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Animal and
Plant Health
Inspection
Service

Permit application 11-052-101rm received from ArborGen

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

Draft Environmental Assessment December 6, 2011

Biotechnology Regulatory Services

Agency Contact

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

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I. Summary

United States Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS) has prepared an environmental assessment (EA) in response to a confined environmental release and movement permit application (APHIS Number 11-052-101rm) received from ArborGen Inc. (ArborGen) to allow the interstate movement, planting and flowering of genetically engineered (transgenic) *Eucalyptus* trees at six confined field site locations in Alabama, Florida, Mississippi, and South Carolina. A total of 14.7 acres (all 6 sites combined) is being requested by the applicant. These plants are a clone[1] coded EH1 derived from a hybrid of *Eucalyptus grandis X Eucalyptus urophylla* and have been genetically engineered with different constructs than the trees previously permitted for environmental release by APHIS. The purpose of the confined environmental release is for ArborGen to assess the effectiveness of introduced gene constructs which are intended to confer cold tolerance; to test the efficacy of genes to alter lignin biosynthesis; to test the efficacy of genes to alter growth; and to test the efficacy of genes designed to alter flowering. In addition the trees have been engineered with a selectable marker gene which confers resistance to the antibiotic kanamycin. ArborGen has also requested under this permit application that all genetically engineered *Eucalyptus* trees authorized under permits 09-070-101rm and 11-041-101rm be incorporated into this new permit and allowed to flower. Trees will also be moved interstate under this permit from ArborGen's nursery and greenhouse facilities in South Carolina to the field test sites identified in the permit application for planting.

In 2010, APHIS completed and EA and FONSI for a permit application from ArborGen to authorize the planting, field testing and flowering of a GE *Eucalyptus* hybrid clone engineered to express various genes on 28 confined field site locations in the southeastern United States, including Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Texas (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf) and (http://www.aphis.usda.gov/brs/aphisdocs/08_014101r_ea.pdf). Pertinent and current information available in the 2010 EA and FONSI has been incorporated by reference into this EA.

APHIS has issued ArborGen permits authorizing the environmental release of transgenic *Eucalyptus* trees, some of which allowed flowering and some of which have not. ArborGen has grown *Eucalyptus* under permits 06-325-111r, 08-011-106rm, 08-014-101rm, 08-039-102rm, 08-151-101r, 09-070-101rm and 11-201-103r. For permits 06-325-111r, 08-011-106rm, 08-014-101rm, 08-151-101r and 11-201-103r the trees have been allowed to flower. Permits 06-325-111r, 08-011-106rm, 08-014-101rm were issued by APHIS upon completion of an EA and subsequent FONSI (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf) and (http://www.aphis.usda.gov/brs/aphisdocs/08_014101r_ea.pdf). Permit 06-325-111r expired on June 27, 2010 and was renewed under 10-112-101r. Permit 08-039-102r expired on April 25, 2011 and was renewed under 11-041-101rm. Permit 08-011-106rm is active and will expire on May 12, 2013. Permit 08-014-101rm is active and will expire

[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.

on May 12, 2013. Permit 08-151-101r expired June 30, 2011and was incorporated into 08-014-101rm. Permit 09-070-101rm is active and will expire on May 11, 2012. Permit 11-201-103r is active and will expire on July 28, 2014.

There are currently six active permits under which ArborGen is authorized by APHIS to grow GE *Eucalyptus* which include 32 unique locations within 7 States: 08-011-106rm, 08-014-101rm, 09-070-101rm, 10-112-101r, 11-041-101rm and 11-201-103r. No plantings at any of the 32 locations are authorized by APHIS to exceed 20 acres in size. Trees are allowed to flower under four permits: 08-011-106rm, 08-014-101rm, 10-112-101r and 11-201-103r (two of the locations in Berkeley and Dorchester Counties, SC trees are not authorized to flower). Under permits 09-070-101rm and 11-041-101rm trees are not authorized to flower. As of September 2011, ArborGen has reported to APHIS that they are growing a total of approximately 67 acres of trees on 18 of the 32 permitted locations.

ArborGen has submitted a new permit application 11-052-101rm to APHIS requesting approval for environmental release of hybrid *Eucalyptus* trees at 6 locations in the States of Alabama, Florida, Mississippi, and South Carolina encompassing a total of 14.7 acres (the subject of this EA). Five of these locations currently have active APHIS permits (08-011-106rm, 08-014-101rm, 09-070-10rm, 10-112-101r, and 11-041-101rm) for environmental release of GE *Eucalyptus* hybrids in Alabama, Florida, Mississippi, and South Carolina. The sixth site in South Carolina has been listed as a holding site for transgenic trees in previous APHIS permits and notifications and is a new location for the release of GE *Eucalyptus*. ArborGen is requesting that trees be allowed to flower at four locations in Alabama, Florida and Mississippi. At the two locations in Berkeley and Dorchester Counties, SC, ArborGen has requested to release trees in containers and have indicated they will not allow these trees to flower at these locations. The size of each individual confined field test sites identified in ArborGen's permit application ranges from 0.5 to 7.7 acres, which is less than the 20 acres analyzed in APHIS EA and subsequent FONSI prepared for Permits 08-011-106rm, 08-014-101rm and 10-112-101r (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf) and (http://www.aphis.usda.gov/brs/aphisdocs/08_014101r_ea.pdf).

II. Purpose and Need

A. Proposed Action

The proposed action is for APHIS to approve ArborGen permit application request (11-052-101rm) and issue a confined environmental release and interstate movement permit for a hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla* with supplemental permit conditions (*see* Appendices VI and VII). The movement authorization would be valid for one year and the release for a three-year period. The permit will need to be renewed by ArborGen and subsequently approved by APHIS to allow the transgenic *Eucalyptus* plants to remain in the ground beyond the three-year time period specified in the permit application.

APHIS would issue a confined environmental release and interstate movement permit to ArborGen in accordance with 7 CFR part 340 to allow the interstate movement, planting, field testing and flowering of a GE *Eucalyptus* hybrid clone (*Eucalyptus grandis* x *Eucalyptus urophylla)* engineered to express various genes at 6 confined field site locations within in the States of Alabama, Florida, Mississippi, and South Carolina encompassing a total of 14.7 acres. The permit would allow all the trees except the trees on two of the sites in South Carolina to flower. Trees would also be authorized to be moved interstate from ArborGen's greenhouse and nursery facilities in Berkeley, Charleston and Dorchester Counties in South Carolina to the field test locations. All genetically engineered *Eucalyptus* trees authorized under permits 09-070-101rm and 11-041-101rm would be incorporated into this new permit and allowed to flower.

B. Purpose of this Environmental Assessment

The purpose of this EA is to assess the potential environmental impacts on the human environment associated with APHIS responding to a confined environmental release and interstate movement permit application (11-052-101rm) received from ArborGen in February 2011 to authorize the planting of GE *Eucalyptus* hybrid clone to support proposed field research studies on six research sites in Alabama, Florida, Mississippi, and South Carolina. The permit application requests APHIS to allow all the trees except the trees on two of the sites in South Carolina to flower. The purpose of the confined environmental release is for ArborGen to assess the effectiveness of introduced gene constructs which are intended to confer cold tolerance; to test the efficacy of genes to alter lignin biosynthesis; to test the efficacy of genes to alter growth; and to test the efficacy of genes designed to alter flowering. In addition the trees have been engineered with a selectable marker gene which confers resistance to the antibiotic kanamycin. ArborGen has also requested under this permit application that all genetically engineered *Eucalyptus* trees authorized under permits 09-070-101rm and 11-041-101rm be incorporated into this new permit and allowed to flower. New trees will also be moved interstate from ArborGen's nursery and greenhouse facilities located in South Carolina to the release locations identified in the permit application where they will be planted.

This EA was conducted pursuant to: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. § 4321 et seq.), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500-1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Generally, issuances of permits for confined field trials of regulated articles are categorically excluded under APHIS NEPA Implementing Procedures. However, if APHIS determines that a confined field release of genetically engineered organisms may have the potential to significantly impact the quality of the human environment, as those terms are defined in 40 CFR 1508.27 and 1508.14, an environmental assessment may be prepared, pursuant to 7 CFR § 372.5(d) to determine if an Environmental Impact Statement is warranted. In this case, APHIS has prepared this EA specifically to address potential environmental impacts associated with new genetic constructs not previously reviewed or permitted for environmental release and interstate movement by APHIS and with allowing existing plantings of genetically engineered *Eucalyptus* trees permitted by APHIS to flower.

C. Need for This Action

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 GE organisms. APHIS' regulations in 7 CFR part 340, which were promulgated pursuant to authority granted by the plant pest provisions of 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 and movement, the application is evaluated to determine whether the environmental release and movement, with appropriate conditions imposed, can be carried out while preventing the dissemination and establishment of plant pests. The receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

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

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

D. Purpose and Description of the Research

The purpose of the ArborGen research is to assess the efficacy of the introduced cold tolerance genes, genes to alter lignin biosynthesis, genes to control flowering, and genes to alter the growth rate 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. The confined release of the trees in different areas of the southeast U.S. will allow the applicant to obtain data on performance of the transgenic trees and the efficacy of the inserted genes in a wide variety of environments. It is important that trees be planted in locations in Florida because this is the only location where a comparison between the transgenic and non-transgenic control trees can be made. In other locations the trees may be damaged by cold which makes a true comparison difficult to impossible.

III. Affected Environment

Biology of *Eucalyptus* and Status in the United States

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

There have been numerous attempts to grow *Eucalyptus* as a commercial forest tree in the southeastern United States, but due to its sensitivity to cold temperatures, these attempts have not met with success. It is only grown in commercial plantations in central and southern Florida, where it normally survives freezing temperatures which are rare and usually not severe. *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* (also known as *E. urograndis*) that ArborGen wishes to allow to mature and flower under this permit has not been categorized as invasive. The *E. grandis* x *E. urophylla* hybrid has been grown for forty years in South America and during this time there has been no evidence of invasiveness by into natural forest areas which are growing as part of an integrated land management system (Luis Silva, International Paper Company, Brazil, comment to docket APHIS-2008-0059). The University of Florida Institute of Food and Agricultural Sciences (IFAS) recently completed a review of the potential invasiveness of *E. urograndis* and found that it is not likely to be invasive and can be a recommended species for planting: http://plants.ifas.ufl.edu/assessment/pdfs/wra/Eucalyptus%20urograndis_WRA.pdf
See also the chart at:
http://plants.ifas.ufl.edu/assessment/pdfs/concl_genus_Feb2011.pdf. Because it is not predicted to be invasive, no specific management practices are recommended for this species, unlike other species of *Eucalyptus* grown in Florida (see: http://plants.ifas.ufl.edu/assessment/pdfs/results_feb2011.pdf.

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 different genes to confer cold tolerance, alter lignin biosynthesis, or alter growth rate along with testing the efficacy of the Barnase gene designed to alter fertility. In addition the trees have been engineered with a common selectable marker gene (*nptII*) which confers resistance to the antibiotic kanamycin. With the exception of the C-Repeat Binding Factor (*CBF)* gene Barnase gene, and *nptII* gene, all genes are claimed as Confidential Business Information (CBI).

