Finding of No Significant Impact and Decision Notice

Animal and Plant Health Inspection Service

Issuance of Permit to Grow Engineered Bahiagrass

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has received a permit application (APHIS number 05-294-02r) from the University of Florida to conduct field tests with bahiagrass plants that are genetically engineered to express glufosinate resistance and neomycin phosphotransferase. The transgenic bahiagrass is part of a field trial to investigate gene flow to non-transgenic bahiagrass. The transgenic grass lines will be evaluated for pollen dispersal, agronomic properties and out-crossing success between different wild-type cultivars of bahiagrass. A description of the field tests may be found in the attached Environmental Assessment (EA), which was prepared pursuant to APHIS regulations (7 CFR 372) promulgated under the National Environmental Policy Act. The field tests are scheduled to begin in 2006 in Jackson County, Florida.

A draft EA was prepared and submitted for public comment for 30 days. No comments were received during the comment period.

APHIS proposed three different actions to take in response to the permit application: the denial of the permit (Alternative A), the granting of the permit with no Supplemental Permit Conditions (Alternative B), and the granting of the permit with Supplemental Permit Conditions containing duplicative safety measures (Alternative C).

Based upon analysis described in the EA, APHIS has determined that the action proposed in Alternative C will not have a significant impact on the quality of the human environment because:

(1) The transgenic bahiagrass lines contain the gene (bar) for expression of the phosphinothricin acetyl transferase enzyme as a screening tool for the applicant. In the unlikely event that the bar gene migrates to bahiagrass outside the field trial, this gene would only confer a selective advantage if glufosinate herbicides are applied to the plants. Phosphinothricin acetyl transferase does not change any ecological or agronomic properties in the plant, apart from having phosphinothricin acetyl transferase enzymatic activity, which confers glufosinate resistance. Therefore, APHIS has determined the presence of the bar gene will have no significant environmental impacts.

(2) To facilitate the selection of transformed plants, the bahiagrass plants were engineered with the NptII gene which encodes for neomycin phosphotransferase, an enzyme which confers tolerance to the antibiotic kanamycin. NptII is an enzyme that inactivates the antibiotic kanamycin

thereby allowing cells containing this gene to grow on medium containing kanamycin. The NptII gene has been given GRAS (Generally Recognized as Safe) status since 1993 and is devoid of inherent plant pest characteristics (Fuchs et al., 1993). Therefore, APHIS has determined the presence of the NptII gene will have no significant environmental impacts.

(3) Pollen gene flow is limited in this study because (a) the small acreage of this study relative to the surrounding pollen sink of non-transgenic plants limits the source of transgenic pollen available to potentially out-cross with nearby wild relatives of sexually compatible bahiagrass, (b) Argentine bahiagrass is not sexually compatible with plant species outside of the *Paspalum* genus, (c) hybrids between diploid and tetraploid Paspalum would all be triploid and sterile and the resulting plants could only propagate by vegetative means. Triploid plants do not grow effectively in the established area and grow 20% more slowly than diploids or tetraploids under optimal conditions; therefore, transgenic pollen movement will not have a significant effect on the environment.

(4) Movement of transgenic bahiagrass seeds is confined. No transgenic seeds will be planted in this experiment, removing an important route for transgenic genes to move beyond the field site boundary. To prevent the spread of seeds from the transgenic plants by insects or animals, wooden cage structures have been built around the entire test plot and covered completely by mosquito netting. Thus, transgenic seed movement is unlikely, and in this case there will not be any significant impact on the environment.

(5) It is unlikely that any transgenic bahiagrass plants will persist within the field site because (a) the applicant will mow a 360 ft border around the field test plot to halt the production of flowers from any renegade bahiagrass plant, (b) seeds will be carefully harvested from transgenic and recipient plants and transported from the field site, and (c) after completion of the field release, the entire field plot will be sprayed with an effective herbicide to remove all grasses involved in the experiment. There should be no persistence of transgenic plants within the field site, and thus no significant impact on the environment.

(6) Bahiagrass is a bunchgrass and the stolons produced do not spread rapidly away from the plant to establish aggressive vegetative growth. The genes inserted into the transgenic bahiagrass plants do not increase vegetative growth; and therefore, weediness potential of the transgenic bahiagrass is not increased. Thus there is no significant impact on the environment.

(7) There has been no intentional genetic change in these plants to affect their susceptibility to plant disease or insect damage. There also is no reason to believe that these or similar characteristics are different between the genetically engineered and non-engineered plants, or that these characteristics will have any significant impact on the environment.

(8) Transfer and expression of DNA from the plant to bacteria is unlikely to occur and thus there is unlikely to be a significant impact on the environment.

(9) Transgenic DNA is no different from other DNA consumed as part of the normal diet; therefore, there is no significant impact on the quality of the human environment.

(10) The proposed field tests are controlled releases of the regulated article into the environment in Jackson County, Florida. There are no listed critical habitats for any threatened and endangered animal species in Jackson County, Florida according to the U.S. Fish and Wildlife database and therefore the release of the regulated article is not expected to affect any critical habitats.

Because APHIS has reached a finding of no significant impact of this field trial of transgenic bahiagrass, no Environmental Impact Statement will be prepared regarding this decision.

Pursuant to its regulations (7 CFR 340) promulgated under the Plant Protection Act of 2000, APHIS has determined that this field trial, following conditions described in Alternative C, will not pose a risk of the introduction or dissemination of a plant pest for the following reasons:

1. Transgenic pollen will be confined to the field site. Transgenic plants are placed in the center of a 2m diameter circle surrounded by a 4m diameter circle of untransformed bahiagrass plants within the 20 acre field site, and it is unlikely that transgenic pollen will find sexually compatible grass species outside the field site because (a) of the apomictic nature of the transgenic bahiagrass and (b) the mowing of any sexually compatible bahiagrass around the field test site. In the highly unlikely event that transgenic pollen finds a receptive plant, the resulting offspring would be a sterile triploid and not likely to survive because of outgrowth by competing wildtype bahiagrass. In addition, the transgenes found in the pollen (gene for kanamycin resistance and a gene for glufosinate resistance) would not cause a selective advantage for the resulting plants outside the field trial and do not confer plant pest characteristics.

2. Movement of transgenic bahiagrass seeds is confined. No transgenic seeds will be planted in this experiment, removing an important route for transgenic genes to move beyond the field site boundary. To prevent the spread of seeds from the transgenic plants by insects or animals, wooden cage structures have been built around the entire test plot and covered completely by mosquito netting. All seedheads within the test plot will be bagged after pollination in order to collect all seeds for propagation in the greenhouse. The bagging and collection of seeds will minimize any seed left in the field. The site will be monitored for three years following the termination of the field trial for the presence of bahiagrass plants.

3. The transgenic plot will be surrounded by a 10 ft fallow (bare ground) border to detect any potential vegetative reproduction by the transgenic bahiagrass. This additional measure will prevent vegetative transgenic propagules from establishing in the field site.

