trees on 28 sites be allowed to flower; including the locations that have already been authorized for flowering. These two combined permits would allow flowering on up to 330 acres across all 28 locations. The size of each field test site ranges from 0.5 to 20 acres. All locations except one would be allowed to flower. 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 permits to allow the planting, field testing and flowering of a *Eucalyptus* hybrid clone engineered to express various genes. 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.

[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.

B. Purpose of this Environmental Assessment

The purpose of this EA is to assess any potential adverse environmental impacts of field research studies being conducted on 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, in order to continue research on *Eucalyptus* hybrid trees originally planted under various notifications, to plant additional trees under the permits, and to allow all the trees except the trees on one of the sites to flower. These are permit application numbers 08-011-106rm and 08-014-101rm.

This environmental assessment (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, issuance of a permit for field trials of regulated articles in categorically excluded from requirements for an environmental assessment. However, when APHIS determines that a confined field release of genetically engineered organisms has the potential to affect significantly the quality of the human environment, as those terms are defined in 40 CFR 1508.27 and 1508.14, an environmental assessment or environmental impact statement will be prepared, pursuant to 7 CFR § 372.5(d). This EA has been prepared because the permittee intends to allow the trees to grow under permit for a number of years and intends to let the trees reach maturity and flower. The actions described in the permit applications involve the release of transgenic *Eucalyptus grandis* x *Eucalyptus urophylla* into the environment. The release of flowering *Eucalyptus* on many sites and in a number of new States and locations needs to be evaluated by APHIS to determine the potential for environmental impacts from such releases. APHIS has prepared this EA because there is a concern that the increased number of locations and size of the releases that would be allowed to flower could potentially result in the release of pollen and/or seed; which would lead to a lack of confinement of the field tests and impacts to the environment. Other impacts such as hydrology, allelopathy and fire are also addressed.

C. Need for This Action

The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Biotechnology Regulatory Services' (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 genetically engineered (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 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 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

The field tests are taking place on land controlled by ArborGen or through contracts for field testing. The exact locations are claimed as CBI. Under the two permits, there are 29 sites where trees have been planted or will be planted, and on 28 sites the trees will be allowed to flower. See below for the States and Counties in which these 29 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 test sites listed in this permit 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.

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 will involve 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. There are existing transgenic *Eucalyptus* field trials of the same clone (EH1) at this location that were planted on approximately 7 acres of test plots under Notifications and Permits 06-325-111r, 08-039-102rm, 05-256-03n, 06-135-01n, 06-150-02n, 07-093-113n and, 07-159-103n. Trees at this location have been allowed to flower under permit 06-325-111r on approximately 6.2 acres. Up to 8,000 additional trees will 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.

Escambia County Alabama Site:

This location has been used as an intensely managed pasture for more than 5 years. The test site is currently planted with grasses suitable for cattle grazing. Site preparation has involved and will involve 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 approximately 30 year-old slash pine, and a re-forested area with less than 7 year-old mixed stands of pine and hardwood species. There are existing transgenic *Eucalyptus* field trials of the same clone (EH1) at this location that were planted on approximately 0.3 acres of test plot under Notification 07-159-103n and are now covered under permit 08-039-102r. Up to 8,000 additional trees will be transferred to this field test site and planted in field plots of up to 10 acres over the next three years.

Evans County Georgia Site:

This location has been a commercial nursery for forest seedling production for over 30 years. Site preparation has and will involve herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of nursery beds of forest tree seedlings, agricultural crops and mixed stands of hardwood and pine. A field trial of the same transgenic *Eucalyptus* clone (EH1) was planted on approximately 0.2 acres at this site under BRS permit # 08-039-102rm. An experimental test of non-transgenic *E.*

[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.

macarthurii is planted within 100 meters of the test plot location. Up to 4,000 additional trees will be transferred to this field test site and planted in field plots of up to 5 acres over the next three years.

Saint Landry's Parrish Louisiana Site:

This location has been used as an experimental agricultural farm for more than 25 years. The location has been used for conducting research experiments with soybean, cotton and wheat. Site preparation has and will involve herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of agricultural fields of rice, sugarcane and millet. There are existing transgenic *Eucalyptus* field trials of the same clone (EH1) at this location that were planted on approximately 1.7 acres of test plots under Notifications 07-145-102n and 07-159-103n; and these are now under Permit #08-039-102rm. Up to 8,000 additional trees will be transferred to this field test site and planted in field plots of up to 10 acres over the next three years.

Marshall County Mississippi Site:

This location has been an agricultural research station for more than 50 years. The location has been used for conducting research experiments with agricultural crops and grasses. The test site was used for experimental planting of grasses. Site preparation involved herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of agricultural fields, and less than 5 year-old pine plantations. There are existing transgenic *Eucalyptus* field trials of the same clone (EH1) at this location that were planted on approximately 0.5 acres of test plot under Notification 07-159-103n and these are now under permit 08-039-102rm. These plantings are under permit application 08-011-106rm. This trial is expected to continue until at least 2011 and would be allowed to flower under the submitted permit application. No additional plantings are anticipated at this site.

Pearl River County Mississippi Site:

This location has been an agricultural research station for more than 5 years. The location has been used for conducting research experiments with agricultural crops and grasses. The test site has been used for experimental planting of grasses. Site preparation has and will involve herbicide application to remove existing grasses, subsoiling, preparation for possible irrigation installation, and planting of trees in flat beds. The surrounding areas of the test site consist of a grape research farm, mixed stands of hardwoods and pine, and a residential area. There are existing transgenic *Eucalyptus* field trials of the same clone (EH1) at this location that were planted on approximately 2.0 acres of test plot under Notification 07-159-103n and these are now included under permit 08-039-102rm. Up to 4,000 additional trees will be transferred to this field test site and planted in field plots of up to 5 acres over the next three years.

