Finding of No Significant Impact and Decision Notice

Animal and Plant Health Inspection Service

Issuance of a permit to continue research on genetically engineered *Eucalyptus* hybrids

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has received a permit application (APHIS number 06-325-111r) from ArborGen, LLC to allow genetically engineered *Eucalyptus* hybrids planted under a previously approved notification (05-256-03n) to flower. A description of this field test may be found in the attached Environmental Assessment (EA) which was prepared pursuant to APHIS regulations at §7 CFR 372, promulgated under the National Environmental Policy Act. The field tests are scheduled to begin in 2007 in Baldwin County, Alabama.

A draft EA was prepared and submitted for public comment for 30 days, as announced in a notice published in the *Federal Register* on April 20, 2007 (Docket No. APHIS-2007-0027, 72 FR 19876-19877). APHIS received 270 comments during the 30-day comment period and addressed the comments, as appropriate, in an attachment to this document.

APHIS proposed three different actions to take in response to this permit application: take no action, which would deny the permit (Alternative A); issue the permit as received (Alternative B); or issue the permit with Supplemental Permit Conditions containing additional environmental safety requirements and a requirement for the filing of field test reports with APHIS (Alternative C).

APHIS has selected the action proposed in Alternative C. APHIS has determined, based on the analysis documented in its EA and response to comments, that the proposed action will not have a significant impact, either directly, indirectly, or cumulatively, on the quality of the human environment; therefore, an Environmental Impact Statement need not be prepared for this action.

Pursuant to its regulations (7 CFR § 340), promulgated under the Plant Protection Act of 2000, APHIS has determined that this field trial will not pose a risk of the introduction or dissemination of a plant pest.

The above determinations are supported by the following:

1. The field test sites are located on secure, private land in Baldwin County, Alabama, and are physically isolated from any sexually compatible *Eucalyptus*. *Eucalyptus* is not native to the United States and only a few ornamental species are planted in the southern States. None of these species are sexually compatible with the hybrid in this field test.

2. There is little probability of asexual spread since this hybrid *Eucalyptus* does not propagate readily without the aid of special environmental conditions. None of

the genes introduced into the transgenic trees are expected to affect asexual propagation.

3. *Eucalyptus* seed is not adapted to wind dispersal so the dispersal of seed is expected to be limited to the proximity of the field test area.

4. It is unlikely that viable seeds will be produced by the *Eucalyptus* hybrids in the field test, and it is unlikely that any seeds produced will be able to germinate and produce viable offspring. Therefore, APHIS concludes that it is not reasonably foreseeable that *Eucalyptus* seeds will be spread by severe wind events and establish outside of the field site.

5. If any seeds were to be formed due to crossing within the field test, there is very little probability that they will germinate since *Eucalyptus* seeds have very limited stored food reserves, are intolerant of shade or weedy competition, and need contact with bare mineral soil to successfully germinate. The agricultural lands surrounding the test site are not conducive to the establishment of *Eucalyptus* seedlings.

6. If any viable seeds were to be produced and grow into seedlings, they will be easily identified by monitoring the field sites and destroyed with herbicide treatment or removed by physical means.

7. The supplemental permit conditions stipulate that an annual report be submitted to APHIS that includes: a map and inventory of the plants in the test, which if any of the plants produced flowers or viable seed, which plants were removed and their disposition, and any unanticipated or adverse effects on plants, nontarget organisms and the environment. 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 no that no shoots are continuing to be produced. Monitoring and mitigation of the field test should be readily accomplished given the small size of the test (~1.1 acres) and the short duration (3 years) of the test.

8. Horizontal movement of the introduced genes is extremely unlikely. The foreign DNA is stably integrated into the plant genome.

9. No adverse consequences to non-target organisms or environmental quality are expected from the field release of these transgenic *Eucalyptus* for the reasons stated below.

- The proteins produced by genes introduced into these *Eucalyptus* lines are not expected to have toxicological or allergenic properties.

- Since *Eucalyptus* is not native to the southeastern United States and due to the confinement conditions imposed on the test, there is a very low probability that the engineered *Eucalyptus* will become established in the environment.

- The trees were transformed in New Zealand and imported into the United States under permit 05-072-03m to South Carolina. *Eucalyptus* is subject to a period of post-entry quarantine when it is imported into the United States. All materials are handled in accordance with the USDA–APHIS requirements for import and quarantine. The Baldwin County site operates under USDA–APHIS PPQ Post-entry quarantine permit # 37-88316.

For the reasons enumerated above, which are consistent with regulations implementing the Plant Protection Act, the field trial of genetically engineered *Eucalyptus* is hereby authorized under APHIS permit 06-325-111r with supplemental permit conditions (Alternative C).

for Rebecca Bech

Rebecca Bech
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: JUN 27 2007

Attachment
Finding of no significant impact
Response to comments
APHIS No. 06-325-111r

In response to a notice published in the Federal Register (Docket No. APHIS-2007-0027, 72FR19876 - 19877) on April 20, 2007, APHIS received 270 comments on the Environmental Assessment, prepared in response to permit application 06-325-111r, during the 30-day comment period. There were 153 respondents that supported the petition. There were 67 respondents who submitted 102 comments opposed to granting the permit.

Respondents supporting granting permit 06-325-111 were foresters, paper and packaging companies, or from related industries, academia, agricultural biotech companies, and individuals. The majority of respondents supporting submitted nearly identical form letters citing President Bush's 2007 State of the Union Address and the need for biomass in the production of alternative fuels to meet the goal of 35 billion gallons by 2017, the sustainability of rural development, local economies, and jobs through dedicated fiber and fuel plantations, a dearth of negative health or environmental impacts, a long history of cultivation in other countries without invasiveness, the absence of wild relatives with which Eucalypts could cross, and the high level of self-incompatibility among *Eucalyptus*.

Comments received from respondents opposing granting permit 06-325-111r were primarily from 13 public interest groups, one of whom submitted a comment in the form of a petition bearing 5,495 signatories, while another submitted 23 separate comments. Other respondents were from academia and individuals. Issues raised by those opposed to granting this permit include: allowing the *Eucalyptus* trees to flower would be precedent setting; the potential of the *Eucalyptus* in the field test to become an invasive species that threatens native plant and animal communities; that APHIS failed to evaluate severe storm events that can occur in the coastal region of Alabama in the EA; global warming and climate change will allow more extensive southern and southeast regions of the U.S. to have weather patterns conducive to the introduction and propagation of escaped GE *Eucalyptus* hybrids, which APHIS failed to consider in the EA; the fact that *Eucalyptus* trees are known to be at high risk of catching fire and tend to burn very hot during forest fires and in regions where droughts occur, such as the Southeastern United States; that *Eucalyptus* plantations have been documented to deplete ground water and cause or exacerbate drought situations, which was not addressed in the EA; the probability that other pests or pathogens associated with *Eucalyptus* will eventually be transported into the southeastern (SE) U.S; that ArborGen was charged with a non-compliance infraction and indicated that giving approval for a previous charge of noncompliance is a clear violation of its own regulations; that the field trial is unnecessary due to the location of the trial and the preexistence of naturally cold-tolerant varieties of *Eucalyptus*; that field test was originally planted under APHIS Notification (05-256-03n) but that permit was for a different organism - *Eucalyptus grandis* -not the hybrid in the current application 06-325-111r; that the *Eucalyptus* field test could be a

source of *Crytococcus neoforman gatti*; the failure to release the gene constructs that were frivolously claimed as confidential business information, and that there was insufficient scientific data and other information on which APHIS could base its evaluation of the environmental impacts that might result should the permit application be approved. APHIS' responses to these and all relevant comments submitted are as follows:

Comment: A number of commenters indicated that allowing the *Eucalyptus* trees to flower would be precedent setting. Some believed that this is the first test to allow flowering and possible seed development in a GE forest tree species and that it will set precedents for risk assessment. "Once this GE tree flowering and seed production is allowed on the U.S. mainland, it will be easier for APHIS to approve outdoor field trial releases of other GE trees for flowering and seed production."

Response: This field test does not set any precedents. Trees have been allowed to flower under several APHIS permits for a number of years, and this information has been made available to the public through APHIS' website. Genetically engineered forest trees, including *Populus* have been allowed to flower under permit. For example poplar trees under permit 95-031-01r, renewed under 00-151-01r, have been flowering in field tests for a number of years. An EA was prepared for the 1995 permit which addressed flowering. In addition apple, plum and papaya have been allowed to flower and produce fruit under both Notifications and Permits. There has been no indication that there has been a loss of confinement or any significant impacts on the environment in any of these field tests.

Comment: There were a number of comments that expressed concern about the potential of the *Eucalyptus* in the field test to become an invasive species that threatens native plant and animal communities. There was a concern that adding the cold tolerance trait would make it more adaptive and invasive in the southeastern U.S. It was commented that APHIS has failed to assess the potential invasiveness and plant pest characteristics of the engineered *Eucalyptus*. It was hypothesized that engineered traits such as cold tolerance could significantly affect the engineered variety's ability to propagate, survive, and impact native ecosystems

Response: In addition to the information presented in the EA on pages 10-12 which led APHIS to conclude that cold tolerant *Eucalyptus* is unlikely to escape from the field test and become invasive in the southeast, there were a number of comments supplied by experts in *Eucalyptus* biology that support APHIS' finding.

