IBC Protocol Form
Form Version: 8

Proposed Containment Level: BSL-2
Original Approval Date: 07/17/2015
Protocol ID: 482
Form ID: 1549

I. Protocol Information

* **List of Form Edits**

In the space below, detail, enumerating by section, all changes/edits made to this form. A summary of any changes to research projects or added projects must be provided in the Research Description in Section II.

June, 2015 Amendment:
Section III. Added two new plant pathogens (Rhizoctonia zeae and Waitea circinata).
Section XI. Attached approved APHIS permits and the pending application for permit amendment to add those new species.

Changes per Matt Anderson's Revision request:
Section I. Changed title to reflect added research goal to assess pathogens for fungicide resistance
Section II: Research description, added new research goal to assess pathogens for fungicide resistance
Section III. Added toxin production warning for Fusarium spp.
Section VI: Added note that the fungicide resistance genes we're studying are unknown and that if our work identifies the genes responsible for the observed resistance, an amendment will be made to list them.
Section X, Risk ASssessment, Safe Operating Procedures: Checked box for "avoid production of biological aerosols"
Section II, Research Description: Deleted an ineffective sentence that attempted to address the dual-use concern

Other comments: It was recommended that we switch our disinfectant agent from 70% ethanol to 5% bleach. Sodium hypochlorite is an oxidant and highly alkaline. Several of the surfaces we work on are metal surfaces, including our biosafety cabinet and high value instruments in the lab, which develop surface corrosion when exposed to bleach. We've already had one issue in our lab where we used bleach and it led to excessive corrosion of a high-sensitivity instrument, which ultimately required we send the instrument back to the company to be serviced, so I would really prefer not to use this disinfectant agent.

* Protocol Title

Fungicide resistance in fungal plant pathogen populations

Legacy Protocol #: (Please list all previous UNL IBC project numbers here if those projects are described in this protocol)

Export Control Compliance Check

☒ * Export Control Program staff have been contacted regarding this research protocol.
(If you have not spoken with the Export Control Program staff, they may be reached at 472-6929 or exportcontrol@unl.edu. Please note that your protocol will not be approved until the IBC receives notification from Export Control Program staff that you are in compliance with Export Control Regulations.)

* Does this research require a Technology Control Plan?
No

Protocol Attributes (select all the apply):

☐ 1. Proprietary matters

☐ 2. HIV/HBV or other bloodborne pathogen
OR
Human blood, tissue, fluids, cells, or cell lines derived therefrom

☒ 3. Human, Animal Or Plant Pathogen

☐ 4. Field Release of Transgenic plants and/or any Plant Pathogen

☐ 5. Animal use *(When selecting this item, please search for and select the applicable IACUC protocols under Question 7)*

☐ 6. Transgenic Organisms (Animals, Plants and Arthropods Only)

☐ 7. Field Collection or Sampling of wild animals

☐ 8. Radioactive material use

☐ 9. Human subjects

☐ 10. Biological agents regulated as "Select Agents"

☒ 11. Regulatory permits are required for possession, use, transfer, import or export of material described in this protocol. Permitting agencies can include USDA, APHIS, CDC, FDA, etc.

* 11a. Note: Please provide the following information below (Permitting Agency, Permit #, Date Issued, & Expiration Date). Also, upload a copy of the permit in Section XI of this form. If your permit has not yet been approved, list the Agency, submitted date, and indicate Pending.

USDA APHIS P526-150604-011 submitted 06/04/15 04:08:54 PM, status: "Pending". This file is provided as a separate attachment for this application.

☐ 12. Experiments involving more than 10 L of culture

☒ 13. Recombinant and/or synthetic nucleic acid molecule use

Major Actions under the NIH Guidelines (Requires NIH review prior to initiation)

☐ 14. Deliberate formation of rDNA (Including synthetic nucleic acid molecules) containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and Shigella dysenteriae neurotoxin) (Section III-B-1)

☐ 15. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture (Section III-A-1)

Dual Use Research of Concern Experimental Categories

☐ 16. Enhance the harmful consequences of a biological agent or toxin.

