ArborGen Inc. Petition (11-019-01p) for Determination of Non-regulated Status of *Eucalyptus* Hybrid Freeze Tolerant Events FTE427 and FTE435

Plant Pest Risk Assessment

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A. Introduction

ArborGen Inc. (referred to hereinafter as ArborGen) has petitioned APHIS (APHIS number 11-019-01p) for a determination that genetically engineered (GE) *Eucalyptus* hybrid (*E. grandis x E. urophylla*) freeze tolerant events FTE427 and FTE435 are unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under APHIS' 7 Code of Federal Regulations (CFR) part 340. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act of 2000[1]. This plant pest risk assessment was conducted to determine if FTE427 and FTE435 are unlikely to pose a plant pest risk.

FTE427 and FTE435 were produced by transformation of *Eucalyptus* tissue using *Agrobacterium tumefaciens.* Because *A. tumefaciens* is a plant pest, and some of the regulatory sequences used to facilitate expression of these genes were also derived from *A. tumefaciens*, these *Eucalyptus* lines have been considered a regulated article under APHIS regulations at 7 CFR part 340.

Potential impacts to be addressed in this risk assessment are those that pertain to the use of FTE427 and FTE435 and their progeny in the absence of confinement. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if these lines are unlikely to pose a plant pest risk. If APHIS determines that a GE organism is unlikely to pose a plant pest risk, then APHIS has no regulatory authority over that organism.
Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk characteristics. The information for consideration of a petition for nonregulated status can be found in APHIS regulation 7 CFR 340.6(c). APHIS will evaluate information submitted by the applicant related to plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, potential weediness of the regulated article, any impacts on the weediness of any other plant with which it can interbreed, changes to agricultural or cultivation practices that may impact diseases and pests of plants, potential effects on non-target organisms, and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology' (51 FR 23302, June 26, 1986). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with the APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies. The EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), regulates the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. The EPA also sets tolerance limits for residues of pesticides on and in food and

[1] Section 403 (14) of the Plant Protection Act (7USC Sec 7702(14)) defines plant pest as:
"Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA). The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its voluntary consultation process. Because the traits engineered into the *Eucalyptus* trees do not fall within the jurisdiction of EPA and FDA no review is needed by these agencies. For some of the construct components we referred to previous consultations conducted by FDA.

ArborGen has indicated in their petition (ArborGen, 2011) that they intend to commercialize FTE427 and FTE435 in USDA plant hardiness zones 8b and above (USDA, 2012b) in the southeastern United States and has provided data from field tests conducted in these States. This assessment has been conducted for the seven States listed in the petition where they indicate they plan to deploy these trees as well as other States at zones 8b and above where the *Eucalyptus* freeze tolerant events (FTEs) could be grown.

B. Development of the Freeze Tolerant *Eucalyptus*

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 Indonesian island of Sulawesi and Ceram, Mindanao in the southern Philippines, northern New Guinea, New Britain and the Timor Islands (Groves, 1994; Ladiges, 1997). There are no wild relatives of *Eucalyptus* that occur naturally in the United States. An overview of the biology of *Eucalyptus grandis* has been published by the US Forest Service (Meskimen and Francis, 1990). *Eucalyptus* has been planted as an ornamental species in the extreme southern United States where mild winters will allow some species to grow. *Eucalyptus* normally propagates in its native range via seeds. It does not spread vegetatively like other trees such as poplar or willow. *Eucalyptus* is usually propagated and sold commercially as rooted stem cuttings (de Assis et al., 2004).

Approximately 90 species of *Eucalyptus* have been introduced into North America, particularly into California and Florida, over the past 150 years (Doughty, 2000). A recent review by Ritter and Yost (2009) indicated that 202 different species of *Eucalyptus* are present in California. There have been numerous attempts to grow *Eucalyptus* as a commercial plantation tree for wood pulp in the southeastern United States, but due to its sensitivity to cold temperatures, these attempts have not met with success. It is only grown in commercial plantations in central and southern Florida, where it normally survives freezing temperatures which are rare and usually not severe. *Eucalyptus* is adapted to live in the mild arid and semi-arid climate of Australia. Severe freezing events that can occur in the southern United States have limited its establishment as a commercial forest tree. There are plantations of *E. grandis*, *E. robusta*, *E. camaldulensis*, *E. tereticornis*, *E. amplifolia* and *E. torelliana* currently grown in south central Florida as short rotation energy crops and for mulch production (Stricker et al., 2000; Rockwood et al., 2004; Rockwood, 2012). These trees are generally planted in areas where severe freezing events are rare.

ArborGen has genetically engineered a commercial clone of the hybrid *E. grandis* × *E. urophylla* (also known as *E. urograndis or E.*× *urograndis*). This particular hybrid, grown primarily in

South America, is fast-growing, but susceptible to freezing temperatures. The purpose of the genetic transformation is to produce trees that are freeze tolerant and male sterile. The freeze tolerant phenotype will allow the trees to be grown in USDA plant hardiness zones up to 8b (See USDA plant hardiness zone map (USDA, 2012b), whereas most commercial *Eucalyptus* have been restricted to the warmer zone 9 in the continental United States. The States with zones 8b and higher where the *Eucalyptus* could potentially be grown include: Alabama, Arizona, California, Florida, Georgia, Louisiana, Mississippi, New Mexico, Oregon, South Carolina, Texas, and Washington. The States of Arkansas, North Carolina, Nevada, and Utah have a few counties at zone 8b but it is highly unlikely that the trees would be grown there since they are far north of the zone where the trees are likely to be grown and are primarily rated at this zone due to microclimate differences (*e.g.* due to certain unique geographic characteristics – such as closeness to a body of water).

<u>Description of the Genetic Modifications</u>

FTE427 and FTE435 were developed through disarmed *Agrobacterium tumefaciens* mediated transformation of a commercial clone of *Eucalyptus* EH1. Leaf explants of EH1 were harvested from actively growing micropropagated shoot clumps and inoculated with *Agrobacterium*. Following inoculation, shoot cultures were cultured on medium containing timentin to kill any remaining Agrobacteria. The disarmed *A. tumefaciens* used for the transformation used a plasmid vector pABCTE01 (ArborGen, 2011, Figure IV.A, p. 34) and contains a *CBF2* (C-Repeat Binding Factor) expression cassette, a *barnase* expression cassette, and an *nptII* selectable marker cassette between the left and right T-DNA border regions. These two lines contain the stably integrated genes *CBF2*, *barnase* and *nptII* and Southern blot analyses show FTE427 and FTE435 contain one complete copy of the *CBF2*, *barnase* and *nptII* genes.

FTE427 and FTE435 have been genetically engineered to contain the following transgene fragments:

RB (right border) - DNA region from *A. tumefaciens* containing the right border sequence used for T-DNA transfer (Barker et al., 1983).

rd29A promoter - rd29A cold-inducible promoter from *Arabidopsis thaliana* (Yamaguchi-Shinozaki and Shinozaki, 1993).

***At CBF2* -** C-repeat binding factor 2 (*CBF2*) from *Arabidopsis thaliana* (Jaglo-Ottosen et al., 1998; Liu et al., 1998; Cook et al., 2004).

E9 terminator - 3' untranslated region from ribulose-1,5-bisphosphate carboxylase small subunit (*RbcS2*) *E9* gene from *Pisum sativum* (Coruzzi et al., 1984).

PrMC2 promoter - PrMC2 male-specific promoter from *Pinus radiata* (Walden et al., 1999).

Barnase - *barnase* from *Bacillus amyloliquefaciens* (Mossakowska et al., 1989; Meiering et al., 1992). This is a mutant *barnase* with reduced toxicity to minimize tissue damage (Rottmann et al., 2008)

RNS2 terminator - RNS2 (Ribonuclease 2) terminator from *Arabidopsis thaliana* (Taylor et al., 1993).

UBQ10 promoter - Polyubiquitin (UBQ10) promoter from *Arabidopsis thaliana* (Norris et al., 1993).

***nptII* -** Neomycin phosphotransferase from Tn5 of *E. coli*. (Rothstein et al., 1981; Fuchs et al., 1993).

nos terminator - 3' untranslated region of nopaline synthase (nos) from TDNA of *Agrobacterium tumefaciens* (Depicker et al., 1982; Bevan et al., 1983).

LB (left border) - DNA region from A. tumefaciens containing the left border sequence used for T-DNA transfer (Barker et al., 1983).

C. Expression of the Gene Product, Enzymes or Changes to Plant Metabolism

USDA-APHIS assessed whether changes in plant metabolism in FTE427 and FTE435 are likely to alter their plant pest risk. The assessment encompasses a consideration of the expressed protein or enzyme and its effect on plant metabolism and if this could lead to an increase plant pest risk.

The C-Repeat Binding Factor (*CBF*) genes are transcription factors that belong to the AP2/EREBP family of DNA binding proteins (Riechmann and Meyerowitz, 1998) and like other transcription factors act as control switches for the coordinated expression of other genes in defined metabolic pathways. CBF protein recognizes and binds to a cold- and drought-responsive DNA regulatory sequence designated as the C-repeat (CRT)/dehydration-responsive element (DRE) (Baker et al., 1994; Yamaguchi-Shinozaki and Shinozaki, 1994), which is found in the promoter regions of many cold-inducible genes (Maruyama et al., 2004). In the majority of studies, *CBF* gene expression appears to be specific to cold induction and does not respond to other stress signals such as abscisic acid (ABA), drought or salt stress (Liu et al., 1998; Medina et al., 1999).

A common observation across experiments in which *CBF* genes are overexpressed in transgenic plants is that constitutive expression of *CBF* negatively impacts a number of other traits (Hsieh et al., 2002). In potato, for example, constitutive expression of *Arabidopsis CBF* genes using the cauliflower mosaic virus (CaMV) 35S promoter was associated with smaller leaves, stunted plants, delayed flowering, and reduction or lack of tuber production (Pino et al., 2007). In contrast, *CBF* genes under the control of a cold-induced promoter, rd29A increased freezing tolerance to the same level as constitutive expression (about 2 °C, or ~3 °F) (Yamaguchi-Shinozaki and Shinozaki, 1993; Kasuga et al., 1999; Narusaka et al., 2003), while restoring growth and tuber production to the levels similar to wild-type plants (Pino et al., 2007). In the rd29A-controlled CBF plants, the same level of freezing tolerance as the CaMV35S versions was observed after only a few hours of exposure to low but non-freezing temperatures. These results suggested that using a stress-inducible promoter to direct *CBF* transgene expression could significantly improve freeze tolerance without negatively impacting other agronomically important traits. In the case of these *Eucalyptus* FTE trees, the *CBF* gene is under the control of a cold-inducible promoter which causes the gene to be expressed under cold temperatures, thus mitigating the potential of reduced growth by overexpression. Under this promoter the trees have exhibited normal plant growth in the field trials conducted to support the petition. Field trials at multiple sites have identified lines of this hybrid with the *CBF2* gene that are able to survive freezing events typically experienced in the southeastern United States in zones 8 and 9. No unusual phenotypic traits have been observed in these trees during the seven years of field trials. The *CBF* gene has not altered the characteristics of the engineered plants other than imparting tolerance to cold (freezing) temperatures.

The purpose of introducing the *barnase* gene into these *Eucalyptus* FTEs is to prevent the formation of pollen in developing flowers. ArborGen's pollen ablation technology was developed based on the principles of tapetum ablation (Mariani et al., 1990). The tapetum is the inner-most layer of the pollen sac and plays a crucial role in the maturation of microspores or pollen (Shivanna et al., 1997). By using a promoter active primarily in the tapetum of the pollen sac (Walden et al., 1999; Höfig et al., 2003) to drive expression of the *barnase* ribonuclease gene; the production of pollen is prevented from forming.

Early experiments at ArborGen (unpublished results) suggested that even very low expression of *barnase* could be detrimental to the plant transformation and regeneration process. They therefore developed a modified form of the *barnase* gene with attenuated activity such that very low levels of expression would not impact overall plant development but would have sufficient activity to obtain ablation of developing pollen. In controlled field trials the two translines FTE427 and FTE435 were accessed for the efficacy of the pollen control construct. Microscopic observations of individual flowers and pooled samples confirmed that no pollen was produced in either transline and the expression was functionally stable in both lines over multiple years, different flowering seasons, different sites, and different physiological ages of plants. No pollen has been produced by either transline during seven years of field testing. There has also been no impact on overall plant development and growth.

The *barnase* gene has been engineered into other crops that have been previously reviewed and addressed in multiple environmental assessments by APHIS. Male sterile corn (USDA APHIS petitions for deregulation 95-288-01p and 98-349-01p), rapeseed (petition 98-278-01p) and chicory (petition 97-148-01p) containing barnase have been reviewed by APHIS and granted non-regulated status. There were no toxicity or allergenicity issues found with this gene in previous FDA reviews (FDA, 1996a; FDA, 1996b; FDA, 1997; FDA 1998a; FDA 2000). The donor for the *barnase* gene is *Bacillus amyloliquefaciens* which is not a plant pathogen.

Neomycin phosphotransferase (*nptII*) from *Escherichia coli* transposon Tn5 was used as a selectable marker during the transformation process. The donor for *nptII* is *Escherichia coli* and is not a plant pathogen. This gene confers resistance to kanamycin is generally accepted as safe (Fuchs et al., 1993) and has been previously used in several deregulated crop plants (e.g. corn, petition 01-137-01p; rapeseed, petition 01-206-02p; cotton, petition 95-045-01p; papaya, petition 04-337-01p; and plum, petition 04-264-01p). The selectable marker gene has had no impact on overall plant development and growth. There have been concerns raised in the past on the possibility of horizontal gene transfer of the *nptII* gene to other organisms. See the section on horizontal gene transfer for further discussion on this topic.

The sequences from *Agrobacterium* T-DNA borders and the nos terminator do not cause plant disease.

Based on all the above noted considerations, APHIS concludes that FTE427 and FTE435 pose no more of a plant pest risk from new gene products or changes to plant metabolism.

D. Potential Impacts on Disease and Pest Susceptibilities

USDA-APHIS assessed whether FTE427 and FTE435 are likely to have significantly increased disease and pest susceptibility because of the introduced genes. This assessment encompasses a consideration of introduced traits, the susceptibility of the engineered trees to pest and diseases, the potential that the *Eucalyptus* grown in new areas of the United States could lead to an increase in the incident of pests and diseases of *Eucalyptus* and other host plants, the ability to manage the pest or disease, and any impacts on APHIS pest control programs.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States of America; and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs or quarantines exist, some of which include restrictions on imports or movement of forest trees or their products.

Eucalyptus is not native to the United States and has been imported into the country for many years as a commercial forest tree as well as an ornamental (Santos, 1997). The *Eucalyptus* that was engineered to produce these two lines 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 PPQ inspectors prior to entry into the U.S. Currently importation of *Eucalyptus* plants into the US is subject to post-entry quarantine as a precaution against the introduction of the exotic pest *Pestalotia disseminata* (also known as *Pestalotiopsis disseminata*) and Leaf Chlorosis Virus (USDA, 2007a). Field tests containing these plants were subjected to inspection by APHIS-PPQ for at least two years and these trees showed no indication of any symptoms for *Pestalotia disseminata* or Leaf Chlorosis Virus, or any other pests and diseases of significant concern.

<u>Results of field tests on the susceptibility of the engineered trees to pests and diseases</u>

After establishment of field tests of EH1 and freeze-tolerant lines 427 and 435 across the southeastern US, the trees were monitored at regular intervals for the occurrence of insect pests and diseases. Trees were monitored for 3 to 5 years, depending on the location (see Appendix C in the petition (ArborGen, 2011)). These observations were made on tests where trees were planted as single tree plot or block plots on 36 different test sites. Following standard forestry practices, the trees were not treated for pests or disease. The results from these observations showed that there were no differences in the occurrence of disease or insect pest susceptibility between freeze tolerant translines and non-transformed control trees of the EH1 hybrid genotype. Observations were recorded on common insect and disease pests of *Eucalyptus*. In a few instances sharpshooters, phyllids, grasshoppers, leaf miners, and *Alternaria* leaf spots were observed. In all cases there was no difference between the transgenic and non-transgenic trees. Where these pests were noted they were not severe, were transient and did not cause any significant injury to the trees. The data presented in the petition support the conclusion that the freeze tolerant lines 427 and 435 show no unexpected phenotypes with respect to disease or pest susceptibility and are not expected to exhibit any increase plant pest risk. The introduction of the genes encoding CBF2, Barnase and NPTII are not expected to affect the susceptibility to pests and diseases due to their mode of action in the plant.

Potential of the trait to allow the establishment of pests and diseases in areas where *Eucalyptus* has not been grown before.

There are a number of insects and diseases of *Eucalyptus* that are already present in the continental U.S.; primarily in Florida and California. There are also pests of *Eucalyptus* grown in Mexico. These pests could possibly expand to new *Eucalyptus* plantings where the freeze tolerance trait could allow the establishment of plantings in areas of the Southeast and Western U.S. where trees have not been previously grown. The following list of insects and disease already present in the United States was compiled from the petition (ArborGen, 2011), and the literature using various sources; primarily a risk assessment for the importation of *Eucalyptus* logs and chips into the United States prepared by the USDA Forest Service (Kliejunas et al., 2001), a publication by Paine et al. (Paine et al., 2011), and chapters from Keane et al. (2000), with updates from various agriculture department websites in California (UCIPM, 2013) and Florida (EDIS, 2013).

Insect pests

Leafcutting ants (*Atta texana*)

Various species of leafcutting ants (*Atta* spp.) can be a significant problem in *Eucalyptus* plantations in other parts of the world (Kliejunas et al., 2001). The principal impact of the ants is growth reduction, although repeated defoliation can kill tree parts or entire seedlings. An indigenous species of *Atta*, Texas leafcutting ant (*Atta texana*) is present in well-drained sites in Texas and western Louisiana (Texas A&M, 2013). These have been seen in the vicinity of one of the field tests sites for the transgenic *Eucalyptus* in Texas. However no damage has been observed in the field tests sites to date. Control of these ants is standard practice in forestry programs where these ants are present and involves the use of a fipronil insecticide-based injection treatment (Drees and Merchant, 2012).

Psyllids:

Multiple psyllids attack *Eucalyptus*, but each typically attacks only one or two species of *Eucalyptus*. Psyllids, sometimes known as jumping plant lice, resemble small cicadas with roof-like wings held above their bodies and strong jumping legs. Psyllids are most abundant in spring. High temperatures reduce the population of some species. *Eucalyptus pulverulenta* is targeted by the bluegum psyllid. Spottedgum lerp psyllid and lemongum psyllid occur on *Eucalyptus citriodora* and *maculata*. The redgum lerp psyllid infests many species, particularly damaging the river red gum *Eucalyptus*. Psyllids secrete honeydew, a sticky substance hosting sooty mold. High populations of this pest cause tip distortion or reduce plant growth but most damage is mild, aesthetic and usually does not warrant concern.

There are eight different psyllid species that have been introduced into North America (Paine et al., 2011). They vary in the degree of harm that they can inflict on various *Eucalyptus* species. All eight species have been reported in California and two have been reported in Florida.

Blue gum psyllid (*Ctenarytaina eucalypti*) is native to Australia. The blue gum psyllid has become an important pest of *Eucalyptus pulverulentus* in California, where the tree is grown for the production of silver foliage for flower arranging. First recorded in California in the 1990s, the psyllid has been the subject of a successful biological control program using the parasitoid, *Psyllaephagus pilosus* Noyes (Chauzat et al., 2002; Purvis et al., 2002; Percy, 2005). *Ctenarytaina eucalypti* was removed as an actionable pest at ports of entry by APHIS in 2011 (Schulze, 2011).

Eucalyptus shoot psyllid (*Blastopsylla occidentalis*) is a pest in several places where *Eucalyptus* is grown commercially. It was reported in California in 1988 (Brennan et al., 1999). It has also been reported in Florida in 2001 but no significant damage has been reported (Halbert et al., 2003).

The tristania psyllid (*Ctenarytaina longicauda*) was first noted in California in 1987 and is present on a variety of *Eucalyptus* hosts but typically does not cause significant damage (Paine et al., 2011).

Eucalyptus psyllid (*Ctenarytaina spatulata*) is native to Australia and was first detected in California in 1991 (Taylor, 1997). No data are available on chemical control for *C. spatulata*. In California, southern France and northern Italy *C. eucalypti* is usually well controlled by an introduced hymenopteran parasitoid *Psyllaephagus pilosus* but no data on the efficacy of this parasitoid against *C. spatulata* are available for the United States. *Ctenarytaina spatulata* was removed as an actionable pest at ports of entry by APHIS in 2011 (Schulze, 2011).

The psyllid *Acizzia uncatoides* was introduced into California in 1954 (Ulyshen and Miller 2007). This psyllid is primarily a pest of *Acacia* spp., but on rare occasions it will feed on some *Eucalyptus* spp. (Paine et al., 2010).

Spotted gum psyllid (*Eucalyptolyma maiden*) is native to Australia. First recorded in California in 2002, this psyllid species is now considered a minor pest on lemon-scented gum (*Eucalyptus citriodora*) and spotted gum (*Eucalyptus maculata*) in California (Percy, 2005).

Red gum lerp psyllid (*Glycaspis brimblecombe*i) is native to Australia. The redgum lerp psyllid was found in Los Angeles in 1998 and has spread throughout much of California (Paine et al., 2006). It was also discovered in Florida in 2001 (Halbert et al., 2003). The red gum lerp psyllid has killed thousands of host trees in California, typically on sites lacking supplementary irrigation (Paine et al., 2011). For control, either systemic insecticides such as Imicide or Merit or biological control with an introduced wasp species (*Psyllaephagus bliteus)* are being used (Paine et al., 2006).

Lemon gum psyllid (*Cryptoneossa triangula*) is native to Australia, and first recorded in California in 2002. This psyllid species is now considered a minor pest on lemon-scented gum (*Eucalyptus citriodora*) and spotted gum (*Eucalyptus maculata*) in California (Percy, 2005).

Epichrysocharis burwellii (lemongum gall wasp)

This wasp was first detected in California in 1991 (Schauff and Garrison, 2000). It is a fairly innocuous pest (Millar et al., 2009). The wasp causes small blister-like galls on the leaves. This wasp is only known to attack Lemon gum Eucalyptus with the damage being cosmetic.

Leptocybe invasa (Blue gum chalcid)

Leptocybe invasa, the blue gum chalcid native to Australia, was first found in Florida in 2008, and to date has been documented in Broward, Dade, Hendry, Glades, Lee, and Palm Beach counties (Wiley and Skelley, 2008; Halbert, 2009a; Halbert, 2009b). Damage from this small wasp occurs through formation of galls on petioles, leaf midribs, and stems of new foliage. Galling causes leaves to curl and may stunt growth and weaken trees. The exact species of *Eucalyptus* that is infected in Florida has not yet been determined (Wiley and Skelley, 2008). There is no known chemical control for this pest but two insect parasitoids (*Quadrastichus mendeli* and *Selitrichodes kryceri*) are being evaluated as potential biological control agents (Kim et al., 2008). ArborGen has conducted surveys for detection and mitigation of this pest in their field trials in cooperation with APHIS-PPQ and the Florida Department of Agriculture and Consumer Services. At this time neither APHIS nor the State of Florida has plans to develop a control program for this insect in Florida (Marzolf, 2013).

Gonipterus scutellatus (Eucalypt weevil)

The genus *Gonipterus* is not endemic to the United States, but *G. scutellatus* was introduced into California in 1994 (Cowles and Downer, 1995) and is now found in Ventura, Los Angeles, and Santa Barbara Counties (Hanks et al., 2000). This species is considered seriously invasive. Both *Gonipterus* spp. feed exclusively on broad-leafed eucalypts. Thus, impacts on other plant species seem unlikely. Defoliation appears to have only a modest impact on a limited number of *Eucalyptus* spp. Evidence in California suggests that defoliation can be severe in *Eucalyptus* windbreaks and urban plantings (Hanks et al., 2000). The removal of foliage will not kill trees but can weaken them and destroy their appearance. *G. scutellatus* does not appear to have achieved its full geographic range in North America (Kliejunas et al., 2001). In Brazil adults have been found overwintering on pines (but not egg laying or feeding on pines). And also in Brazil adults have been observed feeding on the stems of apples when orchards and *Eucalyptus* plantations are adjacent to one another (Kliejunas et al., 2001).

Although *Gonipterus* spp. are acknowledged only as a pest of *Eucalyptus*, major problems with *Gonipterus* spp. occur in regions with frequent frosts. New shoots are most susceptible after frost damage. Thus, *Gonipterus* spp. are likely to be a problem if introduced into temperate climates (Kliejunas et al., 2001). However, the *Eucalyptus* FTEs may not be as susceptible since they are more resistant to frost damage. A very good egg parasite, *Patasson nitens* (=*Anaphes nitens*) (Hymenoptera: Mymaridae), has been introduced into Brazil in an attempt to control *Gonipterus* (Kliejunas et al., 2001). Using this same parasite has led to a successful biological control program for the Eucalyptus weevil in California (Hanks et al., 2000; Millar et al., 2009). By 1997, densities of beetle larvae had dropped to barely detectable levels in California (Hanks et al., 2000).

Phoracantha semipunctata (Eucalyptus longhorned borer)

Eucalyptus longhorned borer (*Phoracantha semipunctata*) was introduced into southern California in the 1980s and rapidly became a pest. In California, the insect spread within 5 years from the southern portion of the state into the San Francisco Bay area (Kliejunas et al., 2001). It currently occurs throughout California wherever *Eucalyptus* trees grow. This beetle readily attacks and kills large numbers of *Eucalyptus* trees. The Eucalyptus longhorned borer is typically associated with trees under moisture stress. The greatest damage occurs in semiarid regions. Trees in other areas are also affected during drought periods. Wood borers infest recently dead trees, freshly cut logs and are also able to kill weakened trees. Natural enemies of the Eucalyptus borer were introduced from Australia, and biological control in combination with improved cultural care of *Eucalyptus* dramatically has reduced the number of trees killed each year by the borer (Paine et al., 2000; Paine et al., 2009).

Phoracantha recurva (Yellow phorancantha borer)

As with *P. semipunctata*, the yellow phoracantha borer is typically associated with trees under moisture stress. In California where the two borers occur together, it has been observed that both species infest large branches and boles of their host trees (Kliejunas et al., 2001). The yellow phoracantha borer may not have as broad a host range as *P. semipunctata* but nonetheless has shown its adaptability by becoming established in several countries around the world. In South Africa, the phoracantha borer has been recorded in non-eucalypt hosts including gum myrtle (*Angophora* sp.), turpentine tree (*Syncarpia* sp.), and *Cupressus lindleyi*. In the southern counties of California, the yellow phoracantha borer has been found in the same eucalypt hosts as those infested by *P. semipunctata* (Kliejunas et al., 2001). Biological control measures are also in place in California to control this wood borer (Paine et al., 2000; Paine et al., 2009). As identified in an EA prepared by APHIS (USDA, 2004) wood products of *Eucalyptus* must be heat treated, fumigated or treated with pesticides before entry into the U.S. due to concerns with importation of both the these borers (Federal Register (69 FR 2289– 2295, Docket No. 02–097–2)).

Trachymela sloanei and Chrysophtharta m-fuscum (Eucalyptus tortoise beetles)

Two species of Eucalyptus leaf beetles from Australia, also called tortoise beetles (family Chrysomelidae), have been introduced into California. *Trachymela sloanei* was found in 1998 in Riverside County and now occurs throughout most areas of California where *Eucalyptus* trees grow. *Chrysophtharta m-fuscum* was discovered in Orange County in 2003 and has spread to at least four nearby counties (Millar et al., 2009). Notched *Eucalyptus* leaves are usually the only obvious indication that trees are infested by tortoise beetles. Well established and properly maintained *Eucalyptus* appear to tolerate extensive leaf feeding. No tortoise beetle control is needed in many landscape situations, despite the tattered appearance of leaves. *Chrysophtharta m-fuscum* is a serious pest of commercially grown Baby Blue Eucalyptus (*Eucalyptus pulverulenta*). The *Eucalyptus* species preferences of these tortoise beetles have not been well documented. However, certain tree species are apparently preferred or avoided by these beetles and other major *Eucalyptus* pests (Millar et al, 2009). These beetles have not been reported in Florida and it is not know to what extent they may be tolerant of cold temperatures. However *Chrysophtharta m-fuscum* was recently found in two counties in South Carolina (Clemson University, 2012). At this time it is not clear if they are overwintering and breeding there.

Trachymela sloanei was removed as an actionable pest at ports of entry by APHIS in 2012 (Cooper, 2012).

Fungal diseases

*Alternaria alternate (*Alternaria leaf spot*)*

Alternaria alternate and other *Alternaria* fungi are widespread throughout the world. In *Eucalyptus*, *Alternaria* species are usually saprophytes on leaves associated with leaf spots (Crous et al., 1989a). As saprophytic fungi they cause mostly cosmetic damage and are not generally pathogenic. ArborGen reported the presence of *Alternaria* leaf spots in some of the field tests in Alabama. No difference was found between the transgenic trees and the controls.

Aulographina eucalypti (Aulographina leaf spot)

Aulographina eucalypti is a common leaf pathogen in natural *Eucalyptus* forests and plantations, causing moderate to severe premature defoliation. In addition to characteristic, roughly circular, corky leaf spots, symptoms also develop on petioles, twigs, and sometimes on fruits and bark. Rain and low temperatures (15°C to 20°C) predispose trees to infection. Splashing rain and blowing wind are the major routes for fungal spore dispersal. Infection occurs primarily in the lower crown. It appears to occur only in Hawaii in the United States (Kliejunas et al., 2001).

Ceratocystis fimbriata (Ceratocystis Canker)

Ceratocystis fimbriata is a wilt disease that occurs in California in fruit trees, but to date has not been found to be significant in *Eucalyptus* plantings (Kliejunas et al., 2001). Sycamore is a host in the United States. The disease can be managed by using clones that have been found to be resistant to the disease (Zauza and Alfenas, 2004). As identified in an EA prepared by APHIS (USDA, 2004) wood products of *Eucalyptus* must be heat treated, fumigated or treated with pesticides before entry into the U.S. due to concerns with importation of this disease (Federal Register (69 FR 2289– 2295, Docket No. 02–097–2)).

Cercospora epicoccoides (Cercospora leaf spot)

This leaf spot is very common on *Eucalyptus cinerea* in Florida and occurs throughout its range (Alfieri and McRitchie, 1975). It is not considered a serious pest.

Colletotrichum gloeosporioides (Anthracnose diseases)

The primary anthracnose disease reported in *Eucalyptus* is *Glomerella cingulate* and its anamorph *Colletotrichum gloeosporioides* (Brown and Ferreira 2000). It is primarily a problem in *Eucalyptus* seed or cutting propagation nurseries. It is distributed worldwide but is more abundant in the tropics and subtropics (Brown and Ferreira 2000). The disease is most serious under conditions of high moisture and temperature. It is fairly easy to control with the use of fungicides in the nursery (Cleary Chemicals, 2011).

Coniothyrium zuluense (Coniothyrium canker)

Coniothyrium canker is an extremely damaging disease of *Eucalyptus* species caused by the pycnidial fungus *Coniothyrium zuluense.* The disease was first discovered in South Africa in 1989 although the causal organism was not characterized and described until later (Wingfield *et al.*, 1997). Infection by *C. zuluense* initially results in measle-like necrotic spots on branches and stems. These develop into large girdling cankers that reduce wood quality and may lead to the death of trees (Roux et al., 2002). The disease first appeared in South Africa in 1990 and much effort has been expended to reduce its impact. The disease although common and damaging, no longer appears to threaten *Eucalyptus* forestry in South Africa (FABI, 2001). Coniothyrium canker was recently discovered in Mexico (Roux et al., 2002) so it is conceivable that it could make its way into the United States. As Roux et al. (2002) note, breeding and selection of disease tolerant clones have been a useful strategy to reduce the impact of the disease in South Africa and it should be equally effective in Mexico. The fungus requires a warm humid climate, such as in subtropical Florida and Hawaii to proliferate and spread (Kliejunas et al., 2001). Areas with cooler climates and lower rainfall may not experience any spread. The need for warm, wet conditions for infection may limit the opportunities for significant disease progression in California (Kliejunas et al., 2001). It is not known if the fungus can survive the colder temperatures in zone 8b. At least one parent of the FTE *Eucalyptus* hybrids, *E. grandis*, is known to be susceptible to this pathogen (Kliejunas et al., 2001; Roux et al. 2002).

Cryphonectria cubensis, *Cryphonectria gyrosa*, *and Botryosphaeria dothidea* (Eucalyptus canker and Bot Canker)

Three canker diseases already present in the U.S. have been found associated with *E. grandis* in Florida *Cryphonectria cubensis* (also known as *Chrysoporthe cubensis), Cryphonectria gyrosa, and Botryosphaeria dothidea* (Eucalyptus canker and Bot Canker) and have resulted in the failure of trees to coppice and regenerate (Barnard et al., 1987). *Cryphonectria* fungi are taxonomically closely related to the chestnut blight fungus, *Cryphonectria parasitica. Botryosphaeria* canker and dieback is one of the most important diseases of *Eucalyptus* spp. in South Africa (FABI, 2001). The fungus, which occurs ubiquitously throughout the southern US, is best known as an opportunistic pathogen that manifests itself under conditions of environmental stress such as drought, late frosts, branch pruning and insect damage. All three canker fungi cause infection through wounds or natural openings in the bark and are favored by high rainfall and humidity distributed throughout the year as well as temperatures that average 23°C or higher (Hodges et al., 1979). Studies in South Africa have shown that there are significant interactions in disease susceptibility between clones and sites so selection of disease resistant clones is important (Heerden and Wingfield, 2002).

The current distribution of the *Cryphonectria* disease in the continental United States appears to be limited to southern Florida. *Botryosphaeria dothidea* has a world-wide distribution on a wide range of trees and shrubs. Because *Cryphonectria* causes heavy losses only in areas where high rainfall occurs most of the year and temperatures average 23°C or higher, damage has not been great in southern Florida (Hodges et al., 1979). Hodges et al. (1979) did not find the disease in southern Georgia or northern Florida. The USDA Forest Service estimates that the potential environmental impact if the canker pathogens were to become established in the United States in areas where it is not already present would be low. Climatic conditions unfavorable to the pathogens would limit their effect on the ecosystem (Kliejunas et al., 2001). As identified in an EA prepared by APHIS (USDA, 2004) wood products of *Eucalyptus* must be heat treated, fumigated or treated with pesticides before entry into the U.S. due to concerns with importation of Botryosphaeria cankers (Federal Register (69 FR 2289– 2295, Docket No. 02–097–2)).

Cryptosporiopsis eucalypti (Cryptosporiopsis leaf spot)

Cryptosporiopsis leaf spot infects leaves and occasionally small twigs. Infection can result in severe defoliation and dieback of young *Eucalyptus* shoots. Infection occurs through stomata or small mechanical wounds. Rain and wind are the major factors involved in localized dissemination of the fungus. It has been reported in Hawaii but not in the continental U.S. (Kliejunas et al., 2001).

Cylindrocladiella camelliae (Cylindrocladiella root rot)

This is a fungal pathogen that causes root rot. It has been reported in *Eucalyptus* in Florida. *Cylindrocladiella* is a ubiquitous fungus distributed throughout the world and is known to be important pathogens of numerous angiosperm and gymnosperm hosts (Crous et al., 1991).

Cylindrocladium spp. (Cylindrocladium leaf spot and blight)

Various species of *Cylindrocladium* (teleomorph = *Calonectria*) cause leaf spots and blight to various degrees on *Eucalyptus* spp. throughout the world. Leaf spots range from small, discrete lesions to irregular necrotic areas. Young stems can be infected and girdled, resulting in shoot blight. These species of *Cylindrocladium* occur in soil and litter as mycelia, hyphae, chlamydospores, and microsclerotia. Foliage and branches are contaminated with vegetative structures and spores by splashed rain, insects, and other microfauna. Frequent precipitation and temperatures ranging between 23°C and 30°C provide favorable conditions for infection. *Calonectria morganii*, *Calonectria ovata*, *Calonectria pteridis*, *Calonectria pyrochroa*, and *Calonectria clavata* occur in Florida and *Calonectria ilicicola* occurs in California (Kliejunas et al., 2001).