4. In addition to any removed transgenic plant material, any non-transgenic plant material removed from the test field plot will be treated as a regulated article. This additional measure will ensure contained movement of the transgenic seeds, plants, and plant parts between greenhouse facilities, laboratory facilities and the field site.

5. After termination of the experiment, the site will be monitored for three years following the termination of the field trial for the presence of bahiagrass plants.

6. The regulatory regions of the genetic constructs inserted into transgenic bahiagrass contain non-coding DNA regulatory sequences that are associated with the introduced genes from cauliflower mosaic virus (CaMV) and *Zea mays* (corn) to facilitate expression in plants. The regulatory sequences from CaMV are the 35S 3' region and 35S promoter and terminator regions. The regulatory sequences from corn are heat shock protein 70 (HSP70), ubiquitin (*ubi*) first intron enhancer sequence, and *ubi* promoter region. None of the DNA regulatory sequences can cause plant disease by themselves or in conjunction with the genes that were introduced into the transgenic bahiagrass lines.

For the reasons enumerated above, which are consistent with regulations implementing the Plant Protection Act, the field trial of bahiagrass plants that are genetically engineered for kanamycin resistance and glufosinate resistance is hereby authorized.

for

Cindy Smith
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date:
5/12/06

USDA/APHIS Environmental Assessment

In response to permit application (05-294-02r)
received from the University of Florida for a field-test to examine
gene flow in genetically engineered Argentine bahiagrass
(*Paspalum notatum*, Flueggé)

U.S. Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

TABLE OF CONTENTS

I. INTRODUCTION

A. Importance and Use of Bahiagrass

Bahiagrass *(Paspalum notatum)* is an important perennial, sod-forming tropical American grass grown in warm regions of the southern United States for forage, soil binding and turf. In Florida, bahiagrass is used on more land area than any other single pasture species, covering an estimated 2-5 million acres (Chambliss, 1991). The primary, commercially used cultivars of *P. notatum* Flueggé in the United States are Argentine, Pensacola, and Tifton-9. The main differences between the different cultivars are leaf width and plant hardiness.

The Argentine cultivar was introduced into the United States from Argentina in 1944. It has wider leaves, is not as cold tolerant as the Pensacola types, and does not initiate growth as early in the spring. It prefers wet soil and can even survive in standing water. It is popular in the sod trade because it produces fewer seedheads than Pensacola (Chambliss, 1991). This variety was initially introduced by the University of Florida for its forage production qualities of superior nutritional value, being able to withstand heavy grazing and having a low incidence of pest problems (Chambliss, 1991). Because the Argentine cultivar has an attractive look with wide broad leaves and a darker green color, it has become the preferred bahiagrass variety for lawns in Florida and other Coastal Southern states. Its susceptibility to cold results in winter kill of stands planted north of approximately Tifton, Georgia (http://www.bahiagrass.com).

Bahiagrass is being genetically engineered to increase its forage production in fall and winter months and improve turf quality, making risk management and risk assessment an integral part of these genetic improvements. The insertion of a marker gene to study possible gene transfer to wild relatives is an important aspect of plant pest risk assessment.

B. Summary of Submitted Permit

1. Proposed Research

Research on pollen movement is critical to addressing the issue of gene flow in transgenic releases, as well as for developing sound risk assessments for wind-pollinated transgenic grass species. Scientists at the University of Florida have been conducting research on the biology of bahiagrass. The purpose of their proposed introduction of two transgenic bahiagrass lines is to investigate the frequency of cross-hybridization between transgenic Argentine bahiagrass with different bahiagrass cultivars under field conditions.

Additionally, the data gathered during this study will be used to assess the confined status of this field release and refine the confinement conditions necessary for future releases of these grass species.

2. Description of the Research

Two herbicide resistant apomictic Argentine bahiagrass lines will be used as the pollen donors to determine gene flow among cultivars of bahiagrass. Twelve plants from different apomictic herbicide resistant lines will be planted in the center of a 2m diameter circle, surrounded by a 4m diameter circle of untransformed bahiagrass plants. This pattern will be used in two experiments. In one experiment, the outer circle will include four different tetraploid wildtypes: Argentine, Paraguay 22, Tifton7, and Batatais. In the second experiment, the outer circle will consist of diploid Pensacola plants. Both experiments have two replications.

To prevent the spread of seeds from the transgenic plants by insects or animals, wooden cage structures have been built covering the entire test plot and covered completely by mosquito netting. The closest wild bahiagrass has been measured at a distance of 280 feet. All wild bahiagrass within the research station boundary (approximately 360 ft from the test plot) will be mowed weekly. Mowing is expected to efficiently prevent flowering, since bahiagrass flower stalks grow to more than two feet tall before shedding pollen. Therefore, no pollen transfer to wild relatives is expected. For a detailed description of the experimental design for this research project, see Appendix I.

3. Description of the Regulated Articles

The proposed research uses two transgenic lines of Argentine bahiagrass (for a detailed description of the regulated articles, see Appendix III):

1) Line ‘B9' has been genetically engineered to express the phosphinothricin acetyl transferase (bar) gene from *Streptomyces hygroscopicus*, which confers resistance to glufosinate herbicides. Expression of this gene is controlled by the polyubiquitin (*ubi*) promoter, mature mRNA and first intron enhancer sequences from *Zea mays*, and the 35S 3' region from cauliflower mosaic virus (CaMV).

2) In addition to the gene sequences above, line ‘P’ has also been genetically engineered to express the neomycin phosphotransferase gene (*NptII*) from *E. coli*, which confers resistance to the antibiotic kanamycin. Expression of this gene is controlled by the enhanced 35S promoter from CaMV, heat shock protein 70 (HSP70), intron from *Zea mays*, and the 35S 3' region from CaMV.

Constructs were inserted into the recipient organisms by microprojectile bombardment.

C. Regulatory Authority

The relevant authorities for regulation of genetically engineered bahiagrass is the Plant Protection Act of 2000, 7 U.S.C. 7701-7772, and USDA, APHIS regulations under 7 CFR § 340, “Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or Which There is Reason to Believe are Plant Pests.” A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxonomic groups listed in the regulation and is also a plant pest, or if there is a reason to believe it is a plant pest. In this submission, bahiagrass has been genetically engineered using the recombinant DNA technique of microprojectile bombardment. The introduced DNA sequence contains regulatory genes from cauliflower mosaic virus (CMV). CMV belongs to a taxonomic group listed in 7

CFR 340.2; therefore, the genetically engineered bahiagrass in this submission is considered a regulated article.

This environmental assessment (EA) was conducted under the authority of the National Environmental Policy Act (NEPA), 42 U.S.C. 4321 and 7 CFR § 372, APHIS' NEPA Implementing Procedures. Except for actions that are categorically excluded, approvals and issuance of permits for proposals involving genetically engineered or non-indigenous species normally require environmental assessments, but not necessarily environmental impact statements (7 CFR § 372.5(b)(4)). The actions described in the application for permit 05-294-02r involves the release of a transgenic bahiagrass species. Analysis by APHIS of the conditions proposed in the permit applications suggests that these actions constitute a confined field release and thus are categorically excluded actions under 7 CFR 372. However, the recent scientific study in creeping bentgrass demonstrating pollen gene flow over large distances (Watrud et al., 2004) creates some uncertainty regarding the confinement of field trials of flowering grasses. APHIS is preparing this Environmental Assessment to address this new confinement issue.