☐ 17. Disrupt immunity or the effectiveness of an immunization

☐ 18. Confer to a biological agent or toxin, resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin, or facilitate their ability to evade detection methodologies

☐ 19. Increase the stability, transmissibility, or the ability to disseminate a biological agent or toxin

☐ 20. Alter the host range or tropism of a biological agent or toxin

☐ 21. Enhance the susceptibility of a host population

☐ 22. Generate a novel pathogenic agent or toxin, or reconstitute an eradicated or extinct biological agent.

NIH Guidelines:

If Recombinant and/or synthetic nucleic acid molecule use, Select All Of The Appropriate Section Citations From NIH Guidelines That Apply

☐ 1. **Exempt**-III-F-1 (synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell. (e.g. oligonucleotides that dont contain an origin of replication or elements that interact with DNA or RNA polymerase), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100ng/kg. If a synthetic nucleic acid molecule meets the criteria of Section III-C, it is not exempt under this Section.

☒ 2. **Exempt**-III-F-2 (recombinant or synthetic nucleic acid molecules that are not in organisms, cells or viruses and that have not been modified or manipulated (e.g. encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes.)

☐ 3. **Exempt**-III-F-3 (recombinant or synthetic nucleic acid molecules that consist solely of the exact sequence from a single source that exists contemporaneously in nature.)

☐ 4. **Exempt**-III-F-4 (recombinant or synthetic nucleic acid molecules that consist entirely of nucleic acids from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means)

☐ 5. **Exempt**-III-F-5 (recombinant or synthetic nucleic acid molecules that consist entirely of nucleic acids from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species))

☐ 6. **Exempt**-III-F-6 (recombinant or synthetic nucleic acid molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent (See Appendices A-I through A-VI, Exemptions Under Section III-F-5--Sublists of Natural Exchangers, for a list of natural exchangers that are exempt from the NIH Guidelines.))

☐ 7. **Exempt**-III-F-7 (genomic DNA molecules that have acquired a transposable element, provided the

transposable element does not contain recombinant and/or synthetic DNA.

☒ 8. **Exempt**-III-F-8 (recombinant or synthetic nucleic acid molecules that do not present risk to health or environment as determined by the NIH Director (Appendix C). E.g., certain recombinant or synthetic nucleic acid molecules containing less than one-half of any eukaryotic viral genome propagated and maintained in tissue culture; certain Escherichia coli K-12 host-vector systems; certain Saccharomyces host-vector systems; certain Bacillus subtilis or Bacillus licheniformis host-vector systems; certain recombinant or synthetic nucleic acid molecules derived entirely from extrachromosomal elements of listed gram positive organisms; purchase or transfer of transgenic rodents; generation of BSL-1 transgenic rodents via breeding)

- ☐ 8a. Appendix C-I (Recombinant or synthetic nucleic acid molecules in Tissue Culture)
- ☒ 8b. Appendix C-II (Escherichia coli K-12 Host-Vector Systems)
- ☐ 8c. Appendix C-III (Saccharomyces Host-Vector Systems)
- ☐ 8d. Appendix C-IV (Kluyveromyces Host-Vector Systems)
- ☐ 8e. Appendix C-V (Bacillus subtilis or Bacillus lichenformis Host-Vector Systems)
- ☐ 8f. Appendix C-VI (Extrachromosomal Elements of Gram Positive Organisms; see guidelines for complete list of exempt organisms)
- ☐ 8g. Appendix C-VII (Purchase and transfer of transgenic rodents that require BL-1 containment)
- ☐ 8h. Appendix C-VIII Generation of BL1 Transgenic Rodents via Breeding

  The breeding of two different transgenic rodents or the breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at BL1 containment will be exempt from the NIH Guidelines if:

  (1) Both parental rodents can be housed under BL1 containment; and

  (2) neither parental transgenic rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and

  (3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.