Diplodia australiae (Diplodia)

Diplodia is a fungal pathogen that causes fungal blight of pines (Diplodia pine) and ear rot of corn (*Diplodia maydis* - synonym *Stenocarpella maydi*). *Diplodia australiae* has been found on *E. globulus* in various locations throughout the world (Duke, 1983) and has been found in California (Kliejunas et al., 2001).

Erythricium salmonicolor (Pink disease)

Pink disease, caused by the fungus *Erythricium salmonicolor*, is widely distributed in the tropics and subtropics of both hemispheres. It is considered one of the most important diseases of *Eucalyptus*, particularly in India, although it has not been identified on *Eucalyptus* in Australia (Jacobs, 1979). This disease has been reported on *Eucalyptus* in Brazil (Gibson, 1975; Jacobs, 1979) and at least one of the FTE hybrid parents (*E. grandis*) is known to be a susceptible host (Kliejunas et al., 2001). It has also been identified in the southeastern United States from Florida to Texas on fig, apple, pear (Tims, 1963), and redbud (Hepting, 1971). In parts of India, *Eucalyptus* plantations in areas with high rainfall (>200 cm annually) have suffered nearly 100% mortality (Seth et al., 1978). It causes stem and branch cankers that can girdle the main stem of young trees causing repeated dieback and possibly tree mortality. Older trees with larger diameter stems can develop non-girdling cankers. A high incidence of pink disease only occurs in climates with very high levels of rainfall (Kliejunas et al., 2001).

Pink disease has a very wide host range including 141 species in 104 genera, including several hosts known to be widespread in the United States. The more suitable environment for colonization to occur is in the Southeast and Hawaii. Western areas are probably too dry for successful colonization to occur. In the Southeast, the areas susceptible to colonization are coastal areas and Florida because of the high amounts of moisture and warmer temperatures. Tree mortality is limited and usually occurs at early ages (Kliejunas et al., 2001). As identified in an EA prepared by APHIS (USDA, 2004) wood products of *Eucalyptus* must be heat treated, fumigated or treated with pesticides before entry into the U.S. due to concerns with importation of this disease (Federal Register (69 FR 2289– 2295, Docket No. 02–097–2)).

Gymnopilus spectabilis (Root rot fungus)

Gymnopilus spectabilis root rot fungus is widespread in the United States and is not considered a problem. In Argentina, it can sometimes cause significant damage in young trees but is generally not considered to be important. *Gymnopilus spectabilis* is associated with old ornamental *Eucalyptus* as a weak pathogen. It has not been a problem in young commercial plantations (Kliejunas et al., 2001).

Heart Rot (caused by numerous fungi)

There are numerous species of fungi that are associated with heart rot in *Eucalyptus*. These have been extensively reviewed by Kile and Johnson (2000). According to Hepting (1971) only a few fungi have been reported to cause trunk or heart rots of eucalypts in the United States, and they are common species that cause such rots in many hardwoods. *Armillaria mellea, Polyporus schweinitzii,* and *Polyporus sulphureus* cause root and butt rots; the former is a soft, white rot and the latter two are brown, carbonizing rots. *Fomes applanatum* (*Ganoderma applanatum*), mainly a wood decay fungus can make some inroads in the heartwood, and *F. robustus* is an effective heart-rotter in living trees of several genera, including *Eucalyptus*. Heather and Griffin (1978) note that young *Eucalyptus* trees can suffer extensive heart rot when grown rapidly in plantations (cited in Kile and Johnson, 2000).

Mycosphaerella suttoniae and *Mycosphaerella walkeri* (Mycosphaerella leaf spot)

Two leaf spot diseases *Mycosphaerella suttoniae* and *Mycosphaerella walkeri* have been found in *Eucalyptus* plantations in South America and *Mycosphaerella walkeri* has been found on dead leaves of *E. globulus* in California (Kliejunas et al., 2001). Pathogenicity of the numerous species in the heterogeneous genus *Mycosphaerella* ranges from minor saprophytes to extremely damaging pathogens. They may cause loss of foliage or leaf spots, and reduced growth. Disease symptoms vary greatly between fungal species and host. Infection of leaves results in necrotic spots or patches and presence of crinkled and distorted foliage. Occurrence is most severe in summer rainfall areas (Kliejunas et al., 2001).

Pestalotia disseminta also known as *Pestalotiopsis disseminata (*Pestalotia or Eucalyptus leafspot)

Eucalyptus leafspot is a serious fungal pathogen that does not currently exist on *Eucalyptus* in the United States. For this reason *Eucalyptus* is subject to post-entry quarantine when imported from all countries except Canada, Europe, Sri Lanka, and Uruguay into the U.S. under 7 CFR § 319.37–2 (USDA, 2007). ArborGen has imported the *Eucalyptus* for field studies under Plant Protection and Quarantine (PPQ) permits (see above).

<u>*Phytophthora* (various species)</u>
A number of *Phytophthora* species are known to affect *Eucalyptus* plantations and these are covered by in a review by Shearer and Smith (2000). *Phytophthora cinnamomi* is most frequently associated with damage and death in *Eucalyptus* forests and plantations in Australia as well as numerous forest trees in North America (Rhoades et al., 2003). *Phytophthora cinnamomi* is one among the most destructive species of *Phytophthora* associated with the decline of forestry, ornamental and fruit species, as well as of some 900 other woody perennial plant species (Ferraris *et al*. 2004). *P. cinnamomi* is causing and has the potential to cause significant ecological damage in native North American biomes from California to the Appalachian mountains (ISSG, 2012). In Australia, death and poor growth of *Eucalyptus* in plantations associated with *Phytophthora* species have occurred in localized areas subject to occasional saturation (Shearer and Smith, 2000). It is often noted that waterlogging increased the severity of *Phytophthora cinnamomi* induced diseases in the field. It is often possible to avoid the disease in plantations by choosing the correct site, species and appropriate management practices (Shearer and Smith, 2000). This is one of most destructive diseases of forest trees worldwide and could impact *Eucalyptus* plantations established in the U.S.

<u>Powdery mildew (various fungal species)</u>
Seven species of powdery mildew have been identified from *Eucalyptus* (Brown and Ferreira, 2000). Powdery mildews occur on *Eucalyptus* growing in greenhouses and nurseries in many countries but are rarely seen in the field (Brown and Ferreira, 2000). This disease can be commonly found on nursery-grown *Eucalyptus* and can cause significant losses if not recognized and properly treated. There are fungicides that can be used to treat the disease in nursery settings (Bayer CropScience, 2011).

<u>*Pseudocercospora eucalyptorum* (Leaf spot disease)</u>

Pseudocercospora eucalyptorum another leaf spot disease was been found on *E. cinerea* in Florida, in 1975 (Crous et al., 1989b). It is not of a major concern in the U.S. at this time (Kliejunas et al., 2001).

<u>*Puccinia psidii* (Eucalyptus rust)</u>

Puccinia psidii is a rust fungus that primarily attacks trees two years of age or younger, including coppiced trees (Kliejunas et al., 2001). The current distribution of Eucalyptus rust in the continental United States is limited to southern Florida. It has also been found in Mexico (Graca et al., 2011). To date, this pathogen has not been a major threat to *Eucalyptus* in the southeastern United States. The pathogen has been damaging on non-*Eucalyptus* hosts, for example, on *Pimenta dioica* (species of allspice grown as ornamentals) and *Melaleuca quinquenervia* in Florida (Burnett and Schubert, 1985). It is also present in Hawaii (USDA, 2010b). The rust fungus has demonstrated the ability to develop races or strains. The fungus targets young leaves and shoots, and infected leaves become deformed and then shrivel. Susceptibility of *E. grandis* varies in different varieties, and *E. urophylla* is reported to be susceptible (Rayachhetry et al., 2001). Host specialization by *P. psidii* is known to occur, where isolates from one host do not infect other hosts that are known to be susceptible (Coutinho et al., 1998). During the field testing of the FTE the petition notes that rust was present on some of the trees (ArborGen, 2011-Appendix C Page 175 and Attachment 2A). This was an unidentified rust, but was not *Puccinia psidii* which causes significant damage to the growing terminals and severe stunting of infected trees (Pearson, 2013).

Costs may be incurred to control the disease if it were to become established on an economically significant host or on *Eucalyptus* species. The Forest Service risk assessment ranks the consequences of introduction as moderate (Kliejunas et al., 2001). Economic or environmental damage following successful establishment of the disease in new locations, or on new hosts are estimated to have a moderate social and political impact (Kliejunas et al., 2001). Presence of the rust in the United States in areas other than its present distribution may also affect export of the host(s) to countries where the rust is not yet present; for example Australia (Kliejunas et al., 2001). It is considered a serious risk to forests in Hawaii due to the potential of infecting various species of the Myrtaceae (USDA, 2010b). Chemical methods have been developed to control *Puccinia* rust in Brazil and are primarily used in nursery settings; because spraying trees in plantations is usually too costly (Masson, 2013).

Sphaerotheca pannosa (Rose powdery mildew)

This fungus has been found in *Eucalyptus* on Florida (Kliejunas et al., 2001) and is fairly ubiquitous in nature. Powdery mildews affects more the 7600 species of hosts worldwide and are ubiquitous in nature (Ridout, 2009).

Stereum albomarginatum (shelf fungus)

Stereum species are saproic fungi that are found to live on all kinds of deadwood or hardwood or dead leaves and have been reported on *Eucalyptus* in California (Kliejunas et al., 2001). Sometimes they appear on live trees.

Pest and disease susceptibility on freeze damaged trees

There might be concern that dieback of the *Eucalyptus* due to extreme freezing events could result in the increase incidence of pests and diseases. Dead or dying trees might be a source of disease and insects. Field observations made by ArborGen of both young and older trees across a large number of sites, where minor or severe dieback occurred as a result of freeze damage, show that there has been no incidence of increased risk of pests and diseases. Where freezing temperatures caused complete dieback of control EH1 trees within the test, but only minor damage to the transgenic events; the dead trees might act as a substrate for pests and diseases that then attack the otherwise healthy trees. However, as stated in the petition, no evidence for increased pests on healthy trees due to the close proximity of multiple dead trees was observed (ArborGen, 2011 page 112). In most cases if a severe freeze results in significant damage to a plantation the landowner will either harvest the trees for the wood (if the trees are large) or will let the trees re-sprout via coppice (if they are small). Dieback in freeze tolerant *Eucalyptus* following occasional extreme winters is expected to be transient and is not expected to have any significant impact on the prevalence of pests or diseases over what typically occurs in managed forests or native forests in the southeastern US.

Summary of insects and diseases

It appears that the more destructive insect pests of *Eucalyptus* that are already present in the United States are the two *Phoracantha* wood borers (*P. semipunctata* and *P. recurva*) and the Eucalyptus weevil *Gonipterus scutellatus* (Kliejunas et al., 2001). If these insects were to become present in *Eucalyptus* plantations in the new areas of the southeast or west where these trees could be grown, they could cause significant damage to the trees. However, the landowners who would grow these trees would need to invest in control of these plant pests, as is currently occurring in South America and other parts of the world. A comment submitted to the Forest Service risk assessment for *Eucalyptus* logs and chips imported into the United States indicates that "Although *Phoracantha semipunctata*, *P. recurva*, and *Gonipterus scutellatus* are found in California, the latter two species are still not widely distributed. Even *P. semipunctata*, the most widely distributed of the three species, is not found throughout the range of *Eucalyptus* in the United States. The Eucalyptus borer is still not reported to occur in Florida and may be of limited distribution in Arizona. Currently, there are active control and management programs in place in the state of California for all three of these exotic pests of *Eucalyptus.*" Therefore there are both biological and chemical means to control these pests and these could be applied to new trees being established in the western U.S.; and potentially in the southeastern U.S. should the pests become established in this region, provided that the chemicals are or can be registered for use there or appropriate approvals are granted for use of biological control agents.

The major fungal pathogens of concern are pink disease, Eucalyptus rust and Coniothyrium canker. They are already present in the U.S. or Mexico and could become more widespread as the plantings of *Eucalyptus* are expanded. Sufficient control methods would need to be put in place if their incidence and severity of infection were to increase. As noted above, the presence of the transgene is not expected to affect the susceptibility of the trees to these insects and diseases. Differences in species and clonal susceptibility would be a much more important factor to consider. Monitoring for these pests and diseases should be conducted as part of good plantation management practices or part of an early detection and rapid response plan. Should these diseases become present in new areas of the U.S. on *Eucalyptus*, control methods would need to be established, for both transgenic and non-transgenic trees.

There is some potential that these insect and disease pests could affect other nearby plants (for example the Eucalypt weevil and Eucalyptus rust could potentially affect other species) but the likelihood is low, as evidenced by the way these pests have behaved in other countries where they have been introduced, e.g. South America (Kliejunas et al., 2001). The introduction of the transgenic *Eucalyptus* should not alter the plant pest relationships between *Eucalyptus* and the surrounding vegetation and crops; compared to what currently exists for trees already grown in Florida and California. The USDA has imposed conditions for the importation of wood products of *Eucalyptus* to prevent the introduction of other insects and diseases of *Eucalyptus* that currently do not occur in the United States or to prevent the further introduction of pests that are already present (Federal Register (69 FR 2289– 2295, Docket No. 02–097–2); USDA, 2004).

Because using pesticides is usually cost prohibitive in large-scale forestry operations, it is likely that at some point breeding for pest- and disease-resistant selections would have to be made with these freeze-tolerant clones in order to find resistant clones as part of a mitigation strategy; as has been practiced in other parts of the world when *Eucalyptus* has been grown for a number of years (Heerden and Wingfield, 2002; Kulkarni, 2002; Zauza and Alfenas, 2004).

E. Potential Effects on Non-target Organisms Beneficial to Agriculture

FTE427 and FTE435 are not engineered for pest resistance, thus there are no 'target' species, and thus no 'non-target' species. APHIS assessed whether exposure or consumption of the GE FTEs would have a direct or indirect adverse effect on species beneficial to agriculture. Organisms considered were representatives of the species associated with production of *Eucalyptus* in a plantation setting. The assessment includes an analysis of new proteins expressed in the GE FTEs compared to the non-GE counterpart, changes in phenotype, and/or any reported impacts on organisms beneficial to agriculture. The *CBF2, barnase* and *nptII* genes would not produce any gene products that would be expected to have a negative effect other organisms.

C-Repeat Binding Factor (*CBF*) genes when overexpressed display improvement in cold tolerance, water retention, higher oil gland density and wax deposition on the leaf cuticle, and over expression of anthocyanin pigments (Navarro et al., 2011). While the effects of *CBF* gene modification can be highly pleiotropic, only endogenous proteins and other compounds already found in *Eucalyptus* are modulated and these are not likely to have any negative effects on other organisms.

As covered above, male sterility is achieved through the localized production of barnase in pollen producing cells. Barnase is a ribonuclease, an enzyme that degrades RNA, thereby regulating protein synthesis. Ribonucleases are highly ubiquitous molecules found in all living cells (Worrall and Luisi, 2007). The transgenic *Eucalyptus* trees covered in the petition were engineered using *barnase* gene mutants with reduced toxicity to minimize tissue damage (Rottmann et al., 2008). The barnase production is controlled by a tissue specific promoter. As with all genes, the gene encoding barnase is present in every cell of the transgenic plant, however, the promoter acts like an "on" switch that controls when and how strongly the gene is expressed. The *PrMC2* promoter used by ArborGen, originally identified in pine (*Pinus radiata*), restricts barnase production to the tapetum, a small layer of cells within the male floral organ, or anther (Walden et al., 1999; Höfig et al., 2003; Rottmann et al., 2008). Within the anther, the tapetum surrounds developing pollen grains. Mariani et al. (1990) developed transgenic tobacco plants using the *barnase* gene also controlled by a tapetum-specific promoter (Tap29). They observed that in transgenic plants, tapetal cells senesced early in their development, preventing pollen formation. As with other ribonucleases, barnase degrades quickly after the destruction of tapetal cells, and does not accumulate within the plant tissues (Mariani et al., 1990; FDA, 1997). Therefore barnase is only produced for a short period of time during floral development, is limited to a discrete cell layer within the anthers of the flower, and is rapidly degraded (Mariani et al., 1990),(Höfig et al., 2003; Rottmann et al., 2008). In previous studies using transgenic radicchio containing the *barnase* gene, researchers were unable to detect accumulation of barnase within floral tissue (FDA, 1997). Therefore it is highly unlikely that consumption of, or exposure to transgenic *Eucalyptus* could contain enough barnase to cause mammalian or insect toxicity. Direct exposure of organ tissue, is also highly unlikely to occur in nature. In addition the FDA has previously reported that consumption of barnase would likely degrade quickly during digestion further reducing the risk of barnase exposure (FDA, 1997).

With regard to toxicity of barnase to bees and other pollinating insects, there is no clear evidence indicating pollinators would be adversely affected by ingestion of barnase. Combined with the fact that barnase is only produced during the short period of tapetum formation, it is quickly degraded, and the lack of pollen produced by the *Eucalyptus* hybrids, it can be concluded the tissue specific production of barnase is unlikely to adversely affect pollinators or other insects. The *barnase* gene has been deregulated previously in three plant species, corn (petitions 95-288-01p, 97-342-01p, 98-349-01p), rapeseed (petitions 98-278-01p, 01-206-01p) and chicory (petition 97-148-01p) since 1995. APHIS is unaware of any reported cases of mammalian or insect toxicity resulting from barnase consumption or exposure occurring within the past 18 years.

Neomycin phosphotransferase (*nptII*) from *Escherichia coli* transposon Tn5 was used as a selectable marker during the transformation process. This gene confers resistance to kanamycin is generally accepted as being safe (Fuchs et al., 1993) and has been previously used in several deregulated crop plants (e.g. corn, petition 01-137-01p; rapeseed, petition 01-206-02p; cotton, petition 95-045-01p; papaya, petition # 04-337-01p; and plum, petition 04-264-01p). As indicated below, the use of the kanamycin resistance gene is not expected to have any effects on non-target organism either directly or through horizontal gene transfer.

Therefore, based on the above analysis of the new gene products expressed in the GE FTE including potential pleiotropic effects, APHIS concludes that exposure to or consumption of the GE FTEs are unlikely to have adverse impacts to organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of FTE427 and FTE435

This weed risk assessment makes use of information available from the petition and the literature on the GE hybrid along with both parents of the hybrid. In addition we conducted a weed risk assessment (WRA) using the APHIS Plant Protection and Quarantine WRA model (Koop et al., 2012).

Weed Risk Assessment Model

The APHIS Plant Protection and Quarantine WRA (Koop et al., 2012) was used to evaluate the GE hybrid, along with both parents and the non-GE hybrid to assess their establishment/spread potential and impact potential (See details in Appendices I, II III and IV). This analysis aids in determining the likelihood of a species becoming weedy or an invasive weed if introduced into the United States. The PPQ WRA is a very robust model to date for evaluating establishment/spread and impact potential that also takes uncertainty into account. The level of uncertainty can show the degree to which data are available (or not) in order to run the model, and it aids in quantifying a weight-of-evidence approach to the risk assessment. Because the risk estimate itself is a likelihood (traditional probability), like any likelihood estimates, the confidence in those estimates depends upon the amount and accuracy of data used to calculate those estimates. Uncertainty is not the same as risk because the unforeseen results may be neutral or beneficial. Because the GE hybrid lines inherit parental traits, and also because reasonable predictions can be made about the hybrid lines' phenotypes based on parental traits (if empirical data are not available for those hybrid-derived traits to run the model), we determined that the model would be appropriate to use for the parents, the non-GE hybrid and the GE hybrid to get a general picture about the weediness of the taxa as a whole.

The figures below show the combined results of both parents and the non-GE hybrid. For the specific detailed analyses see the Appendices.

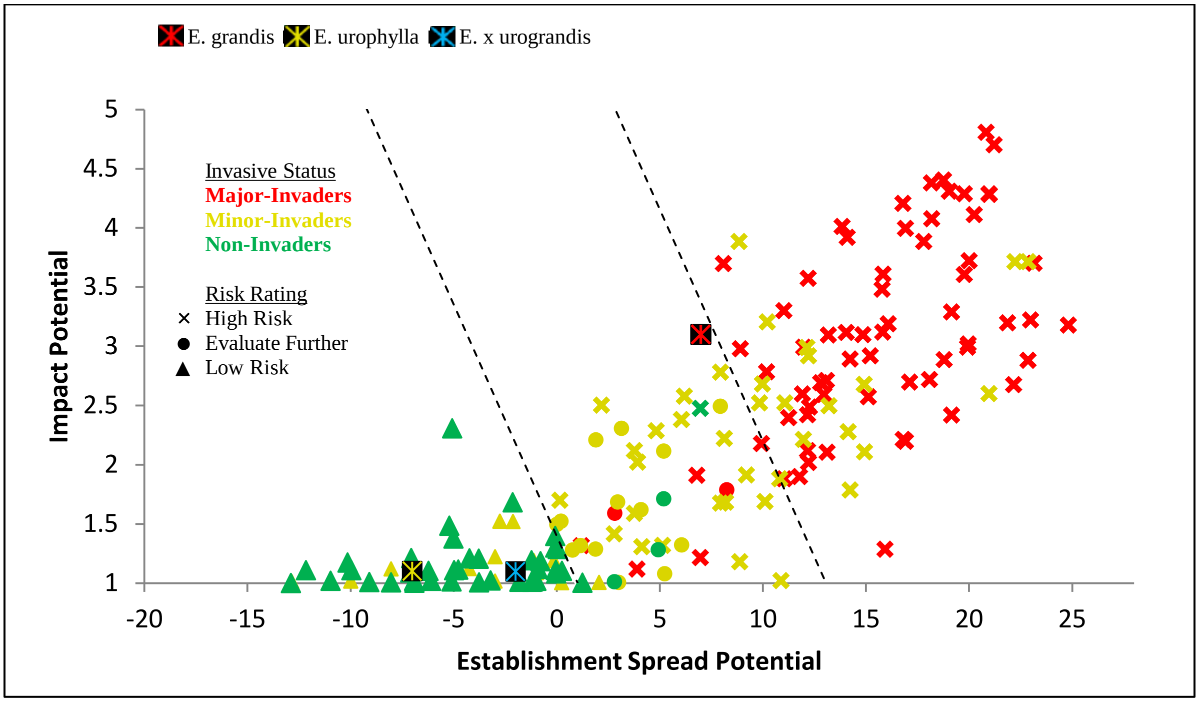

Figure 1. Risk scores (black boxes) of *E. grandis*, *E. urophylla* and non-GE *E.* × *urograndis* relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendices for the complete assessments.

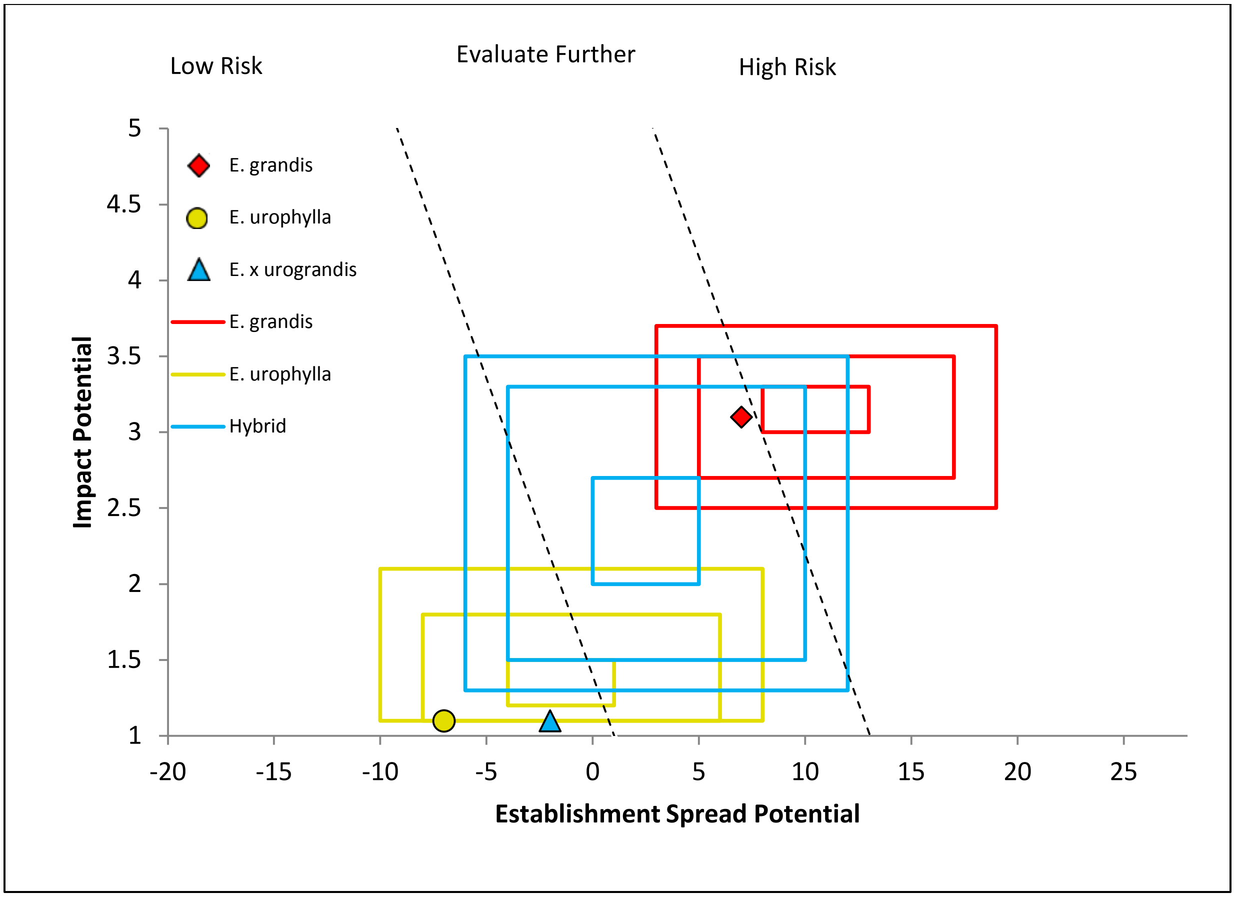

Figure 2. Monte Carlo simulation results (N=5,000) for uncertainty around the risk scores for *E. grandis*, *E. urophylla*, and non-GE *E.* × *urograndis*. For each species, the set of three boxes from the smallest box to the largest contains 50, 95 and 99 percent of the outcomes respectively.

E. grandis **(see Appendix I)**

The risk assessment of one of the parents, *E. grandis*, indicates that it has a 94 percent probability of being an invader and a 6 percent probability of being a non-invader. As shown in Figure 1, *E. grandis*, falls on the border between evaluate further and high risk. Under secondary screening the risk scores were "evaluate further" and "high risk" (Figure 2). When introduced to other areas of the world, *E. grandis* escapes from cultivation and naturalizes in surrounding areas (Booth, 2012; Randall, 2007). It has become invasive in various countries, particularly in South Africa (Booth, 2012; Haysom and Murphy, 2003; Henderson, 2001; Cowling et al., 1997). It invades forests, savannas, and grasslands in South Africa (Cowling et al., 1997) and is a weed of the native flora (Wells et al., 1986). It is a problem species that invades conservation and natural areas. In terms of impact, this species, among others, was cut and felled from South African savannas and grasslands through the Working for Water program to increase water availability for human consumption, agriculture, and ecotourism (Beater et al., 2008; Le Maitre et al., 2002). Therefore this species has shown the ability to spread and establish and to bring about negative impacts in various parts of the world where it has been introduced.

E. urophylla **(see Appendix II)**

The risk assessment of the other parent, *E. urophylla*, indicates that it has a 16.5 percent probability of being an invader and an 83.5 percent probability of being a non-invader. The result of the risk score was "low risk" (see Figure 1). There was an above average amount of uncertainty associated with establishment/spread because of limited information on dispersal mechanisms. However, despite our uncertainty, most of the simulated risk scores resulted in conclusions of low risk. The most important data leading to a conclusion of Low Risk was that there was no strong evidence it has naturalized in areas outside of cultivation, after extensive cultivation for over 75 years in introduced areas.

Non-GE *E.* × *urograndis* (see Appendix III)
The assessment of the hybrid *E.* × *urograndis*, indicated a low level of risk with much higher uncertainty (Figures 1 and 2). The risk analysis indicated that it has a 39 percent probability of being an invader and a 61 percent probability of being a non-invader. The amount of uncertainty was high for both risk elements, but particularly for impact potential because there is very little biological and ecological information available on this human-created hybrid. This hybrid was first created in the 1970s. It has been planted extensively in tropical and subtropical areas and has never been reported to have escaped from plantations. However, evaluation of the hybrid's impact potential is challenging and uncertain because this is not a naturally occurring taxon with a long history of cultivation. The lack of knowledge about how the hybrid will behave in the long-run leads to a high level of uncertainty in the risk assessment. This is reflected in Figure 2 where the distribution of simulated scores does not include the original risk score of the analysis. With additional biological information, and longer-term observations of this taxon's behavior where planted, the uncertainty for this analysis is expected to decrease, and converge onto the risk score (assuming no evidence of escape or naturalization).

Genetically Engineered *E.* × *urograndis* (see Appendix IV)
The assessment of the genetically engineered (GE) hybrid *E.* × *urograndis*, indicates a low level of risk with high uncertainty (Figures 3 and 4). The risk analysis indicates that it has a 71 percent probability of being a non-invader. This compares with a 61 percent likelihood for the non-GE hybrid. Most of the simulated risk scores resulted in conclusions of Evaluate Further. The uncertainty associated with both the establishment/spread and impact potential was above average because there is little biological information available for the non-GE and GE hybrids. The GE hybrid analysis shows a reduction in establishment/spread potential compared to its non-GE counterpart. This lower score is due to the GE hybrid being self-incompatible. Because pollen sterility has been engineered into the tree, the GE trees cannot pollinate themselves. However, they can be pollinated by other non-GE hybrid trees and that is the reason that it continues to produce a limited number of seed. As with the non-GE hybrid, the amount of uncertainty was high and above average. The lack of knowledge about how the hybrid will behave over time, leads to a high level of uncertainty in the risk assessment model. This is reflected in Figures 3 and 4 where the distribution of simulated scores does not include the original risk score of the analysis.

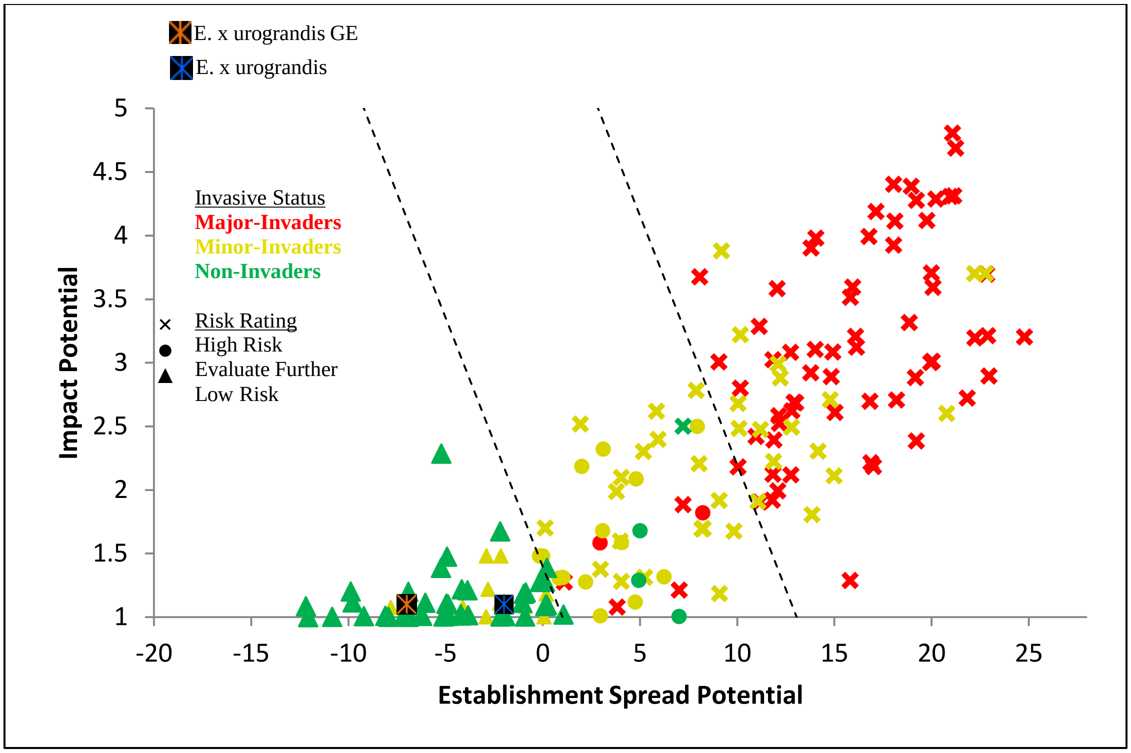

Figure 3. Combined risk scores (black boxes) of Non-GE *E.* × *urograndis* and the GE *E.* × urograndis relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendices the complete assessments.

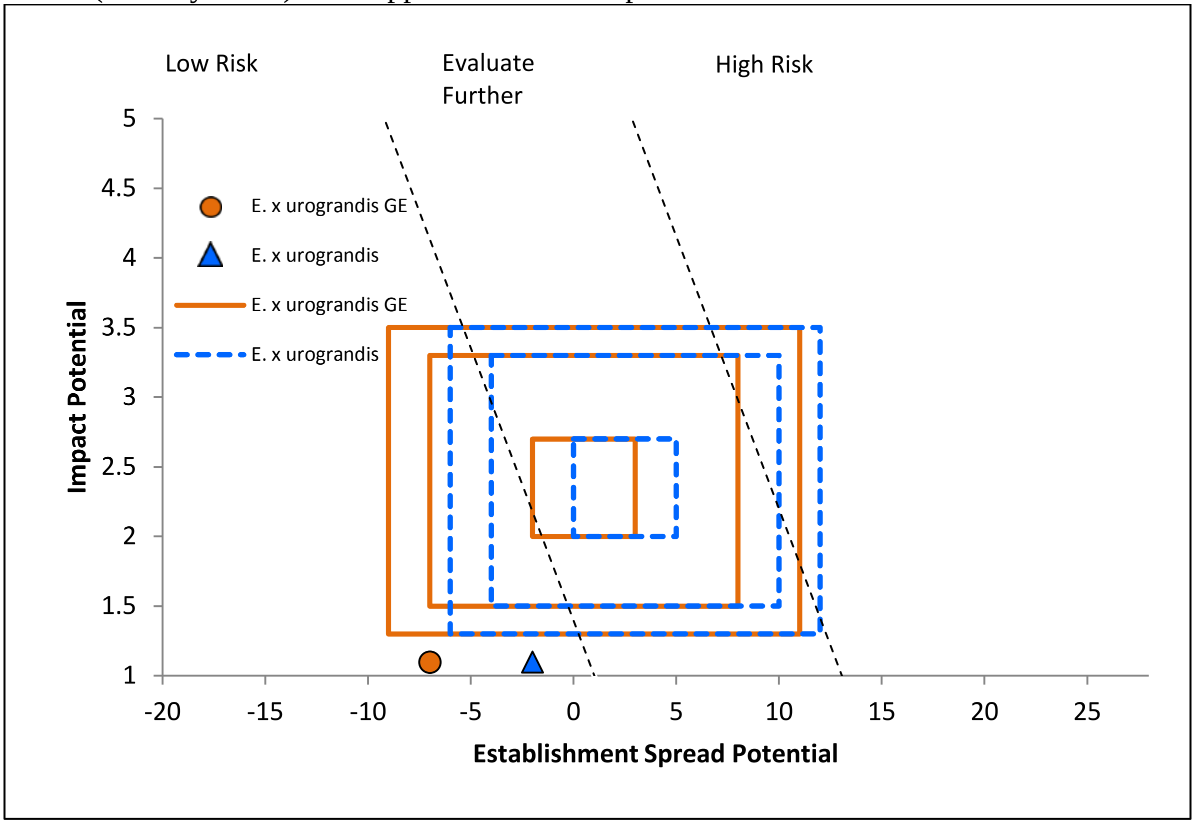

Figure 4. Monte Carlo simulation results (N=5,000) for uncertainty around the risk scores for Non-GE *E.* × *urograndis* and *GE E.* × *urograndis*. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

Overall summary of the WRAs

Table 1. Summary of the of the WRA model results for all four analyses. See appendices for more detail.