II. NEED FOR PROPOSED ACTION

A. Proposed Action

The proposed action is for APHIS, Biotechnology Regulatory Services (BRS), to issue a permit for field-testing Argentine bahiagrass genetically modified to express glufosinate resistance and neomycin phosphotransferase. The transgenic bahiagrass is part of a field trial to investigate gene flow to non-transgenic bahiagrass. The transgenic grass lines will be evaluated for pollen dispersal, agronomic properties and out-crossing success between different wild-type cultivars of bahiagrass.

B. Purpose of this Environmental Assessment

The purpose of this EA is to assess any potential adverse environmental effects of a field research study in Jackson County, Florida. The permit application was received by APHIS, BRS on October 21, 2005. It was submitted by the Dr. Ann Blount, University of Florida, Marianna, Jackson County, Florida. The application number is 05-294-02r.

C. Need for This Action

Under APHIS regulations, the receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

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

III. ALTERNATIVES

A. No Action

Under APHIS/BRS regulations, the Administrator must either grant or deny permits properly submitted under 7 CFR 340. For the purposes of this Environmental Assessment, the No Action alternative would be the denial of permit application 05-294-02r.

The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service, Biotechnology Regulatory Services (APHIS/BRS) has previously allowed the University of Florida (Jackson County, Florida) to plant transgenic glufosinate herbicide tolerant bahiagrass under notification 05-076-12n. The University of Florida proposes to replant the same genetically engineered bahiagrass (*Paspalum notatum* Flueggé) lines under identical conditions specified under notification 05-076-12n. Under the No Action Alternative, if this permit is denied, the transgenic bahiagrass plants will not be planted. Any bahiagrass plants currently planted under notification 05-076-12n will be removed from the field or devitalized before the expiration of the notification, as required by APHIS regulations in 7 CFR 340.3(c).

B. Issue the Permit as Received

Issuing this permit would allow the following research to proceed at a grass field site in Jackson County, FL (see Appendix I for the detailed research plan) under the conditions provided by the applicant (see below, conditions a-i) and the standard permit conditions under 7 CFR §340.4 (see Appendix V). Under this alternative, the field release of the genetically engineered bahiagrass plants would be authorized at the specified location with no additional conditions implemented by APHIS/BRS.

The following redundant mitigation measures are incorporated into the experimental procedures by the applicant to promote a confined field release and ensure the least amount of harm to the environment:

a. The field test site is located on university property in Jackson County, FL. The experimental plot is bordered on two sides by a strip of woodland separating the research center from adjacent agricultural land which is expected to grow perennial peanut. The southern edge of the field, adjacent to the road, is bordered by a 3 ft-high electric, barbed wire fence. The entrance to the field from the road is through a padlocked gate.

b. The test plot site will contain four replications of the following plot design: twelve (12) transgenic plants in a 2m circle surrounded by sixty-four (64) non-transgenic bahiagrass relatives in an outer 4m circle. The small acreage of this study with the surrounding pollen sink of non-transgenic plants limits the source of origin of transgenic pollen available to potentially out-cross with nearby wild relatives of sexually compatible bahiagrass.

c. Argentine bahiagrass does not grow well in the shade, so no bahiagrass is found in the shaded tree canopies in the woodland border strips. The researcher will monitor the shaded wooded areas on the borders of the research station property for any bahiagrass volunteers. Any volunteers that are found will be destroyed by glyphosate herbicide application.

d. In nature, chromosomal genetic material of bahiagrass can only be transferred to other sexually compatible (diploid) plants by cross-pollination. Pollination of a diploid by a tetraploid is expected to produce a sterile triploid plant. Hybrids between diploid and tetraploid Paspalum are all triploid and are sterile. Thus, if hybridization were to occur, the resulting plants could only propagate by vegetative means. Triploid plants do not grow effectively in established areas (Burton, 1990). They grow 20% more slowly than diploids or tetraploids under optimal conditions though they are more effective colonizers in disturbed environments (Bergfeld et al., 2005)

e. The closest wild or cultivated relative of bahiagrass is estimated to be a distance of 200-360 ft to the test plot. These relatives are either apomictic tetraploids or sexual diploids. All wild bahiagrass growing within an area of 360 ft surrounding the test plot will be mowed weekly to prevent the formation of inflorescences; therefore no pollen transfer to wild relatives is expected. Bahiagrass flower stalks become more than two feet tall before flowering; therefore mowing is very effective in eliminating pollen receptors. Seed certification standards for grasses (www.aosca.org) require an isolation distance between diploids and tetraploids of at least 15 ft. The 360 ft isolation distance proposed by the applicant greatly exceeds the AOSCA standard for foundation seed.

f. Bahiagrass is a bunchgrass and the stolons produced do not spread rapidly away from the plant to establish aggressive vegetative growth (as in Bermuda grass). Also, Bahiagrass does not propagate or compete with other established plants in an intact ecosystem (Chambliss, 1991), limiting the spread of any vegetative growth if sterile triploid plants are produced.

g. To prevent the spread of seeds from the transgenic plants by insects or animals, wooden cage structures have been built around the entire test plot and covered completely by mosquito netting.

h. Because this is a gene flow study, all seedheads within the test plot will be bagged after pollination in order to collect all seeds for propagation in the greenhouse. The bagging and collection of seeds will minimize any seed left in the field. The site will be monitored for three years following the termination of the field trial for the presence of bahiagrass plants.

C. Issue Permits with Supplemental Conditions

Issuing this permit would allow the following research to proceed at a grass field site in Jackson County, FL (see Appendix 1 for the detailed research plan) where supplemental permit conditions, based on APHIS scientific analysis of the permit applications, input from the State of Florida, and public comment from this environmental assessment, would be required. If warranted, based on environmental risk of escape of the engineered organism, APHIS will require mitigating measures to prevent spread of the organism outside the field production area.

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

a. Seed heads from the transgenic and recipient plants will be bagged before removal from the plants, and placed into a second bag immediately after cutting seed head from plant.

b. The entire test plot will be surrounded by a 10 ft fallow (bare ground) border to detect any potential vegetative reproduction by the transgenic bahiagrass plants.

c. In addition to any removed transgenic plant material, any non-transgenic plant material removed from the test field plot will be treated as a regulated article.

IV. ENVIRONMENTAL CONSEQUENCES OF THE PROPOSED ACTION AND ALTERNATIVE

A. Deny the Permit

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

B. Issuance of the Permit as Received

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

1. No adverse consequences to non-target organisms or environmental quality are expected from the field release of the transgenic grass lines for the reasons stated below.

