Finding of No Significant Impact and Decision Notice

Animal and Plant Health Inspection Service

Issuance of Permit to Release Genetically-Engineered *Burkholderia glumae*

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has received a permit application (APHIS number 06-111-01r) from Dr. Martin Rush at the Louisiana State University to conduct a field trial using strains of the bacterium *Burkholderia glumae*. Permit application 06-111-01r describes four *Burkholderia glumae* strains: Two wild-type strains endemic to the United States, one of which causes panicle blight on rice and the other naturally non-pathogenic, and two genetically engineered, non-pathogenic strains that share the same avirulent phenotype. A description of the field tests may be found in the attached Environmental Assessment (EA), which was prepared pursuant to APHIS regulations (7 CFR part 372) promulgated under the National Environmental Policy Act. The field tests are scheduled to begin in August 2007 in East Baton Rouge Parish, Louisiana.

An EA was prepared and submitted for public comment for 30 days. One comment that raised 5 issues was received and the issues are addressed in an attachment to this document.

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)
- the granting of the permit with Supplemental Permit Conditions containing duplicative safety measures and reporting requirements (Alternative C)

APHIS chose Alternative C as its Preferred Alternative.

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 *Burkholderia glumae* strains (transgenic strains BGM15 and BGM16, and wild-type strains 336gr-1 and AV) proposed for this release were either derived from or were isolated from blighted rice panicles collected in Louisiana. Thus, the strains to be released are native and prevalent in Louisiana and do not constitute bacteria novel to the proposed area of release.
2. It is highly unlikely that the small additional input of *B. glumae* for this field trial in an area of high *B. glumae* density will provide significantly greater inoculum to the environment, and significantly increase the risk of *B. glumae* infection in susceptible individuals.
3. Transgenic *B. glumae* is not likely to persist in the environment. During the inoculation of the test plants, the applicant proposes an infection strategy that significantly reduces the potential of *B. glumae* leaving the field trial and

persisting in the environment. The infection design utilizes border rows of a tall rice variety and allows inoculation only during non-windy conditions to minimize drift. In the unlikely event that transgenic *B. glumae* strains remain in the environment after the field trial, the transgenic, avirulent *B. glumae* strains are not likely to have a selective advantage over virulent, native strains and, therefore, are unlikely to persist. The applicant will also monitor for and remove volunteer rice plants after the release to ensure that transgenic *B. glumae* does not persist in the local area after the field test is terminated.

4. The transgenic *B. glumae* strains are less virulent than the wild-type, native strain and are unlikely to cause an increase in disease susceptibility to rice plants in the field.
5. There have been no reports of increased insect susceptibility in rice plants during times of increased panicle blight disease pressure.
6. Horizontal gene transfer and expression of DNA from the transgenic *B. glumae* strains to other bacteria species are unlikely to cause an increase in antibiotic resistance as antibiotic resistance genes are derived from and/or widely prevalent in enteric and soil-borne bacteria. *B. glumae* is a rice pathogen and may also inhabit soil. *B. glumae* may naturally transfer the antibiotic resistance genes to other soil bacteria. The antibiotic resistance genes were originally derived from soil-borne bacteria; thus are likely to already be present in Louisiana soils. Therefore, the presence of these genes in *B. glumae* is unlikely to cause an increase in the prevalence of antibiotic resistance genes in the environment because the soil-dwelling microorganisms likely already carry these antibiotic resistance genes.
7. Horizontal gene transfer from microorganisms to animals and plants is highly unlikely under the conditions of this field test.
8. The majority of DNA consumed is degraded in the gastro-intestinal tract although the degradation is not 100 percent efficient. There is evidence that DNA from consumed food can move from the GI tract lumen to other areas of the body and that this is a normal occurrence. No risks have been identified as a result of this movement.
9. Infected rice seeds collected at the termination of the experiment are unlikely to be mixed with any seeds intended for human or animal consumption because they will be contained during movement between field and laboratory, and devitalized after analysis by burning or autoclaving.
10. APHIS has reached a determination that the proposed environmental release under permit 06-111-01r would have no effect on federally listed threatened or endangered species (TES), or species proposed for listing, and no effect on designated critical habitat or habitat proposed for designation in the action area.
11. As the conditions imposed by the applicant and APHIS are expected to confine transgenic *B. glumae* and infected rice plants to the field site, APHIS concludes there would be no significant effect on any native floral species. Additionally, rice is the only known host of *B. glumae* in the United States.
12. Vertebrate animals are not affected by the plant pathogen *B. glumae*. Thus, any accidental consumption of rice leaves or seeds infected with transgenic *B. glumae* during the field trial will not result in animal disease. Additionally, as the bacteria

strains proposed for the field release were collected from Louisiana, typical vertebrate residents have been previously exposed to *B. glumae*. Thus, APHIS concludes there would be no significant effect on any terrestrial vertebrate animal species.

13. The most likely invertebrate animals exposed to the transgenic *B. glumae* would be seed-eating or plant-eating invertebrates. However, *B. glumae* is not an invertebrate pathogen. Additionally, as the bacteria strains proposed for the field release were collected from Louisiana, typical invertebrate residents have been previously exposed to *B. glumae*. Thus, APHIS concludes there would be no significant effect on any invertebrate species.
14. This small field test will not increase the incidence of plant disease and will not have any significant impact on existing agricultural practices.
15. APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create cumulative impacts or in any way reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.) associated with the release site or the ecosystem in which it is situated.

Because APHIS has reached a finding of no significant impact of this field release of transgenic *Burkholderia glumae,* no Environmental Impact Statement will be prepared regarding this decision.

Pursuant to its regulations (7 CFR part 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 *B.glumae* is highly likely to be confined to the field site because
    a. Bacterial liquid inoculum will be doubly contained in water tight containers for transport to and from the field site to ensure that wild-type and transgenic strains of *B. glumae* will remain contained during transport.
    b. After inoculation in the field, Tyvek suits and sprayers used during inoculation will be doubly contained in plastic bags before leaving the field site to ensure that wild-type and transgenic strains of *B. glumae,* that may be present on applicator clothing and in sprayers, remain contained after spraying and during transport to and from the field site.
    c. The field site containing inoculated rice plants and non-inoculated border plants will be located in an isolated levied area and irrigated separately to prevent accidental contamination and release of inoculated seed at harvest.
    d. The field site receiving the transgenic *B. glumae* is separated from other research areas by a 25 ft barren zone.
    e. Plants will be inoculated with the bacteria by spraying under conditions that do not favor drift. Spraying will be at 4 inches above the plants, to further minimize accidental drift.

f. A tall rice variety, Wells, will be planted around each experimental treatment plot at the field site to further minimize drift from the bacterial inoculations.
g. Combines used to harvest seed will be brushed down at the field site before transport to steam-cleaning site to limit the accidental release of seeds potentially infected with transgenic *B. glumae* during transport of the combine from the field site to the steam-cleaning site at the LSU AgCenter Central Research Station.
h. After harvest, the field site will be plowed to bury any unharvested seeds.
i. Infected rice seeds collected at the termination of the experiment are unlikely to be sources of inoculum for future rice infections because they will be contained during movement between field and laboratory, and devitalized after analysis by burning or autoclaving.
j. Harvested seed will be contained via the movement conditions specified in 7 CFR § 340.8 during transport to the weighing facility at the LSU AgCenter Central Research Station to limit the accidental release of seeds potentially infected with transgenic *B. glumae*.
k. All plants within the field site will be treated as regulated articles to ensure the appropriate disposition of rice infected with transgenic *B. glumae*.
l. APHIS inspections will ensure compliance with the Standard and Supplemental Conditions.