☐ 9. III-E (Experiments not included in Sections III-A, III-B, III-C, III-D, or III-F and their subsections are considered in Section III-E. All such experiments are conducted at BSL-1 containment. Example: experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes may be conducted at BSL-1.) [E. coli BL21 stains and Agrobacterium tumefaciens fall under this section]

☐ 10. III-E-1 (recombinant or synthetic nucleic acid molecules containing no more than two-thirds of the genome of any eukaryotic virus may be propagated in maintained in cells in tissue culture using BSL-1 containment so long

as it is demonstrated that the cells lack helper virus for the specific families of defective viruses being used)

☐ 11. III-E-2 Experiments involving Whole Plants (Experiments involving recombinant or synthetic nucleic acid molecules-containing whole plants and/or organisms associated with whole plants not covered by Sections III-A, III-B, III-D, or III-F)

☐ 12. III-E-3 (Experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic rodents). Only experiments that require BL1 containment are covered under this section; experiments that require BL2, BL3, or BL4 containment are covered under Section III-D-4, Experiments Involving Whole Animals.)

13. III-D-1 Experiments using Risk Group 2, Risk Group 3, Risk Group 4 or Restricted agents as Host-Vector Systems

- ☐ 13a. III-D-1-a (Experiments involving the introduction of recombinant or synthetic nucleic acid molecule into RG-2 agents is conducted at BSL-2 containment. Experiments with such agents in whole animals will be conducted at BSL-2 or ABSL-2 containment.)
- ☐ 13b. III-D-1-b (Experiments involving the introduction of recombinant or synthetic nucleic acid molecules into RG-3 agents is conducted at BSL-3 containment. Experiments with such agents in whole animals will be conducted at BSL-3 or ABSL-3 containment.)
- ☐ 13c. III-D-1-c (Experiments involving the introduction of recombinant or synthetic nucleic acid molecules into RG-4 agents is conducted at BSL-4 containment.
- ☐ 13d. III-D-1-d (Containment conditions for experiments involving the introduction of recombinant or synthetic nucleic acid molecules into restricted agents is determined on a case-by-case basis by NIH/OBA review. A USDA permit is required for work with plant or animal pathogens. Experiments with such agents in whole animals will be conducted at BSL-4 or ABSL-4 containment.)

14. III-D-2 Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems

- ☐ 14a. III-D-2-a (Experiments in which DNA from RG-2, RG-3 or RG-4 agents is transferred to nonpathogenic prokaryotes or lower eukaryotes may be performed under BSL-2 containment. [Certain conditions exist for DNA from RG 4 agents, contact the IBC for more information ibc@unl.edu])
- ☐ 14b. III-D-2-b (Containment conditions for experiments in which DNA from restricted agents is transferred to nonpathogenic prokaryotes or lower eukaryotes is determined on a case-by-case basis by NIH/OBA review. A USDA permit is required for work with plant or animal pathogens.)

15. III-D-3 Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in Tissue culture systems

- ☐ 15a. III-D-3-a (Experiments involving the use of infectious or defective RG-2 viruses in the presence of helper virus may be conducted at BSL-2.
- ☐ 15b. III-D-3-b (Experiments involving the use of infectious or defective RG-3 viruses in the presence of helper virus may be conducted at BSL-3.)
- ☐ 15c. III-D-3-c (Experiments involving the use of infectious or defective RG-4 viruses in the presence of helper virus may be conducted at BSL-4.)

- [ ] 15d. III-D-3-d (Experiments involving the use of infectious or defective restricted poxviruses in the presence of helper virus shall be determined on a case-by-case basis following NIH/OBA review. A USDA permit is required for work with plant or animal pathogens.)

- [ ] 15e. III-D-3-e (Experiments involving the use of infectious or defective viruses in the presence of helper virus which are not covered in Sections III-D-3-a through III-D-3-d may be conducted at BSL-1.)

16. III-D-4 Experiments involving Whole Animals

- [ ] 16a. III-D-4-a (Recombinant or synthetic nucleic acid molecules, or DNA or RNA molecules derived therefrom, from any source except greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BSL-1 or ABSL-1 and appropriate to the organisms under study. Similarly, animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under the above referenced containment conditions.)[The PI must demonstrate that the fraction of the viral genome being used does not lead to productive infection.]

- [ ] 16b. III-D-4-b (Experiments involving recombinant or synthetic nucleic acid molecule, or RNA or DNA derived therefrom involving whole animals, including transgenic animals and not covered by Section III-D-1 or III-D-4-a will be conducted at an appropriate containment level determined by the IBC committee)

17. III-D-5 Experiments in Whole Plants (Experiments to genetically engineer whole plants by recombinant or synthetic nucleic acid molecules methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant or synthetic nucleic acid molecules, may be conducted under containment conditions described in III-D-5-a through III-D-5-e.)