2. The proteins produced by genes introduced into these grass lines are not expected to have toxicological or allergenic properties.

3. None of the introduced genes provide the engineered bahiagrass plants with any selective advantage over non-engineered bahiagrass in the ability to be disseminated or to become established in the environment.

C. Issuance of the Permits with Additional Conditions

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

1. No adverse consequences to non-target organisms or environmental quality are expected from the field release of the transgenic grass lines for the reasons stated below.

2. The proteins produced by genes introduced into these grass lines are not expected to have toxicological or allergenic properties.

3. None of the introduced genes provide the engineered bahiagrass plants with any selective advantage over non-engineered bahiagrass in the ability to be disseminated or to become established in the environment.

Under this alternative, APHIS proposes to include the following duplicative safety measures to promote a confined field release and ensure no significant harm to the environment:

a. Seed heads from the transgenic and recipient plants will be bagged before removal from the plants, and placed into a second bag immediately after cutting seed head from plant. This additional measure will further reduce the risk of inadvertent loss of transgenic seeds during harvest.

b. The transgenic plot will be surrounded by a 10 ft fallow (bare ground) border to detect any potential vegetative reproduction by the transgenic bahiagrass plants. This additional measure will prevent vegetative propagules from establishing in the field site.

c. In addition to any transgenic plant material, any non-transgenic plant material removed from the test field plot will be treated as a regulated article. This additional measure will ensure contained movement of the transgenic seeds, plants, and plant parts between greenhouse facilities, laboratory facilities and the field site.

D. Analysis of Issues, Consequences, and Theoretical Risks of Field Research using Transgenic Bahiagrass

1. Possibility of Gene Flow Outside of the Field Test

Genes of bahiagrass may escape from the test plot in two ways. The first pathway of escape is by pollen transfer. The second is by movement of propagative material, *i.e.*, the whole seeds or by vegetative growth.

a. Pollen Movement

Pollen gene flow is expected to be limited in Argentine bahiagrass for the following reasons:

1. The test plot site will contain four replications of the following plot design: twelve (12) transgenic plants in a 2 m circle surrounded by sixty-four (64) non-transgenic bahiagrass relatives in an outer 4 m circle. The small acreage of this study with the surrounding pollen sink of non-transgenic plants limits the source of origin of transgenic pollen available to potentially out-cross with nearby wild relatives of sexually compatible bahiagrass

2. Argentine bahiagrass is not sexually compatible with plant species outside of the *Paspalum* genus.

3. The transgenic Argentine bahiagrass and most of the feral bahiagrass are tetraploid and obligate apomicts (meaning they produce seed without being fertilized by pollen). Hybridization among tetraploid apomicts is negligible because seed development occurs in the absence of fertilization.

4. Hybridization between apomictic tetraploids and sexual diploids appears to occur at low frequency. Argentine bahiagrass produces viable pollen. A small percentage of feral bahiagrass plants are diploid and are capable of being fertilized by pollen derived from tetraploid plants. In laboratory studies, most diploid plants are fertilized by pollen from other diploid plants. However a small percentage of Paspalum hybrids can form between diploids and tetraploids (0.004-0.025% of the progeny formed) (Norrmann et al., 1994).

5. Diploid bahiagrass that can reproduce sexually occur in the nearby wild population; however, hybrids between diploid and tetraploid Paspalum would all be triploid and sterile. Thus, if hybridization were to occur, the resulting plants could only propagate by vegetative means.

6. Triploid plants do not grow effectively in established areas (Burton, 1990). They grow 20% more slowly than diploids or tetraploids under optimal conditions though they are more effective colonizers in disturbed environments (Bergfeld et al., 2005)

Transgenic grass pollen from another grass species, creeping bentgrass, has been shown to hybridize to receptive bentgrass plants over distances as far as 21 km from the source of the transgenic pollen (Watrud et al., 2004), indicating a theoretical risk exists for transgenic pollen movement beyond the field site boundary for any transgenic grass. However these experiments were based on data from 400 acres of transgenic grasses. The bentgrass studies raise some uncertainty with regard to the confinement of field releases of flowering transgenic grasses. Therefore, points **2, 3** and **4** below will address the risks of the transgenes used in this field trial, in the unlikely event that transgenic grass pollen moves beyond the field trial boundaries.

b. Seed Movement

No transgenic seeds will be planted in this experiment, removing an important route for transgenic genes to move beyond the field site boundary; therefore, the only chance for gene flow migration by seed movement will be after seed maturation in the transgenic and recipient plots. Movement of whole seed by animals is the major way for dissemination of bahiagrass seed, for example, cattle may graze bahiagrass seed heads and carry seed to new areas where it can become established, as the seed will germinate after passing through the digestive tract of cattle. To prevent the spread of seeds from the transgenic plants by animals, wooden cage structures have been built around the entire test plot and covered completely by mosquito netting. The applicant is proposing to bag the seed heads after pollination, and collect seeds immediately at maturation to further reduce the potential for mature seed movement by birds and/or rodents. The applicant is also proposing to immediately bag the seed heads after seed maturity, before removing the seed heads from the transgenic plants, to eliminate seed spillage during collection and transportation of transgenic seeds back to the laboratory for cleaning and analysis. To further eliminate the risk of inadvertent seed spillage, APHIS recommends that the applicant bag the seed heads before removal from the plant, and immediately place into a second bag during harvest (under Section III: Alternatives, Subsection: C. Issue Permit with Supplemental Conditions, Condition a). Therefore, because no transgenic seeds are planted during the experiment, large animals, birds and mice are discouraged from entering the transgenic and recipient plots, and seed heads will be monitored daily during the seed ripening period, collected immediately at maturity, and carefully transported from the field site, APHIS is confident that the current permit and supplementary permit conditions will confine the crop and make the risk of gene flow through seed movement.

In a recent workshop hosted by APHIS dealing with gene confinement issues in genetically engineered crops (USDA APHIS, 2004), one of the more likely mechanisms contributing to the breakdown of confinement and movement of seed was identified as human error, and the most reliable means of preventing this is to maintain and reinforce stringent standard operating procedures. In this study, the applicant will follow SOPs to prevent accidental dispersal of the seeds or plants into the environment. In addition, the small scale of this field test (48 transgenic plants total) reduces the likelihood for human error.

2. Risk of the Gene for Glufosinate Resistance to the Environment

The transgenic bahiagrass lines (see Appendix III for more detailed discussion) contain the gene (*bar*) for expression of the phosphinothricin acetyl transferase enzyme as a screening tool for the applicant. In the unlikely event that the *bar* gene migrates to bahiagrass outside the field trial, this gene would only confer a selective advantage if glufosinate herbicides are applied to the plants. Phosphinothricin acetyl transferase does not change any ecological or agronomic properties in the plant, apart from having phosphinothricin acetyl transferase enzymatic activity, which confers glufosinate resistance.