Bamberg County South Carolina Site:

This location has been a managed forest plantation for more than 12 years. The location has been specifically used for short rotation planting of hardwoods and softwood trees for forestry research. The 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 surrounding areas of the test site consist of young pine plantations, mixed stands of hardwoods and pine, and agricultural fields. There are experimental test plots of non-transgenic cold-hardy *Eucalyptus* species (*E. macarthurii, E. benthamii, E. viminalis, E. badjensis*, and *E. dorrigoensis*) planted at least 1000 meters from the test plot location. There are existing transgenic *Eucalyptus* field trials of the same clone (EH1) at this location that were planted on approximately 3.3 acres of test plots under the Notifications 07-093-113n, 07-145-107n, 07-145-106n, 07-159-104n, 07-145-102n, 07-159-103n, and these are now under permit 08-039-102rm. Up to 4,000 additional trees will be transferred to this field test site and planted in field plots of up to 5 acres over the next three years.

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 5 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 5 years. The location has been specifically used for short rotation planting of cottonwood for forestry research. Site preparation has and will involve 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. There are existing transgenic *Eucalyptus* field trials of the same clone (EH1) at this location that were planted on approximately 0.5 acres of test plot under the Notifications 07-145-102n and 07-159-103n and are now under permit 08-039-102rm. These would be allowed to flower under the new permit application. No additional plantings are requested for this site.

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. The 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 test plots adjacent to the field test site include young mixed stands of hardwoods and pines. There are existing transgenic *Eucalyptus* field trials of the same clone (EH1) at this location that were planted on approximately 3.1 acres of test plots under Notifications 07-093-113n,

07-145-107n, 07-145-106n, 07-145-102n, and 07-159-103n and are now under permit 08-039-102rm. These plantings are under permit application 08-011-106rm. These would be allowed to flower under the new permit application. No additional plantings are requested for this site.

Marlboro County South Carolina Site:

This location has been a commercial nursery for forest seedling production for over 30 years. Site preparation has and will involve herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of field plantings of agricultural crops, nursery beds of forest tree seedlings and less than 30 years-old mixed hardwood and pine plantations. There are existing transgenic *Eucalyptus* field trials of the same clone (EH1) at this location that were planted on approximately 0.3 acres of test plot under Notification 07-159-103n and are now under permit 08-039-102rm. These would be allowed to flower under the new permit application. No additional plantings are requested for this site.

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 has been re-bedded by the owner for planting. Site preparation included herbicide application and sub-soiling. The surrounding areas of the test site consist of mixed hardwood stands and managed loblolly pine plantations. Up to 16,000 trees will be transferred to this field test site and planted in field test plots of up to 20 acres over three years.

Jasper County Texas Site 1:

This location has been under managed pine plantations 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 has involved herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of harvest age pine plantations. A field trial of approximately 0.7 acres of transgenic *Eucalyptus* was planted at this site under permit 08-039-102rm. Up to 16,000 trees will be transferred to this field test site and planted in field test plots of up to 20 acres over three years.

Jasper County Texas Site 2:

This location has been under managed pine plantations 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 has involved herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of less than 20 year-old managed hardwood and pine stands. Up to 8,000 trees will be transferred to this field test site and planted in field test plots of up to 10 acres over three years.

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 will 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 area has recently been harvested. Additional site preparation will include herbicide application, plowing to remove stumps, and planting of trees in raised or flat beds. The surrounding areas of the test site consist of managed loblolly pine plantations and mixed hardwood stands. Up to 16,000 trees will 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 harvested by the owner in 2005 and the site was prepared for replanting. The test site is within the areas replanted in 2006.
The surrounding areas of the test site consist of managed loblolly pine plantations and mixed hardwood stands. Up to 16,000 trees will 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 harvested by the owner in 2005. Site preparation 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 mixed hardwood stands. Up to 16,000 containerized trees will 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

area has recently been harvested. Site preparation will include herbicide application, plowing, and planting of trees
in raised or flat beds. The surrounding areas consist of a small cemetery on the property, an area with a few mobile homes to the east and managed loblolly pine plantations to the west, south and north. Up to 16,000 trees will 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 has been used as an intensely managed pasture for more than 5 years. The test site is currently planted with grasses suitable for cattle grazing. Site preparation has and will involve herbicide application to remove existing grasses, subsoiling, preparation for possible irrigation installation, and planting of trees in flat beds. The surrounding areas of the test site consist of agricultural crops and less than 25 years-old hardwoods and pine. There are existing transgenic *Eucalyptus* field trials of the same clone (EH1) at this location that were planted on approximately 1.0 acre of test plot under Notification 07-222-104n that are now covered under permit 08-039-102rm. Up to 4,000 trees will be transferred to this site and planted in field test plots of up to 5 acres over the next three years.

Columbia County Florida Site:

This location has been a 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. Up to 16,000 trees will 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 an agricultural research station for more than 10 years. The location has been used for conducting research experiments on agricultural crops. Site preparation will involve herbicide application, plowing, and planting of trees in flat beds. The surrounding areas of the test site consist of agricultural fields, plantings of horticultural crops and an experimental planting of *Eucalyptus*. 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. A field trial of approximately 0.2 acres of transgenic *Eucalyptus* clone EH1 was planted at this site under BRS permit # 08-039-102rm. Up to 8,000 trees will be transferred to this site and planted in field test plots of up to 10 acres over the next three years.