2. Transgenic *B. glumae* strains are non-pathogenic and do not cause plant disease. The DNA inserted into *B. glumae* will result in the suppression of toxoflavin production, resulting in non-pathogenic strains of transgenic *B. glumae*. The transgenic *B. glumae* strains proposed for release are not expected to cause plant disease, will not result in an increased disease susceptibility in rice plants, and will not result in strains that are more virulent than the endemic *B. glumae* strains already present in the environment.
3. Transgenic *B. glumae* will not persist in the environment. During the inoculation of the test plants, the applicant proposes an infection strategy that significantly reduces the potential of *B. glumae* persistence. The infection design utilizes border rows of a tall rice variety and allows inoculation only during non-windy conditions to minimize drift. In the unlikely event that transgenic *B. glumae* strains remain in the environment after the field trial, the transgenic, avirulent *B. glumae* strains are not likely to have a selective advantage over virulent, native strains and, therefore, are unlikely to persist. The applicant will also monitor for and remove volunteer rice plants after the release to ensure that transgenic *B. glumae* does not persist in the local area after the field test is terminated.
4. Transgenic *B. glumae* will not increase insect susceptibility in rice plants. There have been no reports of increased insect susceptibility in rice plants during times of increased panicle blight disease pressure.
5. The *nptII* and *bla* genes used as selectable markers are devoid of inherent plant pest characteristics.

For the reasons enumerated above, which are consistent with regulations implementing the Plant Protection Act, the field trial of non-pathogenic, transgenic *Burkholderia glumae* strains BGM15 and BGM16 is hereby authorized.

Sidney W. Abel for Rebecca Bech

Rebecca Bech
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U. S. Department of Agriculture
Date: 8/6/2007

Attachment
Finding of no significant impact
Response to comments
APHIS No. 06-111-01r

In response to a notice published in the Federal Register (Docket No. APHIS-2007-0021, 72 FR 33735-33736) on June 19, 2007, APHIS received 1 comment, that raised 5 issues, regarding the Environmental Assessment prepared in response to permit application 06-111-01r, during the 30-day comment period. The submitted comment was against issuing the permit for a field release. APHIS' responses to the issues raised in the submitted comment are as follows:

Issue 1: The genes used as selectable markers may have greater consequences for horizontal gene transfer than the conclusion made by APHIS in the environmental assessment for 06-111-01r. Additionally, APHIS did not address the potential of horizontal transfer of antibiotic resistance to known *Burkholderia* pathogens.

Response: The issue of potential significant environmental consequence(s) of the selectable marker genes in the transgenic *Burkholderia glumae* posed by the submitters were not specifically described within the comment, but a reference was cited (Ho et al. 2007). APHIS recognizes horizontal gene transfer may occur between *B. glumae* and other microorganisms and analyzed the potential risks (page 10 of EA). As antibiotic resistance genes are derived from and/or widely prevalent in enteric and soil-borne bacteria, the use of these genes in this transgenic bacteria species is highly unlikely to cause greater levels of antibiotic resistance (page 10 of EA). These resistance genes are currently widespread in the environment and the release of transgenic *B. glumae* will not increase the risk of antibiotic resistance being passed to other bacteria species (page 10 of EA), including *Burkholderia* species. The reference provided in the comment (Ho et al. 2007) did not address the prevalence of antibiotic resistance in microbial communities or provide scientific evidence contrary to this conclusion.

Issue 2: This is a questionable field trial as the pathogen proposed for release could be spread from the experimental plots to other rice crops.

Response: The comment fails to recognize, as is repeatedly stated in the EA (pages 1, 8, 9, 10, 11, and 12 of the EA) that the wild type strains and the strains from which the transgenic strains were derived are native to Louisiana and were collected from rice planted in Louisiana. The wild-type, pathogenic bacterial strains are already present in Louisiana, and thus the release of the wild-type strain does not constitute a release of novel bacteria.

Issue 3: An alternative research approach may be more effective than those posed by the applicant.

Response: APHIS BRS does not determine *a priori* whether an alternative research approach is more effective than the approach proposed. APHIS will leave it to the applicant to determine the viability of the alternative research direction proposed in the

comment. APHIS considers the potential for the proposed study to impact the human environment. After careful consideration, APHIS concluded that the proposed study will not significantly impact the human environment.

Issue 4: There is concern regarding the potential of negative human effects due to the release of *B. glumae*.

Response: By authorizing the release of transgenic *B. glumae*, APHIS would be authorizing the release of a species of bacteria that is already present and endemic in the state of Louisiana. As stated in the EA, some members of the genus *Burkholderia*, particularly members of the *B. cepacia* complex, are recognized as opportunistic human pathogens in immuno-compromised individuals, (page 8 and Appendix 1 of EA). Also as stated in the EA, *B. glumae* has been shown not to be directly related to *B. cepecia* nor is it considered to be included within the *B. cepacia* complex of known human pathogens (Appendix 1 of EA). APHIS (page 8 of EA) and the commenter both cite the single known human infection by *B. glumae*. As stated on page 8 of the EA, APHIS concluded that the field trial will not significantly increase the risk of *B. glumae* infection in susceptible individuals because the bacterial strains are endemic and prevalent in the release area and the size of the field test is small. In other words, the amount of bacteria being released in this experiment is negligible compared to the amount of *B. glumae* currently resident in the area.

Issue 5: APHIS did not recommend precautions for those working with the pathogen, and may 'take the pathogen to their homes, families and neighbours.'

Response: The comment fails to acknowledge the precautionary measures required in the permit conditions. As stated on page 6 of the EA, personnel applying bacteria to rice plants are required to wear Tyvex suits and masks during inoculation. To minimize the movement of *B. glumae* outside the field site, APHIS recommends a supplemental condition to prevent *B. glumae* transport away from the field due to accidental clothing contamination (page 14 and supplemental condition 2). After inoculation, clothing must be removed within the field test area and double bagged for transport out of the field and destruction in an autoclave (page 14 of the EA and supplemental condition 2). Although the comment acknowledges that epidemics of panicle blight occurred in southern rice states in 1995 and 1998, the commenter fails to recognize that an epidemic results in the occurrence of enormous quantities of *B. glumae*. That this bacteria species is so widely prevalent in the area means that anyone working in rice fields has had extensive exposure to the bacteria. Based on the precautions mandated in the permit and the supplemental conditions, no incremental exposure from the execution of the permit is expected.