Score type	*E. grandis*	*E. urophylla*	*E. grandis x E. urophylla*	GE *E. grandis x E. urophylla*
% Probability Major-invader	35.0	0.6	1.9	1.2
% Probability Minor-invader	59.7	15.9	37.1	27.4
% Probability Non-invader	5.3	83.5	61.0	71.4
* Establishment/ Spread Potential (Uncertainty index)	7 (0.29)	- 7 (0.35)	-2 (0.41)	-4 (0.45)
* Impact Potential (Uncertainty index)	3.1 (0.24)	1.1 (0.13)	1.1 (0.70)	1.1 (0.70)
Model Result	Evaluate Further	Low Risk	Low Risk	Low Risk
Secondary Screening	High Risk	N/A	N/A	N/A

* The Establishment/Spread potential in the model can range from -20 to 25.
The Impact Potential can range from 1 to 5.
Uncertainties range from 0 to 1. Average uncertainty, as defined in the model, is a score of 0.17 (e.g. the average uncertainty among the set of 204 species used to develop the WRA model was 0.17.)

Table 1 summarizes all the data from the WRA model for the four risk assessments. Based on the available evidence, our analysis indicates that the GE hybrid is not likely to escape, establish, and cause harm. One of the parents of the hybrid, *E. grandis*, has shown significant impacts due to its invasiveness, particularly in South Africa. On the other hand, the other parent, *E. urophylla*, has shown no evidence of invasiveness and negative impacts, in spite of having been grown in a number of countries for over 75 years. The scores above show that while both parents are very different, the hybrid is likely to be similar to *E. urophylla*. However, there was much uncertainty with this analysis because of the relatively short duration that the non-GE and GE hybrids have been present in the environment, which leads to a lack of knowledge about how the hybrid will behave over time. The fact that this is a hybrid which was created for increased yield (Foelkel, 2013) may also factor into its invasiveness. Ellstrand and Schierenbeck (2000) have shown that hybridization is sometimes a stimulus for invasiveness in plants.

Naturalization and invasiveness of *Eucalyptus*

What is evident from the analysis above and the literature on *Eucalyptus* naturalization and spread is that some species appear to become naturalized when given enough time (Rejmanek and Richardson, 2011). In numerous reports where *Eucalyptus* has been found to be naturalized and invasive, it has been a slow invader (see below). There is also a continuum of naturalization/ invasiveness that is also reflected in the literature and evident in these results.

Eucalyptus are generally characterized by production of a large number of flowers, fruit and high numbers of seeds (House, 1997; Rejamenek and Richardson, 2011) and although *Eucalyptus* seed is light and very small, it is not adapted to wind dispersal. In eucalypts, mean numbers of viable seed matured per capsule after open pollination in natural stands vary between species, individuals and seasons. Numbers range from 20 up to 100 seeds per capsule (House 1997). As noted in Appendix I, for *E. grandis* a fully mature tree can produce 2 kg of seeds per year and there are approximately 650,000 viable seeds per kg. The dispersal of seed is very limited, generally being confined within a radius of twice the tree or canopy height (approximately 50m for a 25m tall tree at harvest age) (Cremer, 1977; Linacre and Ades, 2004). Research and experience have shown that long distance dispersal of *Eucalyptus* seed and seedling establishment is very rare.

Forsyth et al. (2004) note that *E. grandis* is invasive in South Africa, but also point out that "…in most parts of the world where *Eucalyptus* have invaded, they seldom spread considerable distances from planting sites, and their regeneration is frequently sporadic." Richardson (1998) indicates "*Eucalypts* are also represented on many national or regional weed lists from other parts of the world. Despite this, they have not been nearly as successful in invading alien environments as other widely planted trees such as pines and legumes. Many eucalypts produce large quantities of seeds, so their lack of success as invaders is rather puzzling." This is likely due to the fact that *Eucalyptus* seed are very small, have very limited reserves, and are intolerant of shade or weedy competition. *Eucalyptus* seeds do not have any dormancy barriers to prevent germination (Grose, 1960; Wellington, 1989; Gill, 1997), and seed viability and storage of *Eucalyptus* seeds in soil is typically less than one year (Gill, 1997). This relatively short period of seed viability means that there is a reduced likelihood that seed will persist in the environment. Neither of the parent species or the hybrid store their seeds in capsules for a long period of time that would be the equivalent to a long-term seed bank (see Appendices). Wellington (1989) mentions three other factors limiting successful seedling establishment: insufficient soil moisture, frost, and summer drought.

For successful germination, *Eucalyptus* seed need bare mineral soils with few to no nearby competitors (Meskimen and Francis, 1990; Bell and Williams, 1997). In studying the rate of spread of *Eucalyptus* in California, Kirkpatrick (1977) indicates that the rate of spread of eucalypts from cultivation has been largely restricted by limited dispersal ability combined with the disjunct occurrence of suitable habitats for seedling survival. The major natural expansion of the range of eucalypts has occurred along drainage lines where bare ground occurs almost continuously and where floods may aid dispersal.

It is important to note that in the weed risk assessments (Appendices III and IV), neither the non GE or the GE hybrid have been cultivated for a long period of time. Therefore the results of this analysis should be viewed with the understanding that this is a new taxon that has only been around for about 40 years and the GE hybrid has been field tested for about 7 years. The literature on invasive species has shown that sometimes long lag phases of over 100 years precede the invasion of some plant species (Kowarik, 1995; Ellstrand and Schierenbeck, 2000; Crooks, 2011). Booth (2012) notes that *Eucalyptus* is more of a problem in South Africa perhaps due to the fact that it was introduced and widely planted earlier than in other countries. In a recent publication Callaham et al. (2013) point out there can be significant lag times associated with the naturalization/invasion process, and this underpins concerns over the role of short-term evolution and hybridization as a mechanism for the development of invasive attributes among exotic plants.

In a survey of *Eucalyptus* plantations in Florida and South Carolina, Callaham et al.'s (2013) results indicate that *Eucalyptus* seedlings can establish with some regularity within planted stands, and this shows a strong likelihood that the species used in plantation forestry will become naturalized in the southeastern USA. In their survey *Eucalyptus* seedlings were found in only 4 out of 16 sites surveyed within and proximate to *Eucalyptus* stands in Florida. The most predictive variable evaluated was latitude, with 27ºN being the highest latitude at which seedlings established with regularity. The results of their survey suggested, that under current conditions, the establishment of seedlings near Eucalyptus plantations is a rare event, particularly north of latitude 27ºN. However, south of 27ºN, seedlings were detected more frequently in particular land use types. They also found a positive relationship between plantation age and size the establishment of seedlings in their surveys. Two of the oldest plantations (including *E. grandis*) were established and managed as seed orchards and these sites yielded the highest rates of seedling detections. However, like other workers in subtropical climates (da Silva, et al., 2011), they did not find strong evidence that *Eucalyptus* is or is likely to spread rapidly, or in great numbers, away from the boundaries of plantations. Both Lorentz (2013) and da Silva et al. (2011) conducted studies where plots of *Eucalyptus* were seeded and germination and survival were assessed. Lorentz (2013) distributed seeds from *E. amplifolia*, *E. camaldulensis* and *E. grandis* on plots in Florida to examine the effects of disturbance and seeding density for each species. da Silva (2011) conducted a similar study using seeds of *Eucalyptus grandis* or the *E. urophylla* × *E. grandis* hybrid in Brazil. In both studies seedlings initially germinated but died within a few months. In both cases they noted that in native and modified plant communities proximate to the stands, no *Eucalyptus* recruitment was found and that native flora outcompeted the germinating seedlings. As Forsyth (2004) notes: "Given the history of widespread planting of eucalypts, and the many species involved, we would expect to observe the full range of outcomes in terms of success as aliens. However, in most parts of the world where eucalypts have invaded, they seldom spread considerable distances from planting sites, and their regeneration is frequently sporadic. Their mediocre performance as invaders worldwide is puzzling." It is evident that when *Eucalyptus* naturalizes and becomes invasive it is a very slow process and is often associated with abandoned or failed plantations (Callaham et al., 2013). It is generally not very competitive with native vegetation (Lorentz, 2013; da Silva et al 2011). *Eucalyptus* plantations are typically established using rooted plantlets because of poor establishment using direct seeding methods. Even for rooted plants, removal of competition vegetation is recommended for several months after planting to ensure optimal survival (Meskimen and Francis, 1990).

Kirkpatrick (1977) has shown that *Eucalyptus* is a very slow naturalizer in California. When it does become naturalized it is around waterways and therefore the most likely route to naturalization would be the movement of seeds by water. In this case the GE FTE will likely produce very few viable seeds because this would require cross-pollination from another compatible species due to the expression of the pollen ablation *barnase* gene. Therefore it would be much less likely to become naturalized. Management and oversight of any plantings that might be established in these areas would be advisable in order to monitor for and minimize the establishment and spread of seedlings outside of plantations and along watercourses, particularly those in areas with high impact potential, e.g. that are ecologically sensitive or prone to high water demands for human consumption, agriculture, and ecotourism (Beater et al., 2008; Forsyth et al., 2004; Le Maitre et al., 2002; Lorenz 2013). Lorentz (2013) recommends the use of buffer zones around plantings for the purpose of limiting seed dispersal as well as providing surface water and wildfire protection by limiting the proximity of trees to waterways and by establishing a firebreak around the stand. Where *Eucalyptus* has already invaded Forsyth (2004) recommends removing trees from riparian areas (where water use is likely to be excessive) and nature reserves where all eucalypts have undesirable effects on biodiversity.

Invasiveness between and among species is a continuum (Rejmanek, 2011), and in general *Eucalyptus* tends to be more of a minor invader. This continuum between species within a genus is evident in the literature as well as in the results of the four weed risk assessments conducted in this analysis. One of the parents is considered invasive and the other is not (see Table 1 above). Rejmanek and Richardson (2011) note that *Eucalyptus* in general have been orders of magnitude less successful as invaders than pines and several other widely planted trees including fleshy-fruiting trees. They attribute this limited invasiveness to relatively limited seed dispersal, high mortality of seedlings and possibly a lack of compatible ectomycorrhizal fungi (Rajmanek and Richardson, 2011).

The hybrid used to produce the transgenic trees, *Eucalyptus* EH1, has not shown any evidence of weedy or invasive behavior in the U.S. In Brazil establishment of *E. grandis* plantations began in the 1960s, followed by the establishment of *E. urophylla* and hybrids between *E. grandis* and *E. urophylla* in the 1970s (Wright, 1997). Since the 1970s there have been active breeding programs to examine a number of different hybrids between *E. grandis* and *E. urophylla* (the so called EH series) in Brazil. Therefore *E. grandis, E. urophylla* and their hybrids have been grown for between forty and fifty years in South America (Wright, 1997). According to Silva (2010) "International Paper in Brazil has been planting *Eucalyptus,* primarily *E. grandis* and *E. urophylla* and their hybrids, in Sao Paulo State, Brazil, since the 1960's. Since that time, over 70,000 hectares of *Eucalyptus* plantations have been closely integrated with areas in which native vegetation has been preserved. ….based on field observations made over our 40-year history in South America….. there has been no evidence of invasiveness by *Eucalyptus* into natural forest areas." According to the petition (ArborGen, 2011, page 30) since its introduction in 1994, the genotype EH1 has been planted in Brazil on ~150,000 hectares with no notable indication of its spread beyond plantations. During seven years of field testing in Alabama and Florida, where ArborGen has been allowed to let the engineered trees flower and produce seeds, the trees have not spread beyond planted areas. In spite of some seed production which has occurred in these field tests, there have been no seeds that have germinated and formed seedling volunteers within or adjacent to any of the field test sites (ArborGen 2011, pages 5, 125, 126 and Tables in Appendix C). However, as noted above, naturalization of *Eucalyptus* may take many decades and it may be that there hasn't been enough time and/or propagule pressure for this to be manifested.

<u>Effects on of the introduced genes on naturalization and spread</u>

The gene introduced to affect freeze tolerance has made the engineered *Eucalyptus* more adapted to freezing temperatures in the southern United States. It is documented in the literature that species with larger climatic tolerances (measured as latitudinal range, area of native range, etc.) are more likely to become invasive (Hanspach et al., 2008; Hayes and Barry, 2008). It is suspected that this observation is based on the species tolerance to new environments (Croci et al., 2007). In the PPQ WRA model, increased cold tolerance (greater adaptation across multiple hardiness zones) leads to increased risk (Koop et al., 2012). However, in this assessment the threshold of increased risk was not passed with the addition of one hardiness zone. In the model, thresholds are between zones 3 and 4, and between 9 and 10. Therefore in this case, the ability of the GE hybrid to grow one hardiness zone higher than the non-GE parents did not add to its potential to naturalize and become weedy as far as the risk assessment score; but if the trees can grow in more diverse environments, then that increases the opportunity for seeds to find a suitable environment for establishment and spread.

The freeze-tolerance gene that was engineered into the trees has not affected the reproductive biology such as seed production as shown in the petition (ArborGen 2011, pages 116-121). Therefore there is no evidence that it has affected any phenotypic characteristics other than freeze tolerance that would contribute to enhanced weediness or invasiveness.

The selectable marker gene, when used previously, did not contribute to weediness or invasive phenotypic properties of the genetically engineered plants and there is no evidence that it has done so in this case.

The *barnase* gene that results in pollen ablation should not contribute to weediness or invasive properties and should reduce the ability of the trees to produce progeny. Field test results have shown that the *barnase* gene cassette that has been engineered into these trees is effective at preventing pollen formation. This factor contributed to a lowering of the risk of establishment/spread potential compared to the non-GE hybrid (see Figure 4 above) in the WRA model. Because the *barnase* gene prevents the production of viable pollen, GE trees have to be pollinated by outside pollen in order to produce seed. There is a possibility that other non-transgenic sexually compatible species could be growing near to a plantation in certain areas where cold tolerance is not needed, for example in southern Florida. In this case these could be other hybrids between *E. grandis* and *E. urophylla* as well as the parent *E. grandis* and they could potentially provide pollen that could fertilize these trees (see section below on other species with which it could potentially cross).

<u>Other weed risk assessments</u>

The University of Florida Institute of Food and Agricultural Sciences (IFAS 2012) completed a review of the potential invasiveness of *E. × urograndis* and found that it is not likely to be invasive and can be a recommended species for planting. Because it is not predicted to be invasive, no specific management practices are recommended for this species, unlike other species of *Eucalyptus* grown in Florida (IFAS, 2012). Gordon et al. (2012) recently assessed *E. × urograndis* and determined that it needed to be evaluated further.

<u>Summary of the potential for enhanced weediness</u>

Based on data from the petition, the literature and the weed risk assessments one cannot rule out the possibility that the GE hybrid will become naturalized in the long run if it were to be widely planted. Although it is not likely to become highly invasive, over time it could escape from cultivation and become naturalized and perhaps become a minor invader (with high uncertainty). In cases where *Eucalyptus* has become naturalized and has become invasive, it has done so slowly. It also does not appear to go far beyond established plantations. The most problematic escapes appear to be along water courses where seeds have become distributed by water (Rejmanek and Richardson, 2011). This is particularly true in South Africa (Forsyth et al., 2004; Booth, 2012; ARC, 2011) and has also been found to occur in California (Ritter and Yost, 2009). Trees will tend to spread from failed or abandoned plantations where there appears to be little to no oversight of the trees (Knadler and Sinimbu, 2011). The areas of concern therefore would be if the GE hybrid is planted in areas close to waterways that occur in areas where there is bare mineral soils or if plantations were established and for some reason are later abandoned.

In Brazil, Knadler and Sinimbu (2011) found that that eucalypt species are not a threat to the adjacent undisturbed Cerrado areas; and it is hypothesized that the native grasses inhibit the successful dispersal and germination of *Eucalyptus* seeds in this type of habitat. A similar situation would occur in the southeastern U.S. where grasses and other vegetation would likely shade out any seeds that are released from the plantation. However, management and oversight of any plantations that are established would be advisable to ensure that trees don't escape over time and become naturalized into unwanted areas where undesired impacts could occur. As Stanturf et al. (2013) note, because Callaham et al. (2013) found seedlings in less intensively managed areas such as partially wooded sites, it is important to monitor for potential spread of *Eucalyptus* seedlings into unmanaged areas. Given the slow process by which this occurs with *Eucalyptus*, this would not be particularly labor intensive.

Eucalyptus plantations in general require adequate oversight and management in order to ensure high productivity (Whitesell et al., 1992; Rejmanek and Richardson, 2011). As a part of this oversight, best management practices can be implemented that would reduce invasion risk. Examples for eucalypts may be to avoid cultivation near waterways and cultivation and monitoring practices to control the slow spread from cultivation sites (Gordon, 2012). As noted above, Lorentz (2013) recommends the use of buffer zones around plantings for the purpose of limiting seed dispersal as well as providing surface water and wildfire protection by limiting the proximity of trees to waterways and by establishing a firebreak around the stand. Where *Eucalyptus* has already invaded Forsyth (2004) recommends removing trees from riparian areas (where water use is likely to be excessive) and nature reserves where all eucalypts have undesirable effects on biodiversity). Rejmanek and Richardson (2011) note that because *Eucalyptus* seed do not have dormancy, it would make local eradication an achievable goal. Therefore oversight and management of plantations established with these GE trees, to monitor for any escape of seedlings, would effectively eliminate any inadvertent escape and persistence beyond cultivation. Any seedlings that appear in the vicinity of plantations could be easily controlled with the use of appropriate herbicides.

Due to the short period of time that the hybrid and the GE hybrid have been in cultivation there was a high level of uncertainty in the results of the analysis. As noted above it is important to understand that uncertainty is not the same as risk because the unforeseen results may be neutral or beneficial. Therefore uncertainty does not lead to harm (Raybold, 2012). The uncertainty estimates in the WRA is not due to any calculation errors or limitations in the underlying model, but rather stem from the availability and robustness of the relevant biological and ecological data to run the model.

The potential of the engineered *Eucalyptus* to be weedy was also covered in previous EAs and response to comments for APHIS permits 06-325-111r (USDA, 2007b), 08-011-106rm and 08-014-101rm (USDA, 2012a) and 11-052-101rm (USDA, 2010a) and are herein incorporated by reference.

G. Potential Impacts on the Weediness of Any Other Plants with which FTE427 and FTE435 Can Interbreed

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 be found within approximately 100 meters from the edge of the nearest tree stand (Peters et al. 1990, Linacre and Ades 2004). Pacheco (1987) indicated 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. Other potential pollinators of *Eucalyptus* flowers in the Southeast could be birds that will feed on nectar, such as orioles and some warblers (Forsythe, 2013). In other countries native bats are pollinators of *Eucalyptus* which can lead to gene flow (Southerton, 2013). However the only bats that consume nectar in the continental U.S. occur in the desert Southwest and occur accidently in the Florida Keys (Bat Conservation International, 2013). The bats in the Southwest visit cactus and agave and none of the species in Florida or the Southwest would occur in areas where *Eucalyptus* could be grown. Bees would be the primary method with which pollen could be spread.

There could be two possible routes of gene flow associated with plantations of the GE frost-tolerant *Eucalyptus*. One could be via pollination from nearby *Eucalyptus* plantations of the same clone that is not genetically engineered for pollen sterility; or via pollination from other nearby non-transgenic *Eucalyptus* of a different species which are sexually compatible.

ArborGen has indicated in the petition (ArborGen, 2011 page 4) that the genes introduced into the EH1 hybrid will allow it to be grown in zones 8b and higher. The only sexually compatible species with which the hybrid could cross are other *Eucalyptus* species, growing in zones 8b and higher. Therefore we have considered the possibility of interbreeding with compatible species that are currently growing in these zones commercially or as extant species in the continental U.S. and the potential impacts of the introgression of the transgenes on weediness/invasiveness potential.

A number of publications cover the extent of major *Eucalyptus* species present in North America in California, Florida and other parts of the southeastern U.S. These are summarized below and are shown in Table 2.

Trees grown commercially or under field tests in the southeastern U.S.

The primary species that are being grown commercially in Florida are *E. grandis*, *E. robusta*, *E. camaldulensis*, *E. tereticornis*, *E. amplifolia* and *C. torelliana* (Stricker et al., 2000; Rockwood et al., 2004; Rockwood, 2012). In the lower southeastern States there are also on-going field tests examining the potential of a large number of species. The most promising species in these studies so far are *E. amplifolia*, *E. badjensis*, *E. benthamii*, *E. camaldulensis*, *E. dalrympleana*, *E. dorrigonensis*, *E. dunnii*, *E. grandis*, *E. gunnii*, *E. macarthurii* and *E. viminalis* (Stape, In-press). ArborGen has offered seedlings of *E. benthamii* available for planting in areas of the Southeast (ArborGen, 2012).

Trees present in California and other western States.

On the west coast of the U.S. numerous species of *Eucalyptus* were introduced into California during that State's early history (Santos, 1997), and some of these species have become established. A recent review by Ritter and Yost (2009) indicated that 202 different species of *Eucalyptus* are present in California, represented by one or more mature living trees in the State. In their analysis they present current data on the diversity of *Eucalyptus* in California and which species are spontaneously reproducing, or have the potential to do so. Species were considered naturalized if new propagules met the criteria defined by (Richardson et al., 2000): species establishes new self-perpetuating populations, undergoes dispersal, and becomes incorporated into resident flora. *Eucalyptus* can also be grown as an ornamental tree in parts of Oregon and Washington and a few species are grown by homeowners (Barclay, 2013) but there appear to be no extant populations of *Eucalyptus* present or commercial plantations of *Eucalyptus* grown in those States. For California and other western states, we assessed the sexual compatibility between the transgenic hybrid and the species listed in (Ritter and Yost, 2009; Rockwood, 2012 and Calflora, 2012).

Sexual compatibility between the transgenic hybrids and trees that occur in the U.S.

Before examining the sexual compatibility between species, it is important to understand the taxonomy of the genus. A comprehensive and informal classification proposed by Pryor and Johnson (1971) has been widely used by taxonomists and ecologists. This classification recognized seven subgenera within *Eucalyptus* (Corymbia, Blakella, Eudesmia, Gaubaea, Idiogenes, Monocalyptus and Symphyomyrtus). Recently Brooker (2000) published a formal classification of the genus *Eucalyptus* that assigns all species to a system of subgenra, sections, subsections, series, subseries and supraseries. Among the *Eucalyptus* subgenera, Symphyomyrtus is the largest subgenus and is divided into fifteen major sections (Sejunctae, Racemus, Bolites, Inclusae, Latoangulatate, Similares, Incognitae, Liberivalvae, Exsertaria, Platysperma, Pumilio, Bisectae, Dumaria, Maidenaria, and Adnataria). The classification system developed by Brooker is used to show the taxonomic relationships of the species listed below in Table 2. *E. grandis* and *E. urophylla* the parents of the hybrid belong to closely related series of section Latoangulatate.

Table 2. Taxonomic classification of the major *Eucalyptus* species grown or present in North America (including California, Florida and the southeastern USA)* Classification based on Brooker (Brooker, 2000).

Genus	Subgenus	Section	Series	Major species found in North America
Eucalyptus	Symphyomyrtus	Latoangulatate	Transversae	*E. grandis* (SE and CA)**
				E. saligna (CA)
			Annulares	*E. urophylla* *** *E. resinifera* (CA) *E. robusta* (SE and CA) *E. botryoides* (CA)
			Lepidotae	*E. punctata* (CA)
		Maidenaria	Foveolatae	*E. camphora* (SE) *E. macarthurii* (SE and CA) *E. ovate* (CA)
			Viminales	*E. viminalis* (SE and CA) *E. rubida* (SE and CA) *E. dalrympleana* (SE and CA)

Genus	Subgenus	Section	Series	Major species found in North America
			Neglectae	*E. neglecta* (SE and CA)
			Globulares	*E. nitens* (SE) *E. globulus* (CA)
			Orbiculares	*E. gunnii* (SE and CA) *E. pulverulenta* (CA)
			Argyrophyllae	*E. nova-anglica* (SE) *E. cinerea* (SE)
			Bridgesianae	*E. dunnii* (SE)
			Compactae	*E. badjensis* (SE)
			Benthamianae	*E. benthamii* (SE) *E. parvula* (CA)
			Microcarpae	*E. dorrigoensis* (SE) *E. mannifera* (CA)
			Kitsonianae	*E. kitsoniana* (CA)
			Acaciiformes	*E. nicholii* (CA)
		Exsertaria	Rostratae	*E. camaldulensis* (SE and CA)
			Erythroxylon	*E. tereticornis* (SE and CA) *E. amplifolia* (SE) *E. blakelyi* (CA)
			Singulares	*E. rudis* (CA)
		Sejunctae	Microcorythae	*E. cladocalyx* (CA)
		Bisectae	Lehmanninanae	*E. conferruminata* (CA) *E. megacornuta* (CA) *E. lehmannii* (CA)
			Cornutae	*E. cornuta* (CA)
				E. macrandra (CA)
			Erectae	*E. spathulata* (CA)
		Adnataria	Heterophloiae	*E. polyanthemos* (CA)
			Aquilonares	*E. microtheca* (CA)
			Melliodorae	*E. sideroxylon* (CA) *E. leucoxylon* (CA) *E. melliodora* (CA) *E. microcorys* (CA)
			Rhodoxylon	*E. paniculata* (CA)
		Dumaria	Torquatae	*E. torguata* (CA)
		Inclusae	Inclusae	*E. diversicolor* (CA)
	Eucalyptus	Cineraceae	Pauciflorae	*E. pauciflora* (SE) *E. niphophila = E. pauciflora* subsp. *Niphophila* (SE)
		Eucalyptus	Regnantes	*E. fastigata* (CA) *E. regnans* (CA)
		Aromatica	Insulanae	*E. pulchella* (CA) E. amygdalina (CA)
			Radiatae	*E. dives* (CA) *E. radiata* (CA)
		Longistylus	Preissinanae	E. preissiana (CA)
	Corymbia	Septentrionales	Torellianae	*E. torelliana* (SE)
			Maculatae	*E. citriodora* (CA) *E. maculata* (CA)

Genus	Subgenus	Section	Series	Major species found in North America
		Notiales	Cymbiformes	E. *calophylla* (CA)
			Disjunctae	*E. ficifolia* (CA)
	Eudesmia	Limbatae	Heteropterae	*E. erythrocorys* (CA)

* References for table: Taxonomy: (Brooker, 2000); Species lists: (Rockwood et al., 2004; Ritter and Yost, 2009; Calflora, 2012; Stape, In-press); 11-019-01 petition data.
** SE = present in the southeastern U.S., including Florida (some only in field tests and not planted widely). CA = present in California.
*** *E. urophylla* is currently not grown commercially in the U.S. This species is included in the table as one of the parental species used in the hybrid.

The transgenic EH1 *Eucalyptus* hybrid lines are not likely to be sexually compatible with the vast majority of species that are present in the southeastern U.S. or in California. Natural hybridization among different subgenera and sections within the genus *Eucalyptus* is rare, and hybrid viability decreases with increasing taxonomic distance between parents (Griffin et al., 1988; Potts and Dungey, 2004). In decreasing order of frequency, hybrids are found to occur within series, between series and between sections. Thus, natural hybridization between species from the same section is commonly reported but hybridization between species from major subgenera does not occur (OECD, in press). Even among the closely related species of *Eucalyptus*, hybridization rates are generally very low (Volker, 1995). Where hybridization is possible, it often requires significant human intervention in directed breeding/crossing efforts (Potts and Dungey, 2004). The F_1 hybrids (offspring) generally exhibit poor vigor and reduced fitness compared to open pollinated intraspecific progeny (Lopez et al., 2000). Inviability of these offspring may be expressed at germination, in the nursery and even after planting in the field. Slower germination of hybrid seed often occurs, along with reduced survival of germinants in the nursery, and many seedlings have abnormal phenotypes. Griffin et al. (1988) surveyed natural and manipulated hybrids in the genus *Eucalyptu*s and discussed the challenges of developing even human-made hybrids from such wide crosses (in this case *E. grandis* and *E. globulus* in sections Latoangulatate 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). Several studies of *Eucalyptus* have shown that even if F_1-type hybrids survive to reproductive maturity in natural populations, they exhibit significantly reduced reproductive output compared to competing parental taxa (Drake, 1981; Potts, 1986).

The EHI hybrid was generated as a cross between *E. grandis* and *E. urophylla*. These are both in the Latoangulatate section and in the Transversae and Annulares series, respectively. Most of the other species that are present in the U.S. are in other sections, primarily the Madienaria and Exsertaria sections, but the list includes a number of other sections as well (Table 2). The only likely species with which the hybrid could cross in the environment, if they were grown in the same locations, would be in closely related Series within the same Section. In Table 2 these are species listed in the Latoangulatate Section. Hybrids between *E.* x *urograndis* and other species have been made and are documented. These include manmade hybrids between *E.* x *urograndis* backcrossed to *E. grandis* or *E. urophylla*, or *E.* x *urograndis* crossed to *E. robusta*, *E. saligna*, *E. maidenii* (Dos Santos, 2012) and *E. camaldulensis* (de Assis, 2011). It is important to note that these crosses are normally difficult to make and must be made via hand pollination.

Compatibility with trees grown in the Southeast U.S.

In the southeastern U.S. a few species are being grown commercially and a number are being field tested for cold and freeze tolerance and potential use as plantation forest trees (see above and Table 2). Most other species grown in the southeastern U.S. are members of other distantly related subgenera and sections. Of the species listed, there is a possibility that the hybrid could successfully cross to a couple of these due to close taxonomic relationships. For example species within the same series are more likely to form hybrids than between different series (OECD, in press). The vast majority of the species in the Southeast are in different sections and series so would be less likely to form natural hybrids. Two of the species being grown in Florida could potentially cross with the transgenic hybrid. These are *E. grandis* and *E. robusta*, which are in the same section. The hybrid could backcross with *E. grandis*, one of the parents of the hybrid which exists in the same Section. In addition *E. robusta*, also grown commercially in Florida, is in the same Section and natural hybrids between *E. grandis* and *E. robusta* are known to occur (Meskimen and Francis, 1990; King and Skolmen, 1990). Therefore progeny could be produced from crosses between the transgenic trees and these two species or the hybrids if they were growing in the same location. However, because the transgenic hybrid is not producing pollen, outcrossing would only occur if pollen from nearby commercial trees were to pollinate the transgenic trees. Pollen from *E. grandis* or *E. robusta* could potentially pollinate the hybrid, but based on data from other interspecific hybrids; if any hybrids were to be formed they would likely exhibit poor vigor and reduced fitness (Lopez et al., 2000) and exhibit significantly reduced reproductive output compared to competing parental taxa (Drake, 1981; Potts, 1986). There is a possibility that the hybrid could also be pollinated with pollen from other species in different sections, if they are grown nearby, but the probability would be much lower that natural hybrids would occur. As noted in OECD (in press) plantation species from the Latoangulatae are more likely to hybridize with species from the Exsertaria or Maidenaria than with other sections of Symphyomyrtus and less likely to hybridize with other sections. These sections Esertaria and Maidenaria contain three and fourteen species, respectively, in the Southeast U.S.

A further barrier to potential crossing between the transgenic trees with other species grown commercially in Florida is the expected differences in flowering times between species (Gore and Potts, 1995; Potts et al., 2003). The transgenic hybrid initiates flowers in early summer with expected maturation in mid to late summer, however, *E. grandis* produces mature receptive flowers in the late summer to early fall (Meskimen and Francis, 1990) which does not overlap with the flowering of the hybrid. *Eucalyptus robusta* also flowers in the late fall (King and Skolmen, 1990). Therefore even though the GE hybrid does not produce viable pollen, the asynchrony in flowering times between the transgenic hybrid and with *E. grandis* or *E. robusta*, which are in the same section with the GE hybrid, is likely to prevent the formation of natural hybrids. If in the unlikely event viable hybrids were to be produced and inherit a functional copy of the *CBF2* transgene conferring freeze tolerance, this could potentially expand their northerly range to a more frost hardy zone similar to the effect with the FTE. However, since the *barnase* gene is linked at the same locus with *CBF2* gene, the invasive potential is expected to be lower as in the comparison of the GE FTE *E. x urograndis* with the non-GE *E.* x *urograndis* (Figure 3 above).

Compatibility with trees present in California and other western States

Ritter and Yost (2009) identified 49 different species in California that were either naturalized, expected to naturalize or had no evidence of naturalization. Eighteen of these species are in the category that they considered naturalized. Of these eighteen, two of these (*E. grandis* and *E. robusta*) are in the same section Latoangulatate (known previously as the Transversaria section) as the transgenic hybrid. In the "Expected naturalization" category there are three other species in the Latoangulatate section. These are *E. botryoides*, *E. resinifera* and *E. saligna*. *E. punctata*, also identified by Ritter and Yost is also in the Latoangulatate section and is in the "No evidence of naturalization" category. Therefore, of the species present in California, five could likely have the potential of forming hybrids with the transgenic trees.

Two of the species listed in Ritter and Yost (2009), *E. globulus* (Tasmanian blue gum) and *E. camaldulensis* (Red gum) are now categorized as invasive by the California Invasive Plant Council (CIPC, 2013). *E. globulus* is considered moderately invasive and *E. camaldulensis* is considered limited invasive (Ritter and Yost, 2009). These two species are distantly separated taxonomically from the transgenic hybrid. *E. globulus* is in the section Maidenaria and *E. camaldulensis* is in the section Exsertaria. There is a very low probability that natural hybrids would be formed if they were to occur in the same location, since crossing across sections is rare (OECD, in press).

It is highly unlikely that the transgenic hybrid will be planted in California commercially due to the lack of a significant hardwood plantation forest industry in the State that would use *Eucalyptus*. If Eucalypts were to be used for this purpose, other species or hybrids would most likely be selected that are adapted to this region. Freeze tolerance is not needed because most areas of the State where Eucalyptus is grown is zone 9 and higher so fast growing species are already grown there. Since freeze tolerance is not needed there, there would be no incentive to grow the FTE there.

Also as noted above, because the transgenic hybrid is not producing pollen, outcrossing would only occur if pollen from nearby commercial trees were to pollinate the transgenic trees. Pollen from these species could potentially pollinate the hybrid, but if any hybrids were to be formed they would likely exhibit poor vigor and reduced fitness (Lopez et al., 2000) and exhibit significantly reduced reproductive output compared to competing parental taxa (Drake, 1981; Potts, 1986).

<u>Conclusion: Gene flow and potential for increased weediness of sexually compatible plants.</u>

As noted above, the GE hybrid weed risk analysis shows a reduction in establishment/spread potential compared to its non-GE counterpart. This lower score is due to the GE hybrid being self-incompatible. Because pollen sterility has been engineered into the tree, the GE trees cannot pollinate themselves. However, they can be pollinated by other non-GE hybrid trees and that is the reason that it continues to produce a limited number of seed.

Considering all of the above factors, it is highly unlikely that successful hybridization will occur with any of the species grown commercially in Florida due to distant phylogenetic relationships and/or with temporal separation of flowering times. There is a very low probability that the hybrid will be planted in California and if it were, similar to the conditions in Florida it would be unlikely to form successful hybrids. Therefore there is little evidence that any potential outcrossing with other species of *Eucalyptus* grown in the U.S. is likely to occur and if hybridization were to occur, the resulting hybrids would not likely survive and be weedy or invasive. Importantly, in the case of the GE hybrid trees, only pollen from outside sources could pollinate the trees in plantations. The GE hybrid trees are not producing pollen so would not pollinate other species of trees beyond the planting. If any seedlings were to be formed from these pollinations, they would occur within and adjacent to the plantings and could be detected and removed.