The nearest field of bahiagrass that could be affected by the unlikely event of transgene movement is a 10-acre pastureland approximately 370 ft from the field test site. The privately owned pastureland is composed of a mix of apomictic tetraploid and diploid bahiagrass. The pasture is well outside the AOSCA standards for seed certification requirements that mandate a distance of 15 ft between diploid and tetraploid grass species. This pastureland is currently unused and typically mowed once every three to four weeks, reducing the likelihood of receptive plants during flowering season. No seed or hay is collected for commercial use and it is a well-established pasture (i.e., an intact ecosystem). Further, no glufosinate herbicides are used on this pastureland. Therefore, in the unlikely event that a (sterile) transgenic hybrid plant establishes and survives, the selective pressure of the application of glufosinate herbicides giving an advantage for growth is not present.

3. Risk of the Gene for Kanamycin Resistance to the Environment

The selectable marker neomycin phosphotransferase, *NptII*, also present in these plants, encodes for neomycin phosphotransferase, an enzyme which confers tolerance to the antibiotic kanamycin. *NptII* is an enzyme that inactivates the antibiotic kanamycin thereby allowing cells containing this gene to grow on medium containing kanamycin. The *NptII* gene has been given GRAS (Generally Recognized as Safe) status since 1993 and is devoid of inherent plant pest characteristics (Fuchs et al., 1993). Therefore, APHIS has determined the presence of the *NptII* gene will have no significant environmental impacts.

4. Persistence of Transgenic Grasses

It is highly unlikely that transgenic bahiagrass will persist within the field site. During the experiment, the applicant will mow 360 ft border around the field test plot to halt the production of flowers from any renegade bahiagrass plant. Seeds will be carefully harvested from transgenic and recipient plants, and transported from the field site, reducing the risk of potential germination of a transgenic bahiagrass plant during the next growing season. In addition, after completion of the field release, the entire field plot will be sprayed with an effective herbicide to remove all grasses involved in the experiment. If the applicant removes transgenic or non-transgenic plants from the field site, APHIS proposes to require the applicant to treat all plants removed from the field site as regulated articles (under Section III: Alternatives, Subsection: C. Issue Permit with Supplemental Conditions, Condition c). Thus the removed plants will be transported under contained conditions, eliminating the risk of persistence of a transgenic plant by way of escape during transportation. After completion of the experiment, the field site will be monitored monthly for volunteer plants for three years.

Any transgenic bahiagrass pollen will be tetraploid. In nature, chromosomal genetic material of bahiagrass can only be transferred to other sexually compatible (diploid) plants by cross-pollination. Pollination of a diploid by a tetraploid is expected to produce a sterile triploid plant.

The proposed field experiment will release a relatively small amount of pollen from a total of 48 transgenic plants within 0.25 acres. The previous planting under notification using the identical field design to the one proposed in these permit application found no volunteers outside the field test plot.

Finally, receptive, compatible plant species are available outside the proposed field site. The area surrounding the proposed field site contains both obligate apomictic bahiagrass and sexual diploid bahiagrass. The closest wild or cultivated relative of bahiagrass is estimated to be a distance of 200-360 ft to the test plot. These relatives are either apomictic tetraploids or sexual diploids. All wild bahiagrass growing within an area of 360 ft surrounding the test plot will be mowed weekly to prevent the formation of inflorescences; therefore no pollen transfer to wild relatives is expected. Bahiagrass flower stalks become more than two feet tall before flowering; therefore mowing is very effective in eliminating pollen receptors. Thus, the risk of transgenic pollen reaching a sexually compatible plant is low.

Thus, for successful establishment of bahiagrass outside the field site boundary, transgenic pollen must (1) travel a distance of greater than 360 ft and, (2) find and pollinate remote, isolated, sexually compatible plants within large expanses of non-compatible plants and (3) create a viable triploid plant, and (4) vegetatively propagate successfully in an already established ecosystem. Given the low probability of each of these requirements in regards to transgenic pollen movement, APHIS finds that there is minimal risk of the persistence of transgenic plants outside the proposed field site.

5. Weediness of Bahiagrass

Bahiagrass is a bunchgrass and the stolons produced do not spread rapidly away from the plant to establish aggressive vegetative growth (as in Bermuda grass). In a recent paper, the annual biomasses of guinea grass, Bermuda grass and bahiagrass were compared (Newman et al., 2005). Despite similar crude protein values, the biomass varied significantly among all three grasses with guinea grass being highest at 1.31 mg/ha, followed by Bermuda grass at 0.59 mg/ha, and lastly bahiagrass at 0.14 mg/ha. The annual biomass values demonstrate the amount of vegetative growth of guinea and Bermuda grasses are 9.4 and 4.2 fold higher than bahiagrass. Also, bahiagrass does not propagate or compete with other established plants in an intact ecosystem, limiting the spread of any vegetative growth if triploid plants are produced.

Florida removed bahiagrass from its Category 1 Exotic Pest Species list in 1998 because of questions regarding its ability to invade intact ecosystems (Chambliss, 1991). While it has been observed to rapidly spread vegetatively into disturbed (plowed) soil, it has a difficult time establishing itself beyond areas of disturbed vegetation. Chambliss (Chambliss, 1991) reports that bahiagrass seedlings are small and do not complete well with weeds.

A review of the weediness of bahiagrass can be found in ELEMENT STEWARDSHIP ABSTRACT for *Paspalum notatum* Flueggé Bahia grass, Bahiagrass THE NATURE CONSERVANCY (Violi, 2000).

6. Alteration in Susceptibility to Disease or Insects

There has been no intentional genetic change in these plants to affect their susceptibility to disease or insect damage. Neither the selectable marker gene, neomycin phosphotransferase gene, nor the phosphinothricin acetyl transferase gene is expected to change any plant pest characteristics. There is no reason to believe that these or similar characteristics are different between the genetically engineered and non-engineered plants. The selectable marker gene designed to provide kanamycin resistance is not expected to alter the susceptibility of the transgenic bahiagrass plants to disease or insect damage.

Execution of the prescribed periodic monitoring of the field plots will allow the detection of any unexpected infestation by plant disease organisms or animal pests. The University of Florida is required to report any such unanticipated effects to APHIS under the terms of the permit. See 7 CFR § 340.4(f)(10)(ii).

7. Horizontal Gene Transfer to Other Organisms

Transfer and expression of DNA from the plant to bacteria is unlikely to occur due to several known impediments. First, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression, and the bacteria must be competent to accept DNA. Gebhard and Smalla (Gebhard and Smalla, 1999) and Schluter *et al.*(Schlüter et al., 1995) have studied transgenic DNA movement to bacteria and although theoretically possible, it occurs at extremely low rates (approximately 1 in 10^{-14}). Many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko et al., 2000). There is no evidence that these organisms contain genes derived from plants. Koonin *et al.* (Koonin et al., 2001) and Brown (Brown, 2003) presented reviews based on sequencing data that revealed horizontal gene transfer occurs occasionally on an evolutionary time scale of millions of years.