USDA APHIS Environmental Assessment

In response to a permit application (06-111-01r) received from Dr. Milton Rush of Louisiana State University for a field test of two non-pathogenic, genetically engineered mutant strains of *Burkholderia glumae*

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

TABLE OF CONTENTS

I. INTRODUCTION

A. Summary

Burkholderia glumae Kurita et Tabei is a bacterial plant pathogen that causes bacterial panicle blight in rice (*Oryza sativa*), and is transmitted by infected seed (Sayler et al. 2006). This bacterium was first described in Japan as the cause of grain rotting and seedling blight (Goto and Ohata 1956, Uematsu et al. 1976) and is considered one of the most important rice pathogens in Japan (Azegami et al. 1987). Epidemics of panicle blight occurred in the southern rice producing area of the United States during the 1995 and 1998 growing seasons, with yield losses in some fields estimated to be as high as 40 percent (Rush, M.C., *pers. communication*).

Currently, there is no control method for panicle blight in the United States (Sayler et al. 2006), and most commercially grown rice varieties in the United States are susceptible to the disease (Shahjahan et al. 2000, Sayler et al. 2006). By field testing non-pathogenic, transgenic strains of *B. glumae*, the proposed field release will provide information on bacterial panicle blight infection of rice, and potential routes of pathogen control. The transgenic *B. glumae* strains have been modified to disrupt the disease-causing gene (gene that produces toxoflavin), producing avirulent, non-pathogenic strains. The transgenic *B. glumae* strains also express the genes for kanamycin and ampicillin resistance as selectable markers. The proposed field release will also involve the challenge of rice plants with a wild-type, pathogenic *B. glumae* strain and a naturally-occurring, non-pathogenic *B. glumae* strain, both of which were isolated from and are widely prevalent in Louisiana, the site of the proposed release.

B. Regulatory Authority

The authorities for regulation of genetically engineered (GE) *Burkholderia glumae* are the Plant Protection Act of 2000, 7 U.S.C. 7701-7772, and the United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (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 GE 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. The disease-causing wild-type *B. glumae* is considered the causal pathological agent of panicle blight in rice.

This environmental assessment (EA) was conducted under the authority of the National Environmental Policy Act (NEPA), 42 U.S.C. 4321 and 7 CFR § 372, NEPA Implementing Procedures. Except for actions that are categorically excluded, approvals and issuance of permits for proposals involving GE or non-indigenous species normally require EAs, but not necessarily environmental impact statements (7 CFR § 372.5(b)(4)). The actions described in the application for permit 06-111-01r involve the release of a transgenic, endemic plant pathogen, *B. glumae*. APHIS analysis 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 species being considered has not been previously considered for a release permit, and in its untransformed state is a plant pest of rice. Thus, following 7 CFR 372.5(d)(4) APHIS is preparing an EA to address the confined release of this new species that raises new issues.

II. NEED FOR THE PROPOSED ACTION

The purpose of this EA is to assess any potential adverse environmental effects of a field study involving transgenic *Burkholderia glumae* in East Baton Rouge Parish, Louisiana. The permit application was received by APHIS BRS on April 21, 2006, submitted by Dr. Milton C. Rush, Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, East Baton Rouge Parish, Louisiana. The application number is 06-111-01r. Under APHIS regulations, the receipt of a permit application to introduce a GE 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 part 340. For the purposes of this EA, the No Action alternative would be the denial of permit application 06-111-01r, which would prevent any environmental release of the two transgenic *B. glumae* strains.

B. Issue the Permit as Received

Issuing this permit as received would allow the field release to proceed at the Louisiana State University AgCenter Central Research Station in East Baton Rouge Parish, LA, under the conditions provided by the applicant (see below, conditions a-p) and the standard permit conditions under 7 CFR §340.4. Under this alternative, APHIS BRS would authorize the field release of the GE *B. glumae* at the specified location with no supplemental conditions implemented.

The following redundant mitigation measures are incorporated into the experimental procedures by the applicant to promote a confined field release, contain seed infected with transgenic *B. glumae*, and ensure the least amount of harm to the environment:

a. The field site is on land owned by Louisiana State University (LSU) and is expected to provide adequate physical security. Non-university visitors are monitored.
b. The 0.4 acre field site containing inoculated rice plants and non-inoculated border plants will be located in an isolated levied area and irrigated separately to prevent accidental contamination and release of inoculated seed at harvest.
c. The field site receiving the transgenic *B. glumae* is separated from other research areas by a 25 ft barren zone.
d. Plants will be inoculated with the bacteria by spraying under conditions that do not favor drift. Spraying will be at 4 inches above the plants, to further minimize accidental drift.

e. A tall rice variety, Wells, will be planted around each experimental treatment plot at the field site to further minimize drift from the bacterial inoculations (see Table 1).
f. Plants outside the field site will be monitored for disease during and after the field trial, and if symptoms are observed, samples will be brought back to the lab for testing.
g. Seed heads will be monitored daily for maturity during the seed ripening period.
h. Rice seeds will be mechanically harvested by small-plot combine. After harvest, the combine will be moved by trailer from the field site to the steam cleaning station at the LSU AgCenter Central Research Station.
i. The field site will be left fallow for next cropping season and will continue to be monitored daily until the next growing season begins (approximately March 15th).
j. Harvested seeds will be transferred to the laboratory following container requirements in 7 CFR § 340.8.
k. After harvest, the field site will be plowed to bury any unharvested seeds.
l. Volunteer seedlings that emerge after the field trial will be sprayed with herbicide before flowering.
m. Seed harvested for yield analysis will be burned at the LSU AgCenter Central Research Station burning site.
n. Seed harvested for laboratory analysis will be destroyed by autoclaving.
o. Containers of bacterial cultures will be autoclaved after use in the field. Sprayers will be disinfected with ammonia solution and washed several times for decontamination. Tyvek suits and masks used for bacterial inoculations will also be autoclaved and discarded.
p. Volunteer seedlings that emerge after the field trial will be sprayed with herbicide.
q. No commercial rice is found in East Baton Rouge Parish.

C. Issue the Permit with Supplemental Conditions

Issuing this permit with supplemental conditions would allow the release to proceed at the Louisiana State University AgCenter Central Research Station in East Baton Rouge Parish, LA where supplemental permit conditions would be established based on APHIS' scientific analysis of the permit application, input from the State of Louisiana, and public comment from this EA. 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 release area.