Dr. Tom Ledig, Senior Scientist at the Pacific Southwest Research Station, USDA Forest Service, and an adjunct professor at the University of California, Davis has had a career in forest genetics spanning 41 years. According to Dr. Ledig, "*Eucalyptus grandis*, one of the parents represented in this test, is not a new introduction. It has been grown in Florida for decades and in California for over a century. The other parent, a truly tropical eucalypt, will not grow in the continental United States. Any seed that did germinate from selfing would produce very slow-growing, maladapted plants. The

provision for monitoring and destroying any volunteers after destruction of the test eliminates even that possibility."

Also, according to Dr. Ledig, the popular literature often mentions eucalypts as invasive species. Dr. Ledig comments that "Despite a history of eucalypt planting dating back over a century and a half in California, this has not been my experience. For example, I have seen no seedlings of *Eucalyptus grandis*, one of the parents of the genotype under test, in or around 30-year-old research plantations in central California. Therefore, the ArborGen test, only a few years old at harvest, should pose no threat to native ecosystems."

The reason that *Eucalyptus* is seen all over California is because it was widely planted in the past as an ornamental, and in coastal areas *E. globulus* is invasive due to the presence of favorable conditions for germination and spread such as foggy conditions (see below). This environment does not exist in Alabama.

Professor Yi Li, Department of Plant Science, University of Connecticut, Director and the principal investigator of New England Center for Invasive Plants agrees that the *Eucalyptus* species used by ArborGen, *Eucalyptus grandis* x *Eucalyptus urophyll*a, is not considered invasive.

Richard Bryant, International Paper Company indicated that the company established upwards of 100 acres of research areas with dozens of *Eucalyptus* species since the mid 70s, and the only reproduction ever observed was sprouting. "If these sexually compatible trees failed to reseed their areas, I don't see why anyone would expect this clonal trial to do so."

Dr. Teotônio Francisco de Assis - Assistech Ltda has conducted research on *Eucalyptus* in Brazil for the last 30 years and is considered an expert in *Eucalyptus* biology and breeding. He is the author of a book on cloning and diseases of *Eucalyptus* and has published over 50 scientific articles on *Eucalyptus*. According to the comment submitted by Dr. de Assis, they have learned in Brazil that "successful cultivation of *Eucalyptus* requires careful management. Usually *Eucalyptus* plantations are not established using seeds because the seeds are extremely variable and require very intensively managed conditions to germinate and grow. The majority of current *Eucalyptus* plantations in Brazil are therefore established using rooted cuttings. This process requires cultivation under controlled greenhouse and nursery conditions which was perfected after many years of research in Brazil. *Eucalyptus* does NOT spread naturally through cuttings. Even with the use of rooted cuttings, these are extremely sensitive to weedy competition which requires rigorous weed management in order to establish healthy and vigorous plants. As a breeder of *Eucalyptus* I am very familiar with the limitations of inbreeding. Using a single genotype as is the case for this field trial, it is very unlikely that any viable seed would be produced. It is also well documented that dispersal of *Eucalyptus* pollen is very limited and incompatibility between unrelated species within the genus *Eucalyptus* has been well documented. The experience with growing *Eucalyptus* in Brazil, as well as

my own direct experience, shows that the *Eucalyptus* species can NOT be considered invasive."

Bill Hammond, Forest Research at MeadWestvaco, comments that “the reproductive biology of these species varies but have several characteristics in common. The seeds are very small and wingless. The lack of wings means the seed is not effectively dispersed by the wind. The small size means the germinating seedling has very little carbohydrate reserves and all growing conditions must be favorable for its survival at the time of dispersal. That is, they require bare soil free from competing vegetation and constantly moist conditions as provided by heavy, daily fog. I have been in many stands of *Eucalyptus* in the Southeast that were old enough to be flowering and producing seed and in all my experience I have never seen a seedling that was not planted. There is no evidence of *Eucalyptus* escaping cultivation in this region. Likewise, in places I have visited and worked around the world, I have never seen planted *Eucalyptus* that had escaped cultivation or successfully displaced native vegetation.”

One commenter pointed out that blue gum, *Eucalyptus globulus*, found along the coast from Humboldt to San Diego and in the Central Valley of California is most invasive in coastal locations and easily invades native plant communities. Mr. Hammond points out and reiterates the information that was provided in the EA, that escape has occurred in selected locations such as in the fog belt of California with *E. globulus*. Notably, the seeds of *E. globulus* are more than twice the average size of the species in the permit. There has never been any escape with these species in climates similar to that of Alabama.”

Eucalyptus grandis has been grown commercially in Florida since the 1960s and there has been no evidence that the species has escaped from cultivation and has become invasive. There is no reason to believe that adding cold tolerance to this genetic background would increase the likelihood that the species would become invasive, especially given the fact that the other factors, such as the seed biology and germination characteristics, self-incompatibility issues and lack of appropriate soil and environmental conditions such as fog would be in place. APHIS continues to maintain that the plants being field tested are not invasive nor are any offspring that might be produced likely to be invasive. Other *Eucalyptus* species introduced as an exotic in other countries have rarely become invasive. *E. globulus* introduced into coastal areas of California is the exception more than the rule. Given the experience with the species being grown in this test both in Florida and in Brazil, simply being able to grow the plants in a colder environment in areas where it would not previously grow, will not impart invasive properties. It is highly unlikely that the field test will produce progeny that will become invasive in the area.

The above additional information taken together with the data already presented in the EA support APHIS' finding that the GE hybrid is unlikely to escape from the field test and become invasive.

Comment: One commenter states that "the EA claims that gene flow between field test trees and cold-tolerant *Eucalyptus* that may be growing in Alabama is unlikely because they are not closely related. The EA suggests that this is demonstrated by the grouping if [*sic*] the different species in different taxonomic sections. Although inter-sectional crosses are often less successful than inter-series crosses, as the EA notes, some inter-sectional crosses may nonetheless produce normal seedlings. In particular, species in some different sections in the subgenus *Symphyomytrus* often form successful crosses. This subgenus contains most of the cold-tolerant species noted in the EA (*E. cinerea, E. gunnii, E. neglecta, E. nova-angelica, and E. macarthurii*), which are in the section *Maidenaria*. Species in *Maidenaria* often can be successfully crossed with species in the section that contain the trees of the field test, *Transversaria*. For example, successful crosses between one of the parent species of the field test hybrid, *E. grandis*, and one of the cold-tolerant species, *E. gunnii*, have been accomplished. The progeny varied from low viability to vigorous. Therefore, contrary to the EA's assertion, there is a reasonable chance that cold-tolerant *Eucalyptus* species that may be grown in Alabama could form viable crosses with the field test trees, allowing gene flow to occur."

Response: The commenter fails to point out that crosses between sections are very difficult to make, and even hybrids made between genera within sections require that crosses be made by hand, seed harvested by hand, and grown under very controlled conditions. Even under controlled conditions most of the seeds produced are inviable, abnormal or have poor fitness. Based on the experience of many years of *Eucalyptus* breeders trying to produce hybrids and on the literature, APHIS believes that there are significant barriers to the formation of hybrids with any *Eucalyptus* that could be grown as an ornamental in Alabama. Potts and Dungey 2004 (as referenced in the EA) speak to the high degree of inviability in F_1 hybrids. Inviability of F_1 hybrids 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. While there is potential for natural hybridization between sections Transveraria and Maidenaria this was very rare, in less than 3% of possible combinations at the species level. Griffin (2000) also discussed the challenges of developing even human-made hybrids from such wide crosses (in this case *E. grandis* and *E. globulus* in sections Transveraria 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). Therefore APHIS concludes that the probability of successful hybridization with trees in the field test and any ornamental *Eucalyptus* trees that might be growing in the area is very low.

Comment: Commenters pointed out that APHIS failed to evaluate severe storm events that can occur in the coastal region of Alabama in the EA. They indicated that the Baldwin County, Alabama field trial site is prone to impacts from severe storm events such as tornadoes and hurricanes that could blow GE *Eucalyptus* seeds and vegetative

material over long distances.

Response: The Eucalypts in this field trial are unlikely to produce any seed for the following reasons: First, Eucalypts produce little if any seed from self-pollination. Second the genotype being tested is highly self-sterile. Third, this is a small field trial so the odds of a rare event occurring are that much less likely. In the unlikely event that seed was produced from the field trial, the resulting seeds are expected to produce slow-growing, sickly plants that would have difficulty establishing under optimum circumstances. The likelihood that such seeds would survive severe weather conditions is even less likely. Given the low probability of viable seeds forming that produce fit offspring that could survive dispersal by severe weather, APHIS concludes that it is not reasonably foreseeable that *Eucalyptus* seeds will be spread by severe wind events and establish outside of the field site. Don Rockwood, Professor in the School of Forest Resources and Conservation at the University of Florida, who has been involved in the field testing and production of *Eucalyptus* in Florida for many years points out that "*Eucalyptus* species have been commercially grown on some 20,000 acres in Florida since the 1960s and have not demonstrated any invasive characteristics." There have been no reports of escaped or feral *E. grandis* in Florida, where severe weather events also occur.