Confined Field Test Locations

The confined field tests would take place on land controlled by ArborGen or through private contracts established by ArborGen for field testing. The exact locations are claimed as CBI and have been submitted as part of the APHIS permit application. Under the permit application submitted by ArborGen, there are six research sites where trees would be planted. A total of 14.7 acres (all 6 sites combined) is being requested by the applicant. Five of these locations currently have active APHIS permits (08-011-106rm, 08-014-101rm, 09-070-10rm, 10-112-101r, and 11-041-101rm) for environmental release of GE *Eucalyptus* hybrid clone (*Eucalyptus grandis* x *Eucalyptus urophylla)* in Alabama, Florida, Mississippi, and South Carolina. The remaining site in Dorchester County, South Carolina has been listed in previous APHIS permits/notifications as a holding area for transgenic trees. ArborGen is requesting that trees be allowed to flower at four locations in Alabama, Florida and Mississippi. At the two locations in Berkeley and Dorchester Counties, SC, they have requested to release trees in containers and have indicated they will not allow these trees to flower at these locations. The size of each individual confined field test sites identified in ArborGen's permit application ranges from 0.5 to 7.7 acres, which is less than the 20 acres analyzed in APHIS EA and subsequent FONSI prepared for Permits 08-011-106rm, 08-014-101rm and 10-112-101r (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf) and (http://www.aphis.usda.gov/brs/aphisdocs/08_014101r_ea.pdf). For the five research sites that are currently authorized by APHIS to plant GE *Eucalyptus* hybrid clone (*Eucalyptus grandis* x *Eucalyptus urophylla),* ArborGen has indicated in their permit application that any new planting authorized by APHIS under 11-052-101rm would not expand the current field site locations beyond the areas currently authorized by APHIS. See Table 1 which shows the proposed field test locations and acreage.

All the confined field test sites listed in the permit application by ArborGen are either on privately owned managed plantation forests and agricultural farm lands or experimental research stations managed by academic institutions and industry, and have been in managed agricultural production for more than 5 years. 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 would 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. ArborGen as indicated that the trees would be planted on individual research sites ranging from 0.5 up to 7.7 acres, depending on the location with planting density ranging from 300 - 600 trees per acre[2]. An acre is about the size of a football field.

Table 1. Environmental release locations requested under permit application 11-052-101rm including existing *Eucalyptus* permits at each location.

Location	Existing Permit(s)	Flowering allowed	Acreage requested under the new permit
Baldwin County Alabama	10-112-101r	Yes	2.0
Escambia County Alabama	08-011-106rm	Yes	2.0
Pearl River County Mississippi	08-011-106rm	Yes	2.0
Berkeley County South Carolina*	08-011-106rm 08-014-101rm 09-070-10rm 11-041-101rm	No	0.5
Dorchester County South Carolina*	New location for *Eucalyptus*	No	0.5
Highlands County Florida	08-014-101rm 09-070-10rm 11-041-101rm	Yes No No	7.7
Total			14.7

*Both locations in South Carolina are fenced areas for holding plants in containers prior to planting where no flowering is allowed.

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. Approximately 3.55 acres of field trials of genetically modified *Eucalyptus* trees of some lines in the permit are being grown under APHIS issued permits 08-011-106rm (0.75 acres) and 10-112-101r (2.8 acres). These trees are authorized by APHIS to flower. The oldest of these trees were planted in August 2006. Similar to the current permit authorized by APHIS, site preparation would 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

[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.

orchard. Under the new permit application, APHIS could authorize up to 2.0 acres of field tests to be established and flower at this location (at around 300 - 600 trees per acre) over the next three years and would not expand the current field site location beyond the area currently authorized by APHIS.

Escambia County Alabama Site:

This location had previously been used as an intensely managed pasture for more than 5 years. The test site is currently planted with grasses suitable for cattle grazing. Approximately 0.5 acres of field trials of genetically modified *Eucalyptus* of some lines identified in the permit authorized by APHIS are being grown under permit 08-011-106rm. These trees are authorized by APHIS to flower. The oldest of these trees were planted in July 2007. Similar to the current permit authorized by APHIS, site preparation would 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 greater than 30 years-old slash pine, and a re-forested area with mixed stands of pine and hardwood species. Under the new permit application, APHIS could authorize up to 2.0 acres of field tests to be established and flower at this location (at around 300 - 600 trees per acre) over the next three years and would not expand the current field site location beyond the area currently authorized by APHIS.

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. Approximately 3 acres of field trials of genetically modified *Eucalyptus* trees of some lines identified in the permit authorized by APHIS are being grown under permit 08-011-106rm. These trees are authorized by APHIS to flower. The oldest of these trees were planted in October 2007. Similar to the current permit authorized by APHIS, site preparation would 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. Under the new permit application, APHIS could authorize up to 2.0 acres of field tests to be established and flower at this location (at around 300 - 600 trees per acre) over the next three years and would not expand the current field site location beyond the area currently authorized by APHIS..

Berkeley County South Carolina Site:

This is an extension of a greenhouse facility that has been used for acclimatization of transgenic and non-transgenic *Eucalyptus* tree plants for more than 8 years. Genetically modified *Eucalyptus* trees are being grown in containers under APHIS permits 08-011-106rm, 08-014-101rm, 09-070-10rm and 11-041-101rm. These trees are not authorized by APHIS to flower. Similar to the current permit authorized by APHIS, the proposed 0.5 acre release site is located adjacent to greenhouse facilities and is surrounded by hardwoods and pine plantations and would not expand the current field site location beyond the area currently authorized by APHIS. 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.

Dorchester County South Carolina Site:

This location is an extension of a greenhouse facility and was previously cleared in 2010 for development as a light industrial park, prior to which it had been a managed pine plantation for more than 15 years. The surrounding area consists of man-made ponds, 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. This is a new location that was not assessed in the previous EA. However, this site has previously been listed as a holding area for trees in containers in previous APHIS permits and notifications for other transgenic tree species.

Highlands County Florida Site:

This location has been used for field trials of transgenic *Eucalyptus* trees for at least 5 years. Approximately 5.2 acres of field trials of genetically modified *Eucalyptus* trees are being grown under APHIS permits 08-014-101rm, 09-070-101rm and 11-041-101rm at this location. Trees that are being grown under permit 08-014-101rm are authorized by APHIS to flower. The oldest of these trees were planted in July 2006. Similar to the current permit authorized by APHIS, site preparation would involve herbicide application, plowing, and planting of trees in flat beds. The test site is surrounded by a citrus production area and second-growth pine and hardwood forests. Under the new permit application, APHIS could authorize up to 7.7 acres of field tests to be established and flower at this location (at around 300 - 600 trees per acre) over the next three years and would not expand the current field site location beyond the area currently authorized by APHIS. ArborGen has also requested under this permit application that all genetically engineered *Eucalyptus* trees authorized under permits 09-070-101rm and 11-041-101rm at this site location to be incorporated into this new permit and allowed to flower.

Interstate Movement

As requested in the permit application, APHIS could authorize the movement of genetically engineered *Eucalyptus* trees from nursery and greenhouse sites in Berkeley, Charleston, and Dorchester Counties in South Carolina to the four field release locations identified in the permit application that would allow flowering. As specified in the permit and supplemental permit conditions (*see* Appendix VI), labeled container-grown trees would be placed in an enclosed vehicle (trailer or a van) and transported to the destination test sites.

IV. Alternatives

This EA analyzes the potential environmental consequences of APHIS' response to a confined environmental release and interstate movement permit application (APHIS Number 11-052-101rm) received from ArborGen to allow the movement to, planting and flowering of genetically engineered *Eucalyptus* trees at six confined field site locations in Alabama, Florida, Mississippi, and South Carolina. A total of 14.7 acres (all 6 sites combined) is being requested by the applicant. These plants are a clone[3] coded EH1 derived from a hybrid of *Eucalyptus grandis X Eucalyptus urophylla* and have been genetically engineered with different constructs than the trees previously permitted for environmental release by APHIS. The purpose of the confined environmental release is for ArborGen to assess the effectiveness of introduced gene constructs which are intended to confer cold tolerance; to test the efficacy of genes to alter lignin biosynthesis; to test the efficacy of genes to alter growth; and to test the efficacy of genes designed to alter flowering. In addition the trees have been engineered with a selectable marker gene which confers resistance to the antibiotic kanamycin. ArborGen has also requested under this permit application that all genetically engineered *Eucalyptus* trees authorized under permits 09-070-101rm and 11-041-101rm be incorporated into this new permit and allowed to flower.

Under APHIS regulations, the Administrator must either grant or deny permits properly submitted under 7 CFR part 340. Based upon the permit applications submitted by ArborGen, two alternatives are considered and analyzed in this EA: (1) deny the permit and (2) approve ArborGen permit application request and issue the APHIS permit.

A. No Action – Deny the Permit

Under the No Action alternative APHIS would deny the permit application (11-052-101rm) submitted by ArborGen. ArborGen would not be authorized to move and plant the GE *Eucalyptus* trees with new constructs and allow them to reach maturity and flower. Confined field release under permits 09-070-101rm and 11-041-101rm would continue to not allow flowering.

There are currently six active permits under which ArborGen is authorized by APHIS to grow GE *Eucalyptus* which include 32 unique locations within 7 States: 08-011-106rm, 08-014-101rm, 09-070-101rm, 10-112-101r, 11-041-101rm and 11-201-103r. No plantings at any of the 32 locations are authorized by APHIS to exceed 20 acres in size. Trees are allowed to flower under four permits: 08-011-106rm, 08-014-101rm, 10-112-101r and 11-201-103r (two of the locations in Berkeley and Dorchester Counties, SC trees are not authorized to flower). Under permits 09-070-101rm and 11-041-101rm trees are not authorized to flower. As of September 2011, ArborGen has reported to APHIS that they are growing a total of approximately 67 acres of trees on 18 of the 32 permitted locations. Under the No Action alternative, ArborGen could continue to grow GE *Eucalyptus* as authorized by APHIS under these six active permits.

[3] 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. Preferred Alternative – Issue the APHIS Permit

The Preferred Alternative is to approve ArborGen permit application request (11-052-101rm) and issue the APHIS confined field release and movement permit for a hybrid of *Eucalyptus grandis* X *Eucalyptus urophylla* with supplemental permit conditions (*see* Appendices VI and VII). The movement authorization would be valid for one year and the release would be valid for a three-year period. The permit will need to be renewed by ArborGen and subsequently approved by APHIS to allow the transgenic *Eucalyptus* plants to remain in the ground beyond the three-year time period specified in the permit application.

Under this alternative, APHIS would issue a confined environmental release permit to ArborGen in accordance with 7 CFR part 340 to allow the movement, planting, field testing and flowering of a GE *Eucalyptus* hybrid clone engineered to express various genes at 6 confined field site locations within in the States of Alabama, Florida, Mississippi, and South Carolina encompassing a total of 14.7 acres. The permit would allow all the trees except the trees on two of the sites in South Carolina to flower. All genetically engineered *Eucalyptus* trees authorized under permits 09-070-101rm and 11-041-101rm would be incorporated into this new permit and allowed to flower. This permit would authorize ArborGen to move and plant the GE *Eucalyptus* trees with new constructs and allow them to reach maturity and flower. Under this alternative, the applicant would be allowed to gather data on performance of the transgenic trees over a multiyear period and the efficacy of the genes in a wide variety of environments for multiple years. This alternative would allow the safe development and use of GE organisms under the mission of BRS.