Under APHIS regulations, compliance with all mitigating measures is required:

> *Permit conditions.* A person who is issued a permit and his/her employees or agents shall comply with the following conditions (Standard Permit Conditions, Appendix VI), and any supplemental conditions (Supplemental Permit Conditions, Appendix VII) which shall be listed on the permit, as deemed by the Deputy Administrator to be necessary to prevent the dissemination and establishment of plant pests 7 CFR 340.4(f)

Currently APHIS proposes to include duplicative safety measures to promote a confined field release, contain seed infected with transgenic *B. glumae*, and to ensure no significant harm to the environment. The proposed supplemental conditions are listed at Section V., C. Issuance of the Permit with Additional Conditions, below.

APHIS also proposes to include reporting requirements for the field trial:

a. Activity reports will be submitted 28 days after the release of transgenic *B. glumae* and the termination of the field test. The activity report will include approximate acreage of rice plants inoculated, total acreage of the field test and the actual release and termination date.
b. Disposition of harvested material is to be included in the 6 month Field Test Report.
c. Monitoring reports will be submitted within 3 months after the end of the monitoring period. The monitoring report will include dates of volunteer monitoring, number of volunteers observed, and any action taken to remove, destroy, or test volunteers for *B. glumae* infection.

IV. Research Design for *Burkholderia glumae* Release

A. Purpose of the Research

The field experiment proposed by the applicant will provide information on the pathogenicity of *B. glumae* and work toward developing control methods to reduce yield loss caused by panicle blight. The experiment will test the role of toxoflavin, a potential virulence factor for *B. glumae* (Iiyama et al. 1995). The phytotoxicity of toxoflavin has been demonstrated under greenhouse conditions, but has not yet been studied under field conditions. The transgenic strains of *B. glumae* are engineered to suppress toxoflavin production, causing the transgenic strains to become avirulent (non-pathogenic), and not cause plant disease. By comparing rice infected by transgenic, non-disease causing strains of *B. glumae* to infection by both a native, wild-type strain and an endemic avirulent strain of *B. glumae*, the data gathered during the proposed field trial will help determine if toxoflavin production is required for panicle blight disease.

B. Description of the Research

The experiment will be conducted on a single field site located at the LSU AgCenter Central Research Station in East Baton Rouge Parish, Louisiana. This experiment will examine the pathogenicity associated with the wild-type bacterial pathogen, as compared to two transgenic, avirulent strains and one naturally-occurring, avirulent strain of *B. glumae*. Rice plants will be planted and subjected to one of five experimental treatments: spraying with native, non-transgenic *B. glumae* (strain 336gr-1); spraying with native, non-transgenic, non-pathogenic *B. glumae* (strain AV); spraying with transgenic, non-pathogenic *B. glumae* strain BGM15, spraying with transgenic, non-pathogenic *B. glumae* strain BGM16, and an unsprayed control plot. Each treatment will be tested on two commercial rice varieties, Trenasse and Cocodrie, for a total of 10 treatments (5 inoculation treatments x 2 cultivars). The experimental design will consist of a randomized complete block design with 4 block replications, for a total of 40 plots (10 treatments x 4 replicates of each treatment). Although the total acreage of rice planted for this field trial is 0.4 acres, the total acreage of rice that will receive inoculation of transgenic *B. glumae* strains is 0.02 acres.

The standard treatment plot size for this experiment is 4 x 12 ft, consisting of 7 rows, each with 18 cm spacing. Surrounding each treatment plot will be a 4 x 12 ft border plot of the rice variety, Wells, which is a tall variety planted to minimize drift during the experimental inoculations (see

Table 1). The disease intensity will be recorded at 15 and 30 days after spraying (at panicle emergence). At maturity, grains from the center 4 rows of each plot will be harvested by small-plot combine and grain weight at 12 percent moisture will be recorded.

V. ENVIRONMENTAL CONSEQUENCES OF THE PROPOSED ACTION AND ALTERNATIVES

A. Analysis of Issues, Consequences, and Theoretical Risks of Field Research using Transgenic Burkholderia glumae

1. Risk of Transgenic, Non-Pathogenic *Burkholderia glumae* to the Environment

The *Burkholderia glumae* strains (transgenic strains BGM15 and BGM16, and wild-type strains 336gr-1 and AV) proposed for this release were either derived from or were isolated from blighted rice panicles collected in Louisiana. Thus, the strains to be released are native to Louisiana and do not constitute bacteria novel to the proposed area of release.

Burkholderia glumae is a plant pathogen and causal agent of panicle blight in rice. Most members of the genus *Burkholderia* are soil inhabiting bacteria. Only a few *Burkholderia* species (e.g. *Burkholderia cepacia*) are considered opportunistic human pathogens in immuno-compromised individuals. Recently, a report cited *B. glumae* as the likely disease-causing agent in a single immuno-compromised individual (Weinberg et al. 2006). However, *B. glumae* is a naturally-occurring, ubiquitous organism endemic to and widely prevalent in Louisiana that, prior to the Weinberg et al. (2006) study, had not been implicated as a potential causal agent of adverse effects in humans. It is highly unlikely that releasing both transgenic and non-transgenic *B. glumae* over 0.04 acres during this field trial will significantly increase inoculum levels as these bacterial strains are already present in the environment, Further, the field site proposed for this release is under control of LSU and is not open to the general public. Therefore, it is highly unlikely that this field trial will significantly increase the risk of *B. glumae* infection in susceptible individuals.

The addition of 216 base pairs of the methyltransferase gene inserted into the transgenic strains of *B. glumae* results in the disruption of the gene sequences that produce disease-causing toxoflavin (see Appendix 1: Biology of *Burkholderia glumae* and Appendix 2: Description of the Regulated Article for more information) as shown in greenhouse studies (see 3. Alteration in Susceptibility to Disease and Insects, below). As the addition of this DNA to *B. glumae* will result in the suppression of toxoflavin production, the transgenic *B. glumae* strains proposed for release are not expected to cause plant disease. Consequently, the engineered strains are expected to have no adverse effect on the environment as these strains are non-pathogenic. In the unlikely event that individual transgenic *B. glumae* bacteria revert and begin producing toxoflavin, the resulting *B. glumae* will be no more infectious than the wild-type, native *B. glumae* endemic to Louisiana.

Because the strains to be released are native to and widely prevalent in Louisiana, the inoculum load will not be significantly increased compared to the environmental baseline, and the transgenic modification to the *B. glumae* strains will not result in strains that are more virulent than the endemic *B. glumae* strains already present in the environment, APHIS concludes that the

introduction of the transgenic, non-pathogenic *Burkholderia glumae* strains will not significantly affect the environment.