Comment: A number of commenters mentioned global warming. A standard comment indicated that "Global warming and climate change will allow more extensive southern and southeast regions of the U.S. to have weather patterns conducive to the introduction and propagation of escaped GE *Eucalyptus* hybrids, which APHIS failed to consider in the EA."

Response: This comment is highly speculative and provides no evidence to support the idea that global warming will provide weather patterns conducive to the introduction and propagation of escaped GE *Eucalyptus* hybrids. Florida and Brazil are already much warmer than the southeast U.S. and in neither area is *Eucalyptus grandis* an invasive species. Therefore, APHIS does not consider it reasonably foreseeable that an increase in temperature will overcome the numerous other barriers *Eucalyptus* has to spreading and establishing, namely the trees are unlikely to produce seed, the trees are unlikely to hybridize with any nearby species, any offspring are likely to be sickly, and *Eucalyptus grandis* has difficulty establishing in the wild.

Comment: There were numerous comments concerning the fact that *Eucalyptus* tends to burn very hot during forest fires and in regions where droughts occur, *Eucalyptus* trees are known to be at high risk of catching fire. The Southeast U.S. is currently in the midst of such a drought.

Response: APHIS does not consider a highly managed 1.1 acre test surrounded by agricultural fields to be a serious fire hazard.

Comment: Several commenters indicated that *Eucalyptus* plantations have been documented to deplete ground water and cause or exacerbate drought situations. None of these potential impacts were evaluated in the EA.

Response: At just 1.1 acres, this field trial represents a negligible fraction of the land area in Alabama. APHIS concludes it is not reasonably foreseeable that a field trial of this size could significantly deplete ground water.

Comment: There is a significant probability that other pests or pathogens associated with *Eucalyptus* will eventually be transported into the southeastern (SE) U.S.

Response: The field test has nothing to do with the probability that other pests or pathogens associated with *Eucalyptus* will eventually be transported into the SE U.S. The field test is only a little over 1 acre. *Eucalyptus* already exists in the U.S. in significant amounts in both California and Florida. Adding an additional acre to this amount has no impact on the probability for importation of pests or pathogens. In addition, APHIS has postentry quarantine requirements in place on the importation of *Eucalyptus* (see 7 CFR 319.37-7) specifically aimed at preventing the importation of pests and pathogens associated with *Eucalyptus*. These plants were brought into the U.S. as tissue culture plants after having been inspected in the country of origin and were grown under these postentry requirements after arrival.

Comment: A couple of commenters were concerned that ArborGen was charged with a non-compliance infraction and indicated that giving approval for a previous charge of noncompliance is a clear violation of its own regulation, if not federal law.

Response: Granting a permit to an institution that has a previous compliance infraction is not a violation of APHIS regulations or of Federal law. The problems noted in the past have been addressed and ArborGen has made all the changes in their field testing procedures as recommended by APHIS. APHIS has no reason to believe that the compliance record of ArborGen will compromise the safe execution of the field trial and put the environment at risk. The field test currently under Notification has been inspected by APHIS twice since it was planted and the company remains in full compliance.

Comment: One commenter indicated that "there are several varieties of *Eucalyptus* that are naturally cold-tolerant, at least eight of which could be grown in Southern U.S. states like Alabama. This field trial is not only risky, it is completely unnecessary."

Response: It is not the responsibility of APHIS to assess the necessity of the research being conducted, nor does the need for a particular field test relate to the environmental impacts that might result from that field test.

Comment: The field test was originally planted under APHIS Notification (05-256-03n) but that permit was for a different organism - *Eucalyptus grandis* -not the hybrid in the current application 06-325-111r.

Response: The regulated article under Notification 05-256-03n is the same (*E. grandis* x *E. urophylla*) that is in the permit submission. Some inconsistencies in listing species have arisen since the implementation of ePermits where applicants now enter most of the data rather than BRS staff. BRS is in the process of correcting these inconsistencies.

Comment: A couple of commenters were concerned that the genes and location were claimed as CBI. One commenter claims that APHIS makes frivolous use of Confidential Business Information designations to conceal crucial information for safety evaluation and the persistent regulatory bias towards the uncritical acceptance of GM crops.

Response: APHIS takes the use and designation of CBI very seriously. APHIS operates under the Trades Secrets Act. Information that is claimed as CBI by applicants is evaluated by APHIS to ensure that the claims are legitimate and APHIS evaluated ArborGen's claims for CBI protection in this instance. It would be a clear violation of the Act for APHIS to reveal Confidential Business Information to the public that legitimately falls under the Act. Federal employees can be fined or imprisoned or both and shall be removed from office or employment under Title 18 Crimes and Criminal Procedure, Part 1, Chapter 93 § 1905 of the U.S. Code if confidential information is made available to the public.

Comment: One commenter was concerned over the nature of the selectable marker that is being claimed as CBI. There was a concern that an herbicide-tolerance marker could reduce the ability to control escaped GE *Eucalyptus*, while resistance to an antibiotic like kanamycin would not. Use of a glyphosate-tolerance gene as a marker would carry even greater risk, because glyphosate is widely used to control invasive plants – especially in and near wetlands.

Response: The applicant's claim to keep the selectable marker CBI was evaluated by APHIS and deemed to be a legitimate business reason. Nonetheless APHIS can confirm that the selectable marker will not impact the ability of the agency to control the *Eucalyptus* in the unlikely event that it escapes from the field test.

Comment: One commenter cites a U.S. Forest Service risk assessment done to assess the potential risk of importing *Eucalyptus grandis* http://www.hear.org/Pier/wra/pacific/eucalyptus_grandis_htmlwra.htm. The commenter indicated that the USFS determined that it is an environmental weed, a congeneric weed, a host for recognized pests and pathogens, propagules are adapted to wind dispersal, and hybridizes naturally.

Response: The conclusions from the risk assessment cited were taken out of context by the commenter. An analysis of the risk assessment shows that this was an Australia / New Zealand Weed Risk Assessment adapted for Hawaii, not the mainland U.S. Climatic conditions are very different between Hawaii and the Southeastern U.S. In addition, the hybrids that are referenced in the document are between closely related species, such as *E. grandis* x *E. saligna*, not wide crosses. Interestingly the commenter failed to note that in the Forest Service risk assessment the following statement was

made: "*Eucalyptus grandis*, the fastest growing energywood species in Florida, is not invasive. It has been commercially planted since the 1960's at low density (600 trees/acre) in rotations of 8-12 years on approximately 15,000 acres in Florida with [out] [*sic*] any record of escape [not naturalized in Florida]." The assessment further indicates that *E. grandis* is a naturalized weed in South Africa, but again the climate of South Africa is very similar to that of Australia, where *Eucalyptus* is native, not that of the Southeastern U.S. Therefore APHIS concludes that this study provides no additional data that would alter its determination that the GE hybrids will not be invasive outside of the field test.

The host for recognized pests and pathogens category noted in the Forest Service Risk Assessment indicated that *E. grandis* is a host for *Puccinia psidii*, a tropical rust fungus. This fungus is a recognized pathogen by USDA (http://nt.ars-grin.gov/taxadescriptions/factsheets/index.cfm?thisapp=Pucciniapsidii) and was introduced into Florida in 1977 (http://pestalert.ifas.ufl.edu/tmm-0209.htm). As noted above, *Eucalyptus* must enter the U.S. under postentry quarantine. Imports of wood packaging, logs, and lumber involving tropical hardwood species (including *Eucalyptus*) into the U.S. must be debarked or fumigated (7CFR319.40-5). Imports of living plants are subject to inspection (7CFR319.37). Also as indicated above, the plants in this field test were introduced under postentry quarantine and entered the U.S. as in vitro tissue culture plants so they would not have been harboring *P. psidii.*

Comment: One commenter expressed concern that the *Eucalyptus* field test could be a source of *Cryptococcus neoformans gattii*. The commenter indicates that *C. neoformans gattii* is a very dangerous fungal pathogen that is hosted on a variety of species of *Eucalyptus*. It causes systemic fungal infections in humans, leading to fungal meningitis and death. Cases of the disease resulting from inhalation of spores, have been increasing in number and spreading geographically, likely due to import and export of *Eucalyptus* host species.

According to the commenter, *Cryptococcus 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 *Cryptococcus* in India, Africa, Taiwan, S. America and California. There was an outbreak of cryptococcal disease on the eastern portion of Vancouver Island, British Columbia in 1999. The disease was previously only known to occur in tropical or semi-tropical climates.

Response: APHIS is very familiar with *Cryptococcus neoformans gattii*. APHIS Veterinary Services Center for Emerging Issues prepared an Emerging Disease Notice in 2004 (APHIS, 2004) that covers the outbreak of the disease and reviewed what is known about the situation in British Columbia. Since the onset of the outbreak on Vancouver Island, *C. neoformans* var. *gattii* has been reclassified as a distinct species referred to as *C. gattii* (Meyer, Boekhout, Kwong-Chung, *et al.* 2003).