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 surrounding areas of 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 trees will 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:

This location has been used for hay or vegetable production for at least 5 years. Site preparation has and will involve herbicide application to remove existing vegetation, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of agricultural fields. There are existing transgenic *Eucalyptus* field trials of the same clone (EH1) at this location that were planted on approximately 2.5 acres of test plot under Notification 07-222-104n that are now under permit 08-039-102rm. Up to 8,000 trees will be transferred to this field site and planted in the field plots of up to 10 acres over the next three years.

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 was previously used for field trials of transgenic *Eucalyptus* which were terminated over a year ago and monitored to confirm that all previous transgenic material was destroyed. Site preparation has and will involve herbicide application, plowing, and planting of trees in flat beds. The surrounding areas of the test site consist of less than 5 year-old second-growth pine and hardwood with mixed grasses. The existing field trial of transgenic *Eucalyptus* clone EH1 at this location was planted on approximately 1.4 acres of test plot under Notification 07-145-102n and has been allowed to flower under permit 08-151-101r. It will be transferred to permit 08-014-101rm where it would be allowed to continue to flower. Up to 8,000 trees will 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 an agricultural research station for more than 5 years. The location has been used for conducting research experiments on agricultural crops. Site preparation has and will involve herbicide application, plowing, and planting of trees in flat beds. The surrounding areas of the test site consist of agricultural fields, plantings of horticultural crops and an experimental *Eucalyptus* test plot. An experimental test of non-transgenic *E. amplifolia* is planted approximately 200 meters from the proposed test plot location. An approximately 0.5 acre of test plot of transgenic *Eucalyptus* clone EH1 was planted under Notification 07-222-104n and is now under permit 08-039-102rm at this location. Up to 8,000 trees will be transferred to this site and planted in field test plots of up to 9.5 acres over the next three years.

Taylor County Florida Site 1:

This location has been a managed pine plantation for over 50 years and contains third rotation plantings of pine. The previous plantings were cultivated in beds using standard silvicultural practices. The existing pine plantation at the site was harvested by the owner prior to 2007 and prepared for re-planting. Additional site preparation will involve herbicide application, plowing to remove stumps, and planting of trees in raised beds. The surrounding areas of the test site consist of less than 25 year-old third rotation pine plantings. A field trial of approximately 1.5 acres of transgenic *Eucalyptus* clone EH1 was planted at this site under permit 08-039-102rm. Up to 16,000 trees will be transferred to this site and planted in field test plots of up to 20 acres over the next three years.

Taylor County Florida Site 2:

This location has been a managed pine plantation for over 50 years and contains third rotation plantings of pine. The previous plantings were cultivated in beds using standard silvicultural practices. The existing pine plantation at the site was harvested by the owner prior to 2007 and prepared for re-planting. Additional site preparation will involve herbicide application, plowing to remove stumps, and planting of trees in raised beds. The surrounding areas of the test site consist of less than 25 year-old third rotation pine plantings. A field trial on approximately 1.25 acres of transgenic *Eucalyptus* clone EH1 was planted at this site under BRS permit # 08-039-102rm. Up to 16,000 trees will be transferred to this site and planted in field test plots of up to 20 acres over the next three years.

Taylor County Florida Site 3:

This location has been a managed pine plantation for more than 20 years. Previous pine plantings were cultivated in beds using standard silvicultural practices. The existing pine plantation at this site was harvested by the owner in 2008 and prepared for re-planting. Additional site preparation will involve herbicide application, plowing and planting of trees in raised beds. The test site is within the larger area harvested by the owner and is surrounded by managed stands of pine plantations. Up to 16,000 trees will be transferred to this site and planted in field test plots of up to 20 acres over the next three years.

IV. Alternatives

This EA analyzes the potential environmental consequences of a proposal to issue two permits to allow existing transgenic *Eucalyptus* trees to flower and to plant additional trees that would also be allowed to flower in 28 locations in the southeast U.S. and to plant the trees on a 29th site where trees will not be allowed to flower. Two alternatives are considered in this EA: (1) deny the permit and (2) to issue the permit.

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 Environmental Assessment, the No Action alternative would be the denial of permit applications 08-011-106rm and 08-014-101rm.

Transgenic *Eucalyptus* trees have been previously approved for planting under various Notifications, and permits. Two field tests have been allowed to flower under permits 06-325-111r and 08-151-101r. An EA was prepared for the field test under 06-325-111r. The applicant has now submitted two new permit applications to add additional field sites where more trees will be planted and allowed to flower. Under the No Action Alternative, if these permits are denied, the transgenic *Eucalyptus* plants currently released will 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. Denying the permits would not allow the applicant 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.

B. Preferred Alternative – Issue the Permit

The APHIS-preferred alternative is to issue the permits with supplemental permit conditions for the requested three-year period. The permits will need to be renewed to allow the transgenic plants to remain in the ground beyond this time period. Under this alternative, APHIS would issue the permit to allow the research to proceed at the field test sites in the different States and Counties, with supplemental permit conditions based on APHIS scientific analysis of the permit application, input from the States, and public comment from this environmental assessment. If warranted, based on the environmental risk of escape of the engineered organism, APHIS will require further mitigating measures and monitoring to prevent spread of the organism outside the field production area. If the permit is issued, the trees would remain in the field and be allowed to 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 would allow the safe development and use of genetically engineered (GE) organisms under the mission of BRS.