2. Persistence of Transgenic *Burkholderia glumae*

Burkholderia glumae is transmitted by infected seed (Sayler et al. 2006). Infected rice seeds collected at the termination of the experiment are unlikely to be sources of inoculum for future rice infections because of the numerous confinement measures described in above text and APHIS inspections. During the inoculation of the test plants, the applicant proposes an infection strategy (see III. C. 2. Description of Research, above, and Table 1) that minimizes drift and significantly reduces the potential of *B. glumae* persistence outside the release site. The applicant will also monitor plants that border the release site. If any symptoms are observed, a sample will be brought to the lab and tested for the presence of *B. glumae*.

In Korea, *B. glumae* infection was recently attributed to bacterial wilt symptoms often seen in Korean plants [peppers, tomato, potato, eggplant, sesame, perilla (*Perilla frutescens*) and sunflower] and usually ascribed to *Ralstonia* infection (Jeong et al. 2003). In the United States , *B. glumae* has no known hosts other than rice (Rush, M.C., *pers. communication*). Because East Baton Rouge Parish is not a commercial rice producing Parish, and *B. glumae* is not known to survive in hosts other than rice in the United States, there will be no host available for *B. glumae* infection after the field test. The applicant will also monitor for and remove volunteer rice plants after the release to ensure that transgenic *B. glumae* does not persist in the local area after the field test is terminated.

Thus, APHIS concludes that it is highly unlikely for transgenic strains of *B. glumae* to persist in the environment. Additionally, in the unlikely event that transgenic *B. glumae* strains remain in the environment after the field trial, the transgenic, avirulent *B. glumae* strains are not likely to have a selective advantage over virulent, native strains and, therefore, are unlikely to persist.

3. Alteration in Susceptibility to Disease or Insects

The transgenic strains of *B. glumae* were assessed under greenhouse conditions by artificial inoculation to test their ability to cause panicle blight. The transgenic strains did not show any sheath lesions or panicle blight symptoms, whereas inoculation with the wild-type strain produced typical panicle blight symptoms (Rush, M.C., *pers. communication*). Thus, the transgenic *B. glumae* strains are less virulent than the wild-type, native strain and are unlikely to cause an increase in disease susceptibility to rice plants in the field. There have been no reports of increased insect susceptibility in rice plants during times of increased panicle blight disease pressure.

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

4. Horizontal Gene Transfer to Other Organisms

Besides the gene segment that renders the transgenic *Burkholderia glumae* strains avirulent, these non-pathogenic strains also contain two antibiotic resistance genes as selectable markers. Expression of neomycin phosphotransferase *(nptII)* confers tolerance to the antibiotic kanamycin,

and expression of beta-lactamase (*bla)* confers tolerance to the antibiotic ampicillin. The *nptII* gene is devoid of inherent plant pest characteristics (Fuchs et al. 1993), as is the *bla* gene. The Environmental Protection Agency has granted an exemption from the requirement of a tolerance for NPTII and the nucleic acids necessary for its production in plants (FR 59 49353).

Horizontal gene transfer and expression of DNA from the transgenic *B. glumae* strains to other bacteria species are unlikely to cause an increase in antibiotic resistance as antibiotic resistance genes are derived from and/or widely prevalent in enteric and soil-borne bacteria (Van Dijck and van de Voorde 1976, DeBoy et al. 1980, Jensen et al. 2001, Sengelov et al. 2003). Under natural conditions, these antibiotic resistance genes can be transferred to related microorganisms (Wilson and Salyers 2003). Gene transfer from transgenic *B. glumae*, should not be any different than what occurs naturally. While horizontal gene transfer is widespread between microorganisms, such transfer from microorganisms to animals and plants is highly unlikely under the conditions of this field test (Syvanen 1999).

Therefore, in the unlikely event that the genes for kanamycin resistance and ampicillin resistance would migrate to other bacteria outside the field trial, APHIS concludes there would be no significant impact to the environment.

5. Fate of Transgenic DNA

As rice plants infected with transgenic *Burkholderia glumae* will not be used for food or feed, the information presented in this section is for the unlikely event of accidental consumption.

Transgenic DNA is no different from other DNA consumed as part of the normal diet. GE organisms have been used in drug production and microbial fermentation (cheese and yogurt) since the late 1970's. More than 1.4 billion cumulative acres of engineered food and feed crops have been grown and consumed world wide in the past 7 years (International Service for the Acquisition of Agri-biotech Applications, (ISAAA) at: http://www.isaaa.org/Resources/Publications/briefs/35/executivesummary/default.html). The FDA has not reported any significant concerns with bioengineered food and feed currently on the market. Because of a lack of toxicity, EPA has exempted from the requirement of a tolerance DNA that encodes currently registered plant incorporated protectants (FR 66 37817-37830).

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

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

The *B. glumae* strains used in this experiment are endemic to and widely prevalent in Louisiana. Because the field site is on property owned by Louisiana State University and is a confined field test, the general public will not be exposed to the transgenic bacteria. Due to the small size of the

field trial (0.4 acres, of which only 0.02 acres will be inoculated with transgenic *B. glumae*, see Table 1), and the fact that *B. glumae* is endemic to and prevalent in Louisiana, the amount of *B. glumae* proposed to be released will not result in a significant increase in inoculum load. Infected rice seeds collected at the termination of the experiment are unlikely to be mixed with any seeds intended for human or animal consumption because of the numerous measures described in above text and APHIS inspections during harvesting. The applicant will store all the harvested seeds in dedicated storage bags on site and will transfer seeds to a laboratory setting for seed cleaning and analysis.

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

7. Risks to Threatened and Endangered Species and Designated Critical Habitat

APHIS has reached a determination that the proposed environmental release under permit 06-111-01r would have no effect on federally listed threatened or endangered species (TES), or species proposed for listing, and no effect on designated critical habitat or habitat proposed for designation in the action area. Consequently, consultation under Section 7 of the Endangered Species Act with U.S. Fish and Wildlife Service is not required for the action described in permit 06-111-01r and supplemental conditions proposed by APHIS. Appendix 3 includes the APHIS analysis of TES and critical habitat in the area of the field release.