Only two of the 37 *Cryptococcus* species, *C. neoformans* and *C. gattii*¸ are considered to be major pathogens for animals and humans. *Cryptococcus neoformans* is widespread and has no specific association with *Eucalyptus* (Sorrell and Ellis, 1997). It is found in soil and is commonly spread through bird droppings (Baró, et al 1998).

Most often *C. gattii* has been found in tropical or subtropical regions; however, recent studies suggest that it is more widespread than originally thought. Notable among these studies are investigations of the occurrence of *C. gattii* on and around Vancouver Island in Canada (BCCDC, 2002; Kidd, et al, 2004). A comprehensive analysis of local environmental sources of *C. gattii* in Vancouver demonstrated its association with a variety of native tree species including elder, bitter cherry, cedar, Douglas-fir and Garry oak (Kidd, et al, 2004, 2007a, 2007b). Collectively, these trees represent species that grow over vast expanses of the Pacific Northwest (http://www.na.fs.fed.us/spfo/pubs/silvics_manual/volume_1/silvics_vol1.pdf; http://www.na.fs.fed.us/spfo/pubs/silvics_manual/volume_2/silvics_v2.pdf). The Vancouver studies did not detect *C. gattii* on any of the 23 local samples of *Eucalyptus* material in the Vancouver area (Bartlett *et al.* 2004).

C. gattii has been shown to be associated with four 'red gum' species of *Eucalyptus*, including *E. camaldulensis*, *E. tereticornis*, *E. rudis* and *E. gomphocephala* (Sorrell and Ellis, 1997). Halliday *et al.* (1999) also include *E. grandis* and *E. blakelyi* as additional *Eucalyptus* species for which *C. gattii* has been shown to have a specific ecological association, however citations for this statement were "unpublished data". Krockenberger *et al.* (2002) reported the low grade presence of *C. gattii* isolated from the base of a single dead *E. grandis* (flooded gum) and from *E. microcorys* in Australia. The authors note that "the species-specific importance of these findings should not be overstated because the isolations have been from single trees and could not be replicated in other members of the species (unpublished data)." Furthermore they note that their studies as well as others confirm "that hollows in *E. camaldulensis* trees remain the most reliable and abundant source of *C. n.* var. *gattii*." As cryptococcus has been associated with decaying wood from a wide range of species, including species other than *Eucalyptus*, APHIS does not believe that this single instance is compelling evidence of an ecological association between *C. gattii* and *E. grandis*.

C. gattii has not otherwise been found associated with *E. grandis* or *E. urophylla* or their hybrids. While there is a demonstrated association between *C. gattii* and 'red gum' *Eucalyptus* species, this is not an exclusive association: *C. gattii* has been detected on several native tree species in Canada. *C. gattii* has been isolated from a variety of other environmental sources, including bodies of fresh water and saltwater around Vancouver Island (Kidd et al. 2007b), driftwood in salt water (APHIS 2004), bat feces, insect frass, a wasp's nest, and on other substrates in endemic areas (Baro, Terres-Rodriguez, Mendoza *et al.* 1998). *C. gattii* has been isolated from wood debris from a limited number of *Eucalyptus* species and other tree species (cf. Table 1 in both Halliday et al. 1999 and Bartlett et al. 2004), and chipping and wood chips from infected trees of Douglas fir and/or red alder in British Columbia were found to release very high concentrations of *C. gatti* into the air (Kidd *et al.* 2007b). Cases of *C. gattii* infection have not been reported

in the U.S. other than in California (Chaturvedi et al. 2005), Washington, and Oregon (MacDougall et al. 2007). *C. gattii* is reported as being present in Hawaii (see http://www.cher.ubc.ca/Cryptococcus/new/faq.htm), though there have been no reported cases of *C. gattii* infection of humans in Hawaii. One of the outcomes of the APHIS Veterinary Services review was the conclusion that the "emergence of this "tropical" fungus and its ability to colonize on Vancouver Island stresses the importance of worldwide monitoring of its distribution." It further notes that "Particular focus should be given to those areas that have climactic and ecological attributes similar to eastern Vancouver Island." The notice does not propose monitoring for the pathogen in specific tree species.

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 (Kidd *et al*. 2004). If *C. gattii* spores were present in the tissue culture, contamination, would be evident and the affected lines would be discarded prior to regeneration of trees for introduction into the environment. Another reason it is unlikely that spores could be or were ever present in the hybrid lines used in the field trial is that in the *Eucalyptus* species where *C. gattii* is associated, the pathogen is primarily found colonizing the bark or decaying wood in hollows of older trees and the tissue culture was not derived from woody tissue nor was woody tissue generated during tissue culture. Because the trees were derived from tissues that are not known to be a source of the spores and were derived from sterile tissue culture lines that by all appearances were free from any fungal contamination, APHIS considers there to be a negligible risk that the hybrid trees used in the field trial could be or have been contaminated with *C. gattii*.

Another consideration is whether this field trial will result in a higher incidence of the fungus in the U.S. and thereby pose a risk to human or animal health. APHIS considers this risk 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 field trial is scheduled to be harvested when the trees are still young at about 7 years and in *Eucalyptus species* where there is an association with *C. gattii*, the fungus is associated with older trees. Fourth, the scale of the field test at just over an acre is miniscule compared to the vast expanses of native trees that have been shown to harbor the pathogen. For these reasons, APHIS does not consider that the field trial should lead to a higher incidence of *C. gattii* in the U.S. and therefore should not pose an unnecessary risk to human or animal health.

Comment: Several commenters suggested that there was insufficient scientific data and other information on which APHIS could base its evaluation of the environmental impacts that might result should the permit application be approved.

Response: APHIS disagrees: the information that is currently available is sufficient for APHIS to determine that this field test can be conducted without causing significant impacts to the human environment. This information has been discussed extensively in the EA and in APHIS' response to public comments. To summarize what is known:

- This is a small (1.1 acre) field test.
- The hybrid trees are not invasive.
- The hybrid trees to be tested are unlikely to produce significant numbers of viable seeds.
- Any seeds that may be produced are unlikely to move from the field test site.
- Any seeds that may be produced are unlikely to germinate and survive.
- Any seedlings that may survive are unlikely to be vigorous or to become invasive.
- APHIS requires that the field test site and surrounding fallow zone are monitored for seedling volunteers and any volunteers must be destroyed.
- There are no *Eucalyptus* plantings in the area, and the nearest plantings of significant size are over 100 miles away.
- The field test site will be surrounded by agricultural crops or native, non-*Eucalyptus* tree species.
- It is very unlikely that pollen from one of the field test trees could ever reach another *Eucalyptus* tree, but should it occur, it is extremely unlikely for pollination to occur that would result in the production of viable seed.

Therefore, there is no incomplete or unavailable information "essential to a reasoned choice among alternatives." (40 CFR 1502.22)

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USDA/APHIS Environmental Assessment

In response to permit application (06-325-111r) received from
ArborGen LLC for a field-test of genetically engineered
Eucalyptus grandis X Eucalyptus urophylla

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services

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I. INTRODUCTION

A. Summary

USDA/APHIS has prepared an environmental assessment in response to a permit application (APHIS Number 06-325-111r) received from ArborGen LLC (ArborGen), to continue a field test with genetically engineered (transgenic) *Eucalyptus* trees during which trees included in the test 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 three different constructs. The primary purpose of the test is to test for the effects of two of the constructs which are intended to confer cold tolerance and to test the efficacy of a gene designed to reduce flower development, the exact nature of which is claimed as confidential business information (CBI). In addition the trees have been engineered with a selectable marker gene, also claimed as CBI.

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

The field test was originally planted under an APHIS Notification (05-256-03n) on a site located in Baldwin County, Alabama on November 8, 2005. ArborGen has requested a permit for three years in order to evaluate the expression of the engineered traits.

The bases of confinement for these field tests are:

- The field test sites are located in Baldwin County, Alabama and are physically isolated from any sexually compatible *Eucalyptus*. *Eucalyptus* is not native to the United States and only a few ornamental species are planted in the southern States. None of these species are sexually compatible with the hybrid in this field test.

- There is little probability of asexual spread since this hybrid *Eucalyptus* does not propagate readily without the aid of special environmental conditions. None of the genes introduced into the transgenic trees are expected to affect asexual propagation.

- *Eucalyptus* seed is not adapted to wind dispersal so the dispersal of seed is expected to be limited to the proximity of the field test area.

- If any seeds were to be formed due to crossing within the field test, there is very little probability that they will germinate since *Eucalyptus* seeds have very limited stored food reserves, are intolerant of shade or weedy competition, and need contact with bare mineral soil to successfully germinate.

- If any viable seeds were to be produced and grow into seedlings, they will be easily identified by monitoring of the field sites and destroyed with herbicide treatment or removed by physical means.

- Horizontal movement of the introduced genes is extremely unlikely. The foreign DNA is stably integrated into the plant genome.

The field test is a controlled release of the regulated article into the environment. Procedures for termination of the field test should be sufficient to ensure that none of the transgenic *Eucalyptus* plants persist in the environment. The lack of any sexually compatible species and the inhospitable environment for seedling germination in the field test area make it unlikely that the introduced plants will move from the test area and persist in the environment. The proposed field test should not significantly impact plant or animal populations, including any species that are listed as threatened or endangered species in the test site county.