- [ ] 17a. III-D-5-a (Experiments involving most exotic infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant or synthetic nucleic acid molecule techniques are used with whole plants should be conducted at BL3-P or BL2-P+ biological containment)

- [ ] 17b. III-D-5-b (Experiments involving plants containing cloned genomes of readily transmissible exotic infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems in which it is possible to reconstitute the complete and functioning genome of the infectious agent in planta should be conducted at BL3-P or BL2-P+ biological containment)

- [ ] 17c. III-D-5-c (Experiments with a small number of readily transmissible exotic infectious agents, such as soybean rust fungus and maize streak or other viruses in the presence of their specific arthropod vectors with the potential of being serious pathogens of major US crops should be conducted at BL4-P biological containment)

- [ ] 17d. III-D-5-d (Experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms should be conducted at BL3-P biological containment)

- [ ] 17e. III-D-5-e (Experiments with microbial pathogens of insects or small animals associated with plants if the rDNA-modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems should be conducted at BL3-P or BL2-P+ biological containment)

- [ ] 18. III-D-6 Experiments involving more than 10 L of culture (Containment is determined by the IBC. Appendix K of the NIH Guidelines may be used as a guide where appropriate.)

☐ 19 III-D-7 Experiments involving Influenza Viruses (Experiments with influenza viruses generated by recombinant methods shall be conducted at the biosafety level containment corresponding to the risk group of the source virus for the majority of segments in the recombinant virus.)

☐ 19a. III-D-7-a Human H2N2 (1957-1968) (Experiments with influenza viruses containing the H2 hemagglutinin segment shall be conducted at BSL-3 enhanced. Experiments with H2 HA gene in cold-adapted, live-attenuated vaccine strains may be conducted at BSL-2. Experiments with RG-2 influenza viruses not containing H2 HA gene can be conducted at BSL-2)

☐ 19b. III-D-7-b Highly Pathogenic Avian Influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1) (Experiments involving influenza viruses containing a majority of genes and/or segments from HPAI H5N1 shall be conducted at BSL-3 enhanced containment. Containment level can be reduced for experiments involving influenza viruses containing a minority of genes and/or segments

☐ 19c. III-D-7-c 1918 H1N1 (Experiments involving influenza viruses containing any gene or segment from 1918 H1N1 shall be conducted at BSL-3 enhanced containment.)

☐ 19d. III-D-7-d Antiviral Susceptibility and Containment (Experiments with influenza viruses containing genes from any of the viruses described in III-D-7-a through III-D-7-c may require higher containment if the virus is resistant to both current classes of antiviral agents, adamantanes and neuraminidase inhibitors. Experiments designed to create resistance to neuraminidase inhibitors or other antiviral agents are subject to Section III-A-1.)

III-C. Experiments that require IBC, IRB, and RAC review before research participant enrollment

☐ 20. III-C-1 (Experiments involving the deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into human research participants)

III-B. Experiments that require NIH/OBA and IBC approval before initiation

☐ 21. III-B-1 (Experiments involving the deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 ng/Kg body weight)

☐ 22. **III-B-2** (Experiments that have been Approved (under Section III-A-1-a) as Major Actions under the NIH Guidelines) [Upon receipt and review of an application from a PI, NIH/OBA may determine that a proposed experiment is equivalent to an experiment that has previously been approved by the NIH Director as a Major Action, including experiments approved prior to implementation of these changes. An experiment will only be considered equivalent if, as determined by NIH/OBA, there are no substantive differences and pertinent information has not emerged since submission of the initial III-A-1-a experiment that would change the biosafety and public health considerations for the proposed experiments. If such a determination is made by NIH/OBA, these experiments will not require review and approval under Section III-A.]

III-A. Experiments that require IBC approval, RAC Review, and NIH Director Approval before initiation

☐ 23. III-A-1 (Major Actions, defined as the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by the RAC.)[Consideration is given as to whether the drug resistance trait to be used in the experiment would render that microbe resistant to the primary drug available to and/or indicated for certain populations.]