APHIS proposes to include the following requirements to allow the applicant to conduct a confined field release and to ensure no significant harm to the environment. These are also the current requirements for those permits where trees have already been allowed to flower.

a. Trees in the field test sites will be monitored for flowering and seed formation. Data will be provided to APHIS in an annual report documenting which trees produced flowers and which, if any, produced viable seeds.

b. The field test sites will be monitored for volunteer seedlings. Any volunteers found will be sprayed with herbicide or physically removed. The presence and elimination of any volunteers will be reported to APHIS in an annual report.

c. All non-engineered control *Eucalyptus* trees in the field test plot and any plant material removed from the field site will be treated as regulated articles, i.e., with respect to monitoring for flowering and volunteers, maintaining the identity while in use, and devitalization when no longer in use.

V. Environmental Consequences

A. No Action

Under the no action alternative, the applicant would not be allowed to plant additional acres and let the trees on 28 sites produce flowers. Since preventing flower formation would prove impossible over time, it would mean that the trees will have to be cut down prior to maturity. The *Eucalyptus* trees currently planted at the field test sites would remain in the ground but would be cut down within 2 or 3 years since they would not be allowed to flower. At each of the sites the locations could remain planted in short rotation *Eucalyptus* field tests, or the sites would be returned to forest tree production or agriculture/forestry research. Trees would be cut down and replanted with the same or different trees. 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 the trees produce flowers and would continue to plant more trees at the release sites. This means that the trees would remain in the ground for at least 3 years and most likely longer since the applicant plans to renew the permits. 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

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.

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. However, 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 extent to which the hybrid between *E. grandis* and *E. urophylla* is invasive has not been assessed.

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.

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

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). The prescribed periodic monitoring of the field plots in the permit 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

<u>Gene used as selectable marker</u>

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). Consequently, APHIS has determined the presence of this gene will have no significant environmental impacts.

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. Therefore APHIS has determined the presence of this gene will have no significant environmental impacts.

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). APHIS has therefore determined the presence of this gene will have no significant environmental impacts.

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.

[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.

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 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 the previous EA and response to comments for permit 06-325-111r (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf) and is herein incorporated by reference. Information on the invasiveness of *E. grandis*, received since the publication of the original draft EA on June 3, 2009, has also been included in the Biology section above.

As indicated above, the hybrid *Eucalyptus* EH1 used to produce the transgenic trees has not been shown to be weedy or invasive in the U.S., 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.

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. 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. 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.

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 conditions of the permits will require that all volunteers be reported, found and destroyed to prevent any spread of trees from the field release site.

[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.

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, these seeds would be expected to germinate within a short period. The bordering fields within 100 meters from the edge of the trials will be monitored every six months for germinating seedlings. This distance is twice the 50 meter distance that seeds would be expected to be dispersed from a tree at harvest age. If transgenic seedlings are observed they will be destroyed either by uprooting or by spraying with 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 et al. (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 routes of gene flow outside of the field test. 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, approved under BRS notifications and permits, of the same hybrid *Eucalyptus* variety EH1 are planted adjacent to or at the proposed field test plot locations at the sites in Berkeley, Charleston, Marlboro Counties in South Carolina; Escambia and Baldwin Counties in Alabama; Saint Landry's Parish, Louisiana; Pearl River and Marshall Counties in Mississippi; Jasper County (Site 1) in Texas; and Bay, Glades, Highlands, Marion, Taylor (Site 2) and Gadsden (Site 1) Counties in Florida. The applicant is not aware of any commercial plantings of compatible *Eucalyptus* species within 1000 meters of the test plot location at these sites. Observations by the applicant from self pollination experiments in Brazil showed that the hybrid genotype used in these trials is unable to produce any viable seed from transgenic or non-transgenic trees. However, in the two field trials in Alabama and Florida allowed to flower, the hybrids are producing low numbers of viable seeds, but no volunteer seedlings have occurred in the field trials to date. Any seed production as a result of crossing between trees will result

from trees (including non-transgenic controls) crossing with each other within the test site. There are no other commercial plantings of compatible *Eucalyptus* species within 1000 meters of these test plot locations. So the likelihood of gene flow from the new field tests is virtually nil.

At all test sites in Hardin, Jasper (except Site 1), Jefferson and Newton counties in Texas; Taylor (except Site 2), Columbia and Gadsden (except Site 1) Counties in Florida, there are no 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 test plot location at these sites.

In Bamberg County South Carolina, there are experimental test plots of non-transgenic cold-hardy *Eucalyptus* species (*E. macarthurii*, *E. benthamii*, *E. viminalis*, *E. badjensis*, and *E. dorrigoensis*) planted within 1000 meters of the test plot location. As noted below, these cold hardy *Eucalyptus* species are highly unlikely to be compatible with the hybrid *Eucalyptus* variety because of distant phylogenetic relationship and asynchronous flowering. 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 found these will be reported to APHIS.
In the event that pollen from the non transgenic *Eucalyptus* trees fertilizes the transgenic trees, seed dispersal, if any, is expected within 100 m from the test plot. Should any hybridization and viable seed production occur, the monitoring for and removal of volunteers within 100 m from the edge of transgenic test plot would eliminate any offspring produced.

In Evans County Georgia, there are no existing transgenic field trials at this site. There are no commercial plantings of compatible *Eucalyptus* species within 1000 meters of the proposed test plot location at this site. An experimental test of non-transgenic *E. macarthurii* is planted within 100 meters of the test plot location. As indicated below, this cold-hardy *Eucalyptus* species is unlikely to be compatible with the hybrid *Eucalyptus* variety EH1 because of distant phylogenetic relationship and asynchronous flowering. 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 event that pollen from the non transgenic *Eucalyptus* trees fertilizes the transgenic trees, seed dispersal, if any, is expected within 100 m from the test plot. Should any hybridization and viable seed production occur, the monitoring for and removal of volunteers within 100 m from the edge of transgenic test plot would eliminate any offspring produced.