The APHIS review and analysis of the data indicate that the proposed field test should not present a risk of introduction and dissemination of a plant pest and should not have a significant impact on the quality of the human environment. Therefore, the proposed alternative for APHIS is to issue a permit with supplemental permit conditions.

B. Regulatory Authority

The authorities for regulation of genetically engineered *Eucalyptus* are the Plant Protection Act of 2000, 7 U.S.C. 7701-7772, and USDA–APHIS regulations under 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or Which There is Reason to Believe are Plant Pests." A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxonomic groups listed in the regulation and is also a plant pest, or if there is a reason to believe it is a plant pest. In this submission, the plants have been genetically engineered using disarmed *Agrobacterium tumefaciens*, which is one of the listed taxa in 7 CFR part 340.

This environmental assessment (EA) was conducted under the authority of the National Environmental Policy Act (NEPA), 42 U.S.C. 4321 and 7 CFR § 372, NEPA Implementing Procedures. Generally, issuance of a permit for field trials of regulated articles is categorically excluded from requirements for an environmental assessment (EA) under APHIS NEPA implementing procedures (7 C.F.R. Section 372.5(c)(3)(i)). However, when APHIS determines that a confined field release of genetically engineered organisms has the potential to affect significantly the quality of the human environment, as those terms are defined in 40 C.F.R. §§ 1508.27 and 1508.14, an environmental assessment or environmental impact statement will be prepared, pursuant to 7 C.F.R. § 372.5(d). This EA has been prepared because the permittee intends to allow the trees to grow under permit for a number of years and intends to let the trees flower. The actions described in the application for permit 06-325-111r involve the release of transgenic *Eucalyptus grandis X Eucalyptus urophylla* into the environment. Because the release of flowering Eucalyptus raises new issues, APHIS is preparing an Environmental Assessment.

II. NEED FOR THE PROPOSED ACTION

A. Proposed Action

The proposed action is for APHIS, Biotechnology Regulatory Services (BRS), to issue a permit to allow the continuation of a field-test involving a *Eucalyptus* hybrid clone engineered to express four genes. The genes are all claimed as CBI. Two of the genes are intended to confer increased tolerance to cold temperatures. The third gene is being evaluated for reduced flower development. The fourth gene is a commonly used selectable marker and is claimed as CBI.

B. Purpose of this Environmental Assessment

The purpose of this EA is to assess any potential adverse environmental impacts of a field research study being conducted on a research site in Baldwin County, Alabama. A permit application was received by APHIS–BRS on November 21, 2006 from ArborGen, in order to continue research on *Eucalyptus* hybrid trees originally planted under notification 05-256-03n. The permit application number is 06-325-111r.

C. Need for This Action

The Plant Protection Act directs the USDA to facilitate imports and interstate commerce in agricultural products in ways that will reduce, to the extent practicable, the risk of dissemination of plant pests. Under APHIS regulations, the APHIS Administrator has authority to regulate any organism or product altered or produced through genetic engineering that the Administrator determines is a plant pest or has reason to believe is a plant pest. When APHIS receives an application for a permit for environmental release, the application is evaluated to determine whether the environmental release, with appropriate conditions imposed, can be carried out while preventing the dissemination and establishment of plant pests. The receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

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

D. Purpose and Description of the Research

The focus of the research under this permit is on field evaluation of genes that can promote cold tolerance of *Eucalyptus*. The purpose of the research is to assess the efficacy of the introduced cold tolerance genes. According to the applicant, genetically engineered cold tolerant *Eucalyptus* would enable the production of this hardwood species for pulping and for biofuel applications in managed plantation forests in the southeastern U.S. In addition, the applicant is researching mechanisms for reduced flower development.

III. ALTERNATIVES

A. No Action

Under APHIS–BRS regulations, the Administrator must either grant or deny permits properly submitted under 7 CFR part 340. For the purposes of this Environmental Assessment, the No Action alternative would be the denial of permit application 06-325-111r.

These transgenic *Eucalyptus* trees were previously approved for planting in the fall of 2005 (Notification number 05-256-03n). These plants were planted at the field site with the condition that they not be allowed to flower. An EA was not done for this Notification (05-256-03n) because this non-flowering field trial met the criteria of the categorical exclusion clause in 7 CFR § 372.5 (c)(3)(ii). The applicant has now submitted a permit application for these trees outlining the conditions under which they will be allowed to flower. Under the No Action Alternative, if this permit is denied, the transgenic *Eucalyptus* plants currently released will be not be allowed to flower and the applicant will be required to either remove developing flowers or remove the trees from the field test.

B. Issue the Permit as Received

Issuing this permit would allow the research to proceed at the field site location in Baldwin County, Alabama under the conditions provided by the permittee and the standard permit conditions under 7 CFR § 340.4(f)1-11 (http://www.access.gpo.gov/nara/cfr/waisidx_05/7cfr340_05.html). Under this alternative, the field release of the genetically engineered *Eucalyptus* plants would be authorized at the specified location with no additional conditions imposed by APHIS–BRS.

The following redundant mitigation measures are incorporated into the field test design by the permittee to conduct a confined field release and to ensure minimal impacts to the environment:

a. The test site is located on secure private land and is expected to provide adequate physical security.
b. There are ornamental species of *Eucalyptus* planted in the state, but these are not sexually compatible with the trees in this field test.
c. In the planting area, there is a lack of bare mineral soils devoid of competition that *Eucalyptus* needs for germination and establishment.
d. *Eucalyptus* seeds lack dormancy, are very small and have very limited stored food reserves. If seeds were to be produced, they will germinate, if at all, soon after dispersal. Any seedlings that might happen to germinate will be discovered by monitoring and will be destroyed by rouging or herbicide treatment.

C. Issue Permit with Supplemental Conditions

The APHIS-preferred alternative is to issue the permit with supplemental permit conditions for the requested three-year period. The permit will need to be renewed to allow the transgenic plants to remain in the ground beyond this time period. Under this alternative, APHIS would issue the permit to allow the research to proceed at the field test site in Baldwin County, Alabama with supplemental permit conditions based on APHIS scientific analysis of the permit application, input from the State of Alabama, and public comment from this environmental assessment. If warranted,

based on the environmental risk of escape of the engineered organism, APHIS will require further mitigating measures and monitoring to prevent spread of the organism outside the field production area.

Currently APHIS proposes to include the following measures to conduct a confined field release and to ensure no significant harm to the environment:

a. Trees in the field test sites will be monitored for flowering and seed formation. Data will be provided to APHIS in an annual report documenting which trees produced flowers and which, if any, produced viable seeds.
b. The field test sites will be monitored for volunteer seedlings. Any volunteers found will be sprayed with herbicide or physically removed. The presence and elimination of any volunteers will be reported to APHIS in an annual report.
c. All non-engineered control *Eucalyptus* trees in the field test plot and any plant material removed from the field site will be treated as regulated articles, i.e., with respect to monitoring for flowering and volunteers, maintaining the identity while in use, and devitalization when no longer in use.

IV. ENVIRONMENTAL CONSEQUENCES OF THE PROPOSED ACTION AND ALTERNATIVE

A. Deny the Permit

To deny the permit application would have no expected potential adverse environmental impact, would prevent the field research from proceeding, and would prevent any benefits associated with the knowledge gained from this research study.

B. Issuance of the Permit as Received

The proposed action is not expected to have any adverse environmental impacts for the following biological and physical reasons:

1. No adverse consequences to non-target organisms or environmental quality are expected from the field release of these transgenic *Eucalyptus* for the reasons stated below.
2. The proteins produced by genes introduced into these *Eucalyptus* lines are not expected to have toxicological or allergenic properties.
3. Since *Eucalyptus* is not native to the southeastern United States and due to the confinement conditions imposed on the test, there is a very low probability that the engineered *Eucalyptus* will become established in the environment.
4. The trees were transformed in New Zealand and imported into the United States under permit 05-072-03m to South Carolina. *Eucalyptus* is subject to a period of post-entry quarantine when it is imported into the United States. All materials are handled in accordance with the USDA–APHIS requirements for import and quarantine. The Baldwin County site operates under USDA–APHIS PPQ Post-entry quarantine permit # 37-88316.

C. Issuance of the Permit with Additional Conditions

Under this APHIS-preferred alternative, APHIS will authorize the permit for three years and impose additional measures and monitoring included in the proposed supplemental permit conditions summarized in III. C. above and in detail in Appendix III to further ensure that the field test remains confined and there will be no significant harm to the environment. This alternative is not expected to have any adverse environmental impacts for the same biological and physical reasons as indicated above for issuance of the permit as received.

The proposed monitoring and annual reporting of a) flowering, b) seed formation, and c) the presence and devitalization of volunteers will allow APHIS to assess whether additional monitoring is required, whether the monitoring area should be extended, and whether devitalization methods should be modified in the event that the permit is renewed for a longer duration. In addition to monitoring required by the applicant, all field tests are subject to inspection by APHIS as a standard permit condition.