8. Effects on Native Floral and Faunal Communities

a. Native Floral Communities

The field site proposed in the permit application and the surrounding fields have a history of use as an agricultural research experiment station, in association with Louisiana State University. As the conditions imposed by the applicant and APHIS are expected to confine transgenic *B. glumae* and infected rice plants to the field site, APHIS concludes there would be no significant effect on any native floral species. Additionally, rice is the only known host of *B. glumae* in the United States. In the highly unlikely event that *B. glumae* would move outside the field site, non-transgenic, virulent *B. glumae* is already present in the environment. The addition of non-pathogenic *B. glumae* would not significantly increase the inoculum load already present in the environment, and does not present a plant disease risk.

b. Terrestrial Vertebrate Animals

Except for a single report citing *B. glumae* as the likely disease-causing agent in an immuno-compromised individual, vertebrate animals are not generally affected by the plant pathogen *B. glumae*. Thus, any accidental consumption of rice leaves or seeds infected with transgenic *B. glumae* during the field trial is unlikely to result in animal disease. Additionally, as the bacteria strains proposed for the field release were collected from Louisiana, typical vertebrate

residents have been previously exposed to *B. glumae*. Thus, APHIS concludes there would be no significant effect on any terrestrial vertebrate animal species.

c. Terrestrial Invertebrate Animals

The most likely invertebrate animals exposed to the transgenic *B. glumae* would be seed-eating or plant-eating invertebrates. However, *B. glumae* is not an invertebrate pathogen. Additionally, as the bacteria strains proposed for the field release were collected from Louisiana, typical invertebrate residents have been previously exposed to *B. glumae*. Thus, APHIS concludes there would be no significant effect on any invertebrate species.

d. Aquatic Organisms

The field site, as commonly practiced in rice growing regions, includes a levy irrigation system. However, *B. glumae* is not a water-transmissible pathogen, nor a pathogen of aquatic organisms, and is thus highly unlikely to affect aquatic organisms that inhabit the levies within the proposed release site. APHIS therefore concludes there would be no significant effect on any aquatic species.

9. Impact on Existing Agricultural Practices

The strains of *B. glumae* used for this field trial were collected in Louisiana, where it is widely prevalent. *B. glumae* has also been collected from Texas and Arkansas. The proposal field site is on land owned by LSU, and rice is not commercially produced in East Baton Rouge Parish. The confinement measures as given by the applicant and the supplemental permit conditions proposed by APHIS should confine the transgenic *B. glumae* to the field site. Therefore, APHIS concludes that this small field test will not increase the incidence of plant disease and will not have any significant impact on existing agricultural practices.

10. Cumulative Environmental Effects

The only past, present, and reasonably foreseeable actions associated with the location for the proposed release are those related to the release of avirulent, transgenic *B. glumae*; release of native, non-transgenic *B. glumae*; and agricultural production of rice. As stated previously, APHIS has determined that there is no significant impact to the environment due to the release of transgenic *B. glumae* strains because: (1) the non-transgenic versions of these strains were originally collected from, and are native to, Louisiana; (2) the inoculum load will not be significantly increased compared to the environmental baseline; (3) the transgenic bacteria will not persist in the environment; (4) and the transgenic modification to the *B. glumae* strains will not result in strains that are more virulent than the endemic *B. glumae* strains already present. Additionally, there is no significant impact on disease or insect susceptibility for rice plants inoculated with transgenic *B. glumae*, no effect of the release of transgenic *B. glumae* on federally listed threatened or endangered species, or species proposed for listing, and no effect on designated critical habitat or habitat proposed for designation in the action area, or any animal or plant species in the proposed area of release. The proposed field site is the LSU Ag Center Central Research Station, which has been in agricultural production for the past 20 years, and will likely remain in agricultural cultivation for the foreseeable future. Therefore, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the

proposed action to create cumulative impacts or in any way reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.) associated with the release site or the ecosystem in which it is situated.

11. Consistency of proposal with other environmental requirements

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

B. Analysis of the Alternatives

1. Deny the Permit

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

2. 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:

a. No adverse consequences to non-target organisms or environmental quality are expected from the field release of the transgenic bacteria strains.
b. Neither the genes introduced, nor the proteins produced by these bacteria are expected to have toxicological or allergenic properties.
c. None of the genes introduced, nor the protein produced in these bacteria provide the engineered, non-pathogenic bacteria with any selective advantage over non-engineered bacteria in the ability to be disseminated or to become established in the environment.

3. Issuance of the Permit with Additional Conditions

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

a. No adverse consequences to non-target organisms or environmental quality are expected from the field release of these transgenic bacteria.
b. Neither the genes introduced, nor the proteins produced by these bacteria are expected to have toxicological or allergenic properties.
c. None of the genes introduced, nor the protein produced in these bacteria provide the engineered, non-pathogenic bacteria with any selective advantage over non-

engineered bacteria 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. Bacterial liquid inoculum will be doubly contained in water tight containers for transport to and from the field site to ensure that wild-type and transgenic strains of *B. glumae* will remain contained during transport.
b. After inoculation in the field, Tyvek suits and sprayers used during inoculation will be doubly contained in plastic bags before leaving the field site to ensure that wild-type and transgenic strains of *B. glumae*, that may be present on applicator clothing and in sprayers, remain contained after spraying and during transport to and from the field site.
c. Combines used to harvest seed will be brushed down at the field site before transport to steam-cleaning site to limit the accidental release of seeds potentially infected with transgenic *B. glumae* during transport of the combine from the field site to the steam-cleaning site at the LSU AgCenter Central Research Station.
d. Harvested seed will be contained via 7 CFR § 340.8 during transport to the weighing facility at the LSU AgCenter Central Research Station to limit the accidental release of seeds potentially infected with transgenic *B. glumae* during transport of harvested seeds to the weighing facility at the LSU AgCenter Central Research Station.
e. All plants within the field site will be treated as regulated articles to ensure the appropriate disposition of rice infected with transgenic *B. glumae*.

4. Preferred Alternative

APHIS has chosen Alterative C, issue the permit with supplemental conditions, as the preferred alternative, in order to implement supplemental conditions and reporting requirements.

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Table 1.

The table below is a description of the plot layout for the proposed field site described in 06-111-01r. The rice variety Wells [W] will be planted to surround each of the plots that receive an experimental treatment. No plants will be inoculated in these plots. Two rice varieties are proposed to receive experimental treatments, Tenasse (columns T-I to T-IV) and Cocodrie (columns C-I to C-IV). There are 5 proposed experimental treatments: a non-inoculated control [Cont (T-1)]; inoculation with wild-type, pathogenic *B. glumae* (strain 336gr-1) [Wild (T-2]; inoculation with transgenic, avirulent *B. glumae* strain BGM15 [M-1 (T-3)]; inoculation with transgenic, avirulent *B. glumae* strain BGM16 [M-2 (T-4)]; or inoculation with a naturally-occurring avirulent *B. glumae* strain (AV) [AV (T-5)]. The total acreage of the field test is 0.4 acres, only 0.02 acres of which will be inoculated with transgenic *B. glumae* strains.