V. Environmental Consequences

A. No Action

Under the No Action alternative APHIS would deny the permit application (11-052-101rm) submitted by ArborGen. ArborGen would not be authorized to move, or plant the GE *Eucalyptus* trees with new constructs identified in their permit application and allow them to reach maturity and flower. GE *Eucalyptus* trees would continue to be planted and grown as authorized by APHIS permits (08-011-106rm, 08-014-101rm, 09-070-10rm, 10-112-101r, and 11-041-101rm) in the southeastern United States including the 5 environmental release locations in Alabama, Florida, Mississippi, and South Carolina that are identified in ArborGen's permit request. Confined field release under permits 09-070-101rm and 11-041-101rm would continue to not allow flowering.

There would be no environmental impacts associated with the new constructs identified in the permit application since the trees containing these new constructs would not be authorized by APHIS for interstate movement or environmental release. Under this alternative, the applicant would not be allowed to gather data on performance of the transgenic trees over a multiyear period and the efficacy of the genes in a wide variety of environments for multiple years.

Potential impacts associated with Permits 08-011-106rm, 08-014-101rm and 10-112-101r would not change from those analyzed in the 2010 EA and FONSI prepared for these permits (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf) and (http://www.aphis.usda.gov/brs/aphisdocs/08_014101r_ea.pdf). As specified in the permit conditions for Permits 09-070-101rm and 11-041-101rm, trees at these sites are not authorized to flower and therefore would be required to be cut down prior to maturity. Preventing flower formation would prove impossible over time because the trees grow too tall and too many flowers are produced to remove manually. Subsequently, these site locations could be replanted in short rotation GE *Eucalyptus* field tests if approved by APHIS, or the sites could be returned to other forest tree production, agriculture/forestry research, pasture, or other agricultural activities. Intense management activity including the use of heavy machinery for land preparation, general upkeep, irrigation, and fertilization for the management of tree plantings and grasses and standard silvicultural practices would continue to be used at these sites.

B. Preferred Alternative

Under this alternative, APHIS would issue a confined environmental release permit to ArborGen in accordance with 7 CFR part 340 to allow the movement, planting, field testing and flowering of a GE *Eucalyptus* hybrid clone engineered to express various genes at 6 confined field site locations within in the States of Alabama, Florida, Mississippi, and South Carolina encompassing a total of 14.7 acres with supplemental permit conditions (*see* Appendices VI and VII) for the requested three-year period. The permit would allow all the trees except the trees on two of the sites in South Carolina to flower. All genetically engineered *Eucalyptus* trees authorized under permits 09-070-101rm and 11-041-101rm would be incorporated into this new permit and allowed to flower.

Five of these six locations currently have active APHIS permits (08-011-106rm, 08-014-101rm, 09-070-10rm, 10-112-101r, and 11-041-101rm) for environmental release of GE *Eucalyptus* hybrids in Alabama, Florida, Mississippi, and South Carolina.. The remaining site in Dorchester County, South Carolina has been listed in previous APHIS permits/notifications as a holding area for GE trees. In respect to the five locations that currently have active APHIS permits; the new permit application (11-052-101rm) does not expand the field sites beyond what is current authorized by APHIS or density (number) of trees that could be planted. The holding area in Dorchester County will remain as a holding area for trees. Adding *Eucalyptus* trees to the site will not change any existing conditions. As identified in the new permit application, GE *Eucalyptus* trees with the new constructs would be released at the 5 existing locations in addition to the trees currently approved by APHIS for environmental release. The new permit application does not expand the number of trees or total acreage at any of the 5 confined release sites currently approved by APHIS. Similar to current permits, if approved by APHIS, the trees would remain in the ground for at least 3 years and most likely until maturity because the applicant has indicated in their permit application that they are planning to renew the permits prior to expiration. If the renewal permits are approved by

APHIS, the trees could be allowed to stay in the ground until maturity or when normally harvested (age 7-9). The standard silvicultural practices for land preparation, planting, irrigation, and harvesting of trees would continue to be routinely used on these sites.

Potential Environmental Impact of the Preferred Alternative

Interstate Movement of the trees under permit

Under this Alternative, APHIS could authorize the interstate movement of transgenic *Eucalyptus* trees in accordance with the regulatory requirements identified in 7 CFR 340.4, 340.7 and 340.8, including specific permit conditions (*see* Appendix VI) assigned to this permit that would prevent the dissemination of the trees into the environment. As specified in the supplemental permit conditions, ArborGen would be moving trees in accordance with an APHIS approved variance that only allows trees to be moved in labeled containers within an enclosed vehicle. For the time period between 2005 to 2010, APHIS issued218 permits and notifications that authorized the interstate movement of forest trees. Of these 218 authorized movements, there was one compliance incident associated with a shipment of plants via mail. This compliance incident was associated with a movement that did not have similar APHIS approved variances as those that would be required for ArborGen to move GE *Eucalyptus* trees under this permit request. From 2005 to 2010, no compliance incidents of authorized release were reported to APHIS for moving GE *Eucalyptus* trees using similar APHIS approved variances that would be required to move trees under this permit request.

Considering the specific permit conditions that must be adhered to by the permit requirements identified in 7 CFR 340.4, 340.7 and 340.8, the conditions specified under the APHIS approved variance, the proposed supplemental permit conditions for interstate movement of the regulated article, and past compliance history of similar types of movements, APHIS considers the possibility of unintended exposure from moving GE *Eucalyptus* trees under this Alternative to be negligible to non-existent.

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

Overall impacts on disease or insect susceptibility would be similar to the No Action Alternative.

As presented in the permit application submitted by ArborGen:

- There has been no intentional genetic change in these plants to affect their susceptibility to disease or insect damage.
- None of the genes being engineered into the *Eucalyptus* plants are expected to alter the susceptibility of the transgenic *Eucalyptus* plants to disease or insect damage.

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

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

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

Overall impacts of genes for the selectable marker, cold tolerance, altered flowering, altered lignin and altered growth would be similar to the No Action Alternative. These same types of genes are currently being tested in GE *Eucalyptus* trees and other species being field tested under current APHIS notifications or permits for confined field release.

<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 are no longer subject to the plant pest provisions of the Plant Protection Act and 7 CFR part 340 (e.g. corn, petition 01-137-01p; rapeseed, petition 01-206-02p; cotton, petition 95-045-01p; and papaya, petition 96-051-01p).

<u>Genes conferring cold tolerance</u>

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.

There are also five other genes intended to confer cold tolerance in the permit application that are claimed as CBI. These are all genes derived from other plants. 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 in *Eucalyptus* or other plants have not produced unanticipated phenotypes that would indicate there have been changes to plant metabolism leading to increase plant pest characteristics.

<u>Gene for altered fertility</u>

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 by APHIS and are no longer subject to the plant pest provisions of the Plant Protection Act and 7 CFR part 340. 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 conducted in Alabama under permit 06-325-111r (renewed as 10-112-101r) and Florida under permit 08-151-101r (now covered under 08-014-101rm) confirmed that cold tolerant translines grown in these field test did not produce any pollen (see Appendix I).

Genes for altered lignin

There are three genes identified in the permit application to alter lignin. These genes have been engineered into other crops that have been previously released into the environment under both APHIS notifications and permits. 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. Changes in the lignin biosynthetic pathway could modify interactions between plants and potential pathogens or insect pests on many levels: from the structural integrity of the whole plant to responses at the cellular level and at the interface between the plant and potential pest or beneficial microorganism (Funnell and Pedersen 2006). Pederson et al. (2005) surveyed the literature examining the impact of reduced lignin on plant fitness in a number of species and concluded that there are strong interactions among lignin reducing genes, the genetic background in which they are placed, and the environment in which the resulting plant lines are grown. Taken as a whole, it appears that reducing lignin content of crop plants can negatively impact their agricultural fitness. However, when evaluating individual events of reduced lignin, effect on agricultural fitness may be neutral or even positive (Pedersen et al, 2005, Funnell and Pedersen, 2006). To date the results of field tests with these particular genes have shown no differences in plant pest susceptibility. Growth measurements have indicated that trees had normal to a moderately reduced growth phenotype. The trees have also been 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).

Genes for altered growth

There are four genes identified in the permit application intended to alter growth characteristics of the trees that are all claimed as CBI. These are all genes derived from *Eucalyptus* and other plants. 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 have been field tested in *Eucalyptus* or other plants and have not produced unanticipated phenotypes that would indicate there have been changes to plant metabolism leading to increase plant pest characteristics. Some of these genes being examined by ArborGen in the transgenic *Eucalyptus* have been associated with an increase in growth along with an increase in flowering or seed yield. If this were to occur in these transgenic Eucalyptus, ArborGen is required to report this as an unusual occurrence and must report this as a part of the conditions for field testing.

Non-coding sequences

The transgenic *Eucalyptus* also contains non-coding regulatory sequences[4] derived from plants and plant pathogens. The non-coding regions of the plant pathogens will not result in the production of an infectious entity or cause plant disease symptoms. None of these sequences are expected to pose a plant pest risk.

Method of transformation

The genes were transferred to *Eucalyptus* via well-characterized laboratory techniques that utilize DNA sequences from *Agrobacterium tumefaciens* to transfer introduced genes into the chromosome of the recipient plant (see reviews by (Zambryski 1988, Klee and Rogers 1989) *A. tumefaciens* is a bacterial plant pathogen that can cause crown gall disease on a wide range of dicotyledonous plant species. Although some of the DNA sequences used in the transformation process were derived from the *A. tumefaciens*, the genes that cause crown gall disease are first removed, and therefore the recipient plant does not have crown gall disease. Following transformation, the bacteria are eliminated 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 previous EAs and response to comments for APHIS permit 06-325-111r (http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf) and permits 08-011-106rm, 08-014-101rm (http://www.aphis.usda.gov/brs/aphisdocs/08_014101r_ea.pdf) which are herein incorporated by reference.

The hybrid *Eucalyptus* EH1 used to produce the transgenic trees has not been shown to be weedy or invasive in the U.S. An assessment has been conducted on the weediness or invasiveness potential of the hybrid by The University of Florida IFAS (see the section above Biology of *Eucalyptus* and Status in the United States). In that assessment it was concluded that it is not likely to be invasive and can be a recommended species for planting. None of the genes introduced into the *Eucalyptus* hybrid 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 genes to affect the growth of the trees would not be expected to increase invasiveness or weediness unless they also affect seed release or seed production. The trees could 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

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[4] 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.

15 years, there has been no indication that large numbers of seedlings are being produced and are becoming invasive from the commercial plantations (L. Pearson, ArborGen pers. comm. and Luis Silva, International Paper Company, Brazil, comment to docket APHIS-2008-0059). If the genes to alter growth were to have any effect on seed production, ArborGen is required to report any unusual occurrences, such as increased flowering or seed production to APHIS during the field testing period.

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

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

Possibility of Gene Flow Within the Field Test

All of the trees in the test plots, including control non-transgenic trees, have the same parental genotype EH1. The high level of self-incompatibility in *Eucalyptus* (Campinhos et al. 1998, Pound et al. 2002) is expected to significantly reduce the potential for crossing[5] (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. Controlled seed germination studies have been conducted with seed capsules collected over three years from field

[5] 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.

trials that have been allowed to flower. Results have indicated that either no, or a very low number of viable seeds, are produced in the transgenic as well as in the control trees, most likely as a result of limited self-fertilization by pollen from the fertile control trees.