A person who is issued a permit and his/her employees or agents shall comply with standard permit conditions under 7 CFR § 340.4(f)1-11 http://www.access.gpo.gov/nara/cfr/waisidx_05/7cfr340_05.html and any supplemental conditions (Appendix III) which shall be listed on the permit, as deemed by the Deputy Administrator to be necessary to prevent the dissemination and establishment of plant pests 7 CFR § 340.4(f)

D. Potential Environmental Impact of the Research Using Transgenic Eucalyptus

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. It has not escaped from cultivation in the southeastern United States.

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

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://portal.cal-ipc.org/weedlist). Neither of these species is being proposed to be planted at the permitted field site. The species that ArborGen wishes to allow to flower under this permit have not been categorized as invasive and if engineered with cold tolerance, would be unlikely to become invasive in the southeastern United States.

The applicant anticipates that the trees in this field test will begin to develop flowers during the early summer of 2007. Flower development in *Eucalyptus* typically occurs over several months, and it is anticipated that mature flowers could be observed in this trial beginning in late June or early July of 2007.

1. Possibility of Gene Flow Outside of the Field Test:

The field test consists of 355 transgenic test trees plus 155 non-transgenic trees of clone EH1 (including controls, borders and fillers) on approximately 1.1 acres. It is located in Baldwin County, Alabama. For details on the experimental design see Appendix I.

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. The field trial area at this site is surrounded by research plots of agricultural crops including wheat, corn, cotton and soybeans or other farm crops, a pecan orchard and native tree species. There are no *Eucalyptus* plantings in the surrounding area; the nearest *Eucalyptus* plantings of any significance are over three hundred miles away in the state of Florida. These plantings are primarily *E. grandis* and *E. amplifolia*. It is therefore anticipated that the escape of the regulated article via pollen flow to compatible species is highly unlikely.

There are several species of cold-hardy *Eucalyptus* that can possibly be grown in the Southeast U.S. including the state of Alabama. These species include *E. neglecta, E .niphophila, E. pauciflora, E. camphora, E. nova-anglica, E. macarthurii, E. gunnii* and *E. cinerea*. 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. Several factors, described in the following paragraphs, contribute to the low likelihood that pollen gene flow will occur between the transgenic trees and any of these cold hardy *Eucalyptus*.

First, there are no known *Eucalyptus* trees in the area. The applicant has scouted 500 meters from the field test site and none were found. The USDA plants database (http://plants.usda.gov) does not indicate any significant plantings of these or any other *Eucalyptus* species in Alabama.

Second, the transgenic hybrids are not likely to be sexually compatible with any of the cold hardy species listed above. It is well documented that natural cross-compatibility between different species of eucalypts is limited to closely related series within distinct sections of the *Eucalyptus* genus. For example, *E. grandis* and *E. urophylla*, for which hybrids have been generated in directed breeding programs, are in the Salignae and Resiniferae series, respectively, of section Transversaria (http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/eucclass.pl?gn=Eucalyptus). In contrast, *E. cinerea* and other cold hardy species mentioned above are far removed genetically from the genotype used in this field trial on the evolutionary scale and reside within different Series and Sections of genus *Eucalyptus* (see http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/eucclass.pl?gn=Eucalyptus for details on sections and series in Eucalyptus). Even among the closely related species of *Eucalyptus*, hybridization rates are generally very low (Volker 1995). The published literature supports the fact that natural hybridization among distantly related species within genus *Eucalyptus* is rare and hybrid inviability increases with increasing taxonomic distance between parents (Potts and Dungey 2004). Where hybridization is possible, it often requires significant human intervention in directed breeding/crossing efforts.

Third, 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 clones being tested beings to initiate in early summer of the second year after planting.

Based on the above information, there is little if any significant risk for outcrossing to or from other *Eucalyptus* species because: 1) there are no significant plantings of compatible species close to the site; 2) ornamental species that 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 unlikely event that they could form, would be expected to be of very poor vigor. In addition, as discussed below, the poor competitiveness of *Eucalyptus* seed presents a further limitation for any potential off-site gene flow.

2. Possibility of Crossing within the Field Test:

There are two additional field trials of *Eucalyptus* that were established at the site in 2006 (under Notification numbers 06-135-01n and 06-150-02n), and it is possible that additional plots could be established at this site in the future. All of these test plots, including control non-transgenic trees, have the same parental genotype. 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 within and between test plots.

Seed set from any self pollination is expected to be very poor, and further, the vigor of any selfed progeny is also expected to be greatly reduced. In preliminary experiments conducted in Brazil, the control self-pollinated seed obtained from this genotype had abnormal morphology and failed to germinate (ArborGen, unpublished results). In the unlikely possibility that seed could be produced in the test, several factors in the biology of *Eucalyptus* should be considered which 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 very little possibility that volunteer seedlings could become established in any unmanaged areas that may be close to the site.

Eucalyptus seeds do not have any dormancy barriers to prevent germination of volunteer seeds (Grose 1960, Wellington 1989, Gill 1997) and seed viability and storage of *Eucalyptus* seeds in soil are less than one year (Gill 1997). The *Eucalyptus* species that have become invasive in California are particularly adapted to a Mediterranean climate subject to summer fog, which is conducive to seed germination in those species (http://ucce.ucdavis.edu/datastore/detailreport.cfm?usernumber=48&surveynumber=182). This type of climate does not exist in the Gulf coast area of Alabama. In the unlikely event that any viable seeds are produced, these seeds would be expected to germinate within a short period. The bordering fields within 100 meters from the edge of the trial will be monitored every six months for germinating seedlings. This distance is twice the 50 meter distance that seeds would be expected to be dispersed from a tree at harvest age. If transgenic seedlings are observed they will be destroyed either by uprooting or by spraying with herbicides (e.g., glyphosate or other herbicides to which these trees are susceptible).

3. Possibility of Vegetative Propagation / Persistence Outside of the field test.

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

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

4. Horizontal Gene Transfer to Other Organisms

Horizontal gene transfer and expression of DNA from these plant species to bacteria is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko et al. 2000, Wood et al.

2001, Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al. 2001, Brown 2003). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic (i.e., bacterial) expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, many common transgenes used in plant biotechnology are derived from bacteria commonly found in the environment. The FDA has evaluated horizontal gene transfer from the use of selectable marker genes and concluded that the likelihood of transfer of such genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (http://vm.cfsan.fda.gov/~dms/opa-armg.html). Therefore APHIS concludes that horizontal gene transfer poses no significant environmental risk.

5. Fate of Transgenic DNA in Humans and Animals

The permittee has taken steps to reduce animal access to the transgenic and recipient plots, and there is no intention to use the transgenic *Eucalyptus* for animal feed. Therefore the information presented in this section addresses the unlikely event of accidental consumption by browsing animals.

Transgenic DNA is no different from other DNA consumed as part of the normal diet. Genetically engineered organisms have been used in drug production and microbial fermentation (cheese and yogurt) since the late 1970's. More than 1.4 billion cumulative acres of engineered food and feed crops have been grown and consumed world wide in the past seven years (International Service for the Acquisition of Agri-biotech Applications, (ISAAA) at: http://www.isaaa.org/resources/publications/briefs/35/executivesummary/default.html. The FDA has not reported any significant concerns with bioengineered food and feed currently on the market. The EPA has exempted from a tolerance DNA that encodes currently registered plant incorporated protectants because of a lack of toxicity (FR 66 37817-37830).

There have been several studies in humans and animals following the fate of DNA once consumed (Mercer et al. 1999, Beever and Kemp 2000, Duggan et al. 2000, Einspanier et al. 2001, Chambers et al. 2002, Netherwood et al. 2002, Duggan et al. 2003). The majority of DNA consumed is degraded in the gastrointestinal tract although the degradation is not 100% efficient. There is evidence that DNA from consumed food can move from the GI tract lumen to other areas of the body and that this is a normal occurrence. No risks have been identified as a result of this movement.

6. Risk of the Gene products on the Environment

Gene used as selectable marker

The gene used as a selectable marker is claimed as CBI. In a number of instances, plants transformed with this gene have been deregulated by APHIS. Consequently, APHIS has determined the presence of this gene will have no significant environmental impacts.

Genes conferring cold tolerance

The genes conferring cold tolerance are not expected to produce any toxic substances and are not expected to alter the weediness characteristics of the engineered plants (as described in IV.D.7). Therefore APHIS has determined the presence of this gene will have no significant environmental impacts.

Gene for reduced flower development

This gene has been engineered into other crops that have been previously reviewed and addressed in multiple environmental assessments by APHIS. There is no reason to believe that the function and expression of this gene will be any different from the plants in which it has been previously assessed. There were no toxicity or allergenicity issues found with this gene in previous APHIS reviews. 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. Therefore APHIS has determined the presence of this gene will have no significant environmental impacts.

Non-coding sequences.

The transgenic *Eucalyptus* also contains non-coding regulatory sequences 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.

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 Klee and Rogers 1989; Zambryski 1988). *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.