	T-I		T-II		T-III		T-IV		C-I		C-II		C-III		C-IV	
W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
W	**Cont (T-1)**	W	**M-1 (T-3)**	W	**AV (T-5)**	W	**M-1 (T-3)**	W	**Cont (T-1)**	W	**M-1 (T-3)**	W	**AV (T-5)**	W	**M-1 (T-3)**	W
W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
W	**Wild (T-2)**	W	**AV T-5**	W	**Wild (T-2)**	W	**Cont (T-1)**	W	**Wild (T-2)**	W	**AV T-5**	W	**Wild (T-2)**	W	**Cont (T-1)**	W
W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
W	**M-1 (T-3)**	W	**M-2 (T-4)**	W	**Cont (T-1)**	W	**M-2 (T-4)**	W	**M-1 (T-3)**	W	**M-2 (T-4)**	W	**Cont (T-1)**	W	**M-2 (T-4)**	W
W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
W	**M-2 (T-4)**	W	**Wild (T-2)**	W	**M-1 (T-2)**	W	**AV (T-5)**	W	**M-2 (T-4)**	W	**Wild (T-2)**	W	**M-1 (T-2)**	W	**AV (T-5)**	W
W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
W	**AV (T-5)**	W	**Cont (T-1)**	W	**M-2 (T-4)**	W	**Wild (T-2)**	W	**AV (T-5)**	W	**Cont (T-1)**	W	**M2 (T-4)**	W	**Wild (T-2)**	W
W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W

APPENDIX 1: Biology of *Burkholderia glumae*

Burkholderia glumae are rod-shaped, motile, gram-negative, obligately aerobic bacteria commonly found in soil worldwide. Originally placed in the genus *Pseudomonas*, Urakami et al. (1994) presented morphological, biochemical, and physiological data that moved *Pseudomonas glumae* into the *Burkholderia* genus (Urakami et al. 1994). Further taxonomic analysis established the similarity between *B. glumae*, *B. gladioli*, and *B. plantarii* (Coenye et al. 2001, Maeda et al. 2006). Accordingly, an analysis also established that *B. glumae* is not within the *B. cepacia* complex of human pathogens (Coenye et al. 2001).

In the United States, *B. glumae* causes panicle blight in rice, resulting in sterile spikelets (Sayler et al. 2006) with brown streaks on the paleae and lemmata (Iiyama et al. 1995) and chlorosis of grain on infected panicles (Suzuki et al. 2004). *B. glumae* invades rice spikelets by entering the stomata of the epidermis of the glume (Hikichi et al. 1994) and grows epiphytically through the booting stage (Goto 1992, Sayler et al. 2006). Only plumules in germinating seeds (causing seedling rot in nursery produced rice plants in Asia (Cottyn et al. 1996)) and spikelets during heading (causing panicle blight) are susceptible to *B. glumae* infection (Hikichi et al. 1994). Invasion of one bacterial cell is enough to complete spike infection (Hikichi et al. 1994) and the bacteria also multiplies rapidly on emerging panicles and infects flowers just after emergence (Goto 1992, Sayler et al. 2006). Pathogen transmission is through infected seed (Sayler et al. 2006).

Burkholderia glumae produces a yellowish substance that has been identified as the phytotoxin toxoflavin (Azegami et al. 1987, Suzuki et al. 1998b). Toxoflavin production results in significant rice damage to inoculated plants (Azegami et al. 1987). When non-toxoflavin producing mutants were inoculated on rice plants, no disease symptoms developed, indicating that toxoflavin production by *B. glumae* is a virulence factor for *Burkholderia glumae* (Iiyama et al. 1995) and plays a significant role in disease development (Suzuki et al. 1998b). Toxoflavin production in *B. glumae* is limited to ambient temperatures of 30º to 40º C (Suzuki et al. 1998b), which is consistent with infection occurring predominately during high temperatures and high humidity (Suzuki et al. 2004).

Toxoflavin is produced in *Burkholderia* by an operon consisting of the *tox* gene cluster (*toxABCDE*) and the *toxR* gene (Shingu and Yoneyama 2004, Suzuki et al. 2004). Disruption of the *toxA* gene (methyltransferase) results in mutants that do not produce toxoflavin (Suzuki et al. 1998a) and do not cause chlorosis of the rice grain (Suzuki et al. 2004).

APPENDIX 2: Description of the Regulated Bacteria

Transformed *Burkholderia glumae* strains (BGM15 and BGM16, derived from wild-type strains) were developed in Dr. M. C. Rush's laboratory, Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA. The vector plasmid was purchased from Qiagen, Inc., in Valencia, CA.

The transgenic *B. glumae* strains were created by placing 216 base pairs of the methyltransferase (*toxA*) gene from *B. glumae* into the pDRIVE cloning vector (Qiagen, Inc.). The introduced vector, along with the methyltransferase gene will integrate into the bacterial chromosome by homologous recombination. Disruption of the *tox* operon results in a non-toxoflavin producing strain. The system does not require the use of the plant pathogen, *Agrobacterium tumefaciens*, or other transformation vectors. The cloning vector also contains two selectable markers, the gene (*nptII*) for neomycin phosphotransferase from *Streptomyces kanamyceticus* and the gene (*bla*) for beta-lactamase from *Escherichia coli*. The selectable markers provide resistance to kanamycin and ampicillin, respectively. The promoter for each of the genes is the Bacteriophage T7 promoter, and the terminator for each of the selectable markers is a synthetic TAA codon sequence. The donor DNA sequences are stably and irreversibly integrated into the bacterial genome, where they are maintained and inherited as any other genes of the bacteria cell.

APPENDIX 3: Threatened and Endangered Species Analysis

There is no designated critical habitat or proposed designated critical habitat in the Parish of release (East Baton Rouge Parish, Louisiana) (http://crithab.fws.gov/, accessed 03/30/2007). According to the Fish and Wildlife Service (http://ecos.fws.gov/tess_public/StateListingAndOccurrence.do?state=LA; accessed on 03/30/2007) there are 20 federally listed threatened and endangered animals occurring in Louisiana and 3 threatened and endangered plant species in the state of Louisiana. Of those listed in the state of Louisiana, the following are not found in East Baton Rouge Parish (all websites accessed on 03/30/2007):

Mammals

- Louisiana Black Bear (*Ursus americanus luteolus*) (http://www.fws.gov/endangered/i/a/saa9e.html, http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Ursus+americanus+luteolus)
- Finback Whale (*Balaenoptera physalus*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Balaenoptera+physalus)
- Humpback Whale (*Megaptera novaeangliae*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Megaptera+novaeangliae)

Birds

- Interior Least Tern (*Sterna antillarum*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Sterna+antillarum, http://ecos.fws.gov/speciesProfile/SpeciesReport.do?spcode=B07N)
- Black-capped Vireo (*Vireo atricapillus*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Vireo+atricapilla)
- Red-cockaded Woodpecker (*Picoides borealis*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Picoides+borealis)
- Piping Plover (*Charadrium melodus*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Charadrius+melodus)
- Bald Eagle (*Haliaeetus leucocephalus*)

Fish

- Gulf Sturgeon (*Acipenser oxyrinchus desotoi*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Acipenser+oxyrinchus+desotoi)
- Pallid Sturgeon (*Scaphirhynchus albus*)