At the Marion County Florida site, an experimental test of non-transgenic *E. amplifolia* is planted approximately 200 meters from the proposed test plot location. This *Eucalyptus* species is also unlikely to be compatible with the hybrid *Eucalyptus* variety EH1 because of its distant phylogenetic relationship and asynchronous flowering. As noted above, pollen dispersal is generally limited to 100 m with very low levels beyond that distance. All trees to be planted in this trial have been shown in a previous trial to exhibit the reduced fertility trait. Results from these studies have shown that the engineered trait is

highly effective in preventing the formation of mature pollen. As such they are not expected to fertilize the non transgenic *E. amplifolia* trees. Observations will be made on developing flowers in the test to verify that these trees do have reduced fertility. 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. 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 pollen from the non transgenic *E. amplifolia* trees could fertilize the transgenic trees, seed dispersal, if any, is expected within 100 m from the test plot. Should any hybridization and viable seed production occur, the monitoring for and removal of volunteers within 100m from the edge of transgenic test plot would eliminate any offspring produced.

At the Gadsden County Florida Site 1, 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.

The hybrid genotype used in these studies for transformation may be compatible with *E. grandis* trees grown in Florida. However, *E. grandis* produces mature flowers in the fall whereas the hybrid genotype used in these tests produces mature flowers in mid to late summer. Because of asynchronous flowering, hybridization of transgenic trees with the more common *Eucalyptus* species grown in Florida 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 States where these field test occur. 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.

The transgenic hybrids are not likely to be sexually compatible with any of the cold hardy species listed above. It is well documented that natural cross-compatibility between different species of eucalypts is limited to closely related series within distinct sections of the *Eucalyptus* genus. 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

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. This regrowth will be managed in this field test at termination by devitalizing any sprouts that form from the stumps of harvested trees using 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.

As indicated above 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. 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 to the engineered plants. There are multiple mechanisms in place that would prevent these *Eucalyptus* 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 (see details above and in Appendix 1). Monitoring of seed set and seedling viability will be required during the 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 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.

Impact on Existing Agricultural Practices

The establishment and growth of these small field tests will not have any significant impact on existing agricultural practices because they are solely for research purposes. Current practices will essentially remain the same. The field sites that are being proposed under this permit have been used as forest tree plantations, as 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, Glades, Highlands, Marion, and Taylor counties, Florida. The sites are a mixture of pasture, crop lands and forested areas. If viable seed are formed, these areas

are unsuitable for the establishment of the *Eucalyptus* hybrid clone in this permit. *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 plantations will be cultivated and weeds controlled by herbicides. All sites are intensively managed. The inhospitable conditions for seed germination, in combination with the confinement conditions imposed by the permittee and APHIS, will successfully limit the establishment of any of these species in the surrounding area. Therefore APHIS concludes there would be no significant effect on any native floral species.

Terrestrial animals

The most likely animals to encounter the transgenic *Eucalyptus* trees in this field experiment 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 V - Risk of the Gene products on the Environment). Therefore APHIS concludes there would be no significant effect on any native vertebrate or invertebrate animal species.

Aquatic organisms

Eucalyptus normally grows in areas of bare mineral soils and would not be expected to establish in aquatic or riparian environments away from the field trial. Furthermore, as stated above, there is no expectation of toxicological effects on any organism due to the ingestion of the transgenic plant material in this study. For potential impacts due to *Eucalyptus* effects on hydrology, see section D. APHIS concludes there would be no significant effect on any aquatic species.

Potential Impacts by Fire

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 build up 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 always a risk of forest fire in the southeastern U.S., however, the probability that these field tests 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 highly managed to prevent litter buildup. They are also physically isolated from nearby plantations. If they were to catch fire, the fires should be readily contained.

Potential Impacts to Human Health

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. C. neoformans gattii* is a fungal pathogen that is hosted on a variety of species of Eucalyptus as well as other tree species. It causes systemic fungal infections in humans, leading to fungal meningitis and death. *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. *C. neoformans* infections are found particularly in AIDS patients due to their weak immune systems. 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. The disease was previously only known to occur in tropical or semi-tropical climates. 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)

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 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 and the tissue culture 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 or animal 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 or animal health.

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

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. HGT and expression of DNA from these 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://vm.cfsan.fda.gov/~dms/opa-armg.html). Therefore APHIS concludes that horizontal gene transfer poses no significant 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 from 5 to 50 years. Therefore the land has been in continuous agricultural or forest tree production for years prior to these releases and it is reasonably foreseeable that if the permit were not issued that the sites would continue to be under 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 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 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 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. 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. 2005). 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. 1996). 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. 1997).

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. 2005) 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. 2004, Couch and McDowell 2006).

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

[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.

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*

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

[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.

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 conditions of the permit; including any indications of allelopathic effects.

E. Risks to Threatened and Endangered Species

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 in the action area. Consequently, consultation under Section 7 of the Endangered Species Act 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 area of the field release.

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. The hybrid species of *Eucalyptus* being grown is not considered an invasive species and does not establish itself without human intervention (as described above). 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 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 impact on migratory birds since they would not be expected to inhabit these sorts of field tests.

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 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

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APPENDIX I: Status of Existing Field Tests Allowed to Flower

The applicant has been allowed to let transgenic *Eucalyptus* trees flower under permits 06-325-111r-a2 and 08-151-101r. These trees had the same constructs as those in these two permit applications. All transgenic and non-transgenic trees in these approved tests spanning 5.1 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 viable pollen. The results to date have shown that the barnase gene that has been engineered into these trees is effective at preventing viable 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.

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.