8. Fate of Transgenic DNA

Transgenic DNA is no different from other DNA consumed as part of the normal diet. Genetically engineered organisms have been used in drug production and microbial fermentation (cheese and yogurt) since the late 1970's. More than 1 billion cumulative acres of engineered food and feed crops have been grown and consumed world wide in the past seven years (International Service for the Acquisition of Agri-biotech Applications, (ISAAA) at: http://www.isaaa.org/kc/CBTNews/press_release/briefs30/es_b30.pdf. The EPA has exempted from a tolerance DNA that encodes currently registered plant incorporated protectants because of a lack of toxicity (FR 66 37817-37830).

9. Impacts on Human Health, Including Minorities, Low Income Populations, and Children

Since the field test is on an isolated site on university owned property, the public will not be exposed to the plants. The bahiagrass seeds are unlikely to be mixed with any seeds intended for human or animal consumption because of numerous measures described in above text, in the applicant's SOPs and APHIS inspections during harvesting. All the harvested seeds will be stored in dedicated storage bags on site and seeds transferred to a greenhouse setting for seedling propagation.

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

10. Risks to Threatened and Endangered Species

BRS has reviewed the data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service to determine when a consultation, as required under Section 7 of the Endangered Species Act, is needed. APHIS has reached a determination that the release under the permit 05-294-02r would have no effects on listed threatened or endangered species and consequently, a written concurrence or formal consultation with the Fish and Wildlife Service is not required for this EA. Appendix 4 includes the BRS analysis of threatened and endangered species in the area of the field release.

11. Effects on Native Floral and Faunal Communities

Based on the lack of toxicity of the proteins that will be produced, APHIS concludes that there will be no significant effect on any native floral or faunal species for Jackson County, Florida. The EPA reviewed the safety of phosphinothricin acetyl transferase (40 CFR § 180), and found the *bar* enzyme protein to be non-toxic to mammals. An existing tolerance exemption, CFR 40 Section 180.1151, exists for phosphinothricin acetyl transferase and the genetic material necessary for its production in all plants. APHIS concurs with EPA on this assessment and therefore concludes there would be no significant effect on any vertebrate, invertebrate, or aquatic species. The proposed field test sites are located on land that has been under constant agricultural use for the past 100 years. APHIS concludes there would be no significant effect on any native floral species.

12. Impact on Existing Agricultural Practices

No impact on existing agricultural practices is expected. The University of Florida will employ agricultural practices consistent with growing healthy bahiagrass plants. Weeds will be controlled using herbicide applications. If necessary, insecticides and/or fungicides will be used to control pests such as mole crickets and armyworms that would diminish the health of the plant. Any approved pesticides will be applied by trained personnel in their use and application. The plot will be inspected at least twice weekly during the growing season.

No environmental impacts on nearby crops are expected. No bahiagrass seed production plots are adjacent to the field test area and only perennial peanut from adjacent agricultural land is expected to be grown. The closest commercial bahiagrass seed production field is 8 miles away from the test plot site. A privately owned bahiagrass pastureland area of less than 10 acres is located behind the tree line on the western side of the research station property (approximately 370 ft away from the test plot site). This pastureland will be mowed every 3 weeks by the property owners and no impact from the 12 transgenic plants is expected on this established bahiagrass pastureland. The genetic makeup of the bahiagrass in the pasture is a mix of sexual diploid and apomictic tetraploid cytotypes. The probability of a transgenic sterile triploid being produced and establishing itself in the intact environment is very low (See Section V for more details). Nevertheless, the researcher will be responsible for monitoring this pastureland for concurrent flowering with the transgenic plants, and elimination of wildtype flowering bahiagrass in the pasture during the experimental flowering period.

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X. APPENDICES

Appendix I. Description of the Field Experiment

Plot Design and Location

The field test sites are located on university property in Jackson County, FL. The experimental plot is bordered on two sides by a strip of woodland separating the research center from adjacent agricultural land which is expected to grow perennial peanut. The southern edge of the field, adjacent to the road, is bordered by a 3 ft-high electric, barbed wire fence. The entrance to the field from the road is through a padlocked gate.

Herbicide resistant apomictic Argentine bahiagrass lines B9 and P will be used as the pollen donors to determine gene flow among cultivars of bahiagrass. Twelve plants from different apomictic herbicide resistant lines will be planted in the center of a 2m diameter circle surrounded by a 4m diameter circle of untransformed bahiagrass plants. This pattern will be used in two experiments. In one experiment, the outer circle will include 4 different tetraploid wildtypes: Argentine, Paraguay 22, Tifton7, and Batatais. In the second experiment, the outer circle will consist of diploid Pensacola plants. Both experiments have two replications.

To prevent the spread of seeds from the transgenic plants by insects or animals, wooden cage structures have been built covering the entire test plot and covered completely by mosquito netting.

Agricultural Practices

The regulated 48 bahiagrass plants in the inner circles of the test plots will be allowed to pollinate and the surrounding diploid test plants in the outer circle will be allowed to produce seeds (see above plot design). The plots will be inspected twice weekly to monitor flowering, weeds, and insect or disease damage.

The seed heads from both the transgenic and wildtypes will be checked for anthesis twice a week and bagged 10 days after pollination. Seeds from the wildtype cultivars surrounding the transgenic plants will be collected and germinated in the greenhouse. Seedlings germinated from these seeds will be analyzed for resistance to glufosinate herbicide.

Agricultural practices consistent with growing healthy bahiagrass plants will be used; weeds will be controlled by herbicide applications in plots and between subplots. Weeds within subplots will be removed by hand.

The most serious insect threat to bahiagrass is the mole cricket. These insects burrow through the soil and damage roots, causing rapid wilting of the grass. If necessary, pesticides such as insecticides and/or fungicides will be used to control insect pests and disease that would diminish the health of the plant and subsequent seed yield. EPA registered chemical pesticides are likely to be used to control insect pests on these crops. The only serious disease of bahiagrass is dollar spot, which is expressed as spots several inches in diameter scattered across the turf. A light application of nitrogen (1/2 pound of nitrogen per 1000 square feet) should encourage the grass to outgrow these symptoms. Any pesticides used will be applied by personnel trained in their use and application. The field will be monitored for noxious weeds and other plant pests during the growing season.

During the growing season the plants will be inspected for traits such as weediness, resistance/susceptibility to insects or disease, or unusual differences in plant growth or morphology.

A zone of 360 feet will be maintained and mowed surrounding the field test site to discourage potential out-crossing with wildtype relatives.

Termination of the field test and final disposition of the test plants

The harvested seeds will be placed in dedicated storage bags on site and immediately returned to the laboratory for germination under greenhouse conditions.

After harvest, as soon as possible as the weather allows, the University of Florida will apply the herbicide glyphosate to degrade all plant material in the field to remove and decompose any remaining seed. The field site will be monitored quarterly for three years following the termination of the field test and any volunteer bahiagrass plants will be destroyed by application of glyphosate, removed and autoclaved.