Regular volunteer monitoring of six different trials over 2-5 years have further confirmed the absence of any seeded volunteers in or around the field tests. No seedlings have been found established beneath the trees or in the surrounding areas (Appendix I).

Even if seed are produced in the test, several factors in the biology of *Eucalyptus* would limit the potential for seed dissemination. Although *Eucalyptus* seed is very light and small, it is not adapted to wind dispersal and consequently the dispersal of seed is very limited, generally being confined within a radius of twice the tree or canopy height (approximately 50 meters for a 25 meter tall tree at harvest age)(Cremer 1977, Gill 1997, Linacre and Ades 2004). Another consequence of the very small size of *Eucalyptus* seeds is that they have very limited reserves and are intolerant of shade or weedy competition. In order to successfully germinate and establish, *Eucalyptus* seed needs contact with bare mineral soil and lack of competition either as a result of human intervention or naturally following a fire event (Meskimen and Francis 1990, Bell and Williams 1997). *Eucalyptus* plantations are typically established using rooted plantlets because of poor establishment using direct seeding methods. Even for the rooted plants, competition control is recommended for several months after planting to ensure good survival (Meskimen and Francis 1990). Therefore, there is limited possibility that volunteer seedlings could become established in any unmanaged areas that may be close to the site. However, if they were to appear, the supplemental permit conditions for the permit will require that all volunteers be reported to APHIS, found and destroyed to prevent any spread of trees from the field release site.

Eucalyptus seeds do not have any dormancy barriers to prevent germination of volunteer seeds (Grose 1960, Wellington 1989, Gill 1997) and seed viability and storage of *Eucalyptus* seeds in soil are less than one year (Gill 1997). The *Eucalyptus* species that have become invasive in California (*E. camaldulensis* and *E. globulus*) are particularly adapted to a Mediterranean climate subject to summer fog, which is conducive to seed germination in those species (Sellers 1910). This type of climate does not exist in the Southeastern U.S. In the event that any viable seeds are produced and are deposited on in an area conducive to germination from this field trial, these seeds would be expected to germinate within 7 – 14 days (Meskimen and Francis 1990). In accordance with the supplemental permit conditions for these permits, the bordering fields within 100 meters from the edge of the trials will be monitored every six months for germinating seedlings by the applicant. This distance is twice the 50 meter distance that seeds would be expected to be dispersed from a tree at harvest age (Cremer 1977, Gill 1997, Linacre and Ades 2004). If transgenic seedlings are observed they will be destroyed by the applicant either by uprooting or by spraying with EPA approved herbicides (e.g., glyphosate or other herbicides to which these trees are susceptible) and APHIS will be notified of their occurrence.

Possibility of Gene Flow Outside of the Field Test

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

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

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

There are other species of cold-hardy *Eucalyptus* that can possibly be grown in the Southeast U.S. These species include *E. neglecta, E. niphophila, E. pauciflora, E. camphora, E. nova-anglica, E. macarthurii, E. gunnii* and *E. cinerea*. These could occur in the same states as the proposed field trials . Among these species, *E. cinerea*, also known as the silver dollar tree or Argyle Apple, is the most popular species grown for its ornamental foliage (http://www.australiaplants.com/Eucalyptus_cinerea.htm). These and other ornamentals are likely to be grown as specimen trees (one or few grown together) and not part of large-scale plantations.

The transgenic *Eucalyptus* hybrids that will be grown in the proposed field trials are not likely to be sexually compatible with any of the cold hardy species listed above. For example, *E. grandis* and *E. urophylla*, for which hybrids have been generated in directed breeding programs, are in the Salignae and Resiniferae series, respectively, of section Transversaria (http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/eucclass.pl?gn=*Eucalyptus*). In contrast, *E. cinerea*, and other cold hardy species mentioned above are far removed genetically from the genotype used in this field trial on the evolutionary scale and reside within different Series and Sections of genus *Eucalyptus* (see http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/eucclass.pl?gn=*Eucalyptus* for details on sections and series in *Eucalyptus*). Even among the closely related species of *Eucalyptus*, hybridization rates are generally very low (Volker 1995). The published literature supports the fact that natural hybridization among distantly related species within genus *Eucalyptus* is rare and hybrid inviability increases with increasing taxonomic distance between parents (Potts and Dungey 2004). Where hybridization is possible, it often requires significant human intervention in directed breeding/crossing efforts. Potts and Dungey (2004) make reference to the high degree of inviability in F_1 hybrids (offspring). Inviability of these offspring may be expressed at germination, in the nursery and even after planting in the field. Slower germination of hybrid seed often occurs, along with reduced survival of germinants in the nursery, and many seedlings have abnormal phenotypes. Griffin et al. (1988) surveyed natural and manipulated hybrids in the genus *Eucalyptu*s and discussed the challenges of developing even human-made hybrids from such wide crosses (in this case *E. grandis* and *E. globulus* in sections Transversaria and Maidenaria, respectively), with only 4.4% of seed germinating and only 3.2% of these producing trees that were worthy of further evaluation. To achieve the development of viable hybrids sometimes hundreds of hand pollinations must be made to find a viable hybrid that will grow normally. An example of the procedures required to make these wide-cross hybrids is given in Barbour and Spencer (2000).

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

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

Possibility of Vegetative Propagation / Persistence Outside of the Field Test

Overall impacts on the possibility of vegetative propagation and persistence outside the field test location would be similar to the No Action Alternative.

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

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

There could be a concern that seeds of the hybrid could be widely distributed by severe storms such as hurricanes or tornadoes. The *Eucalyptus* hybrid that is being grown in these proposed field tests produces mature capsules in February and seed fall is shortly after this. Therefore seed release is in late winter / early spring and well outside out of the normal hurricane season which occurs between June and November (http://www.nhc.noaa.gov/). The probability that other storm events might distribute seeds that could survive is extremely low. There would have to be a number of events that would have to combine to have any consequence: seed happens to be shedding at the time a severe storm rolls through a field test site, the seed happens to land on a suitable seed bed of bare soil with no weeds, the site is then not disturbed either by plowing or herbicide treatment, and moisture conditions are suitable for a germinating seedling to survive and grow. Research and experience have shown that long distance dispersal of *Eucalyptus* seeds and seedling establishment is very rare. Forsyth et al (2004) point out that "…in most parts of the world where *Eucalyptus* have invaded, they seldom spread considerable distances from planting sites, and their regeneration is frequently sporadic." Richardson (1998) indicates "Eucalypts are also represented on many national or regional weed lists from other parts of the world. Despite this, they have not been nearly as successful in invading alien environments as other widely planted trees such as pines and legumes. Many eucalypts produce large quantities of seeds, so their lack of success as invaders is rather puzzling." This could likely be due to the fact that *Eucalyptus* seeds are very small, have very limited reserves and are intolerant of shade or weedy competition. In order to successfully germinate and establish, *Eucalyptus* seed needs contact with bare mineral soil and lack of competition either as a result of human intervention or naturally following a fire event (Meskimen and Francis 1990, Bell and Williams 1997). In Brazil where *E. grandis*, *E. urophylla* and their hybrids have been grown since the 1960's and are now planted on several thousand hectares, there is no evidence that wind borne seeds are spreading the trees beyond managed plantations. Over 70,000 hectares of the hybrid has been planted extensively by International Paper, who developed EH1, with no evidence of invasiveness (Luis Silva, International Paper Company, Brazil – comment to the docket to the EA for permits 08-011-106rm and 08-014-101rm). In these environments *Eucalyptus* obviously does not behave like other windblown seeds of grasses, for example, which can be pioneering species. Therefore the likelihood of significant escape and successful establishment of seeds from the field test sites due to storms is negligible. APHIS has determined that the field test will be confined and storms are not expected to compromise this confinement.

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

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

There is no evidence to date that the untransformed clone of the *Eucalyptus* hybrid in these permits is weedy or invasive in the U.S. (L. Pearson, ArborGen pers. comm.) None of the genes introduced into *Eucalyptus* code for traits that would be expected to make the plants more weedy or invasive. The genes introduced to affect cold tolerance could make the engineered *Eucalyptus* more adapted to cold temperatures in the southern United States, but this trait in and of itself would not impart invasive or weediness characteristics (Kolar and Lodge 2001) to the engineered plants.

There may also be a concern that adding genes that affect growth could make the engineered *Eucalyptus* more invasive if they increased the growth rate. However, simply increasing growth without affecting other traits would not lead to increased invasiveness. If the genes that affect growth were to lead to an increase in seed production or release of more seed then this could have an impact on its invasive or weedy potential. If there were to be a significant increase in the number of flowers or seeds produced in these tests, this would be seen as an unusual occurrence and must be reported to APHIS as a part of the conditions of the permit. An increase in seed production would not necessarily lead to an increase in invasiveness unless the seeds were to find a suitable environment for germination.

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

In unlikely event that seeds are formed and seedlings are produced, none of the traits introduced into the transgenic *Eucalyptus* will compromise the ability to control these plants so spread of seedlings and trees from the field test sites is highly unlikely.

Volunteers can be readily identified and controlled. Should any hybridization and viable seed production occur, in accordance with supplemental permit conditions, the monitoring for and removal of volunteers within 100m from the edge of transgenic test plot by the applicant would effectively eliminate any seedlings that may be produced. If transgenic seedlings are observed they will be destroyed by the applicant either by uprooting or by spraying with EPA approved herbicides (e.g., glyphosate or other herbicides to which these trees are susceptible) and APHIS will be notified of their occurrence.

Impact on Existing Agricultural Practices

Overall impacts on existing agricultural practices would be similar to the No Action Alternative.

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

Potential Impacts to Wildlife

<u>Native floral communities</u>

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

Terrestrial animals

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

Aquatic organisms

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

Potential Impacts by Fire

Overall impacts of fire would be similar to the No Action Alternative.

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

There is a historical risk of forest fire in the southeastern U.S., however, the probability that these confined field trials will increase the risk and severity of forest fires in their respective locations is very small. These new plantings are small (none greater than 7.7 acres) and they will be managed by the applicant to prevent litter buildup. Under all of the *Eucalyptus* permits authorized for planting by ArborGen, no location is planted with more than 20 acres. Authorization of this permit will not increase that acreage at any release location. These sites are also physically isolated from nearby plantations. If they were to catch fire, the fires would likely be readily contained. The sites are managed to reduce the risk of fire spreading to or from the study areas by maintaining a firebreak between the test plots and adjacent forested areas. Depending on local conditions at each site the firebreak may be a road, a cultivated strip or a plowed fire line.

Potential Impacts to Human Health

Overall impacts on human health would be similar to the No Action Alternative.

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

C. neoformans gattii is a fungal pathogen that is hosted on a variety of species of *Eucalyptus* as well as other tree species (Upton et al. 2007). It causes systemic fungal infections in humans, leading to fungal meningitis and death (Datta et al., 2009). *C. neoformans gattii* has been found on a number of *Eucalyptus* hosts, some of which are being grown in commercial plantations and imported and exported for ornamental use. People have contracted and died from cryptococcosis in India, Africa, Taiwan, South America and California (Datta e al, 2009). *C. neoformans gattii* infections are found particularly in AIDS patients due to their weak immune systems (Chaturvedi et al, 2005). Infections with this fungus are rare in those with fully functioning immune systems. For this reason, *C. neoformans gattii* is sometimes referred to as an opportunistic fungus. There was an outbreak of cryptococcal disease on the eastern portion of Vancouver Island, British Columbia in 1999 (Datta et al., 2009). The disease was previously only known to occur in tropical or semi-tropical climates.