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

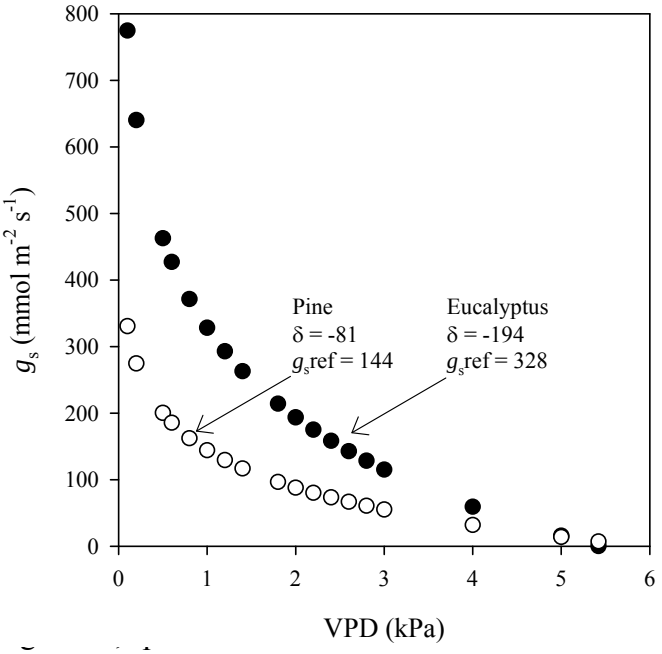

Figure 1: Stomatal conductance response to VPD for pine and *Eucalyptus* (Oren and others 1999)

ill increase

ore sensitive g_s response to VPD than those in more mesic environments (McDowell and others 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 (2005) 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 and others 2004; Couch and McDowell 2006).

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 and others 2007). Mean water table depths typically range 3–8 m (Ford and others 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 and others 1996). The average age of groundwater in the UFA is ~20 years (Happella and others 2006) and groundwater is regularly recharged by surface water in this region (Opsahl and others 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 and others 1997).

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 and others 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.

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APPENDIX IV: Threatened and Endangered Species Analysis

The following analysis was conducted using the following resources:
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
Regional Species lists for FWS offices in Alabama, Georgia, Louisiana, Mississippi, South Carolina, Texas and Florida.
Telephone contact with Field Office personnel with USFWS.

1. 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. Site preparation will involve 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.

Seventeen 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*), Eastern indigo snake (Drymarchon corais couperi), red-cockaded woodpecker (*Picoides borealis*), 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*), inflated heelsplitter mussel (*Potamilus inflatus*), and reticulated flatwoods salamander (*Ambystoma bishopi*). 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 critical habitat listed in the action area.

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 red-cockaded woodpecker could potentially visit the field tests but this species prefers mature pine stands as habitat for nesting. The wood stork primarily inhabits wetland systems notably cypress or mangrove swamps and would not use the field test site. 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 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 approximately 65+ 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. 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 there is any concern. 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 deermouse and the piping plover) is about 65 miles away. The gulf sturgeon has proposed 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.

2. Escambia County, AL (AL-ESC-01)

This location has been used as an intensely managed pasture for more than 5 years. The test site is currently planted with grasses suitable for cattle grazing. Site preparation will involve 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 ~ 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.

3. Evans County, GA (GA-EVA-01)

This location has been a commercial nursery for forest seedling production for over 30 years. Site preparation will involve herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of nursery beds of forest tree seedlings, 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: Eastern Indigo Snake (*Drymarchon 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 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. 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.

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

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

There is one TES animal listed for St. Landry's Parish; the pallid sturgeon (*Scaphirhynchus albus*). 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.

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

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.

5. Marshall County, MS (MS-MAR-01)

This location has been an agricultural research station for more than 50 years. The location has been used for conducting research experiments with agricultural crops and grasses. The test site has been used for experimental planting of grasses. Site preparation will involve herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of 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.

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

This location has been an agricultural research station for more than 5 years. The location has been used for conducting research experiments with agricultural crops and grasses. The test site has been used for experimental planting of grasses. Site preparation will involve herbicide application to remove existing grasses, subsoiling, preparation for possible irrigation installation, and planting of trees in flat beds. The surrounding areas of 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. There is no critical habitat listed for this county. 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*). There is critical habitat for the gulf sturgeon and proposed critical habitat for the Louisiana black bear in Mississippi.

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 critical habitat proposed for the Louisiana black bear is an area lying south of Washington County and west of the main channel of the Mississippi River. The proposed areas are the Tensas River Basin, Atchafalaya River Floodway, Lower Iberia and St. Mary Parishes all of which are well over a hundred miles away. The critical habitat for the gulf Sturgeon occurs 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.

7. Bamberg County, SC (SC-BAM-01)

This location has been a managed forest plantation for more than 12 years. The location has been specifically used for short rotation planting of hardwoods and softwood trees for forestry research. The 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 surrounding areas of the test site consist of 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.

8 & 9. 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 5 years. The release site is located adjacent to greenhouse facilities and is surrounded by hardwoods and pine plantations. The trees will only be held here on a temporary basis until planted in the field. The other location has been under managed forest plantation for more than 5 years. The location has been specifically used for short rotation planting of cottonwood for forestry research. Site preparation will involve 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.

Six 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*), wood stork (*Mycteria americana*), and loggerhead sea turtle (*Caretta caretta*). 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), and loggerhead sea turtle (*Caretta caretta*) occur 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 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. There is proposed critical habitat listed for the West Indian manatee in South Carolina 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.

10. 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. The 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 test plots adjacent to the field test site include young mixed stands of hardwoods and pines.