Security of the field test plot

The test site is expected to provide adequate physical security. The University of Florida is the owner of the field test site. The southern edge of the field, adjacent to the road, is bordered by a 3 ft-high electric, barbed wire fence. The entrance to the field from the road is through a padlocked gate. The site is not prone to flooding. The closest body of water is a small unnamed lake close to Hays Spring which is located 2-3 miles to the east.

Volunteer Monitoring

Volunteer monitoring by the researcher will be done for 3 years after the end of the field test with a focus on any disturbed soil areas in and around a 360 ft radius from the test plot site. Bahiagrass seeds are slow to germinate (28 days) unless subjected to scarification. Under optimal storage conditions (8% moisture and 50% relative humidity), seeds can be stored for 1 yr with little loss in germination (West, 1993). Given this potential for seed dormancy, the field site will be monitored quarterly for three years following the termination of the field test and any volunteer bahiagrass plants will be destroyed by application of glyphosate, removed and autoclaved.

Appendix II. Biology of Bahiagrass

In this section of the environmental assessment, the biology of bahiagrass and plants related to bahiagrass are considered along with potential routes of gene escape. Because the mechanism by which genes are moved from one flowering plant to another is through cross-pollination of sexually compatible plants, the plants with which bahiagrass can cross-pollinate are described. Below is an analysis of the biology of bahiagrass. This review focuses solely on bahiagrass in the United States.

Systematics of Bahiagrass

Bahiagrass (*Paspalum notatum*) is included in the crowngrass genus *Paspalum* of the grass family (Poaceae) and has three recognized varieties, *Paspalum notatum var. latiflorum* J. Döll, *Paspalum notatum var. notatum* Flueggé, and *Paspalum notatum var. saurae* Parodi (USDA, 2005). The primary commercially used cultivars of *P. notatum* Flueggé in the United States are Argentine, Pensacola, and Tifton-9. The main differences between the different cultivars are leaf width and plant hardiness.

Argentine was introduced into the United States from Argentina in 1944. It has wider leaves, is not as cold tolerant as the Pensacola types, and does not initiate growth as early in the spring. It is popular in the sod trade because it produces fewer seedheads than Pensacola (Chambliss, 1991)

Pensacola is the most common cultivar in Florida and covers an estimated 2-5 million acres within the state. Pensacola can be planted farther north than Argentine due to its cold tolerance (Chambliss, 1991).

Tifton-9 is an improved Pensacola bahiagrass variety that was bred and developed by Dr. Glen Burton, Agricultural Research Service, USDA, and the Georgia Coastal Plain Experiment Station. Tifton-9 is a longer-bladed grass than other bahiagrass and produces 30 to 40% more forage per year than its parent Pensacola bahiagrass. Like the Pensacola cultivar, it has greater cold tolerance than the Argentine cultivar (Chambliss, 1991).

Origin and Distribution of Bahiagrass

Bahiagrass (*P. notatum*) is native to the West Indies and South America and was introduced into the USA from Brazil around 1913 (Bennett H.W., 1966). In North America, *P. notatum* can be found from southern California to eastern Texas, from southern Florida to New Jersey, and from central Tennessee to Arkansas (USDA, 2005). It is planted extensively from seed along highways in Florida, North Carolina, and other subtropical and mild temperate areas (USDA, 2005). Bahiagrass is mainly used as pasture grass and it is preferred by ranchers because it withstands close grazing and trampling, is drought tolerant, grows in a variety of soil types and has few insect pests (most notably, the mole cricket) (Chambliss, 1991).

Appendix III. Description of the Regulated Bahiagrass Plant

The University of Florida has engineered Argentine bahiagrass to contain the phosphinothricin acetyl transferase (*bar*) gene from *Streptomyces hygroscopicus* to confer glufosinate resistance and containing the neomycin phosphotransferase gene, *NptII*, as a selectable marker gene. The recipient organism, *Paspalum notatum* Flueggé, cv. Argentine, is a common commercial cultivar used extensively in the southeastern United States, both as a forage crop and as turf. Argentine bahiagrass is an obligate apomictic, perennial bunchgrass, resulting in the exclusive transmission of the entire maternal genotype to the next generation (Vielle Calzada et al., 1996).

The Vectors

The genes were transferred into bahiagrass plants via a vector system using microprojectile bombardment. This process is a well characterized transformation system which integrates the donor genes into the chromosome of the recipient plant cell (Batty and Evans, 1992). The system does not require the use of the plant pathogen, *Agrobacterium tumefaciens*, or other transformation vectors. The donor DNA sequences are stably and irreversibly integrated into the plant's chromosomal DNA, where they are maintained and inherited as any other genes of the plant cell.

Cauliflower mosaic virus (CaMV) and *Zea mays* (corn) are donors for non-coding DNA regulatory sequences that are associated with the introduced genes to facilitate expression in plants. The regulatory sequences from CaMV are the 35S 3' region and 35S promoter and terminator regions. The regulatory sequences from corn are heat shock protein 70 (HSP70), ubiquitin (*ubi*) first intron enhancer sequence, and *ubi* promoter region. None of the DNA regulatory sequences can cause plant disease by themselves or in conjunction with the genes that were introduced into the transgenic bahiagrass lines.

The Selectable Marker

To facilitate the selection of transformed plants, the bahiagrass plants were engineered with the *NptII* gene which encodes for neomycin phosphotransferase, an enzyme which confers tolerance to the antibiotic kanamycin. The selectable marker gene expression cassette consists of the 35S 3' region and promoter from CaMV and the HSP70 intron from corn.

The *NptII* gene was isolated from the donor organism *E. coli*, and encodes the 264-amino acid enzyme, neomycin phosphotransferase (*NptII*). The *NptII* enzyme inactivates a range of aminoglycoside antibiotics such as kanamycin. The presence of *NptII* allows cells to grow on medium containing kanamycin. The *NptII* gene is devoid of inherent plant pest characteristics and is the most commonly used selective marker in plants. This marker has been safely used in many previous field trials and has been shown to be safe (Fuchs et al., 1993).

The Gene of Interest

The bahiagrass plants were engineered to express the gene for the bialaphos resistance (*bar*), phosphinothricin acetyl transferase from *Streptomyces hygroscopicus*, conferring tolerance to glufosinate herbicides. Phosphinothricin, the active ingredient of glufosinate-ammonium herbicide, inhibits the plant enzyme glutamine synthetase, resulting in the accumulation of lethal levels of ammonia in susceptible plants within hours of application. Ammonia is produced by plants as a result of normal metabolic processes.

The *ubi* promoter and *ubi* mature mRNA and first intron enhancer from corn is used to drive the production of *bar* gene, but the promoter sequence itself does not encode a protein.

Appendix IV. Threatened and Endangered Species Analysis

The proposed field tests are controlled releases of the regulated article into the environment in Jackson County, Florida. There are no listed critical habitats for any threatened and endangered animal species in Jackson County, Florida according to the U.S. Fish and Wildlife database (http://criticalhabitat.fws.gov/) and therefore the release of the regulated article is not expected to affect any critical habitats.