Please explain NIH Guidelines section selections if necessary:

We will use PCR for DNA amplification of known sequences. We will also occasionally use Life Technologies' TOPO TA Cloning Kits that contains the Mach1-T1R E. coli strain (modified from the wild-type W strain ATCC #9637, S. A. Waksman) for amplification of unknown DNA sequences so that we can submit them for sequencing.

Grant Information

Please select all grant information applicable to this protocol (start typing the title or ID)

Investigators

Principal Investigator

* Name

Sydney Everhart - everhart@unl.edu - 402 472 2879

* Department

Department of Plant Pathology

Department Head/Chair, Dean, or Director

* Name

James Steadman - jsteadman1@unl.edu - 402 472 3163

Co-Investigator(s) or Collaborator(s)

Personnel

Laboratory Personnel

* Name

Bimal Amaradasa - bamaradasa2@unl.edu - 402 472 2858

* Job Title

Postdoctoral Scholar

* Years of Experience

3

* Please indicate what activities/projects this person will be involved with on this protocol. This will help determine what safety training will be required.

Works with fungi in the lab.

Comments or Additional Information for Part I:

II. Research Description

* In lay language, briefly describe the experimental design and research objectives of the protocol. Your description should allow a non-scientist to understand your work and assess the hazards and risks. Standard procedures can be referred to by common names, but novel procedures and significant modifications to standard procedures should be described. Please avoid or explain acronyms.

Our work aims to better understand factors that may give rise to faster emergence of fungicide resistant strains within pathogen populations. As such, our work uses fungal plant pathogens as a model organism in controlled studies, exposing the fungus to increasing doses of fungicides. The result could yield fungicide resistant strains. If it does, it would help us better understand factors that may cause fungicide-resistance to emerge more rapidly in a population. The benefit is that we can subsequently develop methods that will help to reduce the likelihood of emergence of resistance, thus prolonging efficacy of our current control methods.

June 2015 update: New research in my lab is to survey for fungicide resistance within Rhizoctonia spp. populations from soybean and corn.

Another component of our research is to characterize populations of fungal plant pathogens using genetic markers (PCR-based) and tests for fungicide resistance. This aspect of our work would involve fungal pathogens collected from fields in Nebraska, isolated and stored in the lab. These isolates would be kept for lab use (phenotyping and DNA extraction) and not used for re-deployment in field studies.

III. Microorganism Information

☐ Microorganism Information Not Applicable

Please list all microorganisms being used in your work in this section. This includes archaea, bacteria, viruses (including viral vectors), fungi, and parasites as well as viral vectors. More specific information about viral vectors and other vector types will be asked for in Section VI.

Microorganism(s)

* 1. Microorganism (Genus, species):

Sclerotinia slcerotiorum

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Strain 1980 (used for generation of reference genome), ...

* 3. Human pathogen

No ▼

* 4. Animal pathogen

No ▼

* 5. Plant pathogen

Yes ▼

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

No

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes on this form.

* 6. Toxin Production

No

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

* 1. Microorganism (Genus, species):

Botrytis cinerea

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Fungal Genetics Stock Culture #10316 and 10317.
Other strains from field collection.

* 3. Human pathogen

No

* 4. Animal pathogen

No

* 5. Plant pathogen

Yes

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

Yes

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes on this form.

* 6. Toxin Production

No

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

* 1. Microorganism (Genus, species):

Colletotrichum graminicola

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Fungal Genetics Stock Center #10212

* 3. Human pathogen

No

* 4. Animal pathogen

No

* 5. Plant pathogen

Yes

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

Yes

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes on this form.

* 6. Toxin Production

No

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

* 1. Microorganism (Genus, species):

Stagonospora nodorum

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Fungal Genetics Stock Center #10173

* 3. Human pathogen

No

* 4. Animal pathogen

No

* 5. Plant pathogen

Yes

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

Yes

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes on this form.

* 6. Toxin Production

No

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

* 1. Microorganism (Genus, species):

Magnaporthe grisea

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Fungal Genetics Stock Center #8958

* 3. Human pathogen

No

* 4. Animal pathogen

No

* 5. Plant pathogen

Yes

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

Yes

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes on this form.