Eleven 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*), Bachman's warbler (*Vermivora bachmanii*), red-cockaded woodpecker (*Picoides borealis*), piping plover (*Charadrius melodus*), and flatwoods salamander (*Ambystoma cingulatum*). 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, and West Indian manatee are all aquatic animals and would not be affected by the field test. The birds (Bachman's warbler, 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. Bachman's warbler nests in bushes, blackberry vines, or canes, on swamp palmetto leaf, in densely vegetated watery swamps. The 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: 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 about 43 miles away from the Berkeley county sites and about 57 miles away from the Charleston County site. The proposed critical habitat listed for the West Indian manatee 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 adversely modify designated critical habitat or habitat proposed for designation.

11. Marlboro County, SC

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

There are two TES animals listed for Marlboro County; the red-cockaded woodpecker (*Picoides borealis*) and the shortnose sturgeon (*Scaphirhynchus albus*). 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 (*Acipenser brevirostrum*) 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.

12. 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 has been re-bedded by the owner for planting. Site preparation included herbicide application and sub-soiling. The surrounding areas of 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 animals 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.

13 & 14. Jasper County, TX (TX-JAS-01 and TX-JAS-02)

There are two locations in Jasper County Texas. One location has been under managed pine plantations 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 will involve herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of harvest age pine plantations.

The other location has been under managed pine plantations 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 will involve herbicide application, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of less than 20 years-old managed hardwood and pine stands.

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 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.

15. 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.

16-19. 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 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.

20. Bay County, FL (FL-BAY-01)

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

Sixteen TES animals and 7 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 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*), paper-like whitlow-wort (*Paronychia chartacea*), violet-flowered 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 seven 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.

21. 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 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.

22 & 23. Gadsden County, FL (FL-GAD-01, FL-GAD-02)

There are two planting sites at this location. One location 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 will be used for site preparation, including herbicide application, plowing and planting of trees in raised or flat beds. The surrounding areas of the test site consist of mixed pine-hardwood forests and pine plantations, as well as research plantings of agricultural and horticultural crops.

This other location has also 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 surrounding areas of 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 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 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.

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.

24. Glades County, FL (FL-GLA-01)

This location has been used for hay or vegetable production for at least 5 years. Site preparation will involve herbicide application to remove existing vegetation, subsoiling and planting of trees in flat beds. The surrounding areas of the test site consist of agricultural fields.

Eleven TES animals and two TES plants are listed for Glades County. The animals are: Eastern indigo snake (*Drymarchon couperi*), American crocodile (*Crocodylus acutus*), wood stork (*Mycteria Americana*), snail kite (*Rostrhamus sociabilis plumbeus*), crested caracara (*Caracara cheriway*), Florida scrub-jay (*Aphelocoma coerulescens*), Florida grasshopper sparrow (*Ammodramus savannarum floridanus*), West Indian manatee (*Trichechus manatus*), Florida panther (*Puma concolor coryi*), Ivory-billed woodpecker (*Campephilus principalis*), and Red-cockaded woodpecker (*Picoides borealis*). The plants are Carter's mustard (*Warea carteri*) and Okeechobee gourd (*Cucurbita okeechobeensis ssp. Okeechobeensis*).

The snail kite (*Rostrhamus sociabilis plumbeus*) prefers large, open freshwater marshes and lakes with shallow open waters. 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 even rather wooded areas having associated limited areas of open grassland. The center of its 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 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. 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 six 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. Day-use sites typically are dense patches of saw palmetto surrounded by swamp, pine flatwoods, or hammock. It would shy away from areas with a lot of human activity. The site is surrounded by large areas of land under agricultural production. The American crocodile (*Crocodylus acutus*) inhabits coastal mangrove swamps, brackish and salt water bays, lagoons, marshes, tidal rivers, brackish creeks, abandoned coastal canals and borrow pits. It occupies mostly nonsaline waters in nonbreeding season, moving to saline waters when breeding. The field test site is miles away from any area that the crocodile might inhabit. The same would be true for the West Indian manatee (*Trichechus manatus*) which inhabits an aquatic environment. For the Eastern indigo snake (*Drymarchon couperi*) – According to Brad Rick, (contacted February 27, 2008) of the Vero Beach Field Office USFWS, the species is quite rare, but could be found anywhere. He agreed that the historic and continuous use of the release site and the surrounding

area for hay and vegetable production makes it extremely unlikely that the species would be found in the area, and the appropriate determination would be "no effect". The change from hay and vegetable production to a tree planting could actually benefit the Eastern indigo snake because the current habitat is unsuitable. Given the unlikelihood of the species being in the surrounding area, USFWS does not see a need to consult on this potentially beneficial 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.

For the plants, 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. The Okeechobee gourd (*Cucurbita okeechobeensis ssp. Okeechobeensis*) was originally found in swampy forests and hammocks on muck soils. Today, this species is restricted to disturbed areas that are not cultivated, such as ditch banks and wet road shoulders. It currently persists at a few sites on the shore of Lake Okeechobee in south Florida. It has also been collected in Glades County on an island in Lake Okeechobee and in Broward and Dade counties where it was apparently ephemeral. According to the recovery plans (http://www.fws.gov/verobeach/images/pdfLibrary/waca.PDF, (http://www.fws.gov/verobeach/images/pdflibrary/cuok.PDF) for both of these species, neither is found in the area of the field release.

Critical Habitat: The American crocodile has designated critical habitat but this occurs in the lower parts of Dade County and the Florida keys. There is no critical habitat for this species in this county. There is proposed critical habitat listed for the West Indian manatee but it does not occur in this county. The snail kite has critical habitat in Glades and Hendry Counties, along the western shore of Like Okeechobee which is at least 14 miles from the field test site.