If gene flow were to occur and progeny were to be produced that contained the freeze tolerance gene due to crossing with another species, this gene could give a portion of the progeny the ability to grow in a colder hardiness zone than the original parent. As noted above, in the weed risk assessment, due to the parameters of the model, this trait did not add to its potential to naturalize and become weedy as far as the risk assessment score; however if the trees can grow in more environments, then that increases the opportunity for seeds to find a suitable environment for establishment and spread. Also as noted above, *Eucalyptus* has been shown to be a very slow naturalizer in California and other parts of the world, and any offspring that might occur could be easily controlled with the use of appropriate herbicides. To mitigate the possibility of naturalization and spread into sensitive areas, it would be important to monitor for potential spread of *Eucalyptus* seedlings into unmanaged areas from established plantations.

H. Potential Changes to Agricultural Practices

This section includes an analysis of whether significant changes to agricultural or cultivation practices from adoption of the freeze-tolerant *Eucalyptus* are likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting etc. as they relate to plant pest and diseases. If the freeze-tolerant *Eucalyptus* is a successful commercial species, it is likely that it will replace existing pine plantations and possibly some hardwood plantations in zones 8B and above where the FTE can be grown; primarily in the Southeast U.S. As of 2010 there were around 13,000 acres of *Eucalyptus* being grown commercially in Florida. No other commercial plantings are listed for the other southeastern States (USDA Forest Service, 2013).

There are existing disease and insect pest concerns that occur in the areas where *Eucalyptus* can be grown, particularly in Florida (see section above: Potential of the trait to allow the establishment of pests and diseases in areas where *Eucalyptus* has not been grown before). At present no pests of *Eucalyptus* are subject to APHIS control programs in Florida (Marzolf, 2013). The State of California has biological control programs for the Blue gum psyllid, Red gum lerp psyllid, Eucalypt weevil, Eucalyptus longhorned borer, and the Yellow phorancantha borer.

As new plantings of freeze tolerant *Eucalyptus* are established, existing diseases and insect pests could appear in these new plantings. Climatic conditions are likely to affect how rapidly they might spread to and within these new plantings. Some of these insects and diseases may find the expanded areas of the southeast more or less hospitable, depending on factors such as temperature and rainfall. As noted above, some of these pests might not survive in colder areas whereas others may not be affected. In the field tests conducted by ArborGen, the only pests that were identified were some limited instances of rust, *Alternaria* leaf spot, and insect damage by psyllids (ArborGen, 2011- page 109 and Appendix C). This leaves some uncertainty as to the susceptibility of the FTE to other pests described above. If insect and disease pests were to appear in these plantings, management of these pests is expected to be similar to those practices currently conducted for existing softwood and hardwood plantations including *Eucalyptus* (see previous section and conclusions on Potential Impacts of Genetic Modifications on Disease and Pest Susceptibilities).

In conclusion, APHIS could not identify any significant changes to agricultural or cultivation practices associated with or necessitated by the transgenic phenotype of the FTE compared to conventional *Eucalyptus* that would impact plant pest or diseases or their management. They are not engineered to be more or less resistant to pests or diseases. However, freeze tolerance is expected to expand their range and require adoption of control methods for pests and diseases that currently exist there or those that potentially arrive as a result in cultivation of these FTE. Control methods are expected to be similar to those used in the current range of the pest or disease, but new methods may be needed when they are lacking, such as biological control methods that are used to control pests of *Eucalyptus* in California. Gadgil et al., (2000) note that successful management of diseases in *Eucalyptus* plantations can be achieved by a combination of plant quarantine measures, silvicultural practices and the use of disease resistant planting stock. All these measures would need to be put in place if *Eucalyptus* were to be planted on increasing acreages in the continental U.S.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which FTE427 and FTE435 *Eucalyptus* Cannot Interbreed.

The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al., 1998) and sequencing of large number of genomic sequences (Choi and Kim, 2007). HGT contributed to major transitions in evolution of prokaryotic organisms (Woese, 2002) and has been implicated as a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria and the emergence of increased virulence in viruses, bacteria, and eukaryotes. Although, gene exchange has been documented for nearly all types of genes and between unrelated organisms at an evolutionary scale (Gogarten et al., 2002; Yoshida et al., 2010) the frequency of HGT among higher organisms have been shown to be extremely rare and, consequently, such transfers have not played any major role in their evolution (Kurland et al., 2003).

APHIS examined the potential for the new genetic material inserted into FTE427 and FTE435 *Eucalyptus* to be horizontally transferred to other organisms without sexual reproduction and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants. These two *Eucalyptus* lines only contain inserted DNA sequences from plants and bacteria. The only sequences derived from plant pathogenic organisms are portions of the T-DNA borders and the 3' terminator from the nopaline synthase gene from *A. tumefaciens*, none of which encode gene products. Furthermore, horizontal gene transfer and expression of DNA from a plant species to other fungal, bacterial, or parasitic species or other eukaryotic pests is unlikely to occur based on the following observations.

Although there are many opportunities for plants to directly interact with fungi, bacteria, and parasitic plants (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores), so far there are no reports of significant horizontal gene transfer between evolutionarily distant organisms (as reviewed in (Kurland et al., 2003; Keese, 2008)). Accumulated evidence show that there are universal gene-transfer barriers, regardless of whether transfer occurs among closely or distantly related organisms (Kaneko et al., 2000), (Koonin et al., 2001; Wood et al., 2001; Kaneko et al., 2002; Brown, 2003; Sorek et al., 2007). 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. 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). Examples of HGT between eukaryotes and fungi primarily involve gene acquisition or transfer by fungi to or from other distantly related fungi or bacteria (Keese 2008; Keeling and Palmer 2008). Examples of HGT between plants and invertebrates are extremely rare, and most examples of HGT in insects involves acquisition of genes from their pathogens or endosymbionts (Keese 2008; Acuña et al. 2012; Zhu et al. 2011).

Transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes, and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (FDA;1998b).

Further, to evaluate safety, the European Food Safety Authority (EFSA, 2004) reviewed the antibiotic selection markers used in genetically engineered plants. In this document, various antibiotic resistance genes were assigned into groups based on the criteria of therapeutic use in humans and in animals and presence in the environment; Group I is composed of kanamycin and hygromycin resistance genes. The opinion states that because of the frequency of horizontal transfer plants to other organisms is very rare, previous existence in the environment and the history of use of the kanamycin resistance, that there is no rationale for restricting Group I antibiotics. The antibiotics are rarely used in agriculture or aquaculture and thereby do not provide selective pressure for a possible transfer of the resistance genes from genetically modified plants to soil microorganisms.

Evidence for HGT from plants to other plants is limited to two specific scenarios: (1) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Recently, Yoshida et al. (2010) through a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*) from its monocot host plant. According to this study, the incorporation of the specific genetic sequence (with an unknown function) occurred between sorghum and purple witchweed. However, this HGT occurred before speciation of purple witchweed and related cowpea witchweed (*S. gesnerioides*) from their common ancestor. Likewise, recent studies demonstrated that in a few parasitic species of the Rafflesiaceae family, out of several genetic sequences examined, about 2.1% of nuclear (Xi et al. 2012) and 24%–41% of mitochondrial (Xi et al. 2013) gene transcripts appeared to be acquired from their obligate host species. However, all the above-mentioned instances of HGT between parasitic plants and their hosts were reported to be of ancient origins, on an evolutionary time scale spanning thousand to millions of years ago.

If the GE FTEs were infected by a parasitic plant or were naturally grafted to another plant, there is a very low probability that HGT could result in the other plant acquiring DNA from the FTEs. However, in both scenarios this newly introduced DNA would likely reside in somatic cells, and with little chance of reaching the germ cells, this introduced DNA could not persist in subsequent generations unless the recipient plant reproduced asexually from the affected cells. APHIS therefore concludes that the likelihood of HGT from to another plant, including parasitic plants, is extremely low.

Therefore APHIS concludes that horizontal gene transfer is unlikely to occur from FTE427 and FTE435 *Eucalyptus* to other organisms that could be pests or pathogens, and in the unlikely event of such transfer the transgenes are not likely to be expressed or otherwise result in a greater plant pest risk.

J. Conclusion

APHIS has reviewed the information submitted by the petitioner and conducted a plant pest risk assessment on freeze-tolerant *Eucalyptus* events FTE427 and FTE435.

- There is no plant pest risk from the inserted genetic material.
- There were no atypical responses to disease or plant pests in the field and no indirect plant pest effects on other agricultural products. The transgenic trees have shown no increased incidence of plant pests compared to the non-transgenic control trees. There is the potential that if the trees are commercially successful and there are increased plantings of the *Eucalyptus* in areas of the Southeast where *Eucalyptus* trees are not currently grown, pest and diseases already present in the U.S. could become more widespread as the plantings of *Eucalyptus* are expanded. Sufficient control methods would need to be put in place if their incidence and severity of infection were to increase. Therefore management of plantations for pests would be needed, as for any other forestry species. As noted above, the presence of the transgene is not expected to affect the susceptibility of the trees to these insects and diseases.
- There is also no evidence of deleterious effects on non-targets or beneficial organisms in the agro-ecosystem due to the insertion and expression of the new genes.
- Based on the weed risk assessments conducted, there is a possibility, with high uncertainty, that the transgenic trees could become naturalized over time if widely planted and could become a minor invader if the plantations are not properly managed. Therefore management and oversight of the plantations would be needed to ensure that plants do not inadvertently escape and persist beyond cultivation over time. Due to their slow ability to naturalize this should be easily done. Abandoned plantations could be problematic and measures would need to be taken to either remove the trees or monitor for the escape of seedlings and remove them.
- The trees are not expected to impact the weediness of other plants with which they can interbreed because the formation of natural hybrids is considered unlikely. In the unlikely event that hybrids were to be formed, they would be in the vicinity of established plantations.
- There is also no evidence of deleterious plant pest effects from changes in agricultural/cultivation practices. Horizontal gene transfer is highly unlikely.

Therefore APHIS concludes that FTE427 and FTE435 *Eucalyptus* is highly unlikely to pose a plant pest risk as long as there is proper management and oversight of plantations as they are established and grown.

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Appendix I. Weed Risk Assessment for *Eucalyptus grandis*

Weed Risk Assessment for *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) – Rose gum

United States Department of Agriculture

Animal and Plant Health Inspection Service

May 21, 2013

Version 1

http://www.forestryimages.org
Photo: Edward L. Barnard

http://www.ctahr.hawaii.edu
Photos: J.B. Friday

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Introduction Plant Protection and Quarantine (PPQ) regulates noxious weeds under the authority of the Plant Protection Act (7 U.S.C. § 7701-7786, 2000) and the Federal Seed Act (7 U.S.C. § 1581-1610, 1939). A noxious weed is defined as "any plant or plant product that can directly or indirectly injure or cause damage to crops (including nursery stock or plant products), livestock, poultry, or other interests of agriculture, irrigation, navigation, the natural resources of the United States, the public health, or the environment" (7 U.S.C. § 7701-7786, 2000). PPQ uses weed risk assessment (WRA)—specifically, the PPQ WRA model (Koop et al., 2012)—to evaluate the risk potential of plants, including those newly detected in the United States, those proposed for import, and those emerging as weeds elsewhere in the world.

Biotechnology Regulatory Services (BRS) regulates genetically engineered organisms under the authority of the Plant Protection Act (7 U.S.C. § 7701-7786, 2000) because these organisms my present a pest risk to U.S. plant resources. BRS administers oversight for certain genetically engineered organisms (regulated articles) under the regulations at 7 CFR part 340. A person may petition the agency that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, is no longer regulated under the plant pest provisions of the Plant Protection Act or the regulations at 7 CFR part 340. The petitioner is required to provide information under § 340.6(c)(4) related to plant pest risk that the agency may use to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. A GE organism is no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act when APHIS determines that it is unlikely to post a plant pest risk. Part of the risk assessment that APHIS conducts when reviewing a petition for non-regulated status is the weediness of the regulated article. This risk assessment using the PPQ WRA model was reviewed by weed risk analysts in PPQ.

Because the PPQ WRA model is geographically and climatically neutral, it can be used to evaluate the baseline invasive/weed potential of any plant species for the entire United States or for any area within it. As part of this analysis, we use a stochastic simulation to evaluate how much the uncertainty associated with the analysis affects the model outcomes. We also use GIS overlays to evaluate those areas of the United States that may be suitable for the establishment of the plant. For more information on the PPQ WRA process, please refer to the document, *Background information on the PPQ Weed Risk Assessment*, which is available upon request.

***Eucalyptus grandis* W. Hill ex Maiden – Rose gum or Flooded gum**

Species Information Family: Myrtaceae

Initiation: Several eucalypt species and their hybrids are being examined for their production potential in the United States) for solid wood, pulp for paper production and as potential bioenergy crops (Booth, 2012 ; Stape, In-press). A genetically engineered hybrid (GE) of *E. grandis* and *E. urophylla* is being field tested for its growth potential and field performance in the southeastern United States. As part of the analysis of the weed risk potential of this GE hybrid, we evaluated the weed risk potential of its parents. This is the risk assessment for *E. grandis*.

Foreign distribution: *Eucalyptus grandis* is native to eastern Australia and extends north from near Newcastle in New South Wales to around Bundaberg in Queensland. From there it extends north in scattered disjunct populations, but becomes more common in the wet tropics of northern Queensland (Florabank, 2012). *E. grandis* is probably the most widely used plantation eucalypt, especially for industrial timber production. This species had been planted in the major afforestation countries of Brazil, South Africa, Angola, Argentina, India, Zimbabwe and Malawi. Significant plantings have been made also in its country of origin, Australia and in Cameroon, China, Columbia, Cuba, Fiji, Ghana, Honduras, Kenya, Malaysia, Mozambique, Namibia/South West Africa, Nigeria, Papua New Guinea, Peru, Sri Lanka, Swaziland, Tanzania, Uganda, Uruguay, Zaire and Zambia. Successful trials and woodlots have been established in Costa Rica, Ecuador, Ethiopia, Ghana, Hong Kong, Indonesia, Mexico, Nepal, Portugal, Sierra Leone, Spain and Western Samoa (Schonau, 1984).

U.S. distribution and status: *E. grandis* has been planted in the United States and has naturalized in Florida (NRCS, 2012) and California (Ritter and Yost, 2009).

WRA area[2]: Entire United States, including territories.

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1. *Eucalyptus grandis* analysis

Establishment/Spread Potential *Eucalyptus grandis* is a large tree. It has been introduced as a commercial forest tree for large scale plantings in plantations throughout the world (Schonau, 1984). The species reproduces by seed which are very small, and primarily dispersed by gravity. *Eucalyptus* seeds in general do not appear to form a long term soil seed bank, instead they are stored in the canopy and released in a slow trickle over a period of several years (Wellington, 1989). However, according to House (2013), even though *E. grandis* has woody capsules, it holds its seeds for only 6 months after flowering. They are dropped as they mature. The trait contributing most to the score for this risk element is its invasiveness elsewhere; it has naturalized beyond its native range in Australia (Randall, 2007), and is reported to be invasive in various parts of Southern Africa (Booth, 2012; Haysom and Murphy, 2003; Henderson, 2001; Cowling et al., 1997). It has naturalized in two U.S.

[2] "WRA area" is the area in relation to which the weed risk assessment is conducted [definition modified from that for "PRA area" (IPPC, 2012).

States (California and Florida) where it was introduced in the past (UC, 2012; IRC, 2012; ISB, 2012; NRCS, 2012). There are many other species of *Eucalyptus* that are congeneric weeds which are considered major invaders in other parts of the world (Nyoka, 2003).
Risk score = 7 Uncertainty index = 0.29

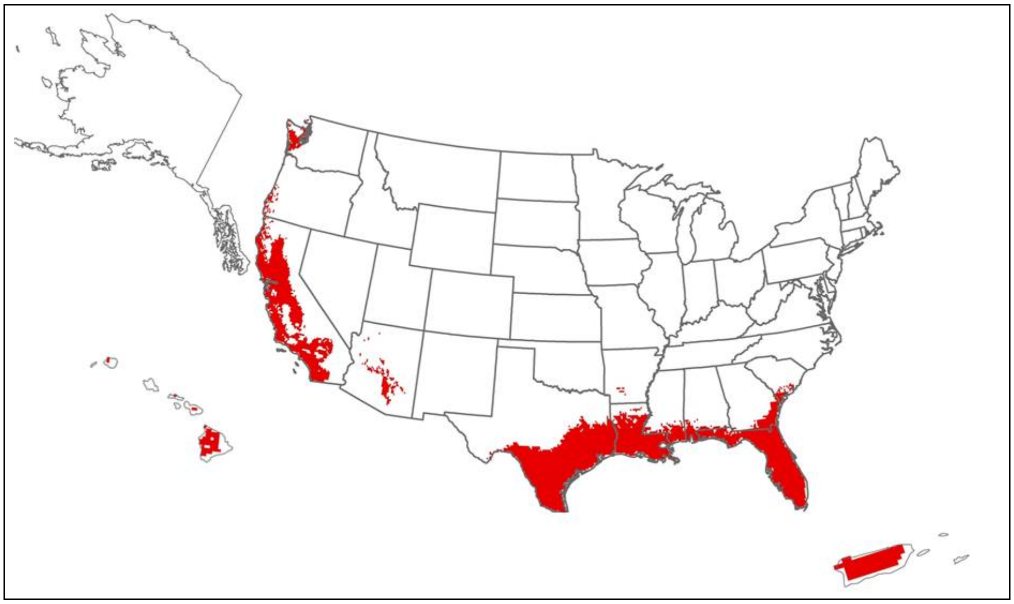
Impact Potential *Eucalyptus grandis* invades forests, savannas, and grasslands in South Africa (Cowling et al., 1997) and is a weed of the native flora (Wells et al., 1986). It is a problem species that invades conservation (Cruickshank, 1988) and natural areas (Daehler, 1998). This species reduces biodiversity, invades natural areas and replaces native vegetation (van Wilgen et al., 2008; Wells et al., 1986). In South Africa grasslands it is reported to reduce stream flow by up to 100% (Beater et al., 2008) and another study reports "that afforestation with *E. grandis* [in South Africa] has reduced annual flow by a maximum of 300-380 mm yr^{-1} " (Van Lill et al., 1980). This species, among others, was cut and felled from South African savannas and grasslands through the Working for Water program to increase water availability for human consumption, agriculture, and ecotourism (Beater et al., 2008; Le Maitre et al., 2002).
Risk score = 3.1 Uncertainty index = 0.24

Geographic Potential Based on three climatic variables, we estimate that about 7.8 percent of the United States is suitable for the establishment of *Eucalyptus grandis* (Fig. 1). This predicted distribution is based on the species' known distribution elsewhere in the world and includes point-referenced localities and areas of occurrence. The map for *Eucalyptus grandis* represents the joint distribution of Plant Hardiness Zones 9-12 (NAPPFAST Global Plant Hardiness Zones: http://www.nappfast.org/), areas with 10-100+ inches of annual precipitation, and the following Köppen-Geiger climate classes: tropical rainforest, tropical savannah, steppe, Mediterranean, humid subtropical, and marine west coast.

The area estimated in Fig. 1 likely represents a conservative estimate as it uses three climatic variables to estimate the area of the United States that is suitable for establishment of the species. Other environmental variables, such as soil and habitat type, may further limit the areas in which this species is likely to establish.

Entry Potential We did not assess *E. grandis*' entry potential because this species is already present in the United States (NRCS, 2012; Ritter and Yost, 2009).

Figure 1. Predicted distribution of *Eucalyptus grandis* in the United States. Map insets for Alaska, Hawaii, and Puerto Rico are not to scale.



2. Results and Conclusion

Model Probabilities: P(Major Invader) = 35.0%
P(Minor Invader) = 59.7%
P(Non-Invader) = 5.3%

Risk Result = Evaluate Further

Secondary Screening = High Risk

Figure 2. *Eucalyptus grandis* risk score (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A for the complete assessment.

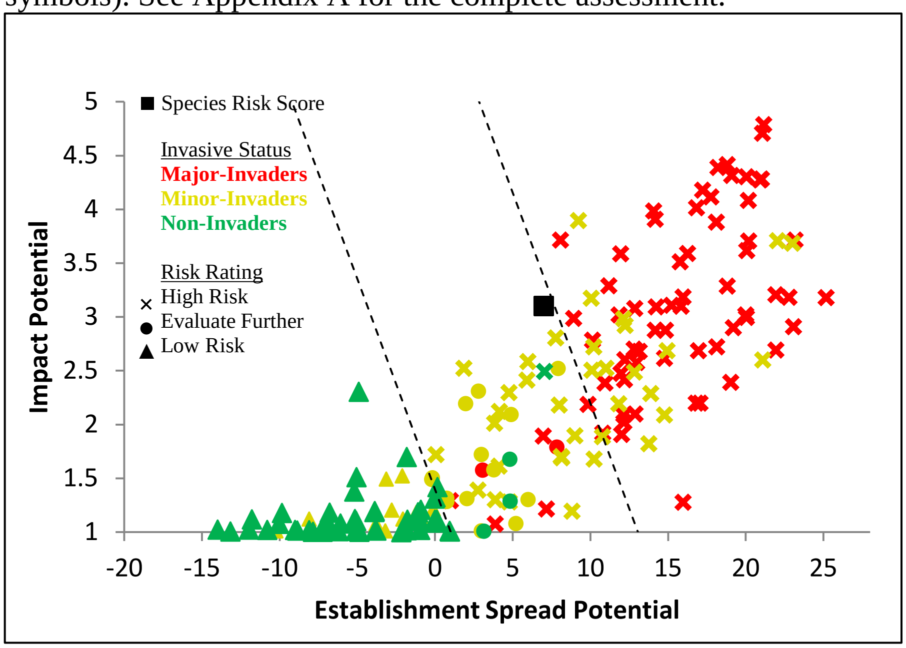
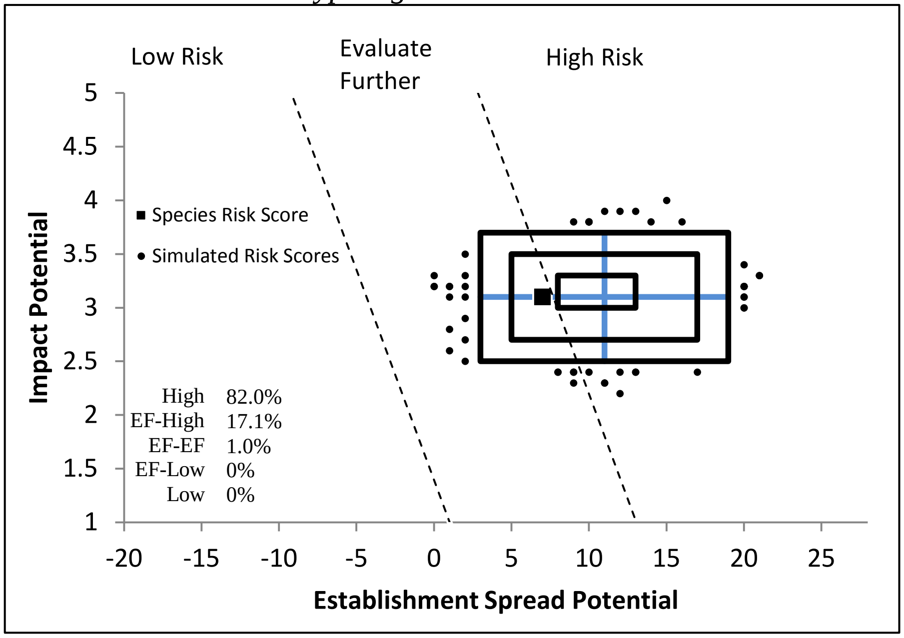


Figure 3. Monte Carlo simulation results (N=5,000) for uncertainty around the risk scores for *Eucalyptus grandis*[a].



[a] The blue "+" symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

<u>3. Discussion</u>

The result of the weed risk assessment for *Eucalyptus grandis* is "Evaluate Further" (Fig 2.) The species' risk scores resulted in a rating of Moderate Risk bordering on High Risk and secondary screening resulted in a conclusion of High Risk because of its history of spread and impacts elsewhere. Our model indicated a 94 percent probability of being an invader and a 6 percent probability of being a non-invader. Two separate assessments done on this species using the Australian weed risk assessment system concluded this species was high risk (Gordon et al., 2011; Daehler et al., 2004). The most important traits leading to a conclusion of Moderate and High Risk were its history of spread and invasion in South Africa and reports that it invades and impacts natural areas. It is reported to have significant impacts on hydrology in parts of the world and is the subject of control and eradication programs because of its historical impact on water resources.

Eucalyptus grandis has naturalized in California and Florida in wooded and preserve areas (UC, 2012; IRC, 2012; ISB, 2012). This species has naturalized in the Mission Valley Preserve of San Diego (UC, 2012; SDRPF, 2012); and in the Fred C. Babcock-Cecil M. Webb wildlife management Area in southern Florida (IRC, 2012. In most cases seedlings are found growing in areas that have likely escaped from trees in cultivation (Callaham et al., 2013; Ritter and Yost, 2009). This species is grown commercially in Florida and is currently sold as planting stock.. There are commercial plantations of *Eucalyptus grandis* currently grown in south central Florida as short rotation energy crops and for mulch production (Rockwood, 2012). Based on the current IFAS WRA, it may be eligible for specified uses if approved by the Florida Invasive Plant

Working Group see: http://plants.ifas.ufl.edu/assessment/pdfs/concl_genus_Feb2011.pdf.

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Appendix A. Weed risk assessment for *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae). The following information was obtained from the species' risk assessment, which was conducted using Microsoft Excel. The information shown in this appendix was modified to fit on the page. The original Excel file, the full questions, and the guidance to answer the questions are available upon request.

Question ID	**Answer - Uncertainty**	**Score**	**Notes (and references)**
Establishment/Spread Potential			
ES-1 (Status/invasiveness outside its native range) Select one: (A) Introduced elsewhere long ago (>75 years) but not escaped; (B) Introduced recently (<75 years) but not escaped; (C) Never introduced elsewhere; (D) Escaped/Casual; (E) Naturalized; (F) Invader.	f - low	5	Native to Australia (NGRP, 2012), and widely introduced and cultivated in other regions. Introduced to Madagascar at an unspecified date and no evidence of escape (Kull et al., 2012). Introduced to India but not reported as invasive (Reddy et al., No Date). Introduced to Papua New Guinea and the Solomon Islands for forestry production (Waterhouse, 1997). The species was introduced to a number of other countries during 1890-1920, including Angola, Zimbabwe, India, Brazil, Argentina, Uruguay and the East African region (AgroForestry, 2012). Introduced to South Australia from its native range in Australia in the 1970s for forestry purposes and not reported to escape (Virtue and Melland, 2003). Beginning to escape (or perhaps naturalized) in two counties of California (UC, 2012). Naturalized in Florida (NRCS, 2012;IRC, 2012;ISB, 2012) and in California (Ritter and Yost, 2009). Naturalized in Australia beyond its native range (Randall, 2007). Introduced into South Africa before 1885 AgroForestry, 2012). Naturalized populations in South Africa are spreading to other areas through long-distance seed dispersal (>100 meters away from parent plants) (Forsyth et al., 2004). Considered "invasive" in South Africa where its spread must be controlled (Macdonald et al., 2003). Several other sources indicate it invades forests, grasslands, and savannas in Southern Africa (Booth, 2012;Cowling et al., 1997; Haysom and Murphy, 2003; Henderson, 2001; Nyoka, 2003). Based on its behavior in South Africa and the description in (Forsyth et al., 2004), answering "f". However, we are using "low" rather than "negl" uncertainty because the other sources reporting it is invasive in southern Africa do not clearly define or categorize the invasion process to lead us to a conclusion "negl" uncertainty. The alternate answers for the Monte Carlo simulation are both "e" because there is no doubt this species has naturalized elsewhere.
ES-2 (Is the species highly domesticated)	n - low	0	This species is cultivated for timber, shade, firewood, and shelter in Africa (Henderson, 2001). It is widely cultivated in tropical and subtropical forest plantations around the world (Meskimen and Francis, 1990). Plants from different clones and provenances are grown (Chaix et al., 2007; Hodgson, 1976b; Horsley and Johnson, 2010; Zhang et al., 2010). In fact, cultivars are selected to increase growth rate and environmental tolerances (McMahon and George, 2010; Meskimen and Francis, 1990), which are positively associated with weediness.

ES-3 (Weedy congeners)	y - negl	1	Three species of *Eucalyptus* are listed in Weber's "Invasive Species of the World". These are *E. cladocalyx*, *E. diversicolor*, and *E. globulus*. They reduce biodiversity, promote fire, reduce water availability (Weber, 2003). In California *E. globulus* and *E. camaldulensis* are listed as invasive by the Invasive Plant Council (Cal-IPC, 2012). An FAO report indicates that numerous other species of Eucalypts have shown a propensity to escape and spread, and cause significant harm (Nyoka, 2003).
ES-4 (Shade tolerant at some stage of its life cycle)	n - low	0	"Rose gum is intolerant of shade. Seedlings can only develop in full or nearly full sunlight; trees must maintain a dominant or codominant canopy position to long survive. Suppressed trees quickly die and intermediate trees must grow to an overstory position or eventually lose vigor and die." (Meskimen and Francis, 1990). In its native range in Australia it grows in mixed eucalypt forests that are relatively open, and sometimes at the edges and interiors of rainforests (Meskimen and Francis, 1990). The evidence strongly suggests this species is intolerant of shade; the one reference to growing in the interior of rainforests (Meskimen and Francis, 1990) may be to individuals colonizing gaps. Answering no, but with low uncertainty to reflect this one reference about rainforest interior.
ES-5 (Climbing or smothering growth form)	n - negl	0	Plant is a tree (Henderson, 2001; Virtue and Melland, 2003).
ES-6 (Forms dense thickets)	n - mod	0	No evidence. Where planted in South Australia, which was recognized to not be the ideal climate, it does not form dense populations (Virtue and Melland, 2003).
ES-7 (Aquatic)	n - negl	0	Plant is a terrestrial tree (McMahon and George, 2010; Henderson, 2001; Virtue and Melland, 2003). Intolerant of swampy conditions (McMahon and George, 2010).
ES-8 (Grass)	n - negl	0	Not a grass. In the Myrtaceae family (NGRP, 2012).
ES-9 (Nitrogen-fixing woody plant)	n - negl	0	Not in plant family known to contain nitrogen fixing species (Martin and Dowd, 1990). This species does not fix nitrogen (McMahon and George, 2010).
ES-10 (Does it produce viable seeds or spores)	y - negl	1	Produces seed (Virtue and Melland, 2003). Reproduces via seeds (Da Silva et al., 2011; Wells et al., 1986). Reported to reproduce extensively (as evidenced by presence of seedlings) in a Santa Cruz arboretum in California (Ritter and Yost, 2009). Propagated by seed (McMahon and George, 2010).
ES-11 (Self-compatible or apomictic)	y - negl	1	Flowers are perfect (Meskimen and Francis, 1990). *Eucalyptus* is generally regarded as outbreeding but is capable of self-pollination (Potts and Wiltshire, 1997). Pollination experiments show that *E. grandis* shows some self-incompatibility as determined by the number of seeds set per flower, and the rate of pollen tube growth in the style, but self-pollinated flowers can still produce seeds (Horsley and Johnson, 2007). In another pollination experiment by the same authors, the authors examined the paternal composition of seeds produced through mixed pollination (outcross and self). In mixed and open (control group) pollination treatments, 100% of the seeds were derived from outcrossed pollen. Overall, these data suggest that some cryptic self-incompatibility and another self-

			incompatibility mechanism is operating, but still some seed can be produced through selfing (Horsley and Johnson, 2010). Self-pollination yields 2 - 47% of the number of seeds that can be produced through outcrossing (Hodgson, 1976c). Nearly all clones of this species are self-fertile to varying degrees, and self-pollination may be more common in out of season flowering (Hodgson, 1976c). In general *Eucalyptus* have high outcrossing rates between 0.69 and 0.84 (cited in Horsley and Johnson, 2010). Inbreeding depression does occur due to selfing in *E. grandis*; with seedlings from selfing demonstrating depressed height growth reaching 8 to 49 percent compared to crossed progenies (Hodgson, 1976c}. Answering yes because it is clear that this species can produce seed through self-pollination, but note that reproductive output may be much higher in mixed populations.
ES-12 (Requires special pollinators)	n - negl	0	This species is entomophilous and honeybees are an important pollinator, although some pollination without bees may occur (Hodgson, 1976b). Insect-pollinated *Eucalyptus* do not express specificity with respect to the insect vector; honeybees can be used to augment pollination success (cited in Chaix et al., 2007). Foraging insects pollinate flowers, including honeybees (Meskimen and Francis, 1990). Eucalypt flowers (referring to the genus) "show no adaptive characteristics that could be particularly attractive to" specialized pollinators (House, 1997).
ES-13 (Minimum generation time) Minimum generative time (A) less than 1 (multiple generations per year), (B) 1 year (annual-1 gen per year), (C) 2or3 years, (D)>3years; (?))	d - high	-1	"The slow growth rate of *E. grandis* in SA [South Australia] would delay time to flowering well beyond 3 years of age" (Virtue and Melland, 2003). Perennial (>5 years) (Cowling et al., 1997). The first flowers appear at 2 to 3 years of age and the first capsules can be obtained 5 to 7 months after anthesis (Hodgson, 1976b). Another report says it can begin flowering within the first year of the plantation (Meskimen and Francis, 1990) but that 97% of *Eucalyptus grandis* families in plantations flower at age 3. However this has no bearing on the actual age of the plants. Seed capsules are mature for harvest 6-7 months after flowering, but capsules remain closed on the tree for at least one year after reaching maturity (Meskimen and Francis, 1990). Flowering can occur as early as 2-3 years of age but capsules remain closed for a few months to years (McMahon and George, 2010). It seems unlikely that a forest tree derived from a germinating seed would begin reproducing by age 2. All of the evidence cited above originates from the forestry literature, where foresters may very likely be basing their observations on plants (i.e., mature seedlings) that have already been growing for a year or two before being planted in the forest plantations. This observation plus the fact that flowering trees retain seeds in enclosed capsules for several months to a year after seed mature, supports the idea that the minimum and effective generation time is probably four years or so. Answering "d", but using high uncertainty. Alternate answers for the Monte Carlo simulation were both "c".
ES-14 (Prolific reproduction)	? - max	0	Unknown. There are no direct estimates of seed production per square meter. A fully mature tree can produce 2 kg of seeds per year and there are approximately 650,000 viable

			seeds per kg (WAC, 2012). Another source states there are approximately 670,000 seeds per kg (cited in Virtue and Melland, 2003). 95-100% of the pollinated flowers set seed, with a range of 2 to 25 seeds set per flower, and an average of 8 seeds per flower (Horsley and Johnson, 2010; Meskimen and Francis, 1990). One study estimates that "the slow release of seeds from capsules means that seed rain is likely to be less than 1000 seeds/m2/year" (Virtue and Melland, 2003). Data for *E. salmonophloia* indicates rates of 1200 seeds per square meter per year (Yates et al., 1995).
ES-15 (Propagules likely to be dispersed unintentionally by people)	? - max	0	No information for *E. grandis*. It is possible that given the very small size of the seeds that under muddy conditions the seeds could readily attach to vehicles, equipment and clothing. Heavy equipment is used to harvest trees and if trees are harvested under wet and muddy conditions seeds could adhere to tires, boots etc. during harvest operations. Long distance dispersal of small seeds without any appendages for adhesion can occur via transport in mud on motor vehicles (Taylor et al., 2011;Clifford, 1959) and clothing (Clifford, 1956). Clifford (1959) noted that small seeds tend to be represented by more individuals in the mud samples than those with large seeds.
ES-16 (Propagules likely to disperse in trade as contaminants or hitchhikers)	? - max	0	No evidence of this occurring for *E. grandis*. It does not seem likely that seeds from a forest plantation tree, which are not well-dispersed, would contaminate a commodity moving in trade. However, it is possible for seeds to adhere to logs in mud and within bark. Therefore answering unknown.
ES-17 (Number of natural dispersal vectors)	1	-2	The following description of the fruit and seeds applies to questions ES17a - ES17e: Seeds produced in capsules (Zhengyi et al., 2012; ARC, 2011; Virtue and Melland, 2003), which are 7-10mm long (Henderson, 2001). Seeds are small, more than 10,000 seeds per 100 grams (Cowling et al., 1997). Seeds measure about 1 mm in length (Meskimen and Francis, 1990). There is no mechanism by which seeds are actively shed by the parent plants (Cremer, 1965).
ES-17a (Wind dispersal)	n - mod		Seeds of *E. grandis* do not have any adaptation that are associated with classic wind dispersal (e.g. plumes or wings) (Boland et al., 1980). According to (Cowling et al., 1997) this species is wind dispersed. Wind aids in passive dispersal of *Eucalyptus* seeds and depends on tree height, crown diameter, wind velocity, and mass and terminal velocity of the seeds; dispersal distances range between 30 to 60 meters (House, 1997). "Seed is mainly dispersed by wind and gravity after release from capsules in the canopy", but most seeds are dispersed near parent trees (Potts and Wiltshire, 1997). Cremer (1977) found that *E. grandis* seeds dispersed an average of 37.3 m from the parent tree when released at 40 m above the ground into a wind speed of 10 km/h. He concluded that few seeds are dispersed greater than twice the height of the tree (cited in Virtue and Melland, 2003). Consequently answering "no" with "mod" uncertainty.