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

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

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

Overall impacts on horizontal gene transfer would be similar to the No Action Alternative.

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

nts/Biotechnology/ucm096135.htm. Therefore APHIS concludes that horizontal gene transfer poses no environmental risk.

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

Potential Effects of Growing *Eucalyptus* on Soil Hydrology

Eucalyptus is recognized as having impacts on hydrology and large widespread plantings could have potential impacts on hydrology in the southeastern United States (Farley et al. 2005a). Since large plantings of *Eucalyptus* have not been grown in many parts of the southeastern US (other than southern Florida) the potential impacts of such plantings on hydrology are unknown. In preparing the EA for permits 08-011-106rm and 08-014-101rm 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 transpiration 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* ranges between 15 and 40 meters, which is a characteristic of a dimorphic rooting pattern (Canadell et al. 1996a). In contrast, mean maximum rooting depths of pine plantation (*P. taeda* and *P. elliottii*) and grass species are 3 meters and 2.6 meters, respectively (Canadell et al. 1996a). According to the Forest Service, conversion to *Eucalyptus* on sites where the water tables are less than 10 meters will likely lower down-slope water tables via direct means (i.e., direct use of ground water by deep roots), affect groundwater-aquifer dynamics, and result in evapotranspiration rates that exceed precipitation input, as have been reported for this species in other locations (Calder et al. 1997a).

Recent research suggests that *Eucalyptus* plantations would reduce stream flow more than pine plantations, and could potentially eliminate low flows. In a review of more than 20 catchment[6] conversion studies, Farley and others (Farley et al. 2005a) showed that converting existing vegetation to *Eucalyptus* plantations reduced stream flow by 20% more than converting it to a pine plantation. This review also showed that the loss of low flows were more complete for *Eucalyptus* plantations compared to pine plantations (100% vs. ~80% reduction of low flows). Elimination of low stream flows could have important ramifications for threatened and endangered aquatic species, such as the gulf strain striped bass, and species of endemic freshwater mussels (Golladay et al. 2004a, Couch and McDowell 2006b).

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

The field test sites requested in the permit application are well dispersed and are limited in size (none are greater than 7.7 acres). Under all of the *Eucalyptus* permits authorized for planting by ArborGen, no location is planted with more than 20 acres and it is anticipated that they are not likely to have significant impacts on hydrology. Authorization of this permit will not increase that acreage. 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 in the permit application occupy more than 0.005% 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.

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[6] 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.

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: http://www.international-allelopathy-society.org/main/home/main.php). 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[7] growth of test crops. Among the three *Eucalyptus* species tested, the soil samples from *E. grandis x urophylla* plantations had an inhibitory effect on germination of maize, bean and watermelon but had a stimulatory effect on squash. The soil from *E. grandis* plantations had an inhibitory effect on squash. The total soluble phenolics varied in different soil samples but did not explain the differential effects on the test crops. The authors concluded that soil samples collected from plantations of *Eucalyptus* species contained allelochemicals that affected germination and early growth of some annual crops but such effects could be avoided by planting crops at a distance of 15 meters away from the edge of plantations.

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

D. Cumulative Effects

As identified in the permit application, the field test sites in this permit application have been in agricultural or forest research, or in agricultural production or forest tree plantations for 5 to 15 years. Therefore the land has been in continuous agricultural or forest tree production at all the proposed field sites for at least 5 years prior to these proposed releases and it is reasonably foreseeable that if the permit were not issued that the sites would continue to be maintained under similar agriculture or forestry

[7] 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.

production. Five of these six locations identified in the permit application currently have active APHIS permits (08-011-106rm, 08-014-101rm, 09-070-10rm, 10-112-101r, and 11-041-101rm) for environmental release of GE *Eucalyptus* hybrids in Alabama, Florida, Mississippi, and South Carolina. In respect to the five locations that currently have active APHIS permits; the new permit application (11-052-101rm) does not expand the field sites beyond what is current authorized by APHIS or density (number) of trees that could be planted. With regard to the Dorchester County, South Carolina site, this location has previously been listed as a holding area for GE trees in previous permits/notifications. This new permit, which allows the release of GE Eucalyptus at this location does not change any conditions applied to that site. The Dorchester County site will remain a holding area for GE trees in containers where flowering is not allowed.

As identified in the new permit application, GE Eucalyptus trees with the new constructs would be released at the 5 existing locations in addition to the trees currently approved by APHIS for environmental release. The new permit application does not expand the number of trees or total acreage that could be planted at any of the 5 confined release sites currently approved by APHIS. It is also reasonably foreseeable that the applicant may request to further extend the permit for this environmental release for additional years beyond the 3 years indicated in the pending permit to observe the growth of these trees to maturity. Moreover, APHIS has received a petition for a determination of nonregulated status of a subset of these transgenic Eucalyptus trees, however, the environmental effects of that petition will be analyzed in a separate NEPA document. The temporary change from agricultural crops to a tree crop may result in a temporary change in resident animal and plant species, but after harvest and termination of the proposed permits, it is reasonably foreseeable that the land will return to agriculture or be replanted to tree production or research. At the end of the field test, transgenic plant material will be removed from the test site and/or destroyed in accordance with supplemental permit conditions established for these permits. The effect of propagation, cross-breeding, and invasiveness on listed species or native ecosystems was not considered a potential impact because of the supplemental permit conditions that prevents these events from occurring. Fire risk to TES was not considered a potential impact because the small size of the field trial combined with the unlikelihood of TES presence within the proposed test area.   Likewise, the effect on hydrology was not considered an impact because of the small size of the field trial combined with the distance from water sources that listed species rely upon.   In addition, the small size of the field plots and the distance between test sites spread over thousands of miles indicate no cumulative effects would result from APHIS issuing the proposed field release permits. 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. Based on the analysis provided in the EA, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create cumulative impacts or reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.) associated with the release sites or the ecosystem in which they are situated. No resources will be significantly impacted due to cumulative impacts resulting from the proposed action.

E. Risks to Threatened and Endangered Species

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

F. Impacts on Unique Characteristics of Protected Areas

Appendix V includes the BRS analysis of the release locations for their proximity to State and Federal protected areas. Based on this analysis, issuance of the permit to allow flowering of these additional constructs is not expected to impact unique characteristics of protected areas such as park lands, wetlands, wild and scenic areas, or ecologically critical areas.

None of the release sites are within protected areas. Common forestry practices that would be carried out in the cultivation of the engineered Eucalyptus are not expected to deviate from current practices. The trees will be grown on agricultural land currently suitable for the field testing of forest trees. There are no proposed major ground disturbances; no new physical destruction or damage to property; no alterations of property, wildlife habitat, or landscapes; and no prescribed sale, lease, or transfer of ownership of any property. This action is limited to issuance of the permit. This action would not convert land use to nonagricultural use. Standard forestry or agricultural practices for land preparation, planting, irrigation, and harvesting of plants would be used on the permitted sites, including the use of EPA registered pesticides. Applicant's adherence to EPA label use restrictions for all pesticides will mitigate potential impacts to the human environment.

G. Other Considerations

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

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

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

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

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

Executive Order 12114, "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories and possessions that result from actions being taken. APHIS has given this due consideration and does not expect an environmental impact outside the United States should APHIS choose any of the two alternatives. These confined field tests are being conducted in the continental U.S. and would not be expected on have environmental effects outside of the U.S.

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

Consistency of Proposal with other Environmental Requirements:

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

H. Conclusion

As outlined under the Purpose and Need sections of this document, this EA was prepared by APHIS to evaluate the potential impacts to the human environment resulting from the inclusion of new constructs in field test releases of flowering *Eucalyptus*, which could potentially lead to a lack of confinement of the field tests and other impacts to the environment. APHIS has evaluated the permit application to determine whether the environmental release and interstate movement, with appropriate conditions imposed by APHIS, can be carried out while preventing the dissemination and establishment of plant pests. After preparing this draft EA, APHIS has concluded that because there is no increase in the number of sites or acreage where trees will be allowed to reach maturity and flower, over those already authorized by APHIS to flower under permits 08-011-106rm, 08-014-101rm, 10-112-101r and 11-201-103r and there is no substantially greater risk of loss of confinement and risk to the environment. Based on the analysis and information provided in this EA and supporting permit application, the new genes that are engineered into the trees should also not pose any greater risk of loss of confinement and risk to the environment. The addition of new genes to increase cold tolerance, alter lignin and growth should not compromise the ability of these to remain confined field tests. Therefore, APHIS concludes that the releases will remain as confined field tests and that the release and interstate movement of genetically engineered trees will not pose a significant plant pest risk. In addition, no threatened and endangered species or critical habitat would be impacted by allowing the trees reach maturity and flower.

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 authorized by APHIS to allow transgenic *Eucalyptus* trees flower under APHIS permits 06-325-111r (renewed as 10-112-101r); 08-151-101r (now covered under 08-014-101rm); 08-011-106-rm and 08-014-101rm. The following is a summary of information that has been collected by the applicant and provided to APHIS as part of their annual reporting requirements under the existing permits that allow flowering.

All transgenic and non-transgenic trees in field tests covered under Permit 06-325-111r (renewed as 10-112-101r), spanning 6.2 acres in Baldwin County, Alabama produced flowers in the late summer months of years 2007 to 2010. The field test from Permit 08-151-101r (now covered under 08-014-101rm) spanning 1.4 acres in Highlands County, Florida produced mature flowers on all transgenic and non-transgenic trees in the late summer months of 2008, to 2010. Three field tests flowered in the late summer months of 2010 under Permit 08-011-106-rm; the field test spanning 0.75 acre in Baldwin County, Alabama; the field test spanning 0.2 acres in Evans County, Georgia; and the field test spanning 0.7 acre in Jasper, County Texas. A field test covered under permit 08-014-101rm in Gadsden County, Florida spanning 0.2 acres flowered in 2010 as well. Where flowering occurred in these tests, several hundred to a few thousand flowers were estimated on each tree. For the field tests in Alabama, Georgia, and Texas that flowered in summer of 2010; the transgenic trees produced more flowers per tree compared to the non-transgenic controls as a result of significant cold damage to non-transgenic trees. In Florida, there was no difference in the number of flowers produced by both transgenic and non-transgenic control trees of the same parental genotype. Observations from the replicated field study conducted in Alabama under permit 06-325-111r (renewed as 10-112-101r) and Florida under permit 08-151-101r (now covered under 08-014-101rm) confirmed that cold tolerant translines grown in these field test did not produce any pollen. The results to date have shown that the barnase gene cassette that has been engineered into these trees is effective at preventing pollen formation.

Mature (but not yet opened) capsules have been collected from non-transgenic and transgenic trees in the following field tests: Baldwin County, Alabama under Permit 06-325-111r (renewed as 10-112-101r) in January 2008 and 2009; Highlands County, Florida under Permit 08-151-101r (now covered under 08-014-101rm) in January 2009 and 2010; Evans County, Georgia; and Jasper County, Texas under Permit 08-011-106rm in January 2011.