* 6. Toxin Production

No

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

* 1. Microorganism (Genus, species):

Fusarium verticillioides

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Fungal Genetics Stock Center #7600, #8961

* 3. Human pathogen

No

* 4. Animal pathogen

No

* 5. Plant pathogen

Yes

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

Yes

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes on this form.

* 6. Toxin Production

Yes

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

* 1. Microorganism (Genus, species):

Fusarium graminearum

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Fungal Genetics Stock Center #9075

* 3. Human pathogen

No

* 4. Animal pathogen

No

* 5. Plant pathogen

Yes

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

Yes

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes on this form.

* 6. Toxin Production

Yes

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

* 1. Microorganism (Genus, species):

Verticillium dahliae

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Fungal Genetics Stock Center #10137, 10138

* 3. Human pathogen

No

* 4. Animal pathogen

No

* 5. Plant pathogen

Yes

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

Yes

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes on this form.

* 6. Toxin Production

No

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

* 1. Microorganism (Genus, species):

Rhizoctonia solani

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Rhs1AP and Rhs1AP-123E

* 3. Human pathogen

No

* 4. Animal pathogen

No

* 5. Plant pathogen

Yes

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

Yes

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes on this form.

* 6. Toxin Production

No

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

* 1. Microorganism (Genus, species):

Macrophomina phaseolina

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Field collected isolates

* 3. Human pathogen

No

* 4. Animal pathogen

No

* 5. Plant pathogen

Yes

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

No

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes on this form.

* 6. Toxin Production

No

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

* 1. Microorganism (Genus, species):

Rhizoctonia zeae

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Collected from diseased plants in Maryland.

* 3. Human pathogen

No

* 4. Animal pathogen

No

* 5. Plant pathogen

Yes

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

Yes

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes on this form.

* 6. Toxin Production

No

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

* 1. Microorganism (Genus, species):

Waitia circinata (also called Rhizoctonia circinata)

* 2. List all strains used and describe their pathogenicity (e.g. virulent, wild type, attenuated, vaccine strain, etc.):

Collected from diseased plants in Maryland.

* 3. Human pathogen

No

* 4. Animal pathogen

No

* 5. Plant pathogen

Yes

* 5.a. Is this microorganism being imported from outside the United States or transferred from another State within the US?

Yes

* 5.b Do you plan to export this microorganism outside the United States?

No

* 5.c Will this pathogen be released to the environment through field trials?

No

If you answered yes to either of these questions please refer to the EHS SOPs on shipping biological materials found at http://ehs.unl.edu/sop/shipping. If you do need a permit, please complete question 11 in Section I: Protocol Attributes

on this form.

* 6. Toxin Production

No

* 7. Used as a Reference or Control?

No

* 8. Administered to animals in vivo

No

* 9. Administered to plants in vivo

No

* 10. Administered to OTCC

No

* 11. Recipient of recombinant and/or synthetic nucleic acid molecules

No

* 12. Recipient of Toxin

No

* 13. Drug/antibiotic resistance introduced

No

Comments or Additional Information for Part III

- Some of these plant pathogens, though rare, have been reported to infect immunocompromised humans: Colletotrichum graminicola, Fusarium verticillioides, and Macrophomina phaseolina.

- Fusarium species are known to produce at least three different classes of mycotoxins, including fumonasins, tricothecenes, and zearaleone, which are known animal toxins when consumed. Both F. verticillioides and F. graminearum are known to produce mycotoxins.

IV. Organ, Tissue, or Cell Cultures Information

☒ OTCC Information Not Applicable

Please list all organs, tissues, body fluids or cell culture lines being used in your work in this section. Include cell lines being used for viral vector packaging, but be aware that more specific information about viral vector packaging lines will be asked for in Section VI.

Comments or Additional Information for Part IV

V. Research Organism Information

☒ Research Organism Information Not Applicable

Please list all research organisms being used in your work in this section. This includes only plants or animals used in your work including both vertebrates and invertebrates. If multiple strains of the same animal or plant are being used, please enter each strain separately unless the answers to the other questions asked are identical for all strains listed.