ES-17b (Water dispersal)	y - mod		*Eucalyptus grandis* is naturalized along riverine systems (Forsyth et al., 2004), suggesting water dispersal is frequent and important for this species. Most Eucalypt invasions in South Africa (where *E. grandis* "invades") happen along watercourses (ARC, 2011), particularly for *E. camaldulensis* (Forsyth et al., 2004). "Seed dispersal in the genus appears to be extremely limited, although there are exceptions where dispersal is enhanced by water transport" (cited in Potts and Wiltshire, 1997). Given the spatial distribution and spread of *E. grandis* in South Africa along riverine systems (ARC, 2011), answering "yes" but with mod uncertainty because it is unknown how important this dispersal vector is for *E. grandis*.
ES-17c (Bird dispersal)	? - max		No evidence for *E. grandis*. Fruit and seeds offer no obvious rewards for frugivores (Meskimen and Francis, 1990;Zhengyi et al., 2012). However, for *Eucalyptus* species in general, some bird predators "mediate some dispersal when they fail to consume a proportion of the seeds they harvest" (House, 1997). Occasionally, some birds like cockatoos may scatter *Eucalyptus* seeds when they crack open living capsules (Cremer, 1965). Without additional evidence on the frequency of seed removal by predacious birds and more specific information *on E. grandis* answering "unknown".
ES-17d (Animal external dispersal)	? - max		No evidence for *E. grandis*. Given the small size of the seeds, it is possible they may get stuck in animal fur. Ant seed predators will disperse some seeds of *Eucalyptus* species if they are not consumed (House, 1997). "Ants are significant harvesters of *Eucalyptus* seed on the ground, removing 60% of fallen seed in tall open-forests" (cited in House, 1997). Harvest of fallen seeds by ants "no doubt results in some effective dispersal" (Potts and Wiltshire, 1997), but probably not for *E. salmonophloia* (Yates et al., 1995). One author reports that even if seeds taken by ants escape predation in ant granaries underground, germinating seeds are usually buried too deep to break through to the soil surface (Wellington, 1989). "Native trigonid bees inadvertently pick up *E. torelliana* seeds while foraging for resin within capsules still in the canopy" (cited in House, 1997). "Seed dispersal in the genus appears to be extremely limited, although there are exceptions where dispersal is enhanced by.....animal transport" (cited in Potts and Wiltshire, 1997). Although there is strong evidence for ant-mediated seed dispersal of *Eucalyptus* we are answering "unknown" because it is not clear whether *E. grandis* is dispersed by ants, how frequently that may or may not occur, and if it did occur, how far ants would disperse the seeds.
ES-17e (Animal internal dispersal)	n - mod		No evidence for *E. grandis*. Fruit and seeds offer no obvious rewards for frugivores (Meskimen and Francis, 1990;Zhengyi et al., 2012). Because we have found no evidence of internal dispersal of *Eucalyptus* seeds by non-avian dispersal agents, answering "no".
ES-18 (Evidence that a persistent (>1yr) propagule bank (seed bank) is formed)	n - high	-1	In general *Eucalyptus* seeds do not have dormancy but a few alpine species require cold , moist pretreatment to break dormancy (Boland et al., 1980). According to Boland

			(1980) no such treatment is required for *E. grandis*. Seed of *E. grandis* can be stored for 20 years at -8oC, or refrigerated at 100C (Meskimen and Francis, 1990). But there is no information on seed longevity in natural populations. In temperate *Eucalyptus* such as, *E. grandis*, seed maturation takes several months and seeds are retained in woody capsules (Hodgson, 1976a). Capsules produce viable seed in 4 - 5 months and are fully mature within 7 months (Hodgson, 1976b). According to House (2013) *E. grandis* does have woody capsules, but holds its seeds for only 6 months after flowering. They are dropped as they mature, and don't need fire to trigger seedfall or regeneration. *Eucalyptus* seeds in general do not appear to form a long term soil seed bank, instead they can be stored in the canopy and released in a slow trickle over a period of several years (Wellington, 1989). However, some species, with fragile capsules, release their seed soon after they mature (Cremer, 1965). Boland (1980) indicates that *E. grandis* seeds should be harvested as soon as they are mature and that it is not a "long duration" species where seeds can be collected during any time of the year. This would indicate that long-term storage of seeds in capsules does not occur with *E. grandis*.
ES-19 (Tolerates/benefits from mutilation, cultivation or fire)	? - max	0	Unknown. *Eucalyptus grandis* coppices when cut (Cowling et al., 1997), but it is unclear if the response is significantly more stronger than in most other tree taxa. Note that *Eucalyptus* seeds in general are retained in the canopy in capsules that open as they desiccate or die (Wellington, 1989). In general forest fires promote the regeneration of Eucalypt forests because it triggers a massive release of canopy stored seed; seeds stored in capsules are well protected from fire and begin to release seeds a few days after the fire has passed (Wellington, 1989). However, seed response to fire or biomass loss is not considered in this question.
ES-20 (Is resistant to some herbicides or has the potential to become resistant)	n - negl	0	No evidence of resistance to herbicides and not listed by Heap (2012). When you consider the long life cycle of this plant, the plantation environments where individuals grow, and silvicultural practices it seems highly unlikely populations will develop herbicide resistance. Although *Eucalyptus* species can and often hybridize (McMahon and George, 2010), there is no evidence that a commercial *Eucalyptus* species has acquired or been genetically engineered for herbicide resistance. Consequently answering "no" with "negl" uncertainty.
ES-21 (Number of cold hardiness zones suitable for its survival)	4	0	
ES-22 (Number of climate types suitable for its survival)	6	2	
ES-23 (Number of precipitation bands suitable for its survival)	10	1	
Impact Potential			
General Impacts			
Imp-G1 (Allelopathic)	y - negl	0.1	Soil samples obtained from *E. grandis* forests inhibited seed

			germination of squash seedlings (Espinosa-García et al., 2008). These were natural concentrations of phenolics found in the soil and not laboratory studies. And the observed effects were correlated with the total amount of phenolics present in the soil. In another study, root extracts and soil from *E. grandis* plantations showed allelopathic effects on seed germination of three different test species; however, the degree of effect and whether it was inhibitory or stimulatory depended on the age of the plantation, among other factors (Zhang et al., 2010). Other *Eucalyptus* species have shown similar allelopathic effects (Espinosa-García et al., 2008). Although not directly considered as evidence for this question, it should be noted that the hybrid *E. grandis* × E. urophylla exhibits some allelopathic effects against some fungi and insects with foliar extracts in a laboratory setting (Liu et al., 2008).
Imp-G2 (Parasitic)	n - negl	0	No evidence. This species does not belong to a family known to contain parasitic plant species (Heide-Jorgensen, 2008; Nickrent, 2009).
Impacts to Natural Systems			
Imp-N1 (Change ecosystem processes and parameters that affect other species)	y - low	0.4	In South Africa grasslands, reduces stream flow by up to 100% (Beater et al., 2008). Another study reports "that afforestation with *E. grandis* [in South Africa] has reduced annual flow by a maximum of 300-380 mm yr-1 " (Van Lill et al., 1980). In a broad study of the impacts of a large number of invasive alien plants in South Africa, this species was classified as affecting surface water in riparian areas (van Wilgen et al., 2008), however, the study does not provide any specific information for *E. grandis*. Relative to several other potential timber species for South East Asia, *E. grandis* had the highest transpiration rates and lowest water use efficiencies (Hu et al., 2012). Some soil properties of agricultural land in China afforested with *E. grandis* changed over time (Zhang et al., 2012), suggesting it may affect soil properties of natural habitats it invades.
Imp-N2 (Change community structure)	y - mod	0.2	The species is considered invasive in Africa, where it is changing a savannah or scrub habitat to open forest or closed forest so there is evidence it changes community structure.
Imp-N3 (Change community composition)	y - low	0.2	Replaces native vegetation (Wells et al., 1986). Categorized as having a high impact on biodiversity (van Wilgen et al., 2008).
Imp-N4 (Is it likely to affect federal Threatened and Endangered species)	y - mod	0.1	Because this species invades natural areas and replaces native vegetation (Wells et al., 1986; van Wilgen et al., 2008), it is likely to affect threatened and endangered species in the United States. This species has naturalized in the Mission Valley Preserve of San Diego (UC, 2012) home to the endangered Least Bell's Vireo (*Vireo bellii pusillus*) (SDRPF, 2012); and in the Fred C. Babcock-Cecil M. Webb wildlife management Area in southern Florida (IRC, 2012 where 7 Federally listed and 11 Florida listed T&E species occur (FFWCC, 2003).
Imp-N5 (Is it likely to affect any globally outstanding ecoregions)	y - high	0.1	Because this species invades and impacts natural areas (Beater et al., 2008) and reduces stream flow in those areas (Beater et al., 2008, it may affect globally outstanding

			ecoregions in the southeastern U.S. and in California (Ricketts et al., 1999).
Imp-N6 (Weed status in natural systems) For conservation/natural areas, choose the best answer. (A) Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control.	c - negl	0.6	Weed of the native flora (Wells et al., 1986). Subject of herbicide registration (Wells et al., 1986). A problem species invading conservation areas (Cruickshank, 1988). Invader of natural areas (Daehler, 1998). This species, among others, was cut and felled from South African savannas and grasslands through the Working for Water program to increase water availability for human consumption, agriculture, and ecotourism (Le Maitre et al., 2002;Beater et al., 2008). Recommended for clearing from riparian areas (Forsyth et al., 2004). Alternate answers for the Monte Carlo simulation were both "b".
Impact to Anthropogenic Systems (cities, suburbs, roadways)			
Imp-A1 (Impacts human property, processes, civilization, or safety)	? - max		*Eucalyptus grandis* has invaded a water catchment region near Cape Town which is important in supplying water for the metropolitan area (Le Maitre et al., 2002). Because it is one of the species targeted for control under the Working for Water program in South Africa and because this species has been documented to reduce stream flow by up to 100% (Beater et al., 2008), it is very likely reducing water for human consumption. However, because we found no documented evidence that it specifically has had this impact in urban areas, we cannot answer "yes". Consequently, answering this question as "unknown". This species is escaping or has naturalized in California under city powerlines (UC, 2012), but it is unknown if it is impacting utilities.
Imp-A2 (Changes or limits recreational use of an area)	n - mod	0	No evidence.
Imp-A3 (Outcompetes, replaces, or otherwise affects desirable plants and vegetation)	n - mod	0	No evidence.
Imp-A4 (Weed status in anthropogenic systems) For urban/suburban areas, choose the best answer. (A) Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control;	c - high	0.4	Considered a ruderal weed in South Africa (Cruickshank, 1988;Wells et al., 1986). Subject of herbicide registration (Wells et al., 1986). This species, among others, was cut and felled from South African savannas and grasslands through the Working for Water program to increase water availability for human consumption, agriculture, and ecotourism (Beater et al., 2008). Due to a lack of strong and specific evidence for control for anthropogenic reasons, using "mod" uncertainty. Alternate answers for the Monte Carlo simulation were both "b".
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product yield)	n - low	0	No evidence.
Imp-P2 (Lowers commodity value)	n - low	0	No evidence.
Imp-P3 (Is it likely to impact trade)	n - mod	0	Regulated in South Africa where all plantings must be permitted and stands controlled to prevent spread (Macdonald et al., 2003). However, there is no evidence suggesting it is likely to follow a trade pathway.
Imp-P4 (Reduces the quality or availability of irrigation, or	? - max		*Eucalyptus grandis* has invaded a water catchment region near Cape Town which is important in supplying water for

strongly competes with plants for water)			irrigation of deciduous fruit trees (Le Maitre et al., 2002), but there is no direct evidence it has affected water availability in this system. Given its ability to reduce streamflow by up to 100% in natural areas (Beater et al., 2008), it seems likely that it could impact production systems.
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - high	0	No evidence of toxicity in *E. grandis*, but cyanogic compounds in two other species (*E. cladocalyx* and *E. viminalis*) have killed goats and guinea fowl (Burrows and Tyrl, 2001). Fresh leaves, leaf-extracts, and growth regulators and their intermediaries (which are endoperoxides) of *Eucalyptus grandis* were fed to goats with experimental infections of *Haemonchus contortus* (the barberpole worm) and found to have positive effects (decreased number of adult worms)(Bennet-Jenkins and Bryant, 1996).
Imp-P6 (Weed status in production systems) For production systems, choose the best answer. (A) Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control.	a - high	0	This species, among others, was cut and felled from South African savannas and grasslands through the Working for Water program to increase water availability for human consumption, agriculture, and ecotourism (Beater et al., 2008). Subject of herbicide registration (Wells et al., 1986). There is no evidence this species is considered a weed of production systems. But because it is being controlled in South Africa for broad reasons related to its impact to affect water resources (Beater et al., 2008;Le Maitre et al., 2002), answering "a" with "high" uncertainty. Alternate answers for the Monte Carlo simulation were "b" and "c".
Geographic Potential			
Plant cold hardiness zones			
Geo-Z1 (Zone 1)	n - negl	N/A	Frost tender.
Geo-Z2 (Zone 2)	n - negl	N/A	Frost tender.
Geo-Z3 (Zone 3)	n - negl	N/A	Frost tender.
Geo-Z4 (Zone 4)	n - negl	N/A	Frost tender.
Geo-Z5 (Zone 5)	n - negl	N/A	Frost tender.
Geo-Z6 (Zone 6)	n - negl	N/A	Frost tender.
Geo-Z7 (Zone 7)	n - negl	N/A	Frost tender.
Geo-Z8 (Zone 8)	n - high	N/A	May be in this zone in South Africa and Sichuan, China, however, this species is frost tender and it may be too cold.
Geo-Z9 (Zone 9)	y - mod	N/A	Australia (GBIF, 2012); South Africa (Henderson, 2001;Cowling et al., 1997;Forsyth et al., 2004); Botswana (Haysom and Murphy, 2003). Includes this zone band, however according to Eldridge (Eldridge et al., 1993) the coldest month should be no lower than 11^0C and mean daily temperature of the coldest month should be no lower than 40^0C for optimum growth of the species..
Geo-Z10 (Zone 10)	y - negl	N/A	United States (CA, FL); Colombia; Australia (GBIF, 2012); Botswana, Zimbabwe (Haysom and Murphy, 2003).
Geo-Z11 (Zone 11)	y - negl	N/A	Dominican Republic, Colombia, South Africa, Australia, Papua New Guinea (GBIF, 2012).
Geo-Z12 (Zone 12)	y - low	N/A	Indonesia, Cuba (Duke, 1983).
Geo-Z13 (Zone 13)	n - low	N/A	No evidence.
Koppen-Geiger climate classes			
Geo-C1 (Tropical rainforest)	y - negl	N/A	United States (FL), Dominican Republic, Colombia,

			Australia (GBIF, 2012).
Geo-C2 (Tropical savanna)	y - negl	N/A	Australia (GBIF, 2012); Indonesia, Cuba (Duke, 1983).
Geo-C3 (Steppe)	y - low	N/A	Botswana, Zimbabwe (Haysom and Murphy, 2003); United States (CA, Riverside County) (UC, 2012).
Geo-C4 (Desert)	n - low	N/A	Escaping in Riverside County, CA, which includes desert, but this is an unlikely climate type (UC, 2012).
Geo-C5 (Mediterranean)	y - low	N/A	United States (CA); South Africa (Western Cape near Cape Town) (ARC, 2011).
Geo-C6 (Humid subtropical)	y - negl	N/A	United States (FL), South Africa, Australia (GBIF, 2012GBIF 2012).
Geo-C7 (Marine west coast)	y - negl	N/A	South Africa, Australia (GBIF, 2012); Zimbabwe (Haysom and Murphy, 2003).
Geo-C8 (Humid cont. warm sum.)	n - low	N/A	No evidence, Frost tender.
Geo-C9 (Humid cont. cool sum.)	n - negl	N/A	Frost tender.
Geo-C10 (Subarctic)	n - negl	N/A	Frost tender.
Geo-C11 (Tundra)	n - negl	N/A	Frost tender.
Geo-C12 (Icecap)	n - negl	N/A	Frost tender.
10-inch precipitation bands			
Geo-R1 (0-10 inches; 0-25 cm)	n - low	N/A	No evidence; likely too dry.
Geo-R2 (10-20 inches; 25-51 cm)	y - low	N/A	United States (CA) (GBIF, 2012); Botswana (Haysom and Murphy, 2003).
Geo-R3 (20-30 inches; 51-76 cm)	y - negl	N/A	South Africa (GBIF, 2012); Zimbabwe (Haysom and Murphy, 2003).
Geo-R4 (30-40 inches; 76-102 cm)	y - negl	N/A	South Africa, Australia (GBIF, 2012).
Geo-R5 (40-50 inches; 102-127 cm)	y - negl	N/A	Australia (GBIF, 2012).
Geo-R6 (50-60 inches; 127-152 cm)	y - negl	N/A	United States (CA), Dominican Republic, Australia (GBIF, 2012); Indonesia, Cuba (Duke, 1983); Grows in 139cm of annual precipitation in Sichuan Province China (Zhang et al., 2012).
Geo-R7 (60-70 inches; 152-178 cm)	y - low	N/A	Indonesia, Cuba (Duke, 1983.
Geo-R8 (70-80 inches; 178-203 cm)	y - negl	N/A	Colombia (GBIF, 2012).
Geo-R9 (80-90 inches; 203-229 cm)	y - low	N/A	This precipitation band falls within two known bands.
Geo-R10 (90-100 inches; 229-254 cm)	y - low	N/A	Colombia (GBIF, 2012).
Geo-R11 (100+ inches; 254+ cm))	y - low	N/A	Indonesia, Cuba (Duke, 1983.
Entry Potential			
Ent-1 (Plant already here)	y -	1	This species has already escaped and naturalized in the United States, in California and Florida (NRCS, 2012; UC, 2012)
Ent-2 (Plant proposed for entry, or entry is imminent)	-	N/A	
Ent-3 (Human value & cultivation/trade status)	-	N/A	
Ent-4 (Entry as a contaminant)			
Ent-4a (Plant present in Canada, Mexico, Central	-	N/A	

America, the Caribbean or China)			
Ent-4b (Contaminant of plant propagative material (except seeds))	-	N/A	
Ent-4c (Contaminant of seeds for planting)	-	N/A	
Ent-4d (Contaminant of ballast water)	-	N/A	
Ent-4e (Contaminant of aquarium plants or other aquarium products)	-	N/A	
Ent-4f (Contaminant of landscape products)	-	N/A	
Ent-4g (Contaminant of containers, packing materials, trade goods, equipment or conveyances)	-	N/A	
Ent-4h (Contaminants of fruit, vegetables, or other products for consumption or processing)	-	N/A	
Ent-4i (Contaminant of some other pathway)	-	N/A	
Ent-5 (Likely to enter through natural dispersal)	-	N/A	

Appendix II. Weed Risk Assessment for *Eucalyptus urophylla*

United States Department of Agriculture

Animal and Plant Health Inspection Service

May 21, 2013

Version 1

Weed Risk Assessment for *Eucalyptus urophylla* S.T. Blake (Myrtaceae) – Timor mountain gum

Eucalyptus urophylla **open forest**
http://www.pbase.com/donfranklin/image/144969226

Agency Contact:

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United States Department of Agriculture
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Introduction Plant Protection and Quarantine (PPQ) regulates noxious weeds under the authority of the Plant Protection Act (7 U.S.C. § 7701-7786, 2000) and the Federal Seed Act (7 U.S.C. § 1581-1610, 1939). A noxious weed is defined as "any plant or plant product that can directly or indirectly injure or cause damage to crops (including nursery stock or plant products), livestock, poultry, or other interests of agriculture, irrigation, navigation, the natural resources of the United States, the public health, or the environment" (7 U.S.C. § 7701-7786, 2000). PPQ uses weed risk assessment (WRA)—specifically, the PPQ WRA model (Koop et al., 2012)—to evaluate the risk potential of plants, including those newly detected in the United States, those proposed for import, and those emerging as weeds elsewhere in the world.

Biotechnology Regulatory Services (BRS) regulates genetically engineered organisms under the authority of the Plant Protection Act (7 U.S.C. § 7701-7786, 2000) because these organisms my present a pest risk to U.S. plant resources. BRS administers oversight for certain genetically engineered plants (regulated articles) under the regulations at (7 CFR part 340). A person may petition the agency that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, is no longer regulated under the plant pest provisions of the Plant Protection Act or the regulations at 7 CFR part 340. The petitioner is required to provide information under § 340.6(c)(4) related to plant pest risk that the agency may use to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. A GE organism is no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act when APHIS determines that it is unlikely to post a plant pest risk. Part of the risk assessment that APHIS conducts when reviewing a petition for non-regulated status is the weediness of the regulated article. This risk assessment using the PPQ WRA model was reviewed by weed risk analysts in PPQ.

Because the PPQ WRA model is geographically and climatically neutral, it can be used to evaluate the baseline invasive/weed potential of any plant species for the entire United States or for any area within it. As part of this analysis, we use a stochastic simulation to evaluate how much the uncertainty associated with the analysis affects the model outcomes. We also use GIS overlays to evaluate those areas of the United States that may be suitable for the establishment of the plant. For more information on the PPQ WRA process, please refer to the document, *Background information on the PPQ Weed Risk Assessment*, which is available upon request.

Eucalyptus urophylla **S. T. Blake – Timor mountain gum**

Species Information Family: Myrtaceae

Initiation: Several eucalypt species and their hybrids are being examined for their production potential in the United States (Booth, 2012; Stape, In-press). A genetically engineered hybrid (GE) of *E. grandis* and *E. urophylla* is being field tested for its growth potential and field performance in the southeastern United States. As part of the analysis of the weed risk potential of this GE hybrid, we evaluated the weed risk potential of its parents. This is the risk assessment for *E. urophylla*.

Foreign distribution: *Eucalyptus urophylla* is native to Indonesia in the Lesser Sunda Islands (NGRP, 2012). *Eucalyptus urophylla* naturally occurs on volcanically derived soils on seven islands in eastern Indonesia (Adonara, Alor, Flores, Lembata (Lomblem), Pantar, Timor and Wetar). It is one of two *Eucalyptus* species (*E. deglupta* and *E. urophylla*) that occur exclusively outside of Australia (Payn, 2008). This species has the greatest altitudinal range in the genus (Wardell-Johnson et al., 1997). It has been introduced extensively throughout tropical areas of the world to be grown in forest plantations for pulp, paper and solid wood products. It was introduced to Java in 1890 and to Brazil in 1919. In 1966, it was introduced to Australia and since then to many other countries, notably Cameroon, China, Congo, French Guiana, Gabon, Ivory Coast, Madagascar, Malaysia and Papua New Guinea (Soerianegara and Lemmens, 1993).

U.S. distribution and status: *E. urophylla* has been planted in Hawaii (Aradhya and Phillips, 1993) but is apparently not being commercialized there. It is not documented as having been introduced into the continental United States. It is not reported to have escaped or naturalized in either Hawaii or the continental U.S.

WRA area[3]: Entire United States, including territories

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1. *Eucalyptus urophylla* analysis

Establishment/Spread Potential

Eucalyptus urophylla is a large tree native to Indonesia. The species reproduces by seed which are very small, and primarily dispersed by gravity. *Eucalyptus* seeds in general do not have dormancy and do not appear to form a long term soil seed bank, instead they are stored in the canopy and released in a slow trickle over a period of several years (Wellington, 1989). However, according to House (2013) even though *E. urophylla* has woody capsules, it holds its seeds for only a few months after flowering. They are dropped as they mature, and don't need fire to trigger seedfall or regeneration. It has been introduced as a commercial forest tree for large scale plantings in plantations throughout the world (Sein and Mitlohner, 2011). Even though the species has been introduced extensively throughout tropical areas of the world, there is no evidence that it is invasive. There are no reports that it has naturalized beyond established plantations in other countries and it has never been reported to be invasive. There was an above average level of uncertainty associated with this risk element, primarily because we could not answer 6 of the questions.
Risk score = -7 Uncertainty index = 0.35

[3] "WRA area" is the area in relation to which the weed risk assessment is conducted [definition modified from that for "PRA area" (IPPC, 2012).

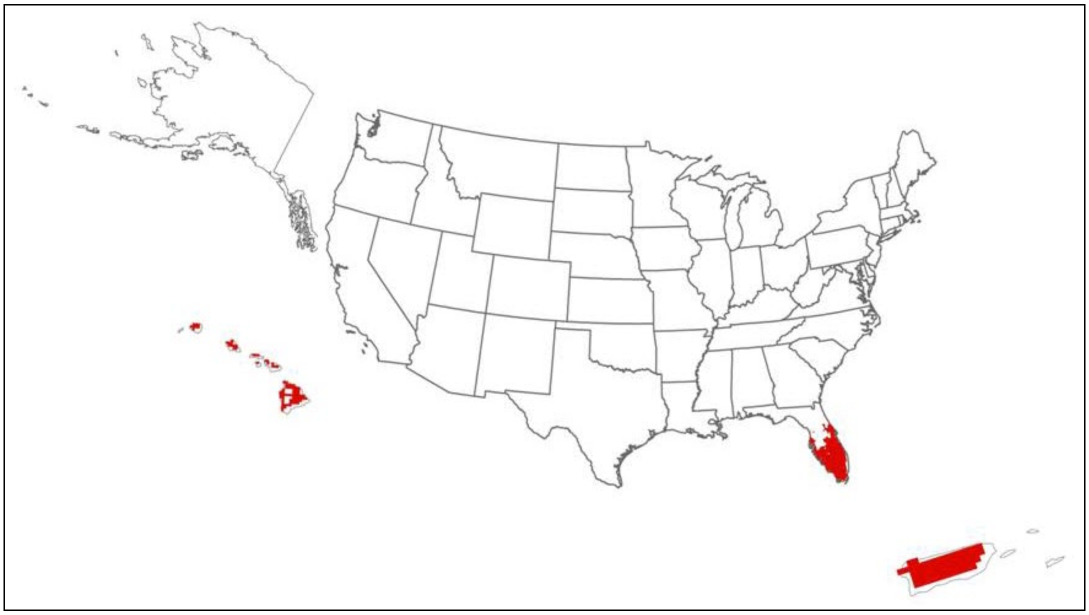
Impact Potential There is no evidence that *Eucalyptus urophylla* is associated with any impacts to or is even considered a weed in natural, anthropogenic, or production systems. There is one study of the species' effects on hydrology, but the study did not examine impacts *per se* (Morris et al., 2004). There are no reports on the impacts of native stands of *E. urophylla* on hydrology or water resources. As with other Eucalypts, *E. urophylla* is reported to be allelopathic (Fang et al., 2009; Qiu et al., 2007; Huang et al., 1997; Zeng and Li, 1997).
Risk score = 1.1 Uncertainty index = 0.13

Geographic Potential Based on three climatic variables, we estimate that about 0.77 percent of the United States is suitable for the establishment of *Eucalyptus urophylla* (Fig. 1). This predicted distribution is based on the species' known distribution elsewhere in the world and includes point-referenced localities and areas of occurrence. The map for *Eucalyptus urophylla* represents the joint distribution of Plant Hardiness Zones 10-13 (NAPPFAST Global Plant Hardiness Zones: http://www.nappfast.org/), areas with 20-100+ inches of annual precipitation, and the following Köppen-Geiger climate classes: tropical rainforest, tropical savannah, humid subtropical. As noted by Harwood (2011), *E. urophylla* is one of the nine most frequently planted species of *Eucalyptus* and is adapted for very warm temperatures in tropical and subtropical areas (a mean annual temperature of 18-28^{0}C).

The area estimated in Fig. 1 likely represents a conservative estimate as it uses three climatic variables to estimate the area of the United States that is suitable for establishment of the species. Other environmental variables, such as soil and habitat type, may further limit the areas in which this species is likely to establish.

Entry Potential We did not assess *E. urophylla* entry potential because this species is already present in the United States in Hawaii (Aradhya and Phillips, 1993).

Figure 1. Predicted distribution of *Eucalyptus urophylla* in the United States. Map insets for Alaska, Hawaii, and Puerto Rico are not to scale.



2. Results and Conclusion

Model Probabilities: P(Major Invader) = 0.6%
P(Minor Invader) = 15.9%
P(Non-Invader) = 83.5%

Risk Result = Low Risk

Secondary Screening = Not Applicable

Figure 2. *Eucalyptus urophylla* risk score (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A for the complete assessment.

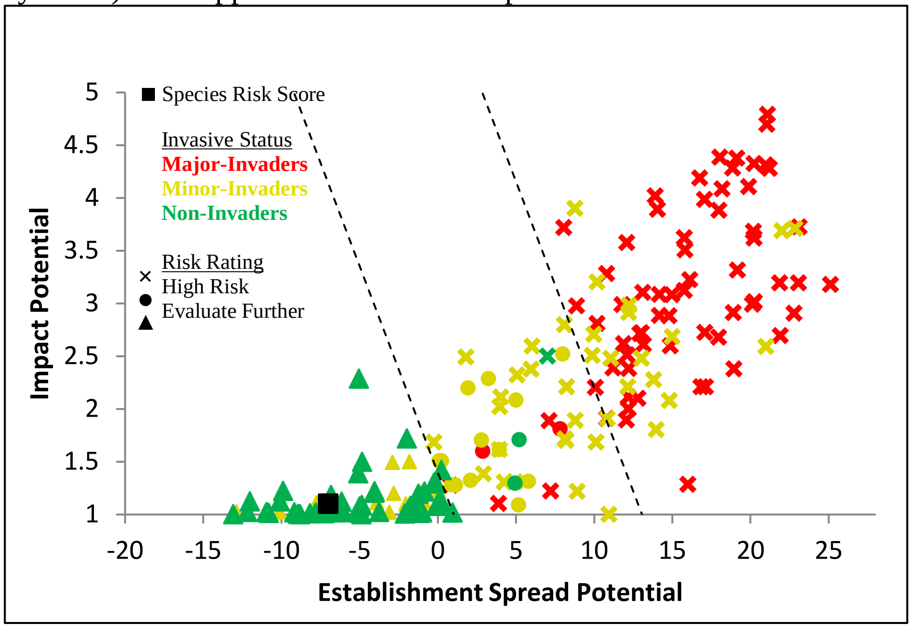
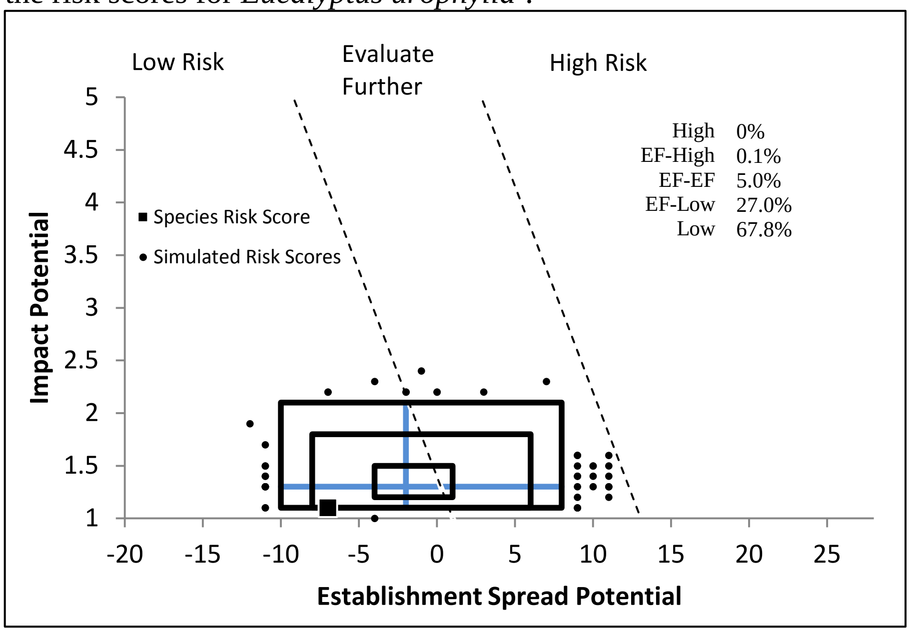


Figure 3. Monte Carlo simulation results (N=5,000) for uncertainty around the risk scores for *Eucalyptus urophylla*[a].



[a] The blue "+" symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

3. Discussion

The result of the weed risk assessment for *Eucalyptus urophylla* is "Low Risk" (Fig 2). Our predictive model indicates there is a 83 percent likelihood this species will not be invasive. There was an above average amount of uncertainty associated with establishment/spread potential. However, despite the uncertainty associated with our analysis, most of the simulated risk scores resulted in conclusions of Low Risk. Two separate assessments done on this species using the Australian weed risk assessment system concluded this species was high risk (Gordon et al., 2011) and a low risk (Daehler et al., 2004).

The most important data leading to an evaluation of Low Risk were the fact that even though it has been planted extensively in plantations for over 75 years, there is only one report that it has naturalized in Ecuador (Randall, 2002); however the data in this report could not be verified. There are no reports of it having escaped from cultivation and becoming invasive. To our knowledge there are still remnant commercial plantations or field tests of *Eucalyptus urophylla* currently growing in Hawaii where it was field tested in the 1990's (Aradhya and Phillips, 1993) (see: http://www.flickr.com/photos/jbfriday/6803487521/) and there are no commercial plantations of *Eucalyptus urophylla* currently grown in southern Florida where the data in our analysis indicate that it could be grown. Based on the current IFAS WRA, it is considered not to be a problem species and may be recommended for planting (see: http://plants.ifas.ufl.edu/assessment/pdfs/concl_genus_Feb2011.pdf).

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Appendix A. Weed risk assessment for *Eucalyptus urophylla* S. T. Blake (Myrtaceae). The following information was obtained from the species' risk assessment, which was conducted using Microsoft Excel. The information shown in this appendix was modified to fit on the page. The original Excel file, the full questions, and the guidance to answer the questions are available upon request.

Question ID	**Answer - Uncertainty**	**Score**	**Notes (and references)**
Establishment/Spread Potential			
ES-1 (Status/invasiveness outside its native range) Select one: (A) Introduced elsewhere long ago (>75 years) but not escaped; (B) Introduced recently (<75 years) but not escaped; (C) Never introduced elsewhere; (D) Escaped/Casual; (E) Naturalized; (F) Invader.	a - mod	-5	Native to Malasia: Indonesia - Lesser Sunda Islands (NGRP, 2012). Introduced extensively throughout tropical areas of the world. It was introduced to Java in 1890 and to Brazil in 1919. In 1966, it was introduced to Australia and since then to many other countries, notably Cameroon, China, Congo, French Guiana, Gabon, Ivory Coast, Madagascar, Malaysia and Papua New Guinea (Soerianegara and Lemmens, 1993). There are no reports except one, of it being naturalized or invasive. It is reported to be naturalized in Ecuador (Randall, 2002) but the data are inconclusive and cannot be verified. The fao.org lists *E. urophylla* as neither invasive or naturalized http://www.fao.org/forestry/24107/en/. The alternative answers for the Monte Carlo simulation are "b" and "e".
ES-2 (Is the species highly domesticated)	n - low	0	*Eucalyptus urophylla* is grown to produce wood for pulp production, fuelwood and charcoal. The wood's other primary use is for boards (Sein and Mitlohner, 2011). Most plantings are from seeds collected from various provenances in Indonesia (Sein and Mitlohner, 2011,Aradhya and Phillips, 1993). It has been used in clonal forestry (Denison and Quaile, 1987) and in breeding programs (CABI, 2012).
ES-3 (Weedy congeners)	y - negl	1	Three species of *Eucalyptus* are listed in Weber's "Invasive Species of the World". These are *E. cladocalyx*, *E. diversicolor*, and *E. globulus*. They reduce biodiversity, promote fire, reduce water availability (Weber, 2003). In California *E. globulus* and *E. camaldulensis* are listed as invasive by the Invasive Plant Council (Cal-IPC, 2012). An FAO report indicates that numerous other species of *Eucalyptus* have shown a propensity to escape and cause harm (Nyoka, 2003).
ES-4 (Shade tolerant at some stage of its life cycle)	n - low	0	Requires very bright light intensity for growth (FAO, 2007) *E. urophylla* is extremely susceptible to competition in the early stages and must be kept weed-free for 6 to 12 months (RISE, 2009). *E. urophylla* is similar to other eucalypts in that it is a vigorous, light demanding species (CABI, 2012).
ES-5 (Climbing or smothering growth form)	n - negl	0	Plant is a tree (AgroForestry, 2012,Zhengyi et al., 2012).
ES-6 (Forms dense thickets)	n - mod	0	No evidence. Does not form dense populations. No evidence that *E. urophylla* forms dense stands in natural settings.
ES-7 (Aquatic)	n - negl	0	Not an aquatic, plant is a terrestrial tree (AgroForestry, 2012).