Replicate capsule samples were collected from transgenic trees and non-transgenic controls in the field tests. For the January 2008 collection only from Baldwin County, AL (Permit 06-325-111r - renewed as 10-112-101r), 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.

Mature seed capsules, prior to opening, were sampled from select trees in three field tests in Baldwin County, Alabama (Permit 06-325-111r - renewed as 10-112-101r); and trees from the single field test in Highlands County, Florida (Permit 08-151-101r - now covered under 08-014-101rm) 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 samples 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 viable seeds produced in these tests is much lower than would be expected for open pollinated mixed stands of *Eucalyptus*.

Mature seed capsules, collected prior to opening, were sampled from the single field test in Highlands County, Florida in early March 2010 (Permit 08-151-101r - now covered under 08-014-101rm). As expected from self-fertilization, a very low number of viable seeds (less than 1) per capsule were observed for transgenic lines and non-transgenic controls trees tested compared to the number of viable seeds (~25 per capsule) expected from an open pollinated mixed stands of *Eucalyptus*.

The controlled seed germination studies with seed capsules collected over three years (2008 to 2010) from field trials allowed to flower have indicated that either no, or a very low number of viable seeds are produced in translines and control EH1 trees compared to what would be expected for an open-pollinating mixed stand of *Eucalyptus*. These 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 where trees have flowered and produced seed capsules. Volunteer monitoring and reporting the presence of volunteers is required in the supplemental permit conditions.

Similar collections of seed capsules have also occurred during 2011 in field tests from Evans County, Georgia and Jasper County, Texas. The seed has not yet been analyzed.

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

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

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

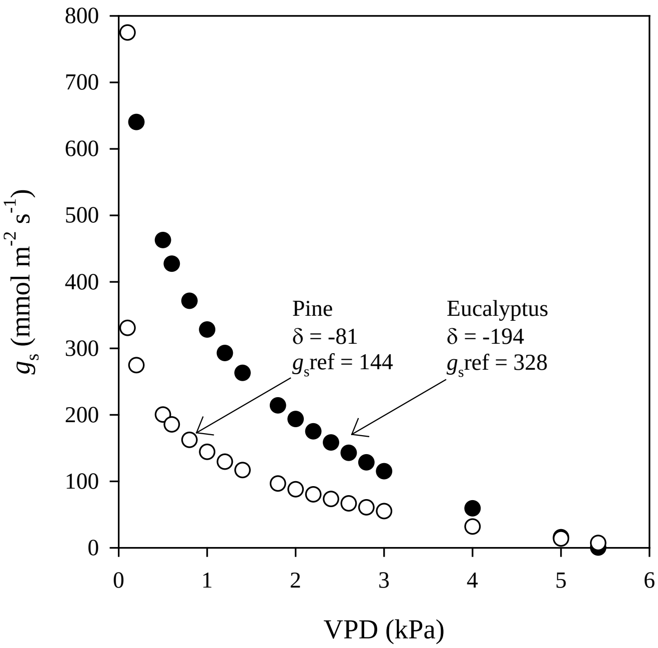

Figure 1: Stomatal conductance response to VPD for pine and *Eucalyptus* (Oren et al. 1999)

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

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

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

The Hydrology document prepared by ArborGen states that afforestation with any tree species may reduce stream flow; and that while *Eucalyptus* plantations might reduce stream flow, the reduction would be comparable to the reduction by pine plantations. Recent research suggests that *Eucalyptus* plantations would reduce stream flow more than pine plantations, and more importantly, *Eucalyptus* plantations could eliminate low flows. In a review of more than 20 catchment conversion studies, Farley and others (2005b) showed that converting existing vegetation to *Eucalyptus* plantations reduced stream flow by 20% more than converting it to a pine plantation. This review also showed that the loss of low flows were more complete for *Eucalyptus* plantations compared to pine plantations (100% vs. ~80% reduction of low flows).

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

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

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

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

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

Management of *Eucalyptus* as coppice stands will affect water use of future rotations

Management practices may create a perennial root stock in *Eucalyptus* plantations. If *Eucalyptus* plantations are managed as coppice stands, the remaining mature, deeplypenetrating 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.

<u>Indirect Measurements</u>:

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

<u>Direct Measurements</u>:

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

Congress passed the Endangered Species Act (ESA) of 1973, as amended, to prevent extinctions facing many species of fish, wildlife, and plants. The purpose of the ESA is to conserve endangered and threatened species and the ecosystems on which they depend as key components of America's heritage. To implement the ESA, the U.S. Fish and Wildlife Service (USFWS) works in cooperation with the National Marine Fisheries Service (NMFS); other Federal, State, and local agencies; Tribes; non-governmental organizations; and private citizens. Before a plant or animal species can receive the protection provided by the ESA, it must first be added to the Federal list of threatened and endangered wildlife and plants.

A species is added to the list when it is determined by the USFWS/NMFS to be endangered or threatened because of any of the following factors:

- The present or threatened destruction, modification, or curtailment of its habitat or range;
- Overutilization for commercial, recreational, scientific, or educational purposes;
- Disease or predation;
- The inadequacy of existing regulatory mechanisms; and
- The natural or manmade factors affecting its survival.

Once an animal or plant is added to the list, in accordance with the ESA, protective measures apply to the species and its habitat. These measures include protection from adverse effects of Federal activities. Section 7 (a)(2) of the ESA requires that Federal agencies, in consultation with USFWS and/or the NMFS, ensure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of designated critical habitat. It is the responsibility of the Federal agency taking the action to assess the effects of their action and to consult with the USFWS and NMFS if it is determined that the action "may affect" listed species or critical habitat.

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

The following resources were used in the analysis:
US Fish and Wildlife Service Endangered Species Homepage: http://www.fws.gov/endangered
US Fish and Wildlife Service Critical Habitat portal: http://criticalhabitat.fws.gov/crithab/
ArcGis Explorer with critical habitat metadata supplied by US Fish and Wildlife Service
County species lists from FWS field offices in Alabama, Mississippi, South Carolina and Florida obtained from http://www.fws.gov/offices/

South Carolina Heritage Trust Database
Telephone contacts with USFWS Field Office personnel
Discussions with property owners of field test sites

1. Baldwin County, AL

This location has been an agricultural research station for more than 20 years. The location has been used for managed production of annual agricultural crops and forest trees. Approximately 3.55 acres of field trials of genetically modified *Eucalyptus* trees of some lines in the permit are being grown under issued permits 08-011-106rm (0.75 acres) and 10-112-101r (2.8 acres) under which these trees are allowed to flower. The oldest of these trees were planted in August 2006. 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. Under the new permit up to 2.0 acres of field tests could be established at this location (at around 300 - 600 trees per acre) over the next three years.

Seventeen TES animals and one TES plant are listed in Baldwin County (list accessed from FWS website 8/30/2011). The TES Animals include loggerhead sea turtle (*Caretta caretta*), green sea turtle, (*Chelonia mydas*), Alabama red-belly turtle (*Pseudemys alabamensis*), Kemp's ridley sea turtle (*Lepidochelys kempii*), Hawksbill sea turtle (*Eretmochelys imbricat*a, Leatherback sea turtle (Dermochelys coriacea), Alabama beach deermouse (*Peromyscus polionotus ammobates*), Perdido Key beach deermouse (*Peromyscus polionotus trissyllepsis*), piping plover (*Charadrius melodus*), West Indian manatee (*Trichechus manatus*), wood stork (*Mycteria americana*), gulf sturgeon (*Acipenser oxyrinchus desotoi*), Alabama sturgeon (*Scaphirhynchus suttkusi*), southern clubshell (*Pleurobema decisum*), Alabama inflated heelsplitter mussel (*Potamilus inflatus*), and eastern indigo snake (*Drymarchon corais couperi*). 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 six turtle species (loggerhead sea turtle, green sea turtle, Alabama redbelly turtle, Kemp’s ridley sea turtle, Hawksbill sea turtle and the leatherback 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 inflated heelsplitter mussel occurs in the Tombigbee and Black Warrior rivers. The southern clubshell mussel occurs in major streams of the Mobile river basin. The closest stream where these mussels could possibly occur is at least a half a mile away from the release site so their habitat does not overlap with the trial site. The wood stork primarily inhabits wetland systems notably cypress or mangrove swamps and would not use the field test site. The piping plover uses sparsely vegetated dunes and coastal beaches in southern Baldwin County, also far away from the field site (about 60 miles). 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 65+ miles from the proposed field trial (distance estimated using ArcGis Explorer). 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, the Alabama beach mouse and the piping plover. There is no designated critical habitat in the immediate environment of the field test. The closest critical habitat (for the Alabama Beach mouse, Perdido Key Beach deermouse and the piping plover) is about 26 miles away. The gulf sturgeon has proposed critical habitat but this does not occur in Baldwin County. (http://crithab.fws.gov/ accessed 8/31/2011)

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

This location had previously been used as an intensely managed pasture for more than 5 years. The test site is currently planted with grasses suitable for cattle grazing. Approximately 0.5 acres of field trials of genetically modified *Eucalyptus* of some lines in the permit are being grown under issued permit 08-011-106rm under which these trees are allowed to flower. The oldest of these trees were planted in July 2007. 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 greater than 30 years-old slash pine, and a re-forested area with mixed stands of pine and hardwood species. Under the new permit up to 2.0 acres of field tests could be established at this location (at around 300 - 600 trees per acre) over the next three years.

Five TES animals are listed for Escambia County (list accessed from FWS website 8/31/2011). The animals are gulf sturgeon, (*Acipenser Oxyrinchus desotoi*), red-cockaded woodpecker

(*Picoides borealis*), wood stork (*Mycteria Americana*), gray bat (*Myotis grisescens*) and eastern indigo snake (*Drymarchon corais couperi*). The listed plant is American chaffseed (*Schwalbea americana*). The USFWS has recently proposed the listing of several mussel species (Federal Register / Vol. 76, No. 192 / 10/04/11), six of which occur in Escambia County. These are the Alabama pearlshell (*Margaritifera marrianae*), round ebonyshell (*Fusconaia rotulata)*, southern kidneyshell ((*Ptychobranchus jonesi)*, Choctaw Bean (*Villosa Choctawensis)*, Narrow Pigtoe (*Fusconaia Escambia)*, and the Fuzzy Pigtoe (*Pleurobema Strodeanum)*. 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 (as measured via ArcGis Explorer). 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. The gray bat might visit the field test location but would not nest there. The eastern indigo snake is highly unlikely to be found at the site, however, the applicant will provide all workers with identifying characteristics of the snake and instructions on what to do if the species is encountered. The American chaffseed occurs on sandy peat, sandy loam, acidic, seasonally moist to dry soils. As noted above 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 and wound not occur in the vicinity of the field test site. None of the proposed mussel species would be found in the release site. Mussels are found on the bottoms of streams and rivers. The closest known location of any of these species is the Conecuh River, approximately 3.5 miles away.