Comments or Additional Information for Part V

VI. Recombinant and/or Synthetic Nucleic Acid Information

☐ Recombinant and/or Synthetic Nucleic Acid Information Not Applicable

Please list all genetic material being used in your work in this section. This includes all types of DNA and RNA as well as synthetic nucleic acid molecules. Do not enter genes associated with viral vector systems, such as gag/pol, Rev, Tat, VSVG, etc. This information is covered by the vector and viral vector subsections.

Genetic Material Information

* 1. Gene Name (e.g., GFP - green fluorescent protein; explain acronyms)

mutated gene or genetic region

* 2. Gene Source (Genus, species, strain; use ATCC nomenclature when possible)

various fungal strain after stress exposure

* 3. Type of Sequence (example: DNA, RNA, siRNA, etc)

DNA

* 4. Function (toxin production, oncogene, marker trait, virulence factor, etc.)

unknown

* 5. Use

Sequencing/PCR only

* 6. Risk Assessment

Low

Vector Description(s)

Please attach plasmid/vector maps in Section XI of the form, if available.

* 1. Vector Type (e.g. bacterial plasmid, phage, etc.)

bacterial plasmid

* 2. Vector Source (Genus, species)

Escherichia coli Mach1™-T1R strain is modified from the wild-type W strain (ATCC #9637, S. A. Waksman)

* 3. Vector Technical Name

pCR 4-TOPO

* 4. Commercial Vendor Name, if applicable

Life Technologies' TOPO-TA Cloning Kit

* 5. Risk Attenuation (e.g. disarmed, replication defective, K-12 derivative, helper dependent, etc.)

The parental strain is generally classified as Biosafety Level 1.

☒ Viral Vectors And Packaging Not Applicable

Comments or Additional Information for Part VI

No specific genes are being targeted for resistance, as our work involves studies to determine environmental stressors that may cause resistance to emerge. If and when resistance is observed to have emerged in the lab, we may or may not pursue further studies to identify genes leading to resistance. If determined, they will be reported.

VII. Toxin Information

☒ Toxin Information Not Applicable

This section only applies to toxins that are expressed in or produced by biological organisms and isolated in the lab. Use of toxins on the US Select Agent list are also to be included in this section.

Comments or Additional Information for Part VII

VIII. Facilities Information

Enter the facilities that will be used to conduct the research described in this form. List each room in a separate entry. If the room use is identical, you can list multiple rooms in one entry.
Research Facilities

* 1. Building

Plant Sciences Building

* 2. Room Number

435 / 434

3. Use of Room (Check all that apply)

- [x] Lab
- [ ] Common Room (shared use)
- [ ] Tissue Culture
- [ ] Viral Vector Work
- [ ] Microscopy
- [ ] Animal Housing
- [ ] Surgery
- [ ] Animal Injections (rDNA, microbe or toxin)
- [ ] Greenhouse
- [ ] Agent Storage Only
- [ ] Other

* 4. Design

BSL-2

* 5. Containment Equipment Use

Yes

(Check all that apply)

- [ ] Laminar Flow Hood/Clean Bench (Horizontal or Vertical Flow)
- [x] Biosafety Cabinet
- [x] Fume Hood
- [ ] Other

* 6. Sharps Use

Yes

* Please list the types of sharps used and the procedures for use:

Dissecting needles for aseptic transfer of fungus.

Comments or Additional Information for Part VIII

The room 434 is actually a walk-in incubator that is accessed from inside 435 (labeled 435A inside lab) and 434 is kept locked at all times.

IX. Specialized Equipment Information

☒ Specialized Equipment Information Not Applicable

Comments or Additional Information for Part IX

X. Risk Assessment/Safety Considerations

* Proposed Containment Level

BSL-2

Facility and/or Practice Enhancements Beyond Proposed Containment Level

Personal Protective Equipment

Options (select all that apply)

- ☒ 1. Lab coats
- ☐ 2. Rear-closing gown
- ☐ 3. Booties
- ☐ 4. Disposable Lab Coat/Gown
- ☒ 5. Eye protection
- ☒ 6. Faceshield
- ☐ 7. Respirator
- ☒ 8. Gloves
- ☐ 9. Double gloves
- ☐ 10. Other