ES-8 (Grass)	n - negl	0	Not a grass, in the Myrtaceae family (NGRP, 2012)
ES-9 (Nitrogen-fixing woody plant)	n - negl	0	Not in plant family known to contain nitrogen fixing species (Martin and Dowd, 1990).
ES-10 (Does it produce viable seeds or spores)	y - negl	1	Reproduces from seeds (Sein and Mitlohner, 2011)(CABI, 2012)
ES-11 (Self-compatible or apomictic)	y - negl	1	Flowers are perfect (House, 1997). There is some degree of self-incompatibility. Studies indicate that *E. urophylla* has an outcrossing rate of 0.91 [0=complete selfing, 1=complete outcrossing in natural populations] (Potts and Wiltshire, 1997). *E. urophylla* can be considered cryptically self-incompatible (Horsley and Johnson, 2007) and late-acting self-incompatibility in *E. urophylla* seems likely on account of the low number of seeds set following self-pollination (Horsley and Johnson, 2007). An 11% reduction in seed set has been shown to result from self-pollination (Potts and Wiltshire, 1997).
ES-12 (Requires special pollinators)	n - negl	0	The bisexual flowers are open to many pollen vectors such as insects, birds or small mammals. Some wind pollination is also possible (AgroForestry, 2012). Eucalypt flowers (referring to the genus) "show no adaptive characteristics that could be particularly attractive to" specialized pollinators (House, 1997).
ES-13 (Minimum generation time) Minimum generative time (A) less than 1 (multiple generations per year), (B) 1 year (annual-1 gen per year), (C) 2or3 years, (D)>3years; (?))	d - high	-1	*Eucalyptus urophylla* first begin to flower when they are 2–3 years old, with seeds being produced abundantly by the age of 4. Flowering occurs during the dry season and within 6 months the seeds reach maturity (Sein and Mitlohner, 2011). *Eucalyptus* flowering in plantations has no bearing on the actual age of the plants. Flowering can occur as early as 2-3 years of age after the trees have been planted (Sein and Mitlohner, 2011). Capsules generally remain closed for a few months to a year before release. It seems unlikely that a forest tree derived from a germinating seed would begin reproducing by age 2. All of the evidence cited above originates from the forestry literature, where foresters may very likely be basing their observations on plants (i.e., mature seedlings) that have already been growing for a year or two before being planted in the forest plantations. This observation plus the fact that flowering trees retain seeds in enclosed capsules for several months after seed mature, supports the idea that the minimum and effective generation time is probably four years or so. Answering "d", but using high uncertainty. Alternate answers for the Monte Carlo simulation are both "c".
ES-14 (Prolific reproduction)	? - max	0	Unknown. There are no direct estimates of seed production per square meter. *E. urophylla* has an average of 450,000 seeds/kg (RISE, 2009) On average there are 400,000–700,000 seeds/kg; with 1000 viable seeds weighing 1.4–2.5 g. (Sein and Mitlohner, 2011). Viable seeds average 210 to 650 per kg. (Nieto and Rodriguez, 2002).
ES-15 (Propagules likely to be dispersed unintentionally by people)	? - max	0	No information for *E. urophylla*. It is possible that given the very small size of the seeds that under muddy conditions the seeds could readily attach to vehicles, equipment and clothing. Heavy equipment is used to

			harvest trees and if trees are harvested under wet and muddy conditions seeds could adhere to tires, boots etc. during harvest operations. Long distance dispersal of small seeds without any appendages for adhesion can occur via transport in mud on motor vehicles (Taylor et al., 2011; Clifford, 1959) and clothing (Clifford, 1956). Clifford (1959) noted that small seeds tend to be represented by more individuals in the mud samples than those with large seeds.
ES-16 (Propagules likely to disperse in trade as contaminants or hitchhikers)	? - max	0	No evidence of this occurring for *E. urophylla*. It does not seem likely that seeds from a forest plantation tree, which are not well-dispersed, would contaminate a commodity moving in trade. However, it is possible for seeds to adhere to logs in mud and within bark. Therefore answering unknown.
ES-17 (Number of natural dispersal vectors)	0	-4	The following description of the fruit and seeds applies to questions ES17a - ES17e: Seeds produced in capsules (Zhengyi et al., 2012. The fruit is a typical *Eucalyptus* capsule: cup shaped and made up of 3–5 valves. It has a double operculum (lid) and the outer operculum is shed early (Sein and Mitlohner, 2011). Seeds are very small, on average there are around 250 seeds per gram (Sein and Mitlohner, 2011). The seed is shed under the influence of gravity and wind. There is no mechanism by which it is actively ejected (Cremer, 1965).
ES-17a (Wind dispersal)	n - mod		In general *Eucalyptus* seeds do not have dormancy but a few alpine species require cold , moist pretreatment to break dormancy (Boland et al., 1980). According to Boland (1980) no such treatment is required for *E. urophylla*. Seeds of *E. urophylla* do not have any adaptations that are associated with classic wind dispersal (e.g., plumes or wings) (Boland et al., 1980). Wind aids in passive dispersal of *Eucalyptus* seeds and depends on tree height, crown diameter, wind velocity, and mass and terminal velocity of the seeds; dispersal distances range between 30 to 60 meters (House, 1997). "Seed is mainly dispersed by wind and gravity after release from capsules in the canopy", but most seeds are dispersed near parent trees (Potts and Wiltshire, 1997). Cremer (1977) found that 12 of 15 species of *Eucalyptus* with wingless seeds dispersed less than 30 m from the parent tree when released at 40 m above the ground into a wind speed of 10 km/h. He concluded that few seeds are dispersed greater than twice the height of the tree (cited in Virtue Virtue and Melland, 2003)). There is no information on dispersal distances for *E. urophylla*. Evidence from the other species in the genus suggest that wind dispersal is limited to areas nearby parent trees. Consequently answering "no" with "mod" uncertainty.
ES-17b (Water dispersal)	? - max		*E. urophylla* grows on mountain slopes and in valleys and there is no indication that in its natural habitat it occurs along riparian areas and is distributed by water. However, given that other species can be dispersed via water and that most Eucalypt invasions in South Africa happen along watercourses (ARC, 2011, Forsyth et al., 2004) it cannot be ruled out that seed of this species

			could be distributed by water as well. Therefore answering unknown.
ES-17c (Bird dispersal)	? - max		No evidence for *E. urophylla*. Fruit and seeds offer no obvious rewards for frugivores (Zhengyi et al., 2012). However, for *Eucalyptus* species in general, some bird predators "mediate some dispersal when they fail to consume a proportion of the seeds they harvest" (House, 1997). Occasionally, some birds like cockatoos may scatter *Eucalyptus* seeds when they crack open living capsules (Cremer, 1965). Without additional evidence on the frequency of seed removal by predacious birds and more specific information on *E. urophylla* answering "unknown".
ES-17d (Animal external dispersal)	? - max		No evidence for *E. urophylla*. Given the small size of the seeds, it is possible they may get stuck in animal fur. Ant seed predators will disperse some seeds of *Eucalyptus* species if they are not consumed (House, 1997). "Ants are significant harvesters of *Eucalyptus* seed on the ground, removing 60% of fallen seed in tall open-forests" (cited in House, 1997). Harvest of fallen seeds by ants "no doubt results in some effective dispersal" (Potts and Wiltshire, 1997), but probably not for *E. salmonophloia* (Yates et al., 1995). One author reports that even if seeds taken by ants escape predation in ant granaries underground, germinating seeds are usually buried too deep to break through to the soil surface (Wellington, 1989). "Native trigonid bees inadvertently pick up *E. torelliana* seeds while foraging for resin within capsules still in the canopy" (cited in House, 1997). "Seed dispersal in the genus appears to be extremely limited, although there are exceptions where dispersal is enhanced by.....animal transport" (cited in Potts and Wiltshire, 1997). Although there is strong evidence for ant-mediated seed dispersal of *Eucalyptus* we are answering "unknown" because it is not clear whether *E. urophylla* is dispersed by ants, how frequently that may or may not occur, and if it did occur, how far ants would disperse the seeds.
ES-17e (Animal internal dispersal)	n - mod		No evidence for *E. urophylla*. Fruit and seeds offer no obvious rewards for frugivores (Zhengyi, 2012 #21}). Because we have found no evidence of internal dispersal of *Eucalyptus* seeds by non-avian dispersal agents, answering "no".
ES-18 (Evidence that a persistent (>1yr) propagule bank (seed bank) is formed)	n - high	-1	Seeds of *E. urophylla* can be kept viable for 5–20 years if they are stored in a sealed container and kept at low humidity (8–10%) and a temperature of 3–5 °C in order to protect against insects and fungi (Sein and Mitlohner, 2011). There are no data available on dormancy of *E. urophylla* seeds and there is no information on seed longevity in natural populations. According to House (2013) *E. urophylla* has woody capsules, but holds its seeds for only a few months after flowering. They are dropped as they mature, and don't need fire to trigger seedfall or regeneration. *Eucalyptus* seeds in general do not appear to form a long term soil seed bank, instead they can stored in the canopy and released in a slow

			trickle over a period of several years (Wellington, 1989). However, some species, with fragile capsules, release their seed soon after they mature (Cremer, 1965). Boland (1980) indicates that *E. urophylla* seeds should be harvested as soon as they are mature and that it is not a "long duration" species where seeds can be collected during any time of the year. This would indicate that long-term storage of seeds in capsules does not normally occur with *E. urophylla*.
ES-19 (Tolerates/benefits from mutilation, cultivation or fire)	? - max	0	Unknown. *Eucalyptus urophylla* coppices when cut (Sein and Mitlohner, 2011), but it is unclear if the response is significantly more stronger than in most other tree taxa. Note that *Eucalyptus* seeds in general are retained in the canopy in capsules that open as they desiccate or die (Wellington, 1989). In general forest fires promote the regeneration of Eucalypt forests because it triggers a massive release of canopy stored seed; seeds stored in capsules are well protected from fire and begin to release seeds a few days after the fire has passed (Wellington, 1989). However, seed response to fire or biomass loss is not considered in this question.
ES-20 (Is resistant to some herbicides or has the potential to become resistant)	n - negl	0	No evidence of resistance to herbicides and not listed by Heap (2012). When you consider the long life cycle of this plant, the plantation environments where individuals grow, and silvicultural practices it seems highly unlikely populations will develop herbicide resistance. Although *Eucalyptus* species can and often hybridize (Potts and Wiltshire, 1997), there is no evidence that a commercial *Eucalyptus* species has acquired or been genetically engineered for herbicide resistance. Consequently answering "no" with "negl" uncertainty.
ES-21 (Number of cold hardiness zones suitable for its survival)	4	0	
ES-22 (Number of climate types suitable for its survival)	3	0	
ES-23 (Number of precipitation bands suitable for its survival)	9	1	
Impact Potential			
General Impacts			
Imp-G1 (Allelopathic)	y - negl	0.1	Soil samples obtained from *E. urophylla* plantations inhibited seed germination of squash seed and bean seeds (Espinosa-García et al., 2008). These were natural concentrations of phenolics found in the soil and not laboratory studies and the observed effects were correlated with the total amount of phenolics present in the soil. Aqueous leaf leachate and leaf volatiles of *Eucalyptus urophylla* were found to have allelopathic effect on seed germination and seedling growth of seven native tree species in China (Fang et al., 2009). Qiu et al. (2007) examined the allelopathic effect of *Eucalyptus urophylla* on four legume tree species, and found that the seedling growth of two species were inhibited by leaf volatiles. Leaf extracts of *Eucalyptus urophylla* were shown to inhibit germination of *Brassica* and root growth

			in mung bean cuttings (Huang et al., 1997). Leaf volatiles had a significant inhibition on seedling growth of *Raphanus sativa*, *Lactuca sativa*, *Leucaena leucocephala* and *Acaia mangium* (Zeng and Li, 1997). There is no evidence from field conditions. Other *Eucalyptus* species have shown similar allelopathic effects (Espinosa-García et al., 2008).
Imp-G2 (Parasitic)	n - negl	0	No evidence. This species does not belong to a family known to contain parasitic plant species (Heide-Jorgensen, 2008; Nickrent, 2009).
Impacts to Natural Systems			
Imp-N1 (Change ecosystem processes and parameters that affect other species)	n - mod	0	No evidence. Has been planted extensively in other countries with large plantings in China (Qi, 2002) and Vietnam (Sein and Mitlohner, 2011). There is only one report that examined effects of *E. urophylla* plantations on hydrology and the results are inconclusive (Morris et al., 2004). There are no reports on impacts of native stands on ecosystem parameters. Because this species has been extensively planted, and because it is not considered an invasive species (there is only one unsubstantiated report of naturalization (Randall, 2002) answering no with mod uncertainty.
Imp-N2 (Change community structure)	n - mod	0	There is no evidence it changes community structure or ecosystem properties.
Imp-N3 (Change community composition)	n - mod	0	There is no evidence it changes community composition. Studies have examined biodiversity in plantations (Hua, 2009; Ping and Xie, 2009 but not naturalized populations.
Imp-N4 (Is it likely to affect federal Threatened and Endangered species)	n - mod	0	No evidence.
Imp-N5 (Is it likely to affect any globally outstanding ecoregions)	n - mod	0	No evidence.
Imp-N6 (Weed status in natural systems) For conservation/natural areas, choose the best answer. (A) Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control.	a - mod	0	No evidence. There is one report of it being naturalized in Ecuador but no evidence that control methods are needed (Randall, 2002).
Impact to Anthropogenic Systems (cities, suburbs, roadways)			
Imp-A1 (Impacts human property, processes, civilization, or safety)	n - mod	0	Has been planted extensively in other countries with large plantings in China (Qi, 2002) and Vietnam (Sein and Mitlohner, 2011). Because this species has been extensively planted, and because it is not considered an invasive species (there is only one unsubstantiated report of naturalization (Randall, 2002) answering no with mod uncertainty.
Imp-A2 (Changes or limits recreational use of an area)	n - mod	0	No evidence.
Imp-A3 (Outcompetes, replaces, or otherwise affects desirable	n - mod	0	No evidence.

plants and vegetation)			
Imp-A4 (Weed status in anthropogenic systems) For urban/suburban areas, choose the best answer. (A) Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control.	a - low	0	No evidence.
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product yield)	n - low	0	No evidence.
Imp-P2 (Lowers commodity value)	n - low	0	No evidence.
Imp-P3 (Is it likely to impact trade)	n - mod	0	No evidence.
Imp-P4 (Reduces the quality or availability of irrigation, or strongly competes with plants for water)	n - mod	0	There are no reports on impacts of native stands *of E. urophyll*a on hydrology or water resources in production systems. Other species of *Eucalyptus* that have invaded native habitats have had significant impacts on hydrology (Le Maitre et al., 2002;Beater et al., 2008). There is only one report that examined effects of *E. urophylla* plantations on hydrology but the study did not examine impacts per se (Morris et al., 2004).
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - high	0	No evidence of toxicity in *E. urophylla*, but cyanogic compounds in 2 other species (*E. cladocalyx* and *E. viminalis*) have killed goats and guinea fowl (Burrows and Tyrl, 2001).
Imp-P6 (Weed status in production systems) For production systems, choose the best answer. (A) Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control.	a - low	0	No evidence.
Geographic Potential			
Plant cold hardiness zones			
Geo-Z1 (Zone 1)	n - negl	N/A	Frost tender (AgroForestry, 2012)
Geo-Z2 (Zone 2)	n - negl	N/A	Frost tender
Geo-Z3 (Zone 3)	n - negl	N/A	Frost tender
Geo-Z4 (Zone 4)	n - negl	N/A	Frost tender
Geo-Z5 (Zone 5)	n - negl	N/A	Frost tender
Geo-Z6 (Zone 6)	n - negl	N/A	Frost tender
Geo-Z7 (Zone 7)	n - negl	N/A	Frost tender
Geo-Z8 (Zone 8)	n - negl	N/A	Frost tender
Geo-Z9 (Zone 9)	n - mod	N/A	Frost tender: Biophysical limit reported to be mean annual temperature: 8-29 deg. C (AgroForestry, 2012)
Geo-Z10 (Zone 10)	y - high	N/A	Grown in the Guangdong Province in South China (Fang et al., 2009) - Based on regional data. Includes this zone band, however the native range does not include this low temperature (Eldridge et al., 1993)

			where the species generally grows from 17-210C to 27-300C.
Geo-Z11 (Zone 11)	y - negl	N/A	Indonesia, Columbia, Dominican Republic, Honduras (GBIF, 2012)
Geo-Z12 (Zone 12)	y - negl	N/A	Indonesia, French Guiana, Philippines (GBIF, 2012)
Geo-Z13 (Zone 13)	y - negl	N/A	Indonesia, Philippines (GBIF, 2012)
Koppen-Geiger climate classes			
Geo-C1 (Tropical rainforest)	y - negl	N/A	Indonesia, Philippines (GBIF, 2012), Hawaii (Aradhya and Phillips, 1993).
Geo-C2 (Tropical savanna)	y - negl	N/A	Indonesia, Honduras, Venezuela (GBIF, 2012),Vietnam (Sein and Mitlohner, 2011), India (Kulkarni, 2010), Minas Gerais, Brazil (Rocha et al., 2008), Bahia, Brazil (Araujo et al., 2012).
Geo-C3 (Steppe)	n - negl	N/A	No evidence
Geo-C4 (Desert)	n - negl	N/A	No evidence
Geo-C5 (Mediterranean)	n - mod	N/A	No evidence. Even though other species of *Eucalyptus* will grow in a Mediterranean climate there is no evidence that this species has been planted and will grow well in these areas. According to Domingues et al., 2011 in the temperate and Mediterranean zones, *E. globulus* and *E. nitens* are the most common planted species while, in sub-tropical and tropical zones, *E. grandis*, *E. urophylla* and their hybrid (*E.*× *urograndis*) are among the preferred ones.
Geo-C6 (Humid subtropical)	y - negl	N/A	South China (Fang et al., 2009;Zhou and Wingfield, 2011), Minas Gerais, Brazil (Rocha et al., 2008), Zululand, S.A. (Gardner et al., 2007) Transvaal, SA (Denison and Quaile, 1987). All regional data but is clear species prefers humid subtropical.
Geo-C7 (Marine west coast)	n - mod	N/A	No evidence Parts of Transvaal, SA have this type of climate. It is unknown if this species is planted there.
Geo-C8 (Humid cont. warm sum.)	n - low	N/A	No evidence, Frost tender (AgroForestry, 2012).
Geo-C9 (Humid cont. cool sum.)	n - negl	N/A	Frost tender
Geo-C10 (Subarctic)	n - negl	N/A	Frost tender
Geo-C11 (Tundra)	n - negl	N/A	Frost tender
Geo-C12 (Icecap)	n - negl	N/A	Frost tender
10-inch precipitation bands			
Geo-R1 (0-10 inches; 0-25 cm)	n - negl	N/A	No evidence, likely too dry.
Geo-R2 (10-20 inches; 25-51 cm)	n - negl	N/A	No evidence
Geo-R3 (20-30 inches; 51-76 cm)	y - low	N/A	Bahia, Brazil (Araujo et al., 2012)
Geo-R4 (30-40 inches; 76-102 cm)	y - low	N/A	Bahia, Brazil (Araujo et al., 2012)
Geo-R5 (40-50 inches; 102-127 cm)	y - low	N/A	Minas Gerais, Brazil (Rocha et al., 2008)
Geo-R6 (50-60 inches; 127-152 cm)	y - negl	N/A	Minas Gerais, Brazil (Rocha et al., 2008), Venezuela (GBIF, 2012), Indonesia (GBIF, 2012)
Geo-R7 (60-70 inches; 152-178 cm)	y - negl	N/A	Minas Gerais, Brazil (Rocha et al., 2008), Honduras (GBIF, 2012)
Geo-R8 (70-80 inches; 178-203 cm)	y - low	N/A	Guangdong Province in South China (Fang et al., 2009)

Geo-R9 (80-90 inches; 203-229 cm)	y - low	N/A	Guangdong Province in South China (Fang et al., 2009), Vietnam (Sein and Mitlohner, 2011) Philippines (GBIF, 2012)
Geo-R10 (90-100 inches; 229-254 cm)	y - low	N/A	Vietnam (Sein and Mitlohner, 2011)
Geo-R11 (100+ inches; 254+ cm))	y - negl	N/A	French Guiana (GBIF, 2012)
Entry Potential			
Ent-1 (Plant already here)	y - negl	1	N/A for this analysis. Has been introduced into Hawaii (Aradhya and Phillips, 1993).
Ent-2 (Plant proposed for entry, or entry is imminent)	-	N/A	
Ent-3 (Human value & cultivation/trade status)	-	N/A	
Ent-4 (Entry as a contaminant)			
Ent-4a (Plant present in Canada, Mexico, Central America, the Caribbean or China)	-	N/A	
Ent-4b (Contaminant of plant propagative material (except seeds))	-	N/A	
Ent-4c (Contaminant of seeds for planting)	-	N/A	
Ent-4d (Contaminant of ballast water)	-	N/A	
Ent-4e (Contaminant of aquarium plants or other aquarium products)	-	N/A	
Ent-4f (Contaminant of landscape products)	-	N/A	
Ent-4g (Contaminant of containers, packing materials, trade goods, equipment or conveyances)	-	N/A	
Ent-4h (Contaminants of fruit, vegetables, or other products for consumption or processing)	-	N/A	
Ent-4i (Contaminant of some other pathway)	-	N/A	
Ent-5 (Likely to enter through natural dispersal)	-	N/A	

Appendix III. Weed Risk Assessment for *Eucalyptus* grandis × *Eucalyptus* urophylla

United States Department of Agriculture

Animal and Plant Health Inspection Service

May 21, 2013

Version 1

Weed Risk Assessment for *Eucalyptus grandis* × *Eucalyptus urophylla*

18 month-old plantation in Florida   23 month-old plantation in Florida

Agency Contact:

Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
United States Department of Agriculture
4700 River Road
Riverdale, MD 20737

Introduction Plant Protection and Quarantine (PPQ) regulates noxious weeds under the authority of the Plant Protection Act (7 U.S.C. § 7701-7786, 2000) and the Federal Seed Act (7 U.S.C. § 1581-1610, 1939). A noxious weed is defined as "any plant or plant product that can directly or indirectly injure or cause damage to crops (including nursery stock or plant products), livestock, poultry, or other interests of agriculture, irrigation, navigation, the natural resources of the United States, the public health, or the environment" (7 U.S.C. § 7701-7786, 2000). PPQ uses weed risk assessment (WRA)—specifically, the PPQ WRA model (Koop et al., 2012)—to evaluate the risk potential of plants, including those newly detected in the United States, those proposed for import, and those emerging as weeds elsewhere in the world.

Biotechnology Regulatory Services (BRS) regulates genetically engineered (GE) organisms under the authority of the Plant Protection Act because these organisms may present a pest risk to U.S. plant resources (7 U.S.C. § 7701-7786, 2000). BRS administers oversight for certain genetically engineered plants (regulated articles) under the regulations at (7 CFR part 340). A person may petition the agency that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, is no longer regulated under the plant pest provisions of the Plant Protection Act or the regulations at 7 CFR part 340. The petitioner is required to provide information under § 340.6(c)(4) related to plant pest risk that the agency may use to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. A GE organism is no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act when APHIS determines that it is unlikely to post a plant pest risk. Part of the risk assessment that APHIS conducts when reviewing a petition for non-regulated status is to assess the weediness of the regulated article. This risk assessment was reviewed by weed risk analysts in PPQ.

Because the PPQ WRA model is geographically and climatically neutral, it can be used to evaluate the baseline invasive/weed potential of any plant species for the entire United States or for any area within it. As part of this analysis, we use a stochastic simulation to evaluate how much the uncertainty associated with the analysis affects the model outcomes. We also use GIS overlays to evaluate those areas of the United States that may be suitable for the establishment of the plant. For more information on the PPQ WRA process, please refer to the document, *Background information on the PPQ Weed Risk Assessment*, which is available upon request.

***Eucalyptus grandis* W. Hill ex Maiden × *Eucalyptus urophylla* S.T. Blake**

Family: Myrtaceae

Species Synonyms: It is commonly known in the trade as *Eucalyptus urograndis*. Although it is called "*E. urograndis*" this name is not a valid species name as determined by: http://www.theplantlist.org/. However, *Eucalyptus* x *urograndis* is a valid name, following the rules of the International Code of Nomenclature of Cultivated Plants: http://www.ishs.org/sci/icracpco.htm.

Information Initiation: Several eucalypt species and their hybrids are being examined for their production potential in the United States) for solid wood, pulp for paper production and as potential bioenergy crops (Booth, 2012);(Stape, In-press). A genetically engineered (GE) hybrid of *E. grandis* and *E. urophylla* is being field tested for its growth potential and field performance in the southeastern United States. As part of the analysis of the weed risk potential of this GE hybrid, we evaluated the weed risk potential of both parents and the non GE hybrid. This is the assessment for the non- GE hybrid.

Foreign distribution: Because this hybrid was artificially created it has no natural range. It is widely planted in Brazil, Venezuela, Colombia, Congo, South Africa and China. It has also been introduced at an experimental level in countries such as Australia, Indonesia, Vietnam, Taiwan, Ecuador, and Mexico (CABI_A, 2012).

U.S. distribution and status: *E. grandis* × *E. urophylla* has been planted in Hawaii (CABI_A, 2012).

WRA area[4]: Entire United States, including territories.

1. *Eucalyptus grandis* × *Eucalyptus urophylla analysis*

Establishment/Spread Potential Hybrids of *Eucalyptus grandis* × *Eucalyptus urophylla* was first introduced into the Congo in 1978 (Vigneron and Bouvet, 2000) and into Brazil in the 1970s (Wright, 1997). The original Brazilian *E.* x *urograndis* hybrid trees were naturally generated because of the proximity of experimental plantation areas (Boland et al., 1980; Ikemori and Campinhos, 1983). The hybrid is planted extensively in tropical and subtropical regions (Bertolucci et al., 1995; Potts and Dungey, 2004) and is reported to be in South Africa, Brazil, Congo, China, Mexico, Colombia and Venezuela (Wright, 1997). It has never been reported to have escaped or having become naturalized or invasive from cultivated plantations in Brazil (Silva, 2010) or elsewhere.
Risk score = -2 Uncertainty index = 0.41

Impact Potential The only evidence of impact or potential damage caused by the hybrid is that it has been documented to be allelopathic (Espinosa-García et al., 2008), as are many other species of *Eucalyptus*, including its parents (Espinosa-García et al., 2008). However, evaluation of the hybrid's impact potential is not straightforward because this is not a naturally occurring taxon and because it was created only about 40 years ago. Aside from being allelopathic, there is no evidence that this taxon has caused any kind of impacts or is considered a weed by anyone. However, whether this reflects the taxon's true biotic potential, or its limited opportunity to establish and cause negative impacts is not clear at this time. Although it has been planted extensively in other countries with large

[4] "WRA area" is the area in relation to which the weed risk assessment is conducted [definition modified from that for "PRA area" (IPPC, 2012).

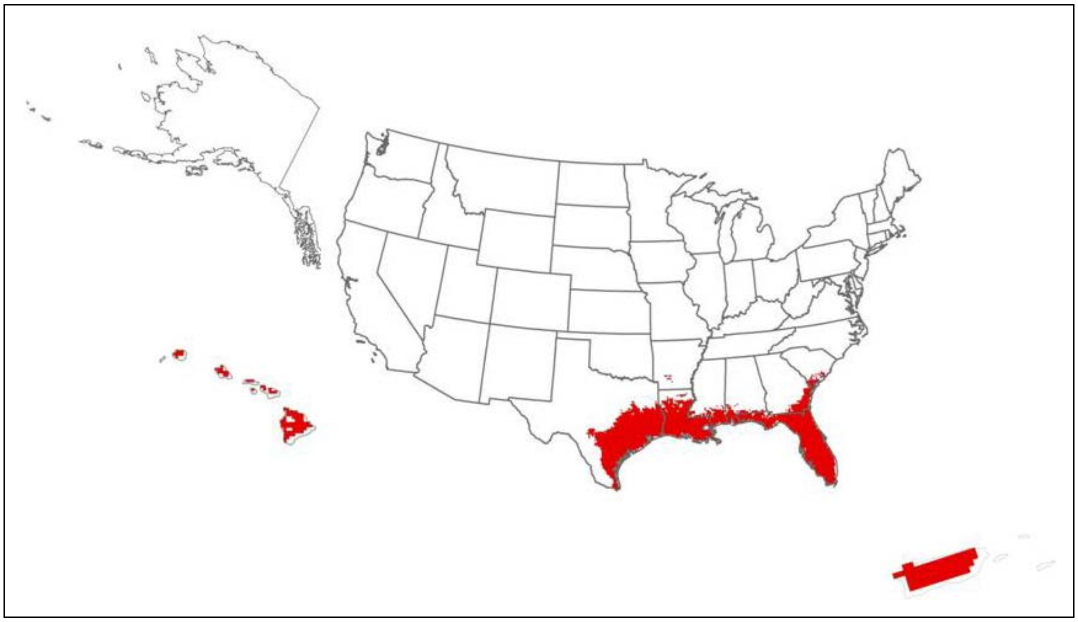
plantings in Brazil, 40 years is likely an insufficient period for evaluation of a woody tree species. The literature on invasive species has shown that sometimes long lag phases of over a 100 years precede the invasion of some plant species (Kowarik, 1995; Ellstrand and Schierenbeck, 2000). For these reasons, we answered the questions in this risk element as either unknown or "no" with high uncertainty. All of the questions on impacts in natural systems were answered as unknown, because one of the parents (*E. grandis*) has been shown to cause a variety of impacts to natural systems.
Risk score = 1.1 Uncertainty index = 0.70

Geographic Potential Based on three climatic variables, we estimate that about 4.9 percent of the United States is suitable for the establishment of *Eucalyptus grandis* × *E. urophylla* (Fig. 1). This predicted distribution is based on the species' known distribution elsewhere in the world and includes areas of occurrence. The map for *Eucalyptus grandis* × *E. urophylla* represents the joint distribution of Plant Hardiness Zones 9-12 (NAPPFAST Global Plant Hardiness Zones: http://www.nappfast.org/), areas with 10-100+ inches of annual precipitation, and the following Köppen-Geiger climate classes: tropical rainforest, tropical savannah, steppe, Mediterranean, humid subtropical, and marine west coast.

The area estimated in Fig. 1 likely represents a conservative estimate as it uses three climatic variables to estimate the area of the United States that is suitable for establishment of the species. Other environmental variables, such as soil and habitat type, may further limit the areas in which this species is likely to establish.

Entry Potential We did not assess they hybrid's entry potential because this species is already present in the United States (CABI_A, 2012; Nehra and Pearson, 2011).

Figure 1. Predicted distribution of *Eucalyptus grandis* × *E. urophylla* in the United States. Map insets for Alaska, Hawaii, and Puerto Rico are not to scale.



2. Results and Conclusion

Model Probabilities: P(Major Invader) = 1.9%
P(Minor Invader) = 37.1%
P(Non-Invader) = 61.0%

Risk Result = Low Risk

Secondary Screening = Not applicable

Figure 2. *Eucalyptus grandis* × *Eucalyptus urophylla* risk score (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A for the complete assessment.

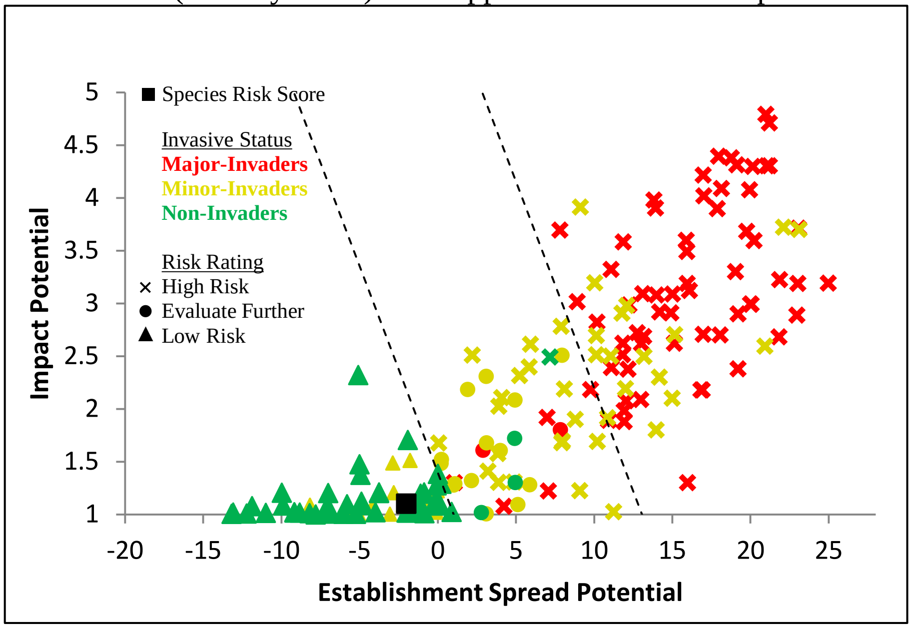
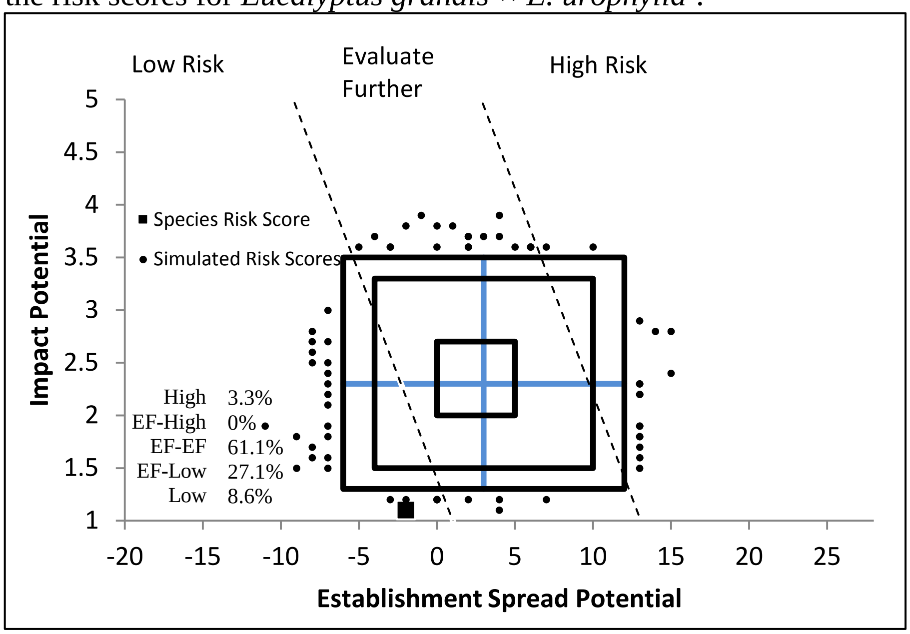


Figure 3. Monte Carlo simulation results (N=5,000) for uncertainty around the risk scores for *Eucalyptus grandis* × *E. urophylla*[a].