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 (http://crithab.fws.gov/ accessed 8/31/2011). There is also proposed critical habitat for a number of freshwater mussel species in Escambia County (Federal Register / Vol. 76, No. 192 / 10/04/11). Six of these habitats occur in Escambia County. These are Alabama pearlshell (*Margaritifera marrianae*), round ebonyshell (*Fusconaia rotulata)*, southern kidneyshell ((*Ptychobranchus jonesi)*, Choctaw Bean (*Villosa Choctawensis)*, Narrow Pigtoe (*Fusconaia Escambia)*, and the Fuzzy Pigtoe (*Pleurobema Strodeanum)*. The proposed critical habitat for these species in Escambia County occurs in Burnt Corn Creek, and in the lower Escambia and Conecuh rivers, and is limited to the creek and river channels up to the ordinary high-water line. The closest CH to the release location is about 3.5 miles away in 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. Highlands County, FL

This location has been used for field trials of transgenic *Eucalyptus* trees for at least 5 years. Approximately 5.2 acres of field trials of genetically modified *Eucalyptus* trees are being grown under issued permits 08-014-101rm, 09-070-101rm and 11-041-101rm at this location. The oldest of these trees were planted in July 2006. Current and future site preparation involves herbicide application, plowing, and planting of trees in flat beds. The test site is surrounded by a citrus production area and second-growth pine and hardwood forests. Under the new permit up to 4.0 acres of additional tests could be established under this permit.

Fifteen TES animals, nineteen TES plants, and one TES lichen are listed for Highlands County (list accessed from FWS website 9/1/2011).. The animals are: American alligator (*Alligator mississippiensis*), wood stork (*Mycteria americana*), Audubon's crested caracara (*Caracara cheriway*), red-cockaded woodpecker (*Picoides borealis*), Florida scrub-jay (*Aphelocoma coerulescens*), Florida grasshopper sparrow (*Ammodramus savannarum floridanus*), Everglade snail kite (*Rostrhamus sociabilis plumbeus*), ivory-billed woodpecker (*Campephilus principalis*), Whooping crane (*Grus americana*) (non-essential experimental population), Florida panther (*Puma concolor coryi*), Puma (*Puma concolor* – similarity of appearance), West Indian manatee (*Trichechus manatus*), Eastern indigo snake (*Drymarchon corais couperi*), bluetail mole skink (*Eumeces egregius lividus*) and sand skink (*Neoseps reynoldsi*).

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

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

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

The bluetail mole skink (*Eumeces egregius lividus*) inhabits sand pine-rosemary scrub or, less frequently, longleaf pine-turkey oak association sandhills. It occupies localized pockets of sufficient leaf litter and moisture to provide abundant food and nesting sites. The sand skink (*Neoseps reynoldsi*) occurs only on Florida's central ridges, at elevations of 27 m or more. It inhabits loose sands of sand pine-rosemary scrub, less often longleaf pine-turkey oak sandhills or turkey oak barrens adjacent to scrub, especially high pine-scrub ecotones. It was determined that the release site is within a geographic area where these two skink species are found. According to Brad Rick, (contacted February 27 and 28, 2008) of the Vero Beach Field Office USFWS, sand skinks and bluetail mole skinks are found in scrub habitat with areas of open sand. The literature indicates that skinks are sometimes found in active and abandoned citrus groves and the applicant confirmed that sandy soils are predominant in the area. It was decided to have the applicant conduct a survey of the species using USFWS protocols to determine if the species is present. The protocols were provided by the Vero Beach Field Office. The USFWS protocol recommends that surveys be conducted between March 1 and May 15 as this is an ideal time to observe evidence of the skinks. A coverboard survey was conducted over a one month period from March 18 to April 15, 2008. The coverboards were checked weekly on March 25, April 1, April 8 and April 15. No evidence indicating the presence of sand skinks or bluetail mole skinks was observed and they are presumed absent. Therefore, the appropriate determination would be "no effect."

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

Scrub plum (*Prunus geniculata*) has a very narrow range and small widely scattered populations. It frequently forms small colonies of several plants but may grow as solitary individuals. It grows in deep, yellow sands of longleaf pine-turkey oak sandhill and white, excessively leached, wind-deposited soils of evergreen scrub oak-sand pine scrub. Carter's mustard (*Warea carteri*) occurs in sandy clearings in sand scrub and sandhills; scattered overstory of sand; longleaf or slash pine and scrub oaks. Florida ziziphus (*Ziziphus celata*) is a scrub that occurs on gently rolling hills with vegetation dominated by *Carya floridana* and *Quercus* species. It prefers open, sunny areas. The Perforate Reindeer Lichen (*Cladonia perforate*) occurs in sandy openings in stabilized sand dunes with Florida scrub vegetation. It is often associated with *Ceratiola*. None of the plants and the lichen listed above would find the field test site as suitable habitat and would not be present given the historic and continuous use of the release site and the surrounding area as a citrus grove and research area used for growing *Eucalyptus*.

Critical Habitat: There is proposed critical habitat listed for the West Indian manatee but none of the proposed habitat occurs in this county. The whooping crane population in this county is a non-essential experimental population and does not have critical habitat (http://crithab.fws.gov/ accessed 9/1/2011).

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. Pearl River County, MS

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. Approximately 3 acres of field trials of genetically modified *Eucalyptus* trees of some lines in the permit are being grown under issued permit 08-011-106rm under which these trees are allowed to flower. The oldest of these trees were planted in October 2007. Current and future site preparation involves 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. Under the new permit up to 2.0 acres of field tests could be established at this location (at around 300 - 600 trees per acre) over the next three years.

There are six TES animals listed in this county and one TES plant (list accessed from FWS website 9/1/2011). The animals are the ringed map turtle (*Graptemys oculifera*), gopher tortoise (*Gopherus polyphemus*), Louisiana black bear (*Ursus a. luteolus*), gulf sturgeon (*Acipenser oxyrhynchus desotoi*), inflated heelsplitter (*Potamilus inflatus*) and red-cockaded woodpecker. The listed plant is Louisiana quillwort (*Isoetes louisianensis*).

The ringed map turtle (*Graptemys oculifera*) inhabits wide rivers with strong currents, adjacent white sand beaches, and an abundance of basking sites in the form of brush, logs, and debris. The field test will not impact this aquatic species which occurs in the Pearl River system – approximately 11 miles from the field test site. The gopher tortoise (*Gopherus polyphemus*) inhabits dry sand ridges dominated by pine and areas maintained by fire. It is common in longleaf pine forests, but its numbers have decreased with the replacement of longleaf pine forests with loblolly pine forests. This field test is located in an agricultural research station that would be an inhospitable environment for the gopher tortoise. According to James Harris (contacted February 6 and 7, 2008), Supervisory Wildlife Biologist with the USFWS in Lacombe, LA, the species is found in a large geographic area that includes the release site. However, the species is not found everywhere within this geographic area. The species is not likely to be on the site because of its location on a facility used for many years as an agricultural research station. The research facility was contacted to determine if gopher tortoises have been observed at the facility. The farm manager has not observed the species at the facility, and no sightings have been reported to him during his nine years as manager. He is familiar with the species and has seen them at another location about 7-8 miles from the release site. Another employee contacted has worked at the site for over 35 years and has never seen a gopher tortoise at the facility but did observe one approximately ten years ago about ¼ mile from the facility. The applicant surveyed the site for the presence of gopher tortoise burrows on January 29, 2008 and none were found. Considering the use of the facility, testimony of the facility employees, and the negative result of the survey, it can be concluded that the species is not present now and would be unlikely to use the site while it operates as an agricultural research station. The Louisiana black bear (*Ursus a. luteolus*) prefers bottomland forests with diverse food resources, including a variety of hard-mast-producing species. Its habitat includes remote areas with little or no human activity so it would not likely be found at the site. The red cockaded woodpecker (*Picoides borealis*) inhabits old growth forests, primarily longleaf pine. It might visit the field test site but would not nest there. The gulf sturgeon, the inflated hellsplitter mussel, and the Louisiana quillwort occur in aquatic environments so would not be affected by the field test.

Critical habitat: The gulf Sturgeon has designated critical habitat in this county in the Pearl River system. The Pearl River is about 11 miles from the field test site. Tributaries of the Pearl River system are approximately 3.5 and 7.7 miles from the test site (http://crithab.fws.gov/ accessed 9/1/2011).

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. Berkeley County, SC

This is an extension of a greenhouse facility that has been used for acclimatization of transgenic and non-transgenic plants for more than 8 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.

Five threatened or endangered animals and three endangered plants are listed in Berkeley County (list accessed from FWS website 9/1/2011). 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*). The TES Plants are pondberry (*Lindera melissifolia*), Canby's dropwort *(Oxypolis canbyi*) and American chaffseed (*Schwalbea Americana*).

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 pondberry 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 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. The site in Berkeley County is a fenced research plot/holding area which would be very inhospitable to these species. Laura Zimmerman (843-727-4707 ext. 226) (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.

The shortnose sturgeon occurs in rivers and estuaries. The West Indian manatee occurs in shallow coastal waters, rivers, bays and lakes; none of which are close to this release location. The red cockaded woodpecker inhabits old growth forests, primarily longleaf pine and might 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. The frosted flatwoods salamander inhabits longleaf or slash pine forests lying between drier land upslope and wetlands and seasonally inhabits wet pine flat-woods with vernal pools. Discussions with Laura Zimmerman (843-727-4707 ext. 226) (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 Francis Marion National Forest. A check of the SC Heritage Trust Database did not identify any occurrences in the area of the release.

Critical Habitat: There is critical habitat for the frosted flatwoods salamander in Berkeley County. This is located in the Francis Marion National Forest which is about 19 miles away from the release location (http://crithab.fws.gov/ accessed 9/1/2011).

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.

6. Dorchester County, SC

This is an extension of a greenhouse facility that is used for acclimatization of transgenic and non-transgenic plants. This location was previously cleared in 2010 for development as a light industrial park, prior to which it had been a managed pine plantation for more than 15 years. The surrounding area consists of man-made ponds, 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.

Five threatened or endangered species (TES) are listed in Dorchester County (TES list dated March 2010 obtained from FWS website 8/16/11). The TES animal species are shortnose sturgeon (*Acipenser brevirostrum*), red-cockaded woodpecker (*Picoides borealis*) and wood stork (*Mycteria americana*). The TES Plants are pondberry (*Lindera melissifolia*) and Canby's dropwort *(Oxypolis canbyi*).

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 pondberry 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 site in Dorchester County is a fenced research plot/holding area which would be very inhospitable to these species. It is located in an industrial park that was established a year ago so no new land is being disturbed.

The shortnose sturgeon occurs in rivers and estuaries. The red cockaded woodpecker inhabits old growth forests, primarily longleaf pine and might visit the release 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: There is no critical habitat or proposed critical habitat list for Dorchester County (http://criticalhabitat.fws.gov/crithab/ accessed 8/16/11)

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.

7. Interstate Movement

APHIS considered the possible effects that interstate movement of GE *Eucalyptus* trees authorized by APHIS could potentially have on threatened or endangered species and their critical habitat. APHIS could authorize the interstate movement of transgenic *Eucalyptus* trees in accordance with the regulatory requirements identified in 7 CFR 340.4, 340.7 and 340.8, including specific permit conditions (*see* Appendices VI) assigned to this permit that would prevent the dissemination of the trees into the environment. As specified in the supplemental permit conditions, ArborGen would be moving trees in accordance with an APHIS approved variance that only allows trees to be moved in labeled containers within an enclosed vehicle. For the time period between 2005 to 2010, APHIS issued 218 permits and notifications that authorized the interstate movement of forest trees. Of these 218 authorized movements, there was one compliance incident associated with a shipment of plants via mail. This compliance incident was associated with a movement that did not have similar APHIS approved variances as those that would be required for ArborGen to move GE *Eucalyptus* trees under this permit request. From 2005 to 2010, no compliance incidents of unauthorized release were reported to APHIS for moving GE *Eucalyptus* trees using similar APHIS approved variances that would be required to move trees under this permit request.