Proposed Vaccination and Medical Monitoring

HEALTH STATUS

Some unusual circumstances warrant special considerations or measures to prevent infection of laboratory personnel by certain microorganisms and other potentially etiological agents:

Please be informed (and ensure that all staff members are similarly informed) that certain medical conditions increase your risk of potential health problems when working with pathogenic microorganisms and/or animals. These conditions can include: pregnancy, certain medications (e.g. anti-TNF agents, corticosteroids, anti-neoplastic drugs), immunosuppression, animal related allergies and chronic skin conditions. If any of these conditions applies, inform your personal physician/health care professional about your work.

☒ Vaccinations and Medical Monitoring Not Applicable

Disinfectants

☐ Surface Disinfectant Not Applicable

Note: If multiple disinfectants are used, please list them separately.

Surface Disinfectant(s)

* Name and Concentration

Ethanol (70%)

* Procedure

Spray surface and allow to air dry.

☒ Equipment Disinfectant Not Applicable

Decontamination and Disposal Methods

☐ Solid Waste Disposal Not Applicable

Solid Waste

☒ Autoclave

* Location

437 Plant Science Hall

* Reference #

437-1, 437-2, 437-3

If you do not know the reference number for the autoclave you have listed, please contact EHS at 472-3784.

☐ EHS Pickup

☐ Incineration

☐ Other

☐ Liquid Waste Disposal Not Applicable

Liquid Waste

☒ Autoclave

* Location

437 Plant Science Hall

* Reference #

437-1, 437-2, 437-3

If you do not know the reference number for the autoclave you have listed, please contact EHS at 472-3784.

☐ EHS Pickup

☐ Incineration

☐ Other

☒ Animal Carcass/Bedding Disposal Not Applicable

Standard Safety Procedures Observed

Listed below are Safe Operation Procedures (SOPs) available on the EHS website. These are provided as recommended guidance and should be used if you have not already established similar procedures in your lab. If you decide to use the EHS SOPs, please keep a copy in your Biosafety Manual. If you choose to use your own procedures, please select "yes" in the next sub-section **"Lab-specific Safety Procedures"** and provide a brief listing of the topics they cover in the comment box and attach a copy of the SOP using the file upload button.

☒ 1. Autoclave Operation and Performance Testing

☒ 2. Avoiding the Production of Biological Aerosols

☒ 3. Biosafety Cabinets

☐ 4. Cell and Tissue Cultures

☒ 5. Disposing of Biohazardous Materials

☒ 6. Spill and Exposure Response for Biohazardous Materials (including Recombinant Nucleic Acids)

☐ 7. Cleaning Up Spills of Bloodborne Pathogens

- [x] 8. Incident Reporting - National Institutes of Health (NIH) Guidance
- [ ] 9. Lentiviral Vectors
- [ ] 10. Necropsy Biosafety
- [x] 11. Sharps - Handling and Disposing
- [x] 12. Standard and Special Microbiological Practices
- [ ] 13. HIV and HBV Research Laboratories/Production Facilities
- [x] 14. Transport of Biohazardous Materials

Lab-specific Safety Procedures

* Safety Procedures Specific to the Lab

No

Security Attributes

- [x] 1. Room is locked when unoccupied
- [x] 2. Freezers, refrigerators, incubators, and other storage devices are locked
- [x] 3. Access to the lab is limited or restricted when actively working with rDNA-containing organisms
- [x] 4. Lab is separated from areas open to unrestricted traffic
- [ ] 5. Other

Additional Information

Use this space to provide additional information about the procedures and practices used in your lab to mitigate the risk presented by the biological materials proposed for use in this form. This should not necessarily duplicate the information already provided in the previous questions, but rather provide context or explanation for the work described in the form as it relates to the safety of the lab workers.

XI. Attachments

Please use this section to attach any additional information about the project that may assist the committee in assessing the risk of the project during review. *Examples include: Grant proposal sections/abstracts, plasmid maps, supplier-provided protocols, etc.*

Attachment

☒Permit_P526P-15-01114_20150302.pdf

☒APHISPermit_10-8-2014_4218266.pdf

☒P526-150604-011_application.pdf