[a] The blue "+" symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

3. Discussion

The result of the weed risk assessment for *Eucalyptus grandis* × *Eucalyptus urophylla* is "Low Risk" (Fig 2.). Our predictive model indicates there is a 61 percent likelihood this species will not be invasive. Most of the simulated risk scores resulted in conclusions of Moderate Risk. The uncertainty associated with impact potential was well above average. The results of this analysis should be viewed with the understanding that this is a new taxon that has only been around for about 40 years. As noted above, the literature on invasive species has shown that sometimes long lag phases of over a100 years precede the invasion of some plant species (Kowarik, 1995; Ellstrand and Schierenbeck, 2000). Booth (2012) notes that *Eucalyptus* is likely more of a problem in South Africa perhaps due to the fact that it has been there longer than in other countries after its introduction. The fact that this is a hybrid which was created for increased yield (Foelkel, 2013) may also factor into its invasiveness. Ellstrand and Schierenbeck (2000) have shown that hybridization is sometimes a stimulus for invasiveness in plants. In a recent publication Callaham et al. (2013) point out that in the case of *Eucalyptus conferruminata*, the species has been extant in California for at least 50 years, but only recently has been observed to produce seedlings (Ritter and Yost, 2009), suggesting that there can be significant lag times associated with the naturalization / invasion process. Two separate assessments done on this hybrid using the Australian weed risk assessment system concluded this taxon was a low risk with evaluate further (Gordon et al., 2011) and a low risk (Chimera, 2012).

To our knowledge there are still remnant demonstration plantings or field tests the hybrid growing in Hawaii where it was field tested in the 1980's (Friday, 2003). There are no commercial plantations of *Eucalyptus grandis* × *E. urophylla* currently grown in the continental U.S. It is being field tested by ArborGen in various locations in the Southeast as a part of their studies with the genetically engineered version of the hybrid (Nehra and Pearson, 2011).

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Appendix A. Weed risk assessment for *Eucalyptus grandis* × *Eucalyptus urophylla* (Myrtaceae). The following information was obtained from the species' risk assessment, which was conducted using Microsoft Excel. The information shown in this appendix was modified to fit on the page. The original Excel file, the full questions, and the guidance to answer the questions are available upon request.

Question ID	Answer - Uncertainty	Score	Notes (and references)
Establishment/Spread Potential			
ES-1 (Status/invasiveness outside its native range) Select one: (A) Introduced elsewhere long ago (>75 years) but not escaped; (B) Introduced recently (<75 years) but not escaped; (C) Never introduced elsewhere; (D) Escaped/Casual; (E) Naturalized; (F) Invader.	b - high	-2	*Eucalyptus grandis* × *Eucalyptus urophylla* is an artificial hybrid created by cross-pollination of the two species. The hybrid was first introduced into the Congo in 1978 (Vigneron and Bouvet, 2000) and into Brazil in the 1970s (Wright, 1997). The original Brazilian *E.* x *urograndis* hybrid trees were naturally generated because of the proximity of experimental plantation areas (Boland et al., 1980; Ikemori and Campinhos, 1983). *E*. x *urograndis* is planted extensively in tropical and subtropical regions (Bertolucci et al., 1995;Potts and Dungey, 2004) and is reported to be in South Africa, Brazil, Congo, China, Mexico, Colombia and Venezuela (Wright, 1997). It has never been reported to have escaped from cultivated plantations in Brazil (Silva, 2010) or elsewhere. Uncertainly is high because the taxon has only been around for around 40 years. Alternative answer is d for both.
ES-2 (Is the species highly domesticated)	n - negl	0	*E. grandis* × *E. urophylla* is not a single hybrid cultivar but a large group of hybrids formed through crossing the two species. *E.* x *urograndis* hybrids are usually deployed as first generation (F1) clones (Potts and Dungey, 2004; Wright, 1997) that can be made from a wide variety of provenances (Potts and Dungey, 2004).
ES-3 (Weedy congeners)	y - negl	1	Three species of *Eucalyptus* are listed in Weber's "Invasive Species of the World". These are *E. cladocalyx, E. diversicolor*, and *E. globulus*. They reduce biodiversity, promote fire, and reduce water availability (Weber, 2003). In California *E. globulus* and *E. camaldulensis* are listed as invasive by the Invasive Plant Council (Cal-IPC, 2012). An FAO report indicates that *E. camaldulensis*, *E. cladocalyx*, *E. diversicolor*, *E. lehmannii*, *E. paniculata* and *E. sideroxylon* are major invaders in South Africa and in Zimbabwe *E. microcorys*, *E. camaldulensis*, *E. tereticornis*, *E. robusta, E. macarthurii*, *E. paniculata*, *E. globulus* and *E. citriodora* are considered major invaders (Nyoka, 2003). One of the parents of the hybrid being assessed, *E. grandis*, is beginning to escape (or is perhaps naturalized) in two counties of California (UC, 2012). Naturalized in Florida (NRCS, 2012; IRC, 2012; ISB, 2012) and in California (Ritter and Yost, 2009). It has naturalized in Australia beyond its native range (Randall, 2007). "invasive" in South Africa where its spread must be controlled (Macdonald et al., 2003). Naturalized populations in South Africa are spreading to other areas through long-distance seed dispersal (>100 meters away from parent plants) (Forsyth et al., 2004). Several other sources indicate it invades forests, grasslands, and savannas in Southern Africa (Booth, 2012; Cowling et al., 1997;Haysom and Murphy, 2003;

			Henderson, 2001 and Nyoka, 2003). For the other parent, *E. urophylla* there are no reports except one, of it being naturalized or invasive. It is reported to be naturalized in Ecuador (Randall, 2012) but the data are inconclusive.
ES-4 (Shade tolerant at some stage of its life cycle)	n - mod	0	Data for the hybrid are unknown. *Eucalyptus* in general are intolerant of shade (http://www.angelfire.com/bc/eucalyptus/selection.html) and urograndis is not listed as shade tolerant. The parent *E. grandis* is intolerant of shade. Seedlings can only develop in full or nearly full sunlight; trees must maintain a dominant or codominant canopy position to long survive. Suppressed trees quickly die and intermediate trees must grow to an overstory position or eventually lose vigor and die" (Meskimen and Francis, 1990). In its native range in Australia, *E. grandis* grows in mixed eucalypt forests that are relatively open, and sometimes at the edges and interiors of rain forests (Meskimen and Francis, 1990). The evidence strongly suggests this species is intolerant of shade; the one reference to growing in the interior of rainforests may be to individuals colonizing gaps. The other parent *E. urophylla* requires very bright light intensity for growth (FAO, 2007) *E. urophylla* is extremely susceptible to competition in the early stages and must be kept weed-free for 6 to 12 months (RISE, 2009). *E. urophylla* is similar to other eucalypts in that it is a vigorous, light demanding species (CABI_B, 2012). Based on the information available from both parents it is not expected that the hybrid would be any more shade tolerant.
ES-5 (Climbing or smothering growth form)	n - negl	0	Plant is a tree (CABI_A, 2012).
ES-6 (Forms dense thickets)	n - mod	0	No evidence. Planted almost exclusively in forest plantations. In general *Eucalyptus* does not form dense thickets.
ES-7 (Aquatic)	n - negl	0	Not an aquatic, plant is a terrestrial tree (CABI_A, 2012).
ES-8 (Grass)	n - negl	0	Not a grass, both parents are in the Myrtaceae family (NGRP, 2012).
ES-9 (Nitrogen-fixing woody plant)	n - negl	0	Not in plant family known to contain nitrogen fixing species (Martin and Dowd, 1990).
ES-10 (Does it produce viable seeds or spores)	y - negl	1	Reproduces from seeds: "Seeds from the *E.* x *urograndis* hybrid are fertile and viable" (Foelkel, 2013). Seeds of the hybrid are offered for sale in Brazil: http://brazilplantseeds.com/index.php/seeds-flowering/eucalyptus-urophylla-x-eucalyptus-grandis-seeds.html. Seed orchards have been used to mass produce *E.* x *urograndis* seeds (CABI_A, 2012).
ES-11 (Self-compatible or apomictic)	y - low	1	Both parents are self- compatible. For *E. grandis*, self-pollination yields 2-47% of the number of seeds that can be produced through outcrossing (Hodgson, 1976c). Nearly all clones of this species are self-fertile to varying degrees, and self-pollination may be more common in out of season flowering (Hodgson, 1976c). Studies indicate that *E. urophylla* has an outcrossing rate of 0.91 [0=complete selfing, 1=complete outcrossing in natural populations] (Potts and Wiltshire, 1997). Given that both parents are self-compatible it is reasonable to assume that the hybrid is also.
ES-12 (Requires special pollinators)	n - negl	0	Eucalypt flowers (referring to the genus) "show no adaptive characteristics that could be particularly attractive to" specialized pollinators (House, 1997). Insect-pollinated *Eucalyptus* do not

			express specificity with respect to the insect vector; honeybees can be used to augment pollination success (cited in Chaix et al., 2007). For the parent *E. grandis* the "species is entomophilous and honeybees are an important pollinator, although some pollination without bees may occur" (Hodgson, 1976b). For the parent *E. urophylla* the bisexual flowers are open to many pollen vectors such as insects, birds or small mammals. Some wind pollination is also possible (AgroForestry, 2012).
ES-13 (Minimum generation time) Minimum generative time (A) less than 1 (multiple generations per year), (B) 1 year (annual-1 gen per year), (C) 2or3 years, (D)>3years; (?))	d - high	-1	The hybrid's minimum generation time is unknown, but it would be expected to be similar to that of both parents. For *E. grandis* the first flowers appear at 2 to 3 years of age and the first capsules can be obtained 5 to 7 months after anthesis (Hodgson, 1976b). Another report says it can begin flowering within the first year of the plantation (Meskimen and Francis, 1990) but that 97% of *Eucalyptus grandis* families in plantations flower at age 3. Seed capsules are mature for harvest 6-7 months after flowering, but capsules remain closed on the tree for at least one year after reaching maturity (Meskimen and Francis, 1990). For *E. urophylla* trees first begin to flower when they are 2–3 years old, with seeds being produced abundantly by the age of 4. Flowering occurs during the dry season and within 6 months the seeds reach maturity (Sein and Mitlohner, 2011). For both parents the evidence cited above originates from the forestry literature, where foresters may very likely be basing their observations on plants (i.e., mature seedlings) that have already been growing for a year or two before being planted in the forest plantations. This observation plus the fact that flowering trees retain seeds in enclosed capsules for several months to a year after seed mature, supports the idea that the minimum and effective generation time is probably four years or so. Answering "d", but using high uncertainty. Alternate answers for the Monte Carlo simulation were both "c".
ES-14 (Prolific reproduction)	? - max	0	Unknown. There are no direct estimates of seed production per square meter. No data can be found for *E.* x *urograndis* seed production in the wild or in seed orchards.
ES-15 (Propagules likely to be dispersed unintentionally by people)	? - max	0	No information is available for *E.* x *urograndis*. It is possible that given the very small size of the seeds that under muddy conditions the seeds could readily attach to vehicles, equipment and clothing. Heavy equipment is used to harvest trees and if trees are harvested under wet and muddy conditions seeds could adhere to tires, boots etc. during harvest operations. Long distance dispersal of small seeds without any appendages for adhesion can occur via transport in mud on motor vehicles (Taylor et al., 2011;Clifford, 1959) and clothing (Clifford, 1956). Clifford (1959) noted that small seeds tend to be represented by more individuals in the mud samples than those with large seeds. Consequently answering as unknown.
ES-16 (Propagules likely to disperse in trade as contaminants or hitchhikers)	? - max	0	No evidence of this occurring for *E.* x *urograndis*. It does not seem likely that seeds from a forest plantation tree, which are not well-dispersed, would contaminate a commodity moving in trade. However, it is possible for seeds to adhere to logs in mud and within bark. Therefore answering unknown.
ES-17 (Number of natural dispersal vectors)	0	-4	0
ES-17a (Wind dispersal)	n - mod		No evidence. Seeds of *Eucalyptus* do not have any adaptation that are associated with classic wind dispersal (e.g plumes or

		wings)(Boland et al., 1980;House, 1997). Wind aids in passive dispersal of *Eucalyptus* seeds and depends on tree height, crown diameter, wind velocity, and mass and terminal velocity of the seeds; dispersal distances range between 30 to 60 meters (House, 1997). "Seed is mainly dispersed by wind and gravity after release from capsules in the canopy", but most seeds are dispersed near parent trees (Potts and Wiltshire, 1997). Cremer (1977) found that 12 of 15 species of *Eucalyptus* with wingless seeds dispersed less than 30 m from the parent tree when released at 40 m above the ground into a wind speed of 10 km/h. He concluded that few seeds are dispersed greater than twice the height of the tree (cited in Virtue (Virtue and Melland, 2003)). There is no information on dispersal distances for *E.* x *urograndis*. Evidence from the other species in the genus suggests that wind dispersal is limited to areas nearby parent trees. Consequently answering "no" with "mod" uncertainty.
ES-17b (Water dispersal)	? - max	No evidence for *E.* x *urograndis*. However, given that other species can be dispersed via water and that most Eucalypt invasions in South Africa happen along watercourses (ARC, 2011; Forsyth et al., 2004) it cannot be ruled out that seed of this species could be distributed by water. Therefore answering unknown.
ES-17c (Bird dispersal)	? - max	No evidence for *E.* x *urograndis*. Fruit and seeds offer no obvious rewards for frugivores. Studies in *Eucalyptus* plantations in the Amazon suggest that *Eucalyptus* stands and secondary growth forests fail to provide suitable foraging habitat for frugivores at any time of the year (Barlow et al., 2007). However, for *Eucalyptus* species in general, some bird predators "mediate some dispersal when they fail to consume a proportion of the seeds they harvest" (House, 1997). Occasionally, some birds like cockatoos may scatter *Eucalyptus* seeds when they crack open living capsules (Cremer, 1965). Without additional evidence on the frequency of seed removal by predacious birds and more specific information on *E.* x *urograndis* answering "unknown".
ES-17d (Animal external dispersal)	? - max	Unknown for *E.* x *urograndis*. Given the small size of the seeds, it is possible they may get stuck in animal fur. Ant seed predators will disperse some seeds of *Eucalyptus* species if they are not consumed (House, 1997). "Ants are significant harvesters of *Eucalyptus* seed on the ground, removing 60% of fallen seed in tall open-forests" (cited in House, 1997). Harvest of fallen seeds by ants "no doubt results in some effective dispersal" (Potts and Wiltshire, 1997), but probably not for *E. salmonophloia* (Yates et al., 1995). One author reports that even if seeds taken by ants escape predation in ant granaries underground, germinating seeds are usually buried too deep to break through to the soil surface (Wellington, 1989). "Native trigonid bees inadvertently pick up *E. torelliana* seeds while foraging for resin within capsules still in the canopy" (cited in House, 1997). "Seed dispersal in the genus appears to be extremely limited, although there are exceptions where dispersal is enhanced by.....animal transport" (cited in Potts and Wiltshire, 1997). Although there is strong evidence for ant-mediated seed dispersal of *Eucalyptus* we are answering "unknown" because it is not clear whether E. x urograndis is dispersed by ants, how frequently that may or may not occur, and if it did occur, how far ants would disperse the

			seeds.
ES-17e (Animal internal dispersal)	n - mod		Unknown for *E.* x *urograndis*. Fruit and seeds of both parents as well as other species of *Eucalyptus* offer no obvious rewards for frugivores (Zhengyi et al., 2012). Because we have found no evidence of internal dispersal of *Eucalyptus* seeds by non-avian dispersal agents, answering "no".
ES-18 (Evidence that a persistent (>1yr) propagule bank (seed bank) is formed)	n - high	-1	Unknown for *E.* x *urograndis*. Seeds of *Eucalyptus* can be stored for a number of years at cold temperatures and remain viable (Meskimen and Francis, 1990; Sein and Mitlohner, 2011). In general *Eucalyptus* seeds do not have dormancy but a few alpine species require cold , moist pretreatment to break dormancy (Boland et al., 1980). And according to Boland (1980) no such treatment is required for either *E. grandis* or *E. urophylla*. Data from both parents indicate that that long-term storage of seeds in soil and capsules likely does not occur with *E. urograndis*. For *E. grandis* there is no information on seed longevity in natural populations. In temperate *Eucalyptus* such as, *E. grandis*, seed maturation takes several months and seeds are retained in woody capsules (Hodgson, 1976a). Capsules produce viable seed in 4 - 5 months and are fully mature within 7 months (Hodgson, 1976b). According to House (2013) *E. grandis* does have woody capsules, but holds its seeds for only 6 months after flowering. They are dropped as they mature, and don't need fire to trigger seedfall or regeneration. Boland (1980) indicates that E. grandis seeds should be harvested as soon as they are mature and that it is not a "long duration" species where seeds can be collected during any time of the year. For the other parent, there are no data available on dormancy of *E. urophylla* seeds and there is no information on seed longevity in natural populations. According to House (2013) *E. urophylla* is similar to *E. grandis*. It also has woody capsules, and holds its seeds for only a few months after flowering. They are dropped as they mature, and don't need fire to trigger seedfall or regeneration. Boland (1980) indicates that *E. urophylla* seeds should be harvested as soon as they are mature and that it is not a "long duration" species where seeds can be collected during any time of the year. *Eucalyptus* seeds in general do not appear to form a long term soil seed bank, instead they can be stored in the canopy and released in a slow trickle over a period of several years (Wellington, 1989). However, some species, with fragile capsules, release their seed soon after they mature (Cremer, 1965). This occurs with both parents and is presumed to be true for the hybrid.
ES-19 (Tolerates/benefits from mutilation, cultivation or fire)	? - max	0	Unknown. *Eucalyptus urograndis* coppices when cut (Bernhard-Reversat et al., 2001) which is a standard practice when managing *E.* x *urograndis* plantations; but it is unclear if the response is significantly more stronger than in most other tree taxa. Note that *Eucalyptus* seeds in general are retained in the canopy in capsules that open as they desiccate or die (Wellington, 1989). In general forest fires promote the regeneration of Eucalypt forests because it triggers a massive release of canopy stored seed; seeds stored in capsules are well protected from fire and begin to release seeds a few days after the fire has passed (Wellington, 1989). However, seed response to fire or biomass loss is not considered in this question.
ES-20 (Is resistant to some herbicides or has the potential	n - negl	0	No evidence of resistance to herbicides and not listed by Heap (Heap, 2012). When you consider the long life cycle of this

to become resistant)			plant, the plantation environments where individuals grow, and silvicultural practices it seems highly unlikely populations will develop herbicide resistance. Although it is possible for this hybrid to hybridize with other species (Potts and Wiltshire, 1997), there is no evidence that a commercial *Eucalyptus* species has acquired or been genetically engineered for herbicide resistance. Consequently answering "no" with "negl" uncertainty.
ES-21 (Number of cold hardiness zones suitable for its survival)	5	0	
ES-22 (Number of climate types suitable for its survival)	4	2	
ES-23 (Number of precipitation bands suitable for its survival)	9	1	
Impact Potential			
General Impacts			
Imp-G1 (Allelopathic)	y - negl	0.1	Soil samples from *E. grandis* × *E. urophylla* plantations had an inhibitory effect on germination of maize, bean and watermelon but had a stimulatory effect on squash (Espinosa-García et al., 2008). These were natural concentrations of phenolics found in the soil and not laboratory studies. Both parents were also included in this study and showed similar results (Espinosa-García et al., 2008). Hao (2011) found that extracts of soil from *E.* x *urograndis* forests decreased levels of chlorophyll, proline and soluble sugars in *Brassica*, *Oryza* and *Raphanus*. In general other *Eucalyptus* species have shown similar allelopathic effects (Espinosa-García et al., 2008).
Imp-G2 (Parasitic)	n - negl	0	No evidence. This species does not belong to a family known to contain parasitic plant species (Heide-Jorgensen, 2008; Nickrent, 2009).
Impacts to Natural Systems			
Imp-N1 (Change ecosystem processes and parameters that affect other species)	? - max		Unknown. The hybrid has been planted extensively in other countries with large plantings in Brazil. It has been planted in other countries such as South Africa, Congo, China, Mexico, Colombia and Venezuela (Wright, 1997). There are no reports on impacts of native stands on ecosystem parameters. However the hybrid is a relatively new taxon. It is cultivated because it grows better than either parent (Foelkel, 2013) and shows resistance to disease (CABI_A, 2012), therefore it is possible that it could outcompete its neighbors. Given that this a relatively new taxon that has only been grown for around 40 years, and one of its parents (*E. grandis*) is known to have impacts on the environment, answering unknown for every question in this sub-element.
Imp-N2 (Change community structure)	? - max		Unknown
Imp-N3 (Change community composition)	? - max		Unknown
Imp-N4 (Is it likely to affect federal Threatened and Endangered species)	? - max		Unknown
Imp-N5 (Is it likely to affect any globally outstanding	? - max		Unknown

ecoregions)			
Imp-N6 (Weed status in natural systems) For conservation/natural areas, choose the best answer. (A) Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control.	? - max		Unknown. Has never been reported to be a weed or needing to be controlled as a weed.
Impact to Anthropogenic Systems (cities, suburbs, roadways)			
Imp-A1 (Impacts human property, processes, civilization, or safety)	? - max		Unknown. Has been planted extensively in other countries with large plantings in Brazil. Planted in other countries such as South Africa, Congo, China, Mexico, Colombia and Venezuela (Wright, 1997). Even though the species has been extensively cultivated it has only been planted for around 40 years so answering unknown.
Imp-A2 (Changes or limits recreational use of an area)	n - high	0	No evidence.
Imp-A3 (Outcompetes, replaces, or otherwise affects desirable plants and vegetation)	n - high	0	No evidence.
Imp-A4 (Weed status in anthropogenic systems) For urban/suburban areas, choose the best answer. (A) Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control.	? - max		Unknown for the hybrid. One of the parents (*E. grandis*) is considered a ruderal weed in South Africa (Cruickshank, 1988;Wells et al., 1986) and is the subject of herbicide registration (Wells et al., 1986). Because the hybrid has only been planted for around 40 years answering unknown.
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product yield)	n - mod	0	No evidence.
Imp-P2 (Lowers commodity value)	n - mod	0	No evidence.
Imp-P3 (Is it likely to impact trade)	n - high	0	No evidence.
Imp-P4 (Reduces the quality or availability of irrigation, or strongly competes with plants for water)	? - max		There are no reports on impacts of native stands of *E.* x *urograndis* on hydrology or water resources in production systems. Other species of *Eucalyptus*, including one of the parents *E. grandis*, have invaded native habitats have had significant impacts on hydrology (Le Maitre et al., 2002;Beater et al., 2008). There are no information available on the hybrid; consequently answering unknown.
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - high	0	No evidence of toxicity in *E.* x *urograndis*, but cyanogic compounds in 2 other species (*E. cladocalyx* and *E. viminalis*) have killed goats and guinea fowl (Burrows and Tyrl, 2001).
Imp-P6 (Weed status in production systems) For production systems, choose the best answer. (A)	a - high	0	No evidence.

Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control.			
Geographic Potential			
Plant cold hardiness zones			
Geo-Z1 (Zone 1)	n - negl	N/A	Frost tender
Geo-Z2 (Zone 2)	n - negl	N/A	Frost tender
Geo-Z3 (Zone 3)	n - negl	N/A	Frost tender
Geo-Z4 (Zone 4)	n - negl	N/A	Frost tender
Geo-Z5 (Zone 5)	n - negl	N/A	Frost tender
Geo-Z6 (Zone 6)	n - negl	N/A	Frost tender
Geo-Z7 (Zone 7)	n - negl	N/A	Frost tender
Geo-Z8 (Zone 8)	n - negl	N/A	Frost tender in zones 8 and lower in the U.S. (Nehra and Pearson, 2011).
Geo-Z9 (Zone 9)	y - mod	N/A	Rio Grande Do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-Z10 (Zone 10)	y - mod	N/A	Espírito Santo, Rio Grande do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-Z11 (Zone 11)	y - mod	N/A	Bahia, Espírito Santo, Rio Grande do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-Z12 (Zone 12)	y - mod	N/A	Bahia, Espírito Santo, Maranhão, Pará: Brazil (CABI_A, 2012).
Geo-Z13 (Zone 13)	y - mod	N/A	Bahia, Maranhão, Pará: Brazil (CABI_A, 2012).
Koppen-Geiger climate classes			
Geo-C1 (Tropical rainforest)	y - mod	N/A	Bahia, Espírito Santo, Maranhão, Pará: Brazil (CABI_A, 2012).
Geo-C2 (Tropical savanna)	y - mod	N/A	Bahia, Espírito Santo, Maranhão, Pará: Brazil (CABI_A, 2012).
Geo-C3 (Steppe)	n - mod	N/A	Bahia: Brazil (CABI_A, 2012). Steppe occurs in Bahia, but most likely too little rainfall to survive.
Geo-C4 (Desert)	n - mod	N/A	No evidence. Desert area occurs in Bahia Brazil but probably too little rainfall to survive.
Geo-C5 (Mediterranean)	n - negl	N/A	No evidence
Geo-C6 (Humid subtropical)	y - mod	N/A	Rio Grande Do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-C7 (Marine west coast)	n - negl	N/A	No evidence
Geo-C8 (Humid cont. warm sum.)	y - mod	N/A	Rio Grande Do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-C9 (Humid cont. cool sum.)	n - negl	N/A	Frost tender
Geo-C10 (Subarctic)	n - negl	N/A	Frost tender
Geo-C11 (Tundra)	n - negl	N/A	Frost tender
Geo-C12 (Icecap)	n - negl	N/A	Frost tender
10-inch precipitation bands			
Geo-R1 (0-10 inches; 0-25 cm)	n - negl	N/A	Too little rainfall to survive
Geo-R2 (10-20 inches; 25-51 cm)	n - mod	N/A	No evidence. Occurs in Bahia Brazil (CABI_A, 2012) (desert area - most likely too little rainfall)
Geo-R3 (20-30 inches; 51-76 cm)	y - high	N/A	Bahia: Brazil (CABI_A, 2012). May be too little rainfall to survive.
Geo-R4 (30-40 inches; 76-102 cm)	y - mod	N/A	Bahia: Brazil (CABI_A, 2012).
Geo-R5 (40-50 inches; 102-127 cm)	y - mod	N/A	Bahia, Espírito Santo, Maranhão: Brazil (CABI_A, 2012).
Geo-R6 (50-60 inches; 127-	y - mod	N/A	Bahia, Espírito Santo, Maranhão, Rio Grande do Sul, São Paulo:

152 cm)			Brazil (CABI_A, 2012).
Geo-R7 (60-70 inches; 152-178 cm)	y - mod	N/A	Maranhão, Rio Grande do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-R8 (70-80 inches; 178-203 cm)	y - mod	N/A	Maranhão, Pará, Rio Grande do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-R9 (80-90 inches; 203-229 cm)	y - mod	N/A	Maranhão, Pará: Brazil (CABI_A, 2012).
Geo-R10 (90-100 inches; 229-254 cm)	y - mod	N/A	Maranhão, Pará: Brazil (CABI_A, 2012).
Geo-R11 (100+ inches; 254+ cm))	y - mod	N/A	Pará: Brazil (CABI_A, 2012).
Entry Potential			
Ent-1 (Plant already here)	y - negl	1	Planted in field tests in Hawaii (CABI_A, 2012) and the continental U.S. (Nehra and Pearson, 2011).
Ent-2 (Plant proposed for entry, or entry is imminent)	-	N/A	
Ent-3 (Human value & cultivation/trade status)	-	N/A	
Ent-4 (Entry as a contaminant)			
Ent-4a (Plant present in Canada, Mexico, Central America, the Caribbean or China)	-	N/A	
Ent-4b (Contaminant of plant propagative material (except seeds))	-	N/A	
Ent-4c (Contaminant of seeds for planting)	-	N/A	
Ent-4d (Contaminant of ballast water)	-	N/A	
Ent-4e (Contaminant of aquarium plants or other aquarium products)	-	N/A	
Ent-4f (Contaminant of landscape products)	-	N/A	
Ent-4g (Contaminant of containers, packing materials, trade goods, equipment or conveyances)	-	N/A	
Ent-4h (Contaminants of fruit, vegetables, or other products for consumption or processing)	-	N/A	
Ent-4i (Contaminant of some other pathway)	-	N/A	
Ent-5 (Likely to enter through natural dispersal)	-	N/A	

Appendix IV. Weed Risk Assessment for genetically engineered *Eucalyptus grandis* × *Eucalyptus urophylla*

United States
Department of
Agriculture

Animal and Plant
Health Inspection
Service

May 21, 2013

Version 1

Weed Risk Assessment for genetically engineered *Eucalyptus grandis* × *Eucalyptus urophylla*

Developing flowers

Field test in Texas

Agency Contact:

Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
United States Department of Agriculture
4700 River Road
Riverdale, MD 20737

Introduction

Plant Protection and Quarantine (PPQ) regulates noxious weeds under the authority of the Plant Protection Act (7 U.S.C. § 7701-7786, 2000) and the Federal Seed Act (7 U.S.C. § 1581-1610, 1939). A noxious weed is defined as "any plant or plant product that can directly or indirectly injure or cause damage to crops (including nursery stock or plant products), livestock, poultry, or other interests of agriculture, irrigation, navigation, the natural resources of the United States, the public health, or the environment" (7 U.S.C. § 7701-7786, 2000). PPQ uses weed risk assessment (WRA)—specifically, the PPQ WRA model (Koop et al., 2012)—to evaluate the risk potential of plants, including those newly detected in the United States, those proposed for import, and those emerging as weeds elsewhere in the world.

Biotechnology Regulatory Services (BRS) regulates genetically engineered (GE) organisms under the authority of the Plant Protection Act because these organisms may present a pest risk to U.S. plant resources (7 U.S.C. § 7701-7786, 2000). BRS administers oversight for certain genetically engineered plants (regulated articles) under the regulations at (7 CFR part 340). A person may petition the agency that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, is no longer regulated under the plant pest provisions of the Plant Protection Act or the regulations at 7 CFR part 340. The petitioner is required to provide information under § 340.6(c)(4) related to plant pest risk that the agency may use to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. A GE organism is no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act when APHIS determines that it is unlikely to post a plant pest risk. Part of the risk assessment that APHIS conducts when reviewing a petition for non-regulated status is to assess the weediness of the regulated article. This risk assessment was reviewed by weed risk analysts in PPQ.

Because the PPQ WRA model is geographically and climatically neutral, it can be used to evaluate the baseline invasive/weed potential of any plant species for the entire United States or for any area within it. As part of this analysis, we use a stochastic simulation to evaluate how much the uncertainty associated with the analysis affects the model outcomes. We also use GIS overlays to evaluate those areas of the United States that may be suitable for the establishment of the plant. For more information on the PPQ WRA process, please refer to the document, *Background information on the PPQ Weed Risk Assessment*, which is available upon request.

Eucalyptus grandis W. Hill ex Maiden × *Eucalyptus urophylla* S.T. Blake

Family: Myrtaceae

Synonyms: It is commonly known in the trade as *Eucalyptus urograndis*. Although it is called "*E. urograndis*" this name is not a valid species name as determined by: http://www.theplantlist.org/. However, *Eucalyptus* × *urograndis* is a valid name, following the rules of the International Code of Nomenclature of Cultivated Plants: http://www.ishs.org/sci/icracpco.htm.

Species Information

Initiation: Several eucalypt species and their hybrids are being examined for their production potential in the United States) for solid wood, pulp for paper production and as potential bioenergy crops (Booth, 2012; Stape, In-press). A genetically engineered (GE) hybrid of *E. grandis* and *E. urophylla* is being field tested for its growth potential and field performance in the southeastern United States. As part of the analysis of the weed risk potential of this GE hybrid, we evaluated the weed risk potential of both parents and the non GE hybrid. This is the assessment for the GE hybrid. The hybrid has been engineered for pollen sterility and for increased tolerance to freezing temperatures. According to the applicant this trait will allow the GE hybrid to be planted in zones 8b and higher in the United States. The non-GE hybrid will survive in zones 9 and higher. This assessment is based on the previous non-GE hybrid analysis. We altered the answers only where the engineered traits that are in the plant were expected to change the underlying biology.

Foreign distribution: Because the non-GE hybrid was artificially created it has no natural range. It is widely planted in Brazil, Venezuela, Colombia, Congo, South Africa and China. It has also been introduced at an experimental level in countries such as Australia, Indonesia, Vietnam, Taiwan, Ecuador, Mexico and Hawaii, USA (CABI_A, 2012).

U.S. distribution and status: The genetically engineered *E. grandis* × *E. urophylla* hybrid is being field tested in the southern States in the continental U.S. (Nehra and Pearson, 2011).

WRA area[5]: Entire United States, including territories.

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1. *Eucalyptus grandis × Eucalyptus urophylla (E. × urograndis)* analysis

Establishment/Spread Potential The GE hybrid started with a base clone of *E. × urophylla* and traits for increase frost tolerance and pollen sterility were engineered into the clone. These traits allow it to be grown in zone 8b and higher and also results in the lack of pollen production in the flowers. In this analysis the risk score for the non-GE hybrid was lower than the non-GE hybrid. The non-GE hybrid risk score was -2 with an uncertainty index of 0.41. This lower score is due to the GE hybrid being self-incompatible. Because pollen sterility has been engineered into the tree, the GE trees cannot pollinate themselves. They can be pollinated from other trees and that is the reason that it continues to produce seed. However it cannot fertilize itself.
Risk score = -4 Uncertainty index = 0.45

Impact Potential The risk score for the GE hybrid remains unchanged from the non-GE hybrid. As with the non-GE hybrid we answered the questions in this risk element as either unknown or "no" with high uncertainty. All of the questions on impacts in natural systems were answered as unknown, because one of the parents (*E. grandis*) has been shown to cause a variety of impacts to natural systems.
Risk score = 1.1 Uncertainty index = 0.70

Geographic Potential Based on three climatic variables, the model estimates that about 13.8 percent of the United States is suitable for the establishment of the GE *Eucalyptus grandis* × *E. urophylla* (Fig. 1). This predicted distribution is based on the species' known distribution elsewhere in the world and includes areas of occurrence. The map for the non GE *Eucalyptus grandis* × *E. urophylla* represents the joint distribution of Plant Hardiness Zones 9-12 (NAPPFAST Global Plant Hardiness Zones: http://www.nappfast.org/), areas with 10-100+ inches of annual precipitation, and the following Köppen-Geiger climate classes: tropical rainforest, tropical savannah, steppe, Mediterranean, humid subtropical, and marine west coast. The distribution increases for

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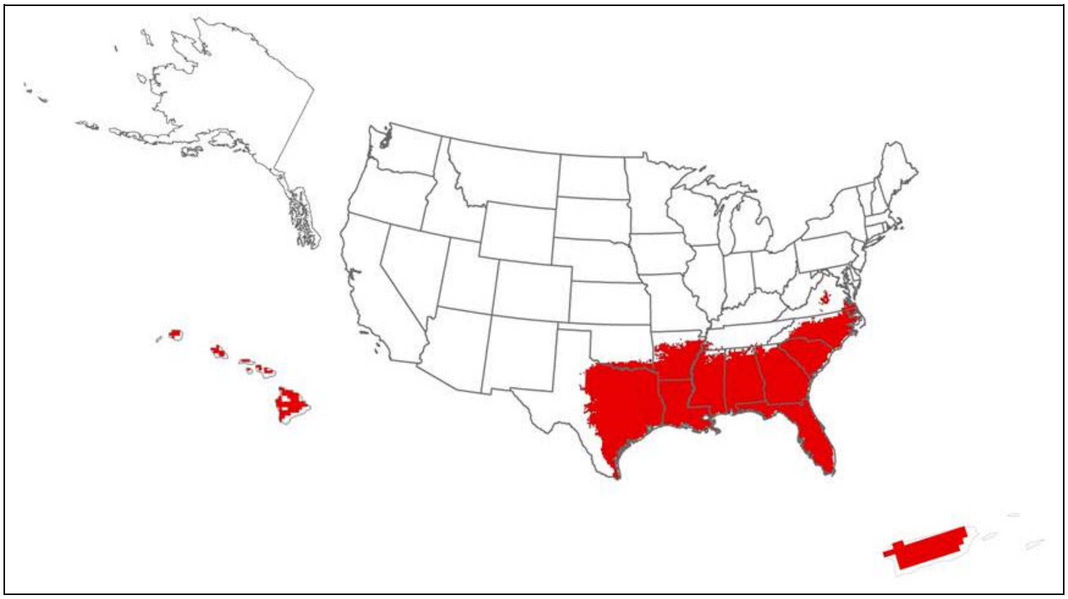
[5] "WRA area" is the area in relation to which the weed risk assessment is conducted [definition modified from that for "PRA area" (IPPC, 2012).

the GE hybrid because the freeze-tolerance trait allows it to be planted in zone 8b which is not the case for the non-GE hybrid which only survives in zones 9 and higher.