Conclusion: Considering the specific permit conditions that must be adhered to by the permit requirements identified in 7 CFR 340.4, 340.7 and 340.8, the conditions specified under the APHIS approved variance, the proposed supplemental permit conditions for interstate movement of the regulated article, and past compliance history of similar types of movements, APHIS has determined that the movement of GE *Eucalyptus* trees in accordance with APHIS approved supplemental permit conditions will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation.

Overall Conclusions

The field test sites in these permit applications have been in agricultural or forest research, or in agricultural production or forest tree plantations for from 5 to 15 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. Potential effects from moving trees would be negligible to non-existent due to the applicants adherence to regulatory requirements identified in 7 CFR 340.4, 340.7 and 340.8, including specific permit conditions (*see* Appendix VI) assigned to this permit that would prevent the dissemination of the trees into the environment. Therefore, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation. Because of this no effect determination, consultation and/or the concurrence of the USFWS and/or the NMFS are not required.

APPENDIX V: Proximity of release sites to Protected Areas

The proposed release locations were accessed for their proximity to State and Federal protected areas and any potential foreseeable impacts to these areas. The analysis used the Protected Areas Database (PAD-US 1.1) (http://www.databasin.org/protected-center/features/PAD-US-CBI) loaded into ArcGis Explorer. Proximity to the protected areas was determined using distance measuring tools in ArcGis Explorer.

Baldwin County, AL

Bon Secour National Wildlife Refuge is located in Baldwin County http://www.fws.gov/bonsecour/. The Bon Secour National Wildlife Refuge comprises provides habitat for the endangered Alabama beach mouse and serves as a nesting site for loggerhead, and includes the Kemp's Ridley sea turtle, the Perdido Key beach mouse and the piping plover. The Baldwin County release location is approximately 21 miles away from the Bon Secour National Wildlife Refuge. Another protected area located in Baldwin County is the Mobile-Tensaw Delta Wildlife Management Area. http://www.dcnr.state.al.us/public-lands/stateLands/foreverwild/FWTracts/mobile-tensawComplex/. It is located approximately 16 miles from the field test location.

Escambia County, AL

Conecuh National Forest is located in Escambia County http://www.stateparks.com/conecuh.html. Clear-cut in the 1930s, the Conecuh was reforested with slash pine that reduced the number of nesting trees for the endangered red-cockaded woodpecker. The forest is currently undergoing a reforestation from slash pine to the native longleaf. In time, this should increase the number of red-cockaded woodpeckers as the trees mature. The Escambia County release location is approximately 8.5 miles from the western edge of the forest. The Perdido River Wildlife Management Area is approximately 35 miles away http://myfwc.com/viewing/recreation/wmas/cooperative/Perdido-River. The northern border of Blackwater River State Forest in Florida is located about a mile away from the release location http://www.fl-dof.com/state_forests/blackwater_river.html. Blackwater River State Forest is known for its longleaf pine/wiregrass ecosystem, which, in combination with the Conecuh National Forest to the north and Eglin Air Force Base to the south, is the largest contiguous ecological community of this type in the world.

Highlands County, FL

Wales Ridge National Wildlife Refuge occurs in Highlands County http://www.fws.gov/lakewalesridge/. The release location is about 7 miles from one section of the refuge. The refuge contains twenty-two different threatened or endangered species of plants. Highlands Hammock State Park is also located in this county and the field test site is adjacent to one border of the State park. http://floridastateparks.org/highlandshammock/. This park is known for its old-growth hammock, cypress swamps and offers a number of recreational activities.

Pearl River County, MS

Bogue Chitto National Wildlife Refuge occurs in Pearl River County http://www.fws.gov/boguechitto/. The refuge's bottomland forests contain sweetgum-water oak stands interspersed with bald cypress-tupelo brakes and overcup oak-water hickory stands. It includes habitat for a number of endangered species. The release site is approximately 9 miles away from the eastern boundary of the refuge.

Berkeley County, South Carolina.

Berkeley County, Georgia contains parts of the Francis Marion National Forest which provides habitat for black bear, alligator, and the endangered red-cockaded woodpecker http://www.fs.usda.gov/scnfs/. The field test site is located approximately 15 miles from Francis Marion National Forest.

Dorchester County, SC.

Dorchester County contains Colonial Dorchester State Historic Site http://www.southcarolinaparks.com/park-finder/state-park/725.aspx and Givhans Ferry State Park http://www.southcarolinaparks.com/park-finder/state-park/1219.aspx. Both of these areas are protected recreational areas maintained by the State of South Carolina. The field test site is located approximately 5 miles from Givhans Ferry State Park and approximately 7 miles from the Colonial Dorchester State Historic Site.

APPENDIX VI: Proposed Supplemental Permit Conditions for Movement

For Movement of *Eucalyptus grandis* x *Eucalyptus urop*hylla under permit 11-052-101rm

1. BRS should be notified in writing of any proposed changes to the permit application (or approved permit) including for example changes in movement protocols, additional transgenic lines or constructs, new destinations, or amount introduced. 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 147, Riverdale, Maryland 20737.

2. The regulated article is to be shipped in containers as specified in the Title 7 Code of Federal Regulation Section 340.8 (7 CFR 340.8) unless a variance request has been reviewed and approved by APHIS/BRS. BRS Notes that the trees would be moved under a previously approved Variance 07-014.

3. While in storage all regulated articles covered under this permit are to be kept in a locked storage facility with limited access to only authorized personnel. Storage containers must be identified as containing a genetically engineered regulated article. At least one sign stating "Authorized Personnel Only" must be posted in each area where the regulated article is stored.

4. Upon completion of research, all regulated articles (except those retained for future studies) should be rendered non-viable by an appropriate method (e.g., heat or steam sterilization, bleach treatment, etc.).

5. There is to be no further distribution of this regulated article under this permit without prior approval from State (intrastate movement) and Federal regulatory officials (interstate movement).

6. APHIS/BRS and/or APHIS/PPQ personnel may conduct inspections of facilities and/or records at any time.

7. Reporting an Unauthorized or Accidental Release

1. According to the regulation in 7 CFR § 340.4(f)(10)(i), 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)
Regulatory Operations Program
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

8. No person shall move a regulated article interstate unless the number of the limited permit appears on the outside of the shipping container.

APPENDIX VII: Proposed Supplemental Permit Conditions for Release

For Release of *Eucalyptus grandis* x *Eucalyptus urop*hylla under permit 11-052-101rm

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.

2. Any unusual flowering or seed set much be reported to APHIS as an unusual occurrence. If there is an overabundance of flowers or seeds produced this must be reported as soon as observed.

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 Code of Federal Regulation 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. Reporting an Unauthorized or Accidental Release

   1. According to the regulation in 7 CFR § 340.4(f)(10)(i), 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)
   Regulatory Operations Program
   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

9. 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)
Regulatory Operations Program
USDA/APHIS
4700 River Rd. Unit 91
Riverdale, MD 20737

10. 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)
Regulatory Operations Program
4700 River Rd. Unit 91
Riverdale, MD 20737
BRS Fax:
Regulatory Operations Program
(301) 734-8910

a. Planting or Environmental Release Report

Planting reports must be submitted to BRS by the 15th of the month following the month in which the environmental release was started and must include the following data:
- Permit number
- Regulated article
- State
- County
- Location Name (Unique ID)
- GPS coordinates of the planting
- Planting Unique ID
- Planting Start Date
- Total acreage of the regulated article planted or otherwise released
- List of all constructs planted
(This list is optional in the planting report but must be included in the Annual and Field Test Reports)

b. Annual Report

Within 30 days after the anniversary date (one-year increments from the effective date listed on the permit) an Annual Report must be submitted to APHIS. If the permit has been amended, the Annual Report is due 30 days from the anniversary date (one year increments from the effective date) of the ORIGINAL permit. 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:

- Permit number
- State
- County
- Location Name(s)
- Location Unique ID(s)
- Any plantings that occurred at each location
- GPS coordinates for each planting
- Size of the plantings (in acres) at each location
- Phenotypic designations (all constructs that were planted)
- Include an accounting of the total acreage that remains in the ground
- Include a detailed map of the plantings
- Indicate if any of the planted material was destroyed before harvest
  If so provide the Pre-Harvest destruction completion date and describe how the pre-harvested material was destroyed
- Indicate if any of the planted material was harvested
  If so provide the harvest completion date
- Describe how the harvested material was terminated
- If the material was terminated in the field and not removed from the field, provide the date the field test was completely terminated and describe the method of termination
- If the material was removed from the field and terminated off site describe how it was disposed and provide the date of off site destruction.
- If material was removed from the field and placed in storage, provide the amount of material that was stored and provide a description of the storage location
- Describe any other disposition methods that may be applicable
- Describe any deleterious effects on plants, non-target organisms, or the environment
- Describe methods of observations and resulting data and analyses
- Indicate if you have submitted any of the following:

1. A report on the accidental or unauthorized release of the regulated article;
2. A report that characteristics of the permitted species are substantially different from those listed in the application; or
3. A report of any unusual occurrence

Also include any reports of any overabundance of flowering or seed set that occurred and how this was handled.

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.

c. Field Test Report

Within six months after the expiration date of the permit, the permittee is required to submit a Field Test Report. Field Test Reports provide the final status and observations at each location and must include:

- Permit number
- State
- County
- Location Name(s)
- Location Unique ID(s)
- Any plantings that occurred at each location
- GPS coordinates for each planting
- Size of the plantings (in acres) at each location
- Phenotypic designations (all constructs that were planted)
    - Indicate if any of the planted material was destroyed before harvest

If so provide the Pre-Harvest destruction completion date and describe how the pre-harvested material was destroyed

- Indicate if any of the planted material was harvested

If so provide the harvest completion date

- Describe how the harvested material was terminated
- If the material was terminated in the field and not removed from the field, provide the date the field test was completely terminated and describe the method of termination
- If material was removed from the field and terminated off site describe how it was disposed and provide the date of off site destruction.
- If material was removed from the field and placed in storage, provide the amount of material that was stored and provide a description of the storage location
- Describe any other disposition methods that may be applicable
- Describe any deleterious effects on plants, non-target organisms, or the environment
- Describe methods of observations and resulting data and analyses
- Indicate if you have submitted any of the following:

1. A report on the accidental or unauthorized release of the regulated article;
2. A report that characteristics of the permitted species are substantially different from those listed in the application; or
3. A report of any unusual occurrence

Also include any reports of any overabundance of flowering or seed set that occurred and how this was handled.

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. Volunteer Monitoring Report

The report must include:

- Permit number
- State
- County
- Location Name(s)
- Location Unique ID(s)
- Dates when the field site and perimeter fallow zone were inspected for volunteers
- Number of volunteers observed
- Any actions taken to remove or destroy volunteers

The final monitoring report is due no later than three months from the end of the volunteer monitoring period.