Reptiles

- Green Sea Turtle (*Chelonia mydas*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Chelonia+mydas)
- Hawksbill Sea Turtle (*Eretmochelys imbricata*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Eretmochelys+imbricata)
- Kemp's Ridley Sea Turtle (*Lepidochelys kempii*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Lepidochelys+kempii)
- Leatherback Sea Turtle (*Dermochelys coriacea*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Dermochelys+coriacea)

- Loggerhead Sea Turtle (*Caretta caretta*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Caretta+caretta)
- Gopher Tortoise (*Gopherus polyphemus*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Gopherus+polyphemus)
- Ringed Map Turtle (*Graptemys oculifera*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Graptemys+oculifera)

Invertebrates

- Pink (Pearlymussel) Mucket (*Lampsilis abrupta*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Lampsilis+abrupta)
- Louisiana Pearl Shell (*Margaritifera hembeli*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Margaritifera+hembeli)

Plants – None of these plants are sexually compatible with rice and *Burkholderia glumae* is not known to infect any species within the 3 plant families to which these species belong.

- American Chaffseed (*Schwalbea americana*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Schwalbea+americana)
- Louisiana Quillwort (*Isoetes louisianensis*) (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Isoetes+louisianensis)
- *Geocarpon minimum* (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Geocarpon+minimum)

Three listed threatened and endangered species are found in East Baton Rouge Parish: The Alabama Heelsplitter is a mussel found only in Louisiana in the Amite River in East Baton Rouge Parish. The Alabama Heelsplitter is not found in the Mississippi River, which is approximately 2 kilometers from the field site. This mussel requires riverine habitat and will not colonize rice levees (http://ecos.fws.gov/speciesProfile/SpeciesReport.do?spcode=F01O). The Pallid Sturgeon is found in East Baton Rouge Parish, within the Mississippi River (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Scaphirhynchus+albus). This sturgeon is a bottom-dwelling river fish, and will not be affected by this small field trial in an area that is already under agricultural production. Additionally, rice levees are not suitable habitat for pallid sturgeon. The Bald Eagle is found in East Baton Rouge Parish (http://www.natureserve.org/explorer/servlet/NatureServe?searchName=Haliaeetus+leucocephalus), and is found primarily in habitats neighboring large bodies of water. However, the Bald Eagle is a carnivore and does not feed on rice plants or seeds. As transgenic *B. glumae* is not an animal pathogen, there are no foreseeable indirect effects on the potential food resources for the Bald Eagle due to this field trial. Thus the field release of non-pathogenic, avirulent *B. glumae* will have no effect on federally listed threatened and endangered species, species proposed for listing, designated critical habitat or habitat proposed for designation.

APPENDIX 4: Supplemental Permit Conditions

1. Bacterial liquid inoculum will be doubly contained in water tight containers for transport to and from the field site.

2. After inoculation in the field, Tyvek suits and sprayers used during inoculation will be doubly contained in plastic bags before leaving the field site.

3. Combines used to harvest seed will be brushed down at the field site before transport to steam-cleaning site.

4. Harvested seed will be contained via 7 CFR 340.8 during transport to the weighing facility at the LSU AgCenter Central Research Station.

5. All plants within the field site will be treated as regulated articles.

6. BRS should be notified in writing of any proposed changes to the permit application (or approved permit) including for example confinement protocols, transgenic lines or constructs, release sites, acreage, etc. Changes usually require amendments to the permit and must be pre-approved by BRS. Requests should be directed to Regulatory Permit Specialist, USDA APHIS BRS, Biotechnology Permit Services, 4700 River Road, Unit 147, Riverdale, Maryland 20737.

7. A BRS Regional Biotechnologist or a Plant Protection and Quarantine Officer may conduct an inspection of the test site at the beginning of the test. Additional inspections may be conducted throughout the permitted release.

8. Harvested plant material may not be used for food or animal feed unless it is first devitalized and approved for such use by the U.S. Food and Drug Administration; and for plant-incorporated protectants, a tolerance for the pesticide must first be established by the U.S. Environmental Protection Agency.

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

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

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

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

A. Activity Report

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

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

B. Field Test Report

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

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

Site name (or GPS)	Crop	Harvest Date	Disposition of Harvested Material

Regarding disposition of harvested material, include the quantity of material harvested and the final location of the harvested material (i.e., destroyed in the field, processed on site or sent to an off-site facility, sent to the laboratory for analysis). If materials were moved, identify the location, what type of facility and quantity transported. Describe methods used to devitalize and dispose of regulated plant materials and what happened to residual material at the field site.

We encourage the inclusion of other types of data if the applicant anticipates submission of a petition for determination of non-regulated status for their regulated article. APHIS considers these data reports as critical to our assessment of plant pest risk and development of regulatory policies based on the best scientific evidence. Failure by an

applicant to provide data reports in a timely manner for a field trial may result in the withholding of permission by APHIS for future field trials.

C. Monitoring Report

The report must include:

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

D. This report may be included as part of the Field Test Report submitted within 6 months of the expiration date of the permit. If the volunteer monitoring period continues beyond the date of submission of the Field Test Report, a separate report should be submitted. The final monitoring report is then due no later than 3 months from the end of the volunteer monitoring period.

10. APHIS shall be notified orally within 24 hours followed by a written notification within 5 days upon discovery in the event of any accidental or unauthorized release of the regulated article.

A. For immediate oral notification, contact APHIS BRS Compliance Staff at (301) 734-7324 and ask to speak to Compliance and Inspection staff member.
B. In the event of an emergency and you are unable to reach the BRS Compliance Staff at the above number, you may call:

The APHIS/BRS Regional Biotechnology Coordinator assigned to the State where the field test occurs.

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

For Eastern Region, contact Ashima SenGupta by phone at (919) 855-7622 or e-mail Ashima.SenGupta@aphis.usda.gov

Or

The APHIS PPQ Regional Biotechnology Coordinator assigned to the State where the field test occurs

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

For Eastern Region, contact Susan Dublinski by phone at (919) 855-7324 or e-mail Susan.G.Dublinski@aphis.usda.gov

Or

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

Louisiana: William Spitzer, Baton Rouge
Phone: (225) 298-5410
Fax: (225) 298-5415
Email: william.e.spitzer@aphis.usda.gov

C. Written notification should be sent:

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

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

11. This approved Biotechnology Permit (APHIS Form 2000) does not eliminate the permittee's legal responsibility to obtain all necessary Federal and State approvals, including: (1) for the use of any non-genetically engineered plant pests or pathogens as challenge inoculum; (2) plants, plant parts or seeds which are under existing Federal or State quarantine or restricted use; (3) experimental use of unregistered chemical; and (4) food or feed use of genetically engineered crops harvested from the field experiment.