The area estimated in Figure 1 likely represents an overestimate of the area where the GE hybrid can be grown. The NAPPFAST data do not subclassify hardiness zones into a and b subzones as does the USDA Hardiness zone map (http://planthardiness.ars.usda.gov/PHZMWeb/). The map in Figure 1 is showing all of zone 8 (including 8a and 8b). The hybrid will not survive in zone 8a (Nehra and Pearson, 2011) so this map is overestimating where it can survive. However, because the PPQ WRA model uses the NAPPFAST dataset we are including this information in this document with the understanding that other data more accurately indicate where it is capable of growing.

Entry Potential We did not assess they hybrid's entry potential because this species is already present in the United States (Nehra and Pearson, 2011).

Figure 1. Predicted distribution of *Eucalyptus grandis* × *E. urophylla* in the United States. Map insets for Alaska, Hawaii, and Puerto Rico are not to scale.



2. Results and Conclusion

Model Probabilities: P(Major Invader) = 1.2%
P(Minor Invader) = 27.4%
P(Non-Invader) = 71.4%

Risk Result = Low Risk
Secondary Screening = Not applicable

Figure 2. *Eucalyptus grandis* × *Eucalyptus urophylla* risk score (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A for the complete assessment.

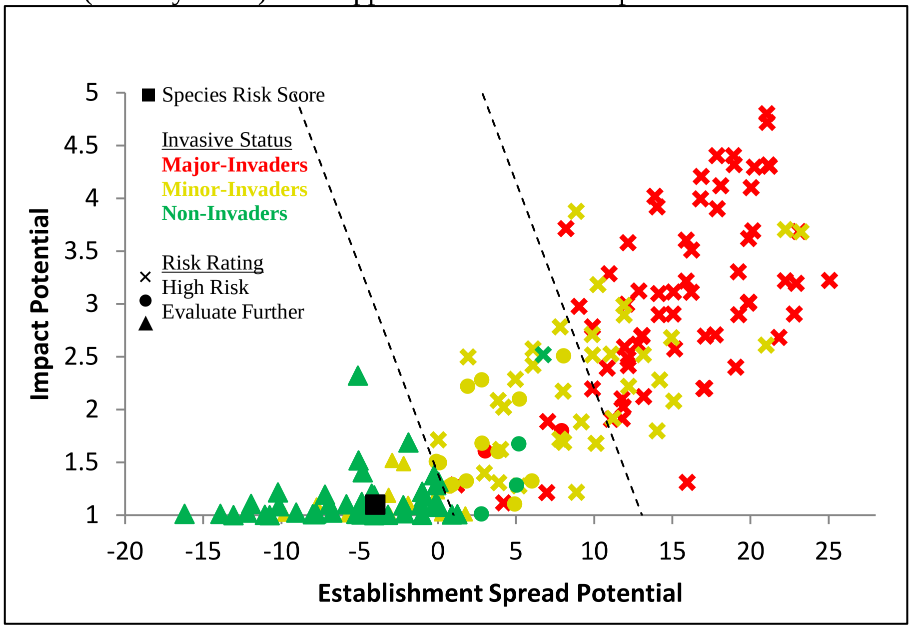
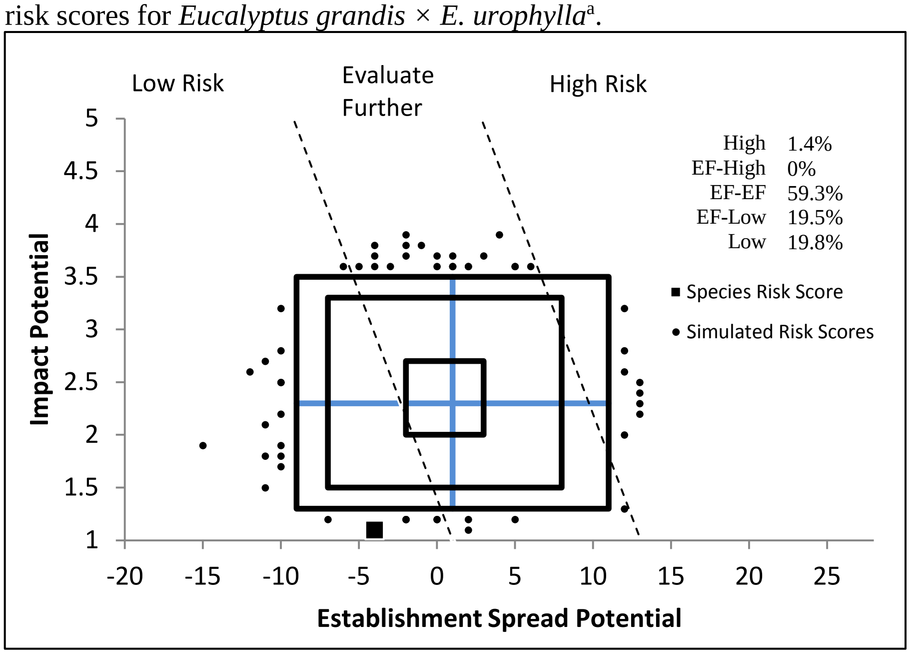


Figure 3. Monte Carlo simulation results (N=5,000) for uncertainty around the risk scores for *Eucalyptus grandis* × *E. urophylla*[a].



[a] The blue "+" symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

3. Discussion

The result of the weed risk assessment for the GE hybrid of *Eucalyptus grandis* × *Eucalyptus* *urophylla* is "Low Risk" (Fig 2.). Our predictive model indicates there is a 71 percent likelihood this species will not be invasive. This compares with a 61 percent likelihood for the non-GE hybrid. Most of the simulated risk scores resulted in conclusions of Evaluate Further. The uncertainty associated with both the establishment/spread and impact potential were well above average because there is little biological information available for the non-GE and GE hybrids. Furthermore, the non-GE hybrid is a relatively new taxon that has had a relatively short time to express potential evidence of escape, spread and impact. 'History elsewhere' is often one of the most important traits used to predictive invasiveness in plants. As with the non-GE hybrid, the results of this analysis should be viewed with the understanding that this is a new taxon that has only been around for about 40 years and the GE version of the hybrid has been under field tests since 2006. As noted for the non-GE hybrid, the literature on invasive species has shown that sometimes long lag phases of over a 100 years precede the invasion of some plant species (Kowarik, 1995; Ellstrand and Schierenbeck, 2000). Booth (2012) notes that *Eucalyptus* is more of a problem in South Africa perhaps due to the fact that it has been there longer than in other countries. The fact that this is a hybrid which was created for increased yield (Foelkel, 2013) may also factor into its invasiveness. Ellstrand has shown that hybridization is sometimes a stimulus for invasiveness in plants (Ellstrand and Schierenbeck, 2000). In a recent publication Callaham et al (2013) point out that in the case of *Eucalyptus conferruminata,* the species has been extant in California for at least 50 years, but only recently has been observed to produce seedlings (Ritter and Yost, 2009), suggesting that there can be significant lag times associated with the naturalization / invasion process.

4. Literature Cited

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Appendix A. Weed risk assessment for GE *Eucalyptus grandis* × *Eucalyptus urophylla* (Myrtaceae). The following analysis is almost identical to the one done for the non GE hybrid. Except for the questions relating to plant hardiness zone suitability (Geo-Z8), and self-compatibility (ES-11), all other answers/uncertainties are the same. For many of the questions below, we added some information to the notes column that is specific to the GE hybrid, but these did not lead to a change in the original answer. Except for the two identified questions, we assumed that genetic modification did not or will not change any other attributes of the non-GE hybrid. The following information was obtained from the species' risk assessment, which was conducted using Microsoft Excel. The information shown in this appendix was modified to fit on the page. The original Excel file, the full questions, and the guidance to answer the questions are available upon request.

Question ID	Answer - Uncertainty	Score	Notes (and references)
Establishment/Spread Potential			
ES-1 (Status/invasiveness outside its native range) Select one: (A) Introduced elsewhere long ago (>75 years) but not escaped; (B) Introduced recently (<75 years) but not escaped; (C) Never introduced elsewhere; (D) Escaped/Casual; (E) Naturalized; (F) Invader.	b - high	-2	*Eucalyptus grandis* × *Eucalyptus urophylla* is an artificial hybrid created by cross pollination of the two species. The hybrid was first introduced into the Congo in 1978 (Vigneron and Bouvet, 2000) and into Brazil in the 1970s (Wright, 1997). The original Brazilian *E.* × *urograndis* hybrid trees were naturally generated because of the proximity of experimental plantation areas (Boland et al., 1980;Ikemori and Campinhos, 1983).. *E.* × *urograndis* is planted extensively in tropical and subtropical regions (Bertolucci et al., 1995;Potts and Dungey, 2004) and is reported to be in South Africa, Brazil, Congo, China, Mexico, Colombia and Venezuela (Wright, 1997). It has never been reported to have escaped from cultivated plantations in Brazil (Silva, 2010) or elsewhere. The genetically engineered version of the hybrid was created by transforming a single clone (EH1) that was introduced into Brazil in 1994. The EH1 clones has not been reported to have escaped from cultivation anywhere since its introduction (Nehra and Pearson, 2011). The GE trees have been field tested since 2006 under permit and no seedlings have been produced or found in the area of the field test sites (Nehra and Pearson, 2011). Alternative answer is d for both.
ES-2 (Is the species highly domesticated)	n - negl	0	The *E. grandis* × *E. urophylla* is not a single hybrid cultivar but a large group of hybrids formed through crossing the two species. *E.* × *urograndis* hybrids are usually deployed as first generation (F1) clones (Potts and Dungey, 2004; Wright, 1997) that can be made from a wide variety of provenances (Potts and Dungey, 2004).
ES-3 (Weedy congeners)	y - negl	1	Three species of *Eucalyptus* are listed in Weber's "Invasive Species of the World". These are E. cladocalyx, *E. diversicolor*, and *E. globulus*. They reduce biodiversity, promote fire, reduce water availability (Weber, 2003). In California *E. globulus* and *E. camaldulensis* are listed as invasive by the Invasive Plant Council (Cal-IPC, 2012). An FAO report indicates that *E. camaldulensis*, *E. cladocalyx*, *E. diversicolor*, *E. lehmannii*, *E. paniculata* and *E. sideroxylon* are major invaders in South Africa and in Zimbabwe *E. microcorys*, *E. camaldulensis*, *E. tereticornis*, *E. robusta*, *E.*

			macarthurii, *E. paniculata*, *E. globulus* and *E. citriodora* are considered major invaders (Nyoka, 2003). One of the parents of the hybrid, *E. grandis*, is beginning to escape (or is perhaps naturalized) in two counties of California (UC, 2012). Naturalized in Florida (NRCS, 2012; IRC, 2012; ISB, 2012) and in California (Ritter and Yost, 2009). It has naturalized in Australia beyond its native range (Randall, 2007). "invasive" in South Africa where its spread must be controlled (Macdonald et al., 2003). Naturalized populations in South Africa are spreading to other areas through long-distance seed dispersal (>100 meters away from parent plants) (Forsyth et al., 2004). Several other sources indicate it invades forests, grasslands, and savannas in Southern Africa (Booth, 2012; Cowling et al., 1997;Haysom and Murphy, 2003; Henderson, 2001 and Nyoka, 2003). For the other parent, *E. urophylla* there are no reports except one, of it being naturalized or invasive. It is reported to be naturalized in Ecuador (Randall, 2012) but the data are inconclusive. The fao.org lists *E. urophylla* as neither invasive or naturalized http://www.fao.org/forestry/24107/en/.
ES-4 (Shade tolerant at some stage of its life cycle)	n - mod	0	Data for the hybrid are unknown. *Eucalyptus* in general are intolerant of shade (http://www.angelfire.com/bc/eucalyptus/selection.html) and *urograndis* is not listed as shade tolerant. The parent *E. grandis* is intolerant of shade. Seedlings can only develop in full or nearly full sunlight; trees must maintain a dominant or codominant canopy position to long survive. Suppressed trees quickly die and intermediate trees must grow to an overstory position or eventually lose vigor and die." (Meskimen and Francis, 1990). In its native range in Australia *E. grandis* grows in mixed eucalypt forests that are relatively open, and sometimes at the edges and interiors of rain forests (Meskimen and Francis, 1990). The evidence strongly suggests this species is intolerant of shade; the one reference to growing in the interior of rainforests may be to individuals colonizing gaps. The other parent *E. urophylla* requires very bright light intensity for growth (FAO, 2007) *E. urophylla* is extremely susceptible to competition in the early stages and must be kept weed-free for 6 to 12 months (RISE, 2009). *E. urophylla* is similar to other eucalypts in that it is a vigorous, light demanding species (CABI_B, 2012). Based on the information available from both parents it is not expected that the hybrid would be any more shade tolerant. The traits engineered into the hybrid would not be expected to alter this characteristic.
ES-5 (Climbing or smothering growth form)	n - negl	0	Plant is a tree (CABI_A, 2012).
ES-6 (Forms dense thickets)	n - mod	0	No evidence. Planted almost exclusively in forest plantations. In general *Eucalyptus* does not form dense thickets.
ES-7 (Aquatic)	n - negl	0	Not an aquatic, plant is a terrestrial tree (CABI_A, 2012).
ES-8 (Grass)	n - negl	0	Not a grass, both parents of the hybrid are in the Myrtaceae family (NGRP, 2012).
ES-9 (Nitrogen-fixing woody plant)	n - negl	0	Not in plant family known to contain nitrogen fixing species (Martin and Dowd, 1990).
ES-10 (Does it produce viable seeds or spores)	y - high	1	"Seeds from the *E. urograndis* hybrid are fertile and viable" (Foelkel, 2013). A gene for pollen sterility was introduced to the GE version to limit reproduction. Therefore the trees do not

			produce viable pollen. However, it is still possible for outside pollen to fertilize female ovules in the flowers. The GE hybrid is producing a limited number of viable seeds in the field tests (Nehra and Pearson, 2011). This is presumably due to fertile pollen entering the field test. Even though thus far it is rare, it seems that the GE hybrid can still produce seed. Using "high" uncertainty.
ES-11 (Self-compatible or apomictic)	n - high	-1	Both parents of the hybrid are self-compatible and can produce some seed from self-pollination (Potts and Wiltshire, 1997; Horsley and Johnson, 2007). The non-GE hybrid is presumed to also be self-compatible. However, a pollen sterility gene was introduced into the GE hybrid to limit seed production and none of the trees are producing viable pollen in field tests (Nehra and Pearson, 2011). Thus, assuming this gene is functioning properly, the GE hybrid is not self-compatible. Because field tests show some very minor seed production we are answering with high uncertainty. It is possible that these seeds were produced from outside pollen donors.
ES-12 (Requires special pollinators)	n - negl	0	Eucalypt flowers (referring to the genus) "show no adaptive characteristics that could be particularly attractive to" specialized pollinators (House, 1997). Insect-pollinated *Eucalyptus* do not express specificity with respect to the insect vector; honeybees can be used to augment pollination success (cited in Chaix et al., 2007). For the parent *E. grandis* the "species is entomophilous and honeybees are an important pollinator, although some pollination without bees may occur" (Hodgson, 1976b). For the parent *E. urophylla* the bisexual flowers are open to many pollen vectors such as insects, birds or small mammals. Some wind pollination is also possible (AgroForestry, 2012).
ES-13 (Minimum generation time) Minimum generative time (A) less than 1 (multiple generations per year), (B) 1 year (annual-1 gen per year), (C) 2or3 years, (D)>3years; (?))	d - high	-1	The hybrid's minimum generation time is unknown, but it would be expected to be similar to that of both parents. For *E. grandis* the first flowers appear at 2 to 3 years of age and the first capsules can be obtained 5 to 7 months after anthesis (Hodgson, 1976b). Another report says it can begin flowering within the first year of the plantation (Meskimen and Francis, 1990) but that 97% of *Eucalyptus grandis* families in plantations flower at age 3. Seed capsules are mature for harvest 6-7 months after flowering, but capsules remain closed on the tree for at least one year after reaching maturity (Meskimen and Francis, 1990). For *E. urophylla* trees first begin to flower when they are 2–3 years old, with seeds being produced abundantly by the age of 4. Flowering occurs during the dry season and within 6 months the seeds reach maturity (Sein and Mitlohner, 2011). For both parents the evidence cited above originates from the forestry literature, where foresters may very likely be basing their observations on plants (i.e., mature seedlings) that have already been growing for a year or two before being planted in the forest plantations. This observation plus the fact that flowering trees retain seeds in enclosed capsules for several months to a year after seed mature, supports the idea that the minimum and effective generation time is probably four years or so. Answering "d", but using high uncertainty. Alternate answers for the Monte Carlo simulation were both "c".
ES-14 (Prolific reproduction)	? - max	0	There are no direct estimates of seed production per square

			meter. No data can be found for the non GE *E.* × *urograndis* in the wild or in seed orchards. The pollen ablation gene engineered into the GE trees should result in less seed production. However, the transgenic hybrid is producing a very low number of viable seeds in field tests. Germination rates are about 0.5 seed per capsule that germinate from the transgenic trees. The non-transgenic hybrid when grown in Florida, where it does not get cold damaged, produces about 1viable seed per capsule (Nehra and Pearson, 2011). Therefore in the existing field tests, the GE trees are producing a reduced number of viable seeds which is apparently the result of pollination from nearby non GE trees. The extent to which this would occur on a large scale is unknown. Therefore answering unknown.
ES-15 (Propagules likely to be dispersed unintentionally by people)	? - max	0	No information is available for *E.* × *urograndis*. It is possible that given the very small size of the seeds that under muddy conditions the seeds could readily attach to vehicles, equipment and clothing. Heavy equipment is used to harvest trees and if trees are harvested under wet and muddy conditions seeds could adhere to tires, boots etc. during harvest operations. Long distance dispersal of small seeds without any appendages for adhesion can occur via transport in mud on motor vehicles (Taylor et al., 2011; Clifford, 1959) and clothing (Clifford, 1956). Clifford (1959) noted that small seeds tend to be represented by more individuals in the mud samples than those with large seeds. Consequently answering as unknown.
ES-16 (Propagules likely to disperse in trade as contaminants or hitchhikers)	? - max	0	No evidence of this occurring for *E.* × *urograndis*. It does not seem likely that seeds from a forest plantation tree, which are not well-dispersed, would contaminate a commodity moving in trade. However, it is possible for seeds to adhere to logs in mud and within bark. Therefore answering unknown.
ES-17 (Number of natural dispersal vectors)	0	-4	0
ES-17a (Wind dispersal)	n - mod		No evidence. Seeds of *Eucalyptus* do not have any adaptation that are associated with classic wind dispersal (e.g. plumes or wings)(Boland et al., 1980;House, 1997). Wind aids in passive dispersal of *Eucalyptus* seeds and depends on tree height, crown diameter, wind velocity, and mass and terminal velocity of the seeds; dispersal distances range between 30 to 60 meters (House, 1997). "Seed is mainly dispersed by wind and gravity after release from capsules in the canopy", but most seeds are dispersed near parent trees (Potts and Wiltshire, 1997). Cremer (1977) found that 12 of 15 species of *Eucalyptus* with wingless seeds dispersed less than 30 m from the parent tree when released at 40 m above the ground into a wind speed of 10 km/h. He concluded that few seeds are dispersed greater than twice the height of the tree (cited in Virtue (Virtue and Melland, 2003)). There is no information on dispersal distances for *E.* × *urograndis*. Evidence from the other species in the genus suggests that wind dispersal is limited to areas nearby parent trees. Consequently answering "no" with "mod" uncertainty.
ES-17b (Water dispersal)	? - max		No evidence for *E.* × *urograndis*. However, given that other species can be dispersed via water and that most Eucalypt invasions in South Africa happen along watercourses (ARC, 2011; Forsyth et al., 2004) it cannot be ruled out that seed of

			this species could be distributed by water. Therefore answering unknown.
ES-17c (Bird dispersal)	? - max		No evidence for *E.* × *urograndis*. Fruit and seeds offer no obvious rewards for frugivores. Studies in *Eucalyptus* plantations in the Amazon suggest that *Eucalyptus* stands and secondary growth forests fail to provide suitable foraging habitat for frugivores at any time of the year (Barlow et al., 2007). However, for *Eucalyptus* species in general, some bird predators "mediate some dispersal when they fail to consume a proportion of the seeds they harvest" (House, 1997). Occasionally, some birds like cockatoos may scatter *Eucalyptus* seeds when they crack open living capsules (Cremer, 1965). Without additional evidence on the frequency of seed removal by predacious birds and more specific information on *E.* x *urograndis* answering "unknown".
ES-17d (Animal external dispersal)	? - max		Unknown for *E.* × *urograndis*. Given the small size of the seeds, it is possible they may get stuck in animal fur. Ant seed predators will disperse some seeds of *Eucalyptus* species if they are not consumed (House, 1997). "Ants are significant harvesters of *Eucalyptus* seed on the ground, removing 60% of fallen seed in tall open-forests" (cited in House, 1997). Harvest of fallen seeds by ants "no doubt results in some effective dispersal" (Potts and Wiltshire, 1997), but probably not for *E. salmonophloia* (Yates et al., 1995). One author reports that even if seeds taken by ants escape predation in ant granaries underground, germinating seeds are usually buried too deep to break through to the soil surface (Wellington, 1989). "Native trigonid bees inadvertently pick up E. torelliana seeds while foraging for resin within capsules still in the canopy" (cited in House, 1997). "Seed dispersal in the genus appears to be extremely limited, although there are exceptions where dispersal is enhanced by.....animal transport" (cited in Potts and Wiltshire, 1997). Although there is strong evidence for ant-mediated seed dispersal of *Eucalyptus* we are answering "unknown" because it is not clear whether *E.* × *urograndis* is dispersed by ants, how frequently that may or may not occur, and if it did occur, how far ants would disperse the seeds.
ES-17e (Animal internal dispersal)	n - mod		Unknown for *E.* × *urograndis*. Fruit and seeds of both parents as well as other species of *Eucalyptus* offer no obvious rewards for frugivores (Zhengyi et al., 2012). Because we have found no evidence of internal dispersal of *Eucalyptus* seeds by non-avian dispersal agents, answering "no".
ES-18 (Evidence that a persistent (>1yr) propagule bank (seed bank) is formed)	n - high	-1	Unknown for *E.* × *urograndis*. Seeds of *Eucalyptus* can be stored for a number of years at cold temperatures and remain viable (Meskimen and Francis, 1990; Sein and Mitlohner, 2011). In general *Eucalyptus* seeds do not have dormancy but a few alpine species require cold , moist pretreatment to break dormancy (Boland et al., 1980). And according to Boland (1980) no such treatment is required for either *E. grandis* or *E. urophylla*. Data from both parents indicate that that long-term storage of seeds in soil and capsules likely does not occur with *E.* × *urograndis*. For *E. grandis* there is no information on seed longevity in natural populations. In temperate *Eucalyptus* such as *E. grandis*, seed maturation takes several months and seeds are retained in woody capsules (Hodgson, 1976a). Capsules produce viable seed in 4 - 5 months and are fully

			mature within 7 months (Hodgson, 1976b). According to House (2013) *E. grandis* does have woody capsules, but holds its seeds for only 6 months after flowering. They are dropped as they mature, and don't need fire to trigger seedfall or regeneration. Boland (1980) indicates that *E. grandis* seeds should be harvested as soon as they are mature and that it is not a "long duration" species where seeds can be collected during any time of the year. For the other parent, there are no data available on dormancy of *E. urophylla* seeds and there is no information on seed longevity in natural populations. According to House (2013) *E. urophylla* is similar to *E. grandis*. It also has woody capsules, and holds its seeds for only a few months after flowering. They are dropped as they mature, and don't need fire to trigger seedfall or regeneration. Boland (1980) indicates that *E. urophylla* seeds should be harvested as soon as they are mature and that it is not a "long duration" species where seeds can be collected during any time of the year. *Eucalyptus* seeds in general do not appear to form a long term soil seed bank, instead they can be stored in the canopy and released in a slow trickle over a period of several years (Wellington, 1989). However, some species, with fragile capsules, release their seed soon after they mature (Cremer, 1965). This occurs with both parents and is presumed to be true for the hybrid.
ES-19 (Tolerates/benefits from mutilation, cultivation or fire)	? - max	0	Unknown. *Eucalyptus* × *urograndis* coppices when cut (Bernhard-Reversat et al., 2001) which is a standard practice when managing *E. urograndis* plantations; but it is unclear if the response is significantly stronger than in most other tree taxa. Note that *Eucalyptus* seeds in general are retained in the canopy in capsules that open as they desiccate or die (Wellington, 1989). In general forest fires promote the regeneration of Eucalypt forests because it triggers a massive release of canopy stored seed; seeds stored in capsules are well protected from fire and begin to release seeds a few days after the fire has passed (Wellington, 1989). However, seed response to fire or biomass loss is not considered in this question.
ES-20 (Is resistant to some herbicides or has the potential to become resistant)	n - negl	0	No evidence of resistance to herbicides and not listed by Heap (Heap, 2012). When you consider the long life cycle of this plant, the plantation environments where individuals grow, and silvicultural practices it seems highly unlikely populations will develop herbicide resistance. Although it is possible for this hybrid to hybridize with other species (Potts and Wiltshire, 1997), there is no evidence that a commercial *Eucalyptus* species has acquired or been genetically engineered for herbicide resistance. Consequently answering "no" with "negl" uncertainty.
ES-21 (Number of cold hardiness zones suitable for its survival)	6	0	
ES-22 (Number of climate types suitable for its survival)	4	2	
ES-23 (Number of precipitation bands suitable for its survival)	9	1	
Impact Potential			
General Impacts			

Imp-G1 (Allelopathic)	y - negl	0.1	Soil samples from *E. grandis* × *urophylla* plantations had an inhibitory effect on germination of maize, bean and watermelon but had a stimulatory effect on squash (Espinosa-García et al., 2008). These were natural concentrations of phenolics found in the soil and not laboratory studies. Both parents were also included in this study and showed similar results (Espinosa-García et al., 2008). Hao (2011) found that extracts of soil from *E. urograndis* forests decreased levels of chlorophyll, proline and soluble sugars in *Brassica, Oryza* and *Raphanus*. In general other *Eucalyptus* species have shown similar allelopathic effects (Espinosa-García et al., 2008). The traits engineered into the hybrid would not be expected to affect its allelopathic potential.
Imp-G2 (Parasitic)	n - negl	0	No evidence. This species does not belong to a family known to contain parasitic plant species (Heide-Jorgensen, 2008; Nickrent, 2009).
Impacts to Natural Systems			
Imp-N1 (Change ecosystem processes and parameters that affect other species)	? - max		Unknown. The hybrid has been planted extensively in other countries with large plantings in Brazil. It has been planted in other countries such as South Africa, Congo, China, Mexico, Colombia and Venezuela (Wright, 1997). There are no reports on impacts of native stands on ecosystem parameters. However the hybrid is a relatively new taxon. It is cultivated because it grows better than either parent (Foelkel, 2013) and shows resistance to disease (CABI_A, 2012), therefore it is possible that it could outcompete its neighbors. Given that this a relatively new taxon that has only been grown for around 40 years, and one of its parents (*E. grandis*) is known to have impacts on the environment, answering unknown for every question in this sub element.
Imp-N2 (Change community structure)	? - max		Unknown
Imp-N3 (Change community composition)	? - max		Unknown
Imp-N4 (Is it likely to affect federal Threatened and Endangered species)	? - max		Unknown
Imp-N5 (Is it likely to affect any globally outstanding ecoregions)	? - max		Unknown
Imp-N6 (Weed status in natural systems) For conservation/natural areas, choose the best answer. (A) Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control.	? - max		Unknown. Has never been reported to be a weed or needing to be controlled as a weed.
Impact to Anthropogenic Systems (cities, suburbs, roadways)			
Imp-A1 (Impacts human property, processes, civilization, or safety)	? - max		Unknown. Has been planted extensively in other countries with large plantings in Brazil. Planted in other countries such as South Africa, Congo, China, Mexico, Colombia and Venezuela (Wright, 1997). Even though the species has been extensively planted it has only been cultivated for around 40

			years so answering unknown. The GE version has been in cultivation for only 7 years.
Imp-A2 (Changes or limits recreational use of an area)	n - high	0	No evidence.
Imp-A3 (Outcompetes, replaces, or otherwise affects desirable plants and vegetation)	n - high	0	No evidence.
Imp-A4 (Weed status in anthropogenic systems) For urban/suburban areas, choose the best answer. (A) Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control.	? - max		Unknown for the hybrid. One of the parents (*E. grandis*) is considered a ruderal weed in South Africa (Cruickshank, 1988;Wells et al., 1986) and is the subject of herbicide registration (Wells et al., 1986). Because the hybrid has only been planted for around 40 years answering unknown. The GE version has been planted for 7 years.
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product yield)	n - mod	0	No evidence.
Imp-P2 (Lowers commodity value)	n - mod	0	No evidence.
Imp-P3 (Is it likely to impact trade)	n - high	0	No evidence.
Imp-P4 (Reduces the quality or availability of irrigation, or strongly competes with plants for water)	? - max		There are no reports on impacts of native stands of *E.* × *urograndis* on hydrology or water resources in production systems. Other species of *Eucalyptus*, including one of the parents *E. grandis*, have invaded native habitats have had significant impacts on hydrology (Le Maitre et al., 2002;Beater et al., 2008). There are no information available on the hybrid or the GE version; consequently answering unknown.
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - high	0	No evidence of toxicity in *E.* × *urograndis*, but cyanogic compounds in 2 other species (*E. cladocalyx* and *E. viminalis*) have killed goats and guinea fowl (Burrows and Tyrl, 2001). The traits engineered into the hybrid would not be expected to alter these properties.
Imp-P6 (Weed status in production systems) For production systems, choose the best answer. (A) Plant not a weed; (B) Plant a weed but no evidence of control by people; (C) Plant a weed and evidence of control.	a - high	0	No evidence.
Geographic Potential			
Plant cold hardiness zones			
Geo-Z1 (Zone 1)	n - negl	N/A	Frost tender
Geo-Z2 (Zone 2)	n - negl	N/A	Frost tender
Geo-Z3 (Zone 3)	n - negl	N/A	Frost tender
Geo-Z4 (Zone 4)	n - negl	N/A	Frost tender
Geo-Z5 (Zone 5)	n - negl	N/A	Frost tender
Geo-Z6 (Zone 6)	n - negl	N/A	Frost tender
Geo-Z7 (Zone 7)	n - negl	N/A	Frost tender
Geo-Z8 (Zone 8)	y - mod	N/A	The transgenic trait allows the tree to be grown in zones 8b and

			higher in the U.S. (Nehra and Pearson, 2011). This is based on the USDA hardiness zone map.
Geo-Z9 (Zone 9)	y - mod	N/A	Rio Grande Do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-Z10 (Zone 10)	y - mod	N/A	Espírito Santo, Rio Grande do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-Z11 (Zone 11)	y - mod	N/A	Bahia, Espírito Santo, Rio Grande do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-Z12 (Zone 12)	y - mod	N/A	Bahia, Espírito Santo, Maranhão, Pará: Brazil (CABI_A, 2012).
Geo-Z13 (Zone 13)	y - mod	N/A	Bahia, Maranhão, Pará: Brazil (CABI_A, 2012).
Koppen-Geiger climate classes			
Geo-C1 (Tropical rainforest)	y - mod	N/A	Bahia, Espírito Santo, Maranhão, Pará: Brazil (CABI_A, 2012).
Geo-C2 (Tropical savanna)	y - mod	N/A	Bahia, Espírito Santo, Maranhão, Pará: Brazil (CABI_A, 2012).
Geo-C3 (Steppe)	n - mod	N/A	Bahia: Brazil (CABI_A, 2012). Steppe occurs in Bahia, but most likely too little rainfall to survive.
Geo-C4 (Desert)	n - mod	N/A	No evidence. Desert area occurs in Bahia Brazil but probably too little rainfall to survive.
Geo-C5 (Mediterranean)	n - negl	N/A	No evidence
Geo-C6 (Humid subtropical)	y - mod	N/A	Rio Grande Do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-C7 (Marine west coast)	n - negl	N/A	No evidence
Geo-C8 (Humid cont. warm sum.)	y - mod	N/A	Rio Grande Do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-C9 (Humid cont. cool sum.)	n - negl	N/A	Frost tender
Geo-C10 (Subarctic)	n - negl	N/A	Frost tender
Geo-C11 (Tundra)	n - negl	N/A	Frost tender
Geo-C12 (Icecap)	n - negl	N/A	Frost tender
10-inch precipitation bands			
Geo-R1 (0-10 inches; 0-25 cm)	n - negl	N/A	Too little rainfall to survive
Geo-R2 (10-20 inches; 25-51 cm)	n - mod	N/A	No evidence. Occurs in Bahia Brazil (CABI_A, 2012) (desert area - most likely too little rainfall)
Geo-R3 (20-30 inches; 51-76 cm)	y - high	N/A	Bahia: Brazil (CABI_A, 2012). May be too little rainfall to survive.
Geo-R4 (30-40 inches; 76-102 cm)	y - mod	N/A	Bahia: Brazil (CABI_A, 2012).
Geo-R5 (40-50 inches; 102-127 cm)	y - mod	N/A	Bahia, Espírito Santo, Maranhão: Brazil (CABI_A, 2012).
Geo-R6 (50-60 inches; 127-152 cm)	y - mod	N/A	Bahia, Espírito Santo, Maranhão, Rio Grande do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-R7 (60-70 inches; 152-178 cm)	y - mod	N/A	Maranhão, Rio Grande do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-R8 (70-80 inches; 178-203 cm)	y - mod	N/A	Maranhão, Pará, Rio Grande do Sul, São Paulo: Brazil (CABI_A, 2012).
Geo-R9 (80-90 inches; 203-229 cm)	y - mod	N/A	Maranhão, Pará: Brazil (CABI_A, 2012).
Geo-R10 (90-100 inches; 229-254 cm)	y - mod	N/A	Maranhão, Pará: Brazil (CABI_A, 2012).
Geo-R11 (100+ inches; 254+ cm))	y - mod	N/A	Pará: Brazil (CABI_A, 2012).
Entry Potential			
Ent-1 (Plant already here)	y - negl	1	Planted in field tests in Hawaii (CABI_A, 2012) and the continental U.S. (Nehra and Pearson, 2011).

Ent-2 (Plant proposed for entry, or entry is imminent)	-	N/A
Ent-3 (Human value & cultivation/trade status)	-	N/A
Ent-4 (Entry as a contaminant)		
Ent-4a (Plant present in Canada, Mexico, Central America, the Caribbean or China)	-	N/A
Ent-4b (Contaminant of plant propagative material (except seeds))	-	N/A
Ent-4c (Contaminant of seeds for planting)	-	N/A
Ent-4d (Contaminant of ballast water)	-	N/A
Ent-4e (Contaminant of aquarium plants or other aquarium products)	-	N/A
Ent-4f (Contaminant of landscape products)	-	N/A
Ent-4g (Contaminant of containers, packing materials, trade goods, equipment or conveyances)	-	N/A
Ent-4h (Contaminants of fruit, vegetables, or other products for consumption or processing)	-	N/A
Ent-4i (Contaminant of some other pathway)	-	N/A
Ent-5 (Likely to enter through natural dispersal)	-	N/A