7. Alteration in Weediness characteristics

None of the genes introduced into *Eucalyptus* code for traits that would be expected to make the plants more weedy or invasive. The genes introduced to affect cold tolerance could make the engineered *Eucalyptus* more adapted to cold temperatures in the southern United States, but this trait in and of itself would not impart invasive or weediness characteristics to the engineered plants because as mentioned in section IV.D.2.and 3., the species of *Eucalyptus* at issue in this permit has difficulty establishing without human intervention even in warmer climates. The cold-tolerance genes are not expected to affect the reproductive biology such as seed production or vegetative reproduction capabilities. The selectable marker gene, when used previously, did not contribute to

weediness or invasive properties of the genetically engineered plants. APHIS also concludes that the gene for reduced flower development should not contribute to weediness or invasive properties and could reduce the ability of the tree to produce progeny. None of the traits introduced into the transgenic *Eucalyptus* will compromise the ability to control these plants as weeds.

8. Alteration in Susceptibility to Disease or Insects

There has been no intentional genetic change in these plants to affect their susceptibility to disease or insect damage. The permittee has observed no changes in the incidence of pests, beneficial insects or pathogens between the transgenic and non-transgenic controls in the existing field tests. 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.

Execution of the prescribed 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 under Notification.

9. Effects on Native Floral and Faunal Communities

a. Native Floral Communities

The field site in the permit application is located in Baldwin County, Alabama. It is a mixture of crop lands and forested areas. These areas are unsuitable for the establishment of the *Eucalyptus* hybrid clone in this permit. *Eucalyptus* is intolerant of shade or weedy competition. In order to successfully germinate and establish, *Eucalyptus* seed need contact with bare mineral soil and the removal of competing plants, either as a result of human intervention or naturally following a fire event. With the exception of the field test area, the agricultural areas surrounding the field site 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 lands nearby are frequently tilled and cultivated. The plantations will be cultivated and weeds controlled by herbicides.

The inhospitable conditions for seed germination, in combination with the confinement conditions imposed by the permittee and APHIS, will successfully limit the establishment of any of these species in the surrounding area. Therefore APHIS concludes there would be no significant effect on any native floral species.

b. Terrestrial Animals

The most likely animals to encounter the transgenic *Eucalyptus* trees in this field experiment would be browsing mammals (e.g., deer), burrowing animals (such as rodents), and leaf consuming insects (considered plant pests). The browsing by deer should be minimized since the test site is fenced to discourage deer. In the unlikely event of accidental 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. Therefore APHIS concludes there would be no significant effect on any native vertebrate or invertebrate animal species.

c. Aquatic Organisms

The closest body of water is a tidal creek within about a half mile from the field site. As noted above, seed is not expected to disperse more than 50 meters from a mature *Eucalyptus* tree, and thus would not be expected to establish in aquatic environments away from the field trial. Furthermore, as stated above, there is no expectation of toxicological effects on any organism due to the ingestion of the transgenic plant material in this study. APHIS therefore concludes there would be no significant effect on any aquatic species.

10. Risks to Threatened and Endangered Species

APHIS has reached a determination that the proposed environmental release will have no effect on federally listed threatened or endangered species or species proposed for listing, and no effect on designated critical habitat or habitat proposed for designation in the action area. Consequently, consultation under Section 7 of the Endangered Species Act with the United States Fish and Wildlife Service is not required for the action described in the preferred alternative of this EA. Appendix II includes the BRS analysis of threatened and endangered species in the area of the field release.

11. Cumulative impacts

The applicant has grown these trees under Notifications since October, 2005 and wishes to grow these an additional 3 years under permit. Prior to the establishment of this field test the site was used as an experimental farm for agricultural crops and forest trees for over ten years. It is reasonably foreseeable that the applicant may request to further extend the permit for this environmental release for additional years to observe the growth of these trees to maturity. The temporary change from agricultural crops to a tree crop may result in a temporary change in resident animal and plant species, but after harvest it is reasonably foreseeable that the land will return to agriculture or be replanted to tree research. The only past, present, and reasonably foreseeable actions associated with the location for the proposed release are those related to agricultural production. Because the proposed field test will have no significant effects on the human environment, 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 site or the ecosystem in which it is situated. No resources will be significantly impacted due to cumulative impacts resulting from the proposed action.

Considering the organism and traits introduced, the limited duration of the trial, and the manner in which the trial must be conducted, the size and location of the proposed field releases are unlikely to impact the capacity of the release to significantly affect the quality of the human environment.

12. Impact on Existing Agricultural Practices

This small field test will not have any significant impact on existing agricultural practices because this test is solely for research purposes. It is located in an area specifically allocated and designed for field testing crop plants and forest trees.

13. Potential Impacts on Humans, Including Minorities, Low Income Populations, and Children

Because the field test is on an isolated property controlled by the permittee's contractor, the public will not be exposed to these transgenic plants. The trees will remain in the ground for several years and will be isolated from the public. When the test is terminated the trees will be cut down and chipped on site. Herbicide applications will be used to control volunteers from stump sprouts. None of the regulated material will leave the test site other than as samples taken to the laboratory for analysis. All the harvested material will be stored in dedicated storage containers on site and transferred to a laboratory setting for analysis.

Consideration of these potential impacts are specified in Executive Orders 13045 and 12898 and address the identification of health or safety risks that might disproportionately affect children or have adverse impacts on minorities and low-income populations. The proposed actions are not expected to adversely affect any of these groups.

14. 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.C § 4321 et seq.); (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR §§ 1500-1508); (3) USDA regulations implementing NEPA (7 CFR § 1b); and (4) APHIS NEPA Implementing Procedures (7 CFR part 372).

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APPENDIX I: Description of the Field Experiments

The regulated field trial is planted in a randomized complete block design with 8 replicated blocks. Each block consists of single tree plots representing a single ramet (vegetatively propagated tree) for each line of transgenic cold tolerant *Eucalyptus* and gene to reduce flower development, transgenic marker gene control lines and non-transgenic control Eucalyptus trees of the same genotype (EH1) used for transformation. The test plot is surrounded by a single border row of non-transgenic *Eucalyptus* trees on all four sides. The trees are planted at 10x6 ft spacing. There are currently 355 transgenic test trees plus 155 non-transgenic EH1 trees (including controls, borders and fillers) on approximately 1.1 acres. All required silvicultural practices are applied to the test plot for fertilization and weed control. The plants in the trial are being observed regularly for general growth and vigor. Quantitative data have been and will continue to be recorded periodically on growth (height, diameter, tree form), as well as data on winter damage (estimated branch/tip dieback, recovery/regrowth after freeze damage) and on the efficacy of the gene aimed to reduce flower development.

APPENDIX II: Threatened and Endangered Species Analysis

Action Area

The regulated article is a clone of a *Eucalyptus* hybrid (*Eucalyptus grandis* x *Eucalyptus urophylla)*. The proposed field trial is located in Baldwin County, Alabama. The total area of the test plot is approximately 1.1 acres with about 510 trees, including non-transgenic controls and borders, planted in 8 replicated blocks.

Pollination and prevention measures for the escape of the regulated article

The escape of the regulated article via pollen flow to compatible species is highly unlikely because 1) the potential for pollen dispersal is limited in space to a relatively short distance (about 100 meters) while the nearest *Eucalyptus* plantings of any significance are over a hundred miles away from the action area; 2) other cold-hardy *Eucalyptus* species that could possibly be grown in the action area are unlikely to be compatible; 3) it is unlikely that flowering time in these other species (spring flowering) will overlap with the hybrid used in the test (flowering in mid to late summer); 4) viable hybrids (if any) as well as seeds set from any self-pollination would be of a very poor vigor, and would likely not germinate; and 5) *Eucalyptus* seed is very small (with limited reserves) and not adapted to wind dispersal and weed competition.

In the unlikely event that any viable seed would be produced, any transgenic seedlings that might germinate will be destroyed by the permittee using methods such as uprooting and/or treatment with registered herbicides. Preventative measures consist of regular monitoring of the field site and nearby fields within a 100 meter range.

Federal TES within the Action Area

The USFWS websites was accessed on 12/20/06 to analyze the TES for Baldwin County, Alabama. There are 79 animal species and 17 plant species identified as threatened and endangered in the state of Alabama (USFWS[1]) of which 18 TES animals and two TES plants are identified in Baldwin County (USFWS[2]). These include:

Animals

- Mouse, Alabama (*Peromyscus polionotus ammobates*)
- Mouse, Perdido (*Peromyscus polionotus trissyllepsis*)
- Plover, piping (*Charadrius melodus*)
- Least tern (*Sterna antillarum*)
- Stork, wood (*Mycteria americana*)
- Eagle, bald (*Haliaeetus leucocephalus*)
- Woodpecker, red-cockaded (*Picoides borealis*)
- Flatwoods salamander (*Ambystoma cingulatum*)
- Snake, eastern indigo (*Drymarchon corais couperi*)
- West Indian manatee (*Trichechus manatus*)

- Sea turtle, loggerhead (*Caretta caretta*)
- Turtle, Alabama red-belly (*Pseudemys alabamensis*)
- Sea turtle, green (*Chelonia mydas*)
- Sea turtle, Kemp's ridley (*Lepidochelys kempii*)
- Sturgeon, Alabama (*Scaphirhynchus suttkusi*)
- Sturgeon, gulf (*Acipenser oxyrinchus desotoi*)
- Heelsplitter, Alabama inflated (*Potamilus inflatus*)
- Pigtoe, heavy (*Pleurobema taitianum*)

Plants

- Chaffseed, American (*Schwalbea americana*)
- Panhandle lily (*Lillium iridollae*)