For the state of Florida, there are fifty-seven animals and fifty-four plants listed on the threatened and endangered species list (http://ecos.fws.gov/) from the U.S. Fish and Wildlife database. Of the fifty-seven animals listed, only seven animals potentially reside in Jackson County, Florida:

- Gray Bat (*Myotis grisescens*)
- Bald Eagle (*Haliaeetus leucocephalus*)
- Piping Plover (*Charadrius melodus*)
- Flatwoods Salamander (*Ambystoma cingulatum*)
- Eastern Indigo Snake (*Drymarchon corais couperi*)
- Wood Stork (*Mycteria americana*)
- Red-cockaded Woodpecker (*Picoides borealis*)

The Gray Bat only resides in limestone caves, so the field test will not endanger this species as there are no limestone caves in the direct vicinity of the field test site. The Bald Eagle (*Haliaeetus leucocephalus*) lives near large bodies of open water such as lakes, marshes, seacoasts and rivers, where there are plenty of fish to eat and tall trees for nesting and roosting. Since the nearest source of water is an unnamed lake 2-3 miles away from the field test site, it is unlikely that this species would be impacted by the field test. The Piping Plover (*Charadrius melodus*) over-winters in dune areas on the shores of northwest Florida coasts, so the field trial is not expected to affect this bird species. The Flatwoods Salamander (*Ambystoma cingulatum*) is thought to be extinct in Jackson County and no sightings have been reported in over 10 years (http://myfwc.com/imperiledspecies/). The Eastern Indigo Snake (*Drymarchon corais couperi*) is widespread throughout the state, but nowhere are they abundant. They occur in hardwood forests, moist hammocks, pine flatwoods, prairies, and around cypress ponds. The nearest source of water is an unnamed lake 2-3 miles away from the field test site where the eastern indigo snake would likely reside, so the field test is not expected to impact this endangered species. The Wood Stork (*Mycteria americana*) is found nesting in wetlands such as cypress, hardwood, and mangrove swamps. No wetland areas are found around the field test site so no expected impact for this species is expected. The Red-cockaded Woodpecker (*Picoides borealis*) makes its home in mature pine forests. Since the land involved in the field trial and adjacent land has been used for agricultural purposes for over 100 years, this species would not have established itself in the area; therefore no impact is expected to occur with this species.

Of the fifty-four plants listed, only four potentially reside in Jackson County, Florida (http://www.virtualherbarium.org/ and http://www.fws.gov/):

- Fringed Campion (*Silene polypetala*)
- Gentian Pinkroot (*Spigelia gentianoides*)
- Florida Torreya (*Torreya taxifolia*)
- Crystal Lake Nailwort (*Paronychia chartacea minima*)

The Fringed Campion (*Silene polypetala*) is only found the Apalachicola/Chattahoochee River in the Florida Panhandle over 25 miles away from the field test site, so the field trial is not expected to impact this endangered species. The Gentian Pinkroot plant (*Spigelia gentianoides*) only exists in one small population on State-managed land 20-23 miles away at the Three Rivers State Recreation Area, Lake Seminole, Jackson County, so the field trial is not expected to impact this endangered species (http://www.centerforplantconservation.org/). The Florida Torreya (*Torreya taxifolia*) is also a rare endemic found mainly along the Apalachicola/Chattahoochee River (http://www.efloras.org/) and therefore not expected to be impacted by the proposed field trial. According to The Center for Plant Conservation (http://www.centerforplantconservation.org/), the Crystal Lake Nailwort (*Paronychia chartacea minima*) is found in the white sand margins of karst ponds in Bay and Washington Counties of Florida. No impact on this species is expected in this field test site in Jackson County, Florida because the plants will be maintained in an enclosed structure surrounded by mosquito netting and no animals will have access to the plants.

Appendix V. Standard Permit Conditions for APHIS Form 2000 (7 CFR 340.4)

(f) *Permit conditions*. A person who is issued a permit and his/her employees or agents shall comply with the following conditions, and any supplemental conditions which shall be listed on the permit, as deemed by the Administrator to be necessary to prevent the dissemination and establishment of plant pests:

(1) The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

(2) All packing material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner so as to prevent the dissemination and establishment of plant pests.

(3) The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit;

(4) The regulated article shall be maintained only in areas and premises specified in the permit;

(5) An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article;

(6) The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article;

(7) The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article;

(8) The regulated article shall be subject to the application of remedial measures (including disposal) determined by the Administrator to be necessary to prevent the spread of plant pests;

(9) A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, non-target organisms, or the environment;

(10) APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

(i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

(ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms);

(11) A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only at a port of entry which is designated by an asterisk in 7 CFR 319.37-14(b);

(ii) Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and

(iii) Mark and identify the regulated article in accordance with 340.5 of this part.

Appendix VI. Supplemental Permit Conditions for APHIS Form 2000 (7 CFR 340.4)

Field Observation and Monitoring

The applicant has thoroughly described field site monitoring and management practices that should provide the necessary degree of biological and physical confinement. Confinement practices under the permit include the following:

- The applicant has provided APHIS and State regulatory officials a map of the proposed test site. One month after planting the applicant will submit a detailed map of the planted test site. Borders of the site will be described with GPS coordinates.

- To prevent the spread of seeds from the transgenic plants by insects or animals, wooden cage structures have been built covering the entire test plot and covered completely by mosquito netting.

- A zone of 360 feet will be maintained and mowed surrounding the field test site to discourage potential out-crossing with wildtype relatives. A detailed map of the locations of feral bahiagrass within the 360 ft zone has been given. This zone will be mowed weekly during the transgenic bahiagrass flowering period. Should weather or environmental conditions prevent mowing, and flowering of feral bahiagrass occurs during that time, all flowering stalks on the 48 transgenic bahiagrass will be immediately removed to prevent any potential pollen outflow. Should this event occur, it must be reported immediately to the Compliance Branch of USDA/APHIS.

- During the field test, the plot will be monitored at least twice weekly during the growing season to monitor flowering, insect or disease damage, and weeds.

- When the flower stalks begin to grow on the transgenic bahiagrass, the USDA/APHIS Compliance Branch will be notified promptly so they can schedule an inspection of the premises during flowering.

- All plants remaining at the termination of the experiment will be sprayed with the herbicide glyphosate. Bahiagrass seeds are slow to germinate (28 days) unless subjected to scarification. Under optimal storage conditions (8% moisture and 50% relative humidity), seeds can be stored for 1 yr with little loss in germination (West, 1993). Given this potential for seed dormancy, the field site will be monitored quarterly for three years following the termination of the field test and any volunteer bahiagrass plants will be destroyed by application of glyphosate, removed and autoclaved.

- The University of Florida will use equipment dedicated to this field test as outlined in their SOPs. This equipment will not be used for any other purposes during the course of the field test, and after the field test is completed, all equipment will be thoroughly cleaned at the field site and inspected to ensure that all genetically-engineered seed and other plant material has been removed and destroyed. Any seeds discovered will be removed and autoclaved.