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 adverse 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.

25. 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 was previously used for field trials of transgenic *Eucalyptus* which were terminated more than 12 months ago and monitored to confirm that all previous transgenic material was destroyed. Site preparation will involve herbicide application, plowing, and planting of trees in flat beds. The surrounding areas of the test site consist of less than 5 year-old second-growth pine and hardwood with mixed grasses.

Fourteen 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), 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*), Florida Panther (*Puma concolor coryi*), West Indian manatee (*Trichechus manatus*), Eastern Indigo Snake (*Drymarchon couperi*), Bluetail Mole Skink (*Eumeces egregius lividus*) and Sand Skink (*Neoseps reynoldsi*).

The plants are: Large-flowered Bonamia (*Bonamia grandiflora*), Pygmy Fringetree (*Chionanthus pygmaeus*), Pigeon Wings (*Clitoria fragrans*), Short-leaved Rosemary (*Conradina brevifolia*), Avon Park Rabbit-bells (*Crotalaria avonensis*), Yellow Scrub Balm (*Dicerandra christmanii*), Scrub Mint (*Dicerandra frutescens*), Scrub Buckwheat (*Eriogonum longifolium var. gnaphalifolium*), Wedge-leaved Button-snakeroot (*Eryngium cuneifolium*), Highlands Scrub St. John's-wort (*Hypericum cumulicola*), Florida Gayfeather (*Liatris ohlingerae*), Britton's Bear-grass (*Nolina brittoniana*), Paper-like Whitlow-wort (*Paronychia chartacea ssp. Chartacea*), Lewton's Polygala (*Polygala lewtonii*), Wireweed (*Polygonella basiramia*), Small's Jointweed (*Polygonella myriophylla*), Scrub Plum (*Prunus geniculata*), Carter's Mustard (*Warea carteri*), and Scrub Ziziphus (*Ziziphus celata*).

The lichen is: Perforate Reindeer Lichen (*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 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 large-flowered 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. Park Rabbit-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. Yellow Scrub Balm (*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. Wedge-leaved Button-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 St. John's-wort (*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 the rare wedge-leaved button snakeroot (*Eryngium cuneifolium*). Florida Gayfeather (*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. Paper-like 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*). Small's Jointweed (*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. Scrub 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 and the Everglade snail kite but none of the proposed habitat occurs in this county. The whooping crane has designated critical habitat but it does not occur in this county. The 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.

26. Marion County, FL (FL-MAR-01)

This location has been an agricultural research station for more than 5 years. The location has been used for conducting research experiments on agricultural crops. Site preparation will involve herbicide application, plowing, and planting of trees in flat beds. The surrounding areas of the test site consist of agricultural fields, plantings of horticultural crops and an experimental *Eucalyptus* test plot.

Nine TES animals and five TES plants are listed for Marion County. The animals are: sand skink (*Neoseps reynoldsi*), Eastern indigo snake (*Drymarchon couperi*), American alligator (*Alligator mississippiensis*), wood stork (*Mycteria Americana*), snail kite (*Rostrhamus sociabilis plumbeus*), red-cockaded (*Woodpecker Picoides borealis*), Florida scrub-jay (*Aphelocoma coerulescens*), West Indian manatee (*Trichechus manatus*), hoary bat (*Lasiurus cinereus*). The plants are: large-flowered bonamia (*Bonamia grandiflora*), longspurred mint (*Dicerandra cornutissima*), scrub buckwheat (*Eriogonum longifolium var. gnaphalifolium*), Britton's bear-grass (*Nolina brittoniana*), Lewton's polygala (*Polygala lewtonii*).

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 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. The hoary bat prefers deciduous and coniferous forests and woodlands. It roosts usually in tree foliage 3-5 m above ground, with dense foliage above and open flying room below, often at the edge of a clearing and commonly in hedgerow trees. It sometimes roosts in rock crevices and rarely uses caves in most of its range. It would be unlikely to find the release site hospitable habitat since there are no trees in the plot itself. Once the trees are established and growing, if the trees were to reach maturity, it would not likely find the trees suitable habitat since they do not have dense foliage and the plot is in the middle of an agricultural research area, not in a deciduous or coniferous forest. For the Eastern indigo snake (*Drymarchon 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 large-flowered 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. 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 sandhill. *Nolina* is entirely dependent on fire or some other mechanism to maintain an open successional stage in the scrub or sandhill. 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 six 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 does not occur 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.

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

There are three release site locations in Taylor County.

Two of the locations have been under managed pine plantations for over 50 years and contain third rotation plantings of pine. The previous plantings were cultivated in beds using standard silvicultural practices. The existing pine plantation at this site was harvested by the owner prior to 2007 and prepared for re-planting. Additional site preparation will involve herbicide application, plowing to remove stumps, and planting of trees in raised beds. The surrounding areas of the test site consist of less than 25 year-old third rotation pine plantings.

The third location has been under managed pine plantation for more than 20 years. Previous pine plantings were cultivated in beds using standard silvicultural practices. The existing pine plantation at this site was harvested by the owner in 2008 and prepared for re-planting. Additional site preparation will involve herbicide application, plowing and planting of trees in raised beds. The test site is within the larger area harvested by the owner and is surrounded by managed stands of pine plantations.

There are eleven TES animals listed for Taylor County. There are no listed plants. The animals are: Eastern indigo snake (*Drymarchon couperi*), American alligator (*Alligator mississippiensis*), 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*).

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. 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 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.

Overall Conclusions

The field test sites in this permit application 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.

APPENDIX V: Proposed 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, Glades FL, 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.