Analysis of the TES animals

Species of concern and types of habitats used

The first two mouse species listed above (*Alabama Beach mouse* and *Perdido Key Beach mouse*) 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 *Eucalyptus* trial (distance estimated from the USFWS MAPPER). The shorebirds *piping plover* and *least tern* are known to use sparsely vegetated dunes and coastal beaches in southern Baldwin County, also far away from the field site where the regulated article site is located (about 60 miles). The *wood stork* primarily inhabits wetland systems notably cypress or mangrove swamps while the *bald eagle* would preferably utilize inland waterways and estuarine areas nesting and roosts near large water bodies such as lakes, marshes, seacoasts and rivers. It is unlikely that the *Eucalyptus* trial will interfere with these species' natural habitats. The *red-cockaded woodpecker* could potentially use the *Eucalyptus* plantations but this bird has a tendency to prefer mature pine forests instead. Similarly, the *Flatwoods salamander* uses the wet pine flat-woods associated with vernal pools. Even though the *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[3]). From the list above, the remaining TES of concern (*West Indian manatee, sturgeons, mussels,* and *sea turtles*) are adapted to aquatic habitats, and their habitat systems (bays, lagoons, salt marshes, creeks, ship channels, and other freshwater environments) would be unlikely to overlap with the proposed transgenic *Eucalyptus* trial. The closest body of water is more than 100 meters from the trial site, and plants from this trial are not expected to disperse or establish beyond this distance.

Critical habitats

It appears that most of the TES animals within the action area use inshore or wetland systems most of which are concentrated essentially in the southern and southeastern coastal beaches of Baldwin County. There is no designated critical habitat in the immediate environment of the *Eucalyptus* trials. The closest critical habitat is about 65 miles away. As stated above it is extremely unlikely

that any *Eucalyptus* seeds would escape from the field planting and become established in the critical habitats for these species.

Analysis of the TES plants

Species of concern and Critical habitats status

Only two plant species are listed as TES in Baldwin County, AL. The *American chaffseed* generally occurs in seasonally moist to dry sandy and acidic soil, within open grass-sedge systems while the *panhandle lily* usually thrives in poorly drained soil along streams and bays. Although listed as TES, both plants have no critical habitats listed in the action area.

Toxin production

The transgenic modification of the regulated article is not intended and is unlikely to result in the production, or increase the production, of a toxin, natural toxicant, allelochemical, pheromone, hormone, etc. that could result in killing or interfering with the normal growth, development, or behavior of a federally listed TES analyzed above.

Summary and Conclusions

The analysis for the permit planting location indicates that about 23% of all TES identified in the state of Alabama reside in Baldwin County, of which none has been listed as having a critical habitat in an immediate environment of the transgenic *Eucalyptus* trials. The proposed action area does not include, or interfere with, the critical habitats of the TES of concern. APHIS has reached a determination of no effect on TES and no adverse modification of designated critical habitat for the following reasons: 1) the transgenic *Eucalyptus* trees are not sexually compatible with any threatened or endangered plant species in the action area; 2) no TES plants are located in habitat that would be disturbed or otherwise affected as a result of the conduct of the trial and no critical habitat is present in that location; 3) none of the TES animal species utilizes *Eucalyptus* plantations as a feeding area, cover, or nesting site; and 4) the transgenic modification is not intended to result in the production of any chemical that could directly or indirectly result in killing or interfering with the normal growth, development, or behavior of a TES in the action area.

APHIS has reached a determination that the proposed environmental release will have no effect on federally listed threatened or endangered species or species proposed for listing, and no effect on designated critical habitat or habitat proposed for designation in the action area. Consequently, consultation under Section 7 of the Endangered Species Act with the United States Fish and Wildlife Service is not required for the action described in the preferred alternative of this EA.

Literature cited (websites accessed on 12/20/06)

USFWS MAPPER: *http://criticalhabitat.fws.gov/*
USFWS[1]: *http://ecos.fws.gov/tess_public/StateListingAndOccurrence.do?state=AL*
USFWS[2]: http://www.fws.gov/daphne/es/specieslst.html#Baldwin

US Forest Services[3]:
http://www.forestry.state.al.us/publication/TF_publications/tffall00/threatened_and_endangered_species.pdf

APPENDIX III: Supplemental Permit Conditions for Permit 06-325-111r

1. Please note that transportation of all test and plant materials to and from the field test site must be done in accordance with APHIS/USDA regulations outlined in "Container requirements for the movement of regulated articles", 7CFR340.8(b)(I & ii) unless a shipping container variance has been approved by APHIS-BRS.

2. 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 sites, 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 147, Riverdale, Maryland 20737.

3. 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).

4. 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 chemicals; and (4) food or feed use of genetically engineered crops harvested from the field experiment.

5. APHIS/BRS and/or APHIS/PPQ personnel may conduct inspections of the test site, facilities, and/or records at any time.

6. 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.

7. 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 no that no shoots are continuing to be produced.

8. All non-engineered control trees in the field test plot and any plant material removed from the field site will be treated as regulated articles.

9. Reporting Unauthorized Releases: 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 oral notification, contact APHIS/BRS Compliance Staff at (301) 734-5690 and ask to speak to a Compliance and Inspection staff member.
- In the event of an emergency and you are unable to reach the BRS Compliance Staff at the above number, you may call:

The APHIS/BRS Regional Biotechnology Coordinator assigned to the state, where the field test occurs:

For Western Region, contact Ralph Stoaks by phone at (970) 494-7573 or e-mail Ralph.D.Stoaks@aphis.usda.gov

Or

The APHIS/PPQ Regional Biotechnology Coordinator assigned to the state where the field test occurs:

For Western Region, contact Stacy Scott by phone at 970-494-7577 or e-mail Stacy.E.Scott@aphis.usda.gov

Or

The APHIS State Plant Health Director for the state where the field test occurs. The list of APHIS State Plant Health Director is available at http://ceris.purdue.edu/napis/names/sphdXstate.html.

10. Reporting Unintended Effects:

According to the regulation in 7 CFR § 340.4(f)(10)(ii), APHIS shall be notified in writing as soon as possible but within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the permit application or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

Written notification should be sent by one of the following means:

By e-mail:
BRSCompliance@aphis.usda.gov

By mail:
Biotechnology Regulatory Services (BRS)
Compliance and Inspection Branch
USDA/APHIS
4700 River Rd. Unit 147
Riverdale, MD 20737

11. Reports and Notices:

Send notices and all reports (CBI and CBI-deleted or non-CBI copies) to BRS by e-mail, mail, or fax.

BRS E-mail:
BRSCompliance@aphis.usda.gov

BRS Mail:
Animal and Plant Health Inspection Service (APHIS)
Biotechnology Regulatory Services (BRS)
Compliance and Inspection Branch
4700 River Rd. Unit 147
Riverdale, MD 20737

BRS Fax, Compliance and Inspection Branch:
(301) 734-8669

The following reports are required:

a. Activity Report

Within 28 calendar days after planting, submit a report, in paper format or electronically, that includes the following information for each field test site:

i. Permit number;
ii. Regulated article;
iii. Release site [provide state, county, internal identification number (if available), and either a single GPS coordinate as a reference point (center of plot or specify corner) or specific address];
iv. Approximate number of seeds or plants planted per construct (transformation code);
v. Total acreage of regulated articles planted and border rows;
vi. The actual planting date

b. Annual Report

Each year during the permitted field trial an Annual Report must be submitted to BRS. The submission of an annual report is due 60 days prior to the anniversary of the effective date. The annual report shall include the APHIS reference number for the permit, site location information, a map of the plantings, an inventory of what is in the ground (number of individuals or acres of each species/trait combination, date of planting, list of line numbers), a list of plants (genotype and quantity) that were removed during the previous year, the method of disposition of the plants that were removed, and a narrative of unanticipated or adverse events on plants, nontarget organisms, or the environment.

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

c. Final Field Test Report

Within 6 months after the expiration date of the permit, the permittee is required to submit a Field Test Report.

Field Test Reports shall include:

i. Constructs and specific transformed lines (event) planted;
ii. Planting and harvest dates;
iii. Total acreage of the test;
iv. The methods of observation;
v. The resulting data and analysis regarding all deleterious effects on plants, non-target organisms, or the environment. This should include, but not be limited to, data on insect damage, disease susceptibility, gross morphology and any indications of weediness; and
vi. A table with the following information for each line and gene released.

The disposition table should contain the following information: site name (or GPS), crop, gene, harvest date, and disposition of harvested material. The disposition table is a formal record of how the regulated material was removed from the environment. An accounting of the harvested material should be provided with regards to what material is harvested, how much material is harvested per site, what is done to devitalize residual and harvested material at the site, where the harvested material is transported, stored and further processed up to the time it is taken to a contained facility.

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. Final Monitoring Report

The report must include:

i. Dates when the field site and perimeter 100 meter zone were inspected for volunteers;
ii. Number of volunteers observed; and
iii. Any actions taken to remove or destroy volunteers.

This report may be included as part of the Field Test Report submitted within 6 months of the expiration date of the permit. The final monitoring report is then due no later than 3 months from the end of the volunteer monitoring period.