THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 25, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents are described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits. The form must also be completed if any work is proposed involves plants or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

This form must also be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, OHS (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR Assistant Professor Graham J Thompson
SIGNATURE
DEPARTMENT Biology
ADDRESS Health Science Building 413
PHONE NUMBER 86570
EMAIL graham.thompson@uwo.ca

Location of experimental work to be carried out: Building(s) BIOTRON Room(s) microbiology module

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: NSERC DISCOVERY
GRANT TITLE(S): Evolutionary genetics of sociality : experimental tests using termites and bees

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

Ms Catherine Gao
Mr Michael Newsoglium

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ⊘YES O NO

If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES ⊘NO

If YES, please give the name of the species. _____

What is the origin of the microorganism(s)? _____

Please describe the risk (if any) of escape and how this will be mitigated:

_____

_____

_____

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

_____

_____

_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	Health Canada or CFIA Containment Level
Metarhizium anisopliae	O Yes ⊘No	⊘Yes O No	O Yes ⊘No		Dr Mike Bidochka (Brock University)	⊘1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3

*Please attach a Material Safety Data Sheet or equivalent from the supplier. – See Attachment

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? O YES ⊘NO

If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes O No		Not applicable
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes O No		
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate HC or CFIA containment level required O 1 O 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES O NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**HC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? O YES O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
				.

* Please attach a Material Data Sheet or equivalent if available.

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below O NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

4.6 Will virus be infectious to humans or animals? O YES O NO

4.7 Will this be expected to increase the containment level required? O YES O NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES Ø NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O YES Ø NO If no, please proceed to section 7.0

6.2 Name of animal species to be used insects only (termites)

6.3 AUS protocol # _____

6.4 Will any of the agents listed be used in live animals O YES, specify: _____ O NO

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES O NO
- Non- Human Primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES O NO
- Others (wild or domestic) O YES, please specify _____ O NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES O NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ~~O~~✓NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-off" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

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10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES O NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will the agent be imported? O YES, please give country of origin_____ Ø NO
If no, please proceed to Section 10.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

} training is in progress during winter 2011

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. ⊘1 O2 O3

13.2 Has the facility been certified by OHS for this level of containment?
⊘YES, permit # if on-campus not available for this work specifically.
O NO
⊘NOT REQUIRED Biotron as a whole is designed for this.

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: 20 January 2019

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

Biohazardous Agents Registry Form
Principle Investigator - Graham J Thompson
20 January 2009

Brief description of work.

Our lab will be using an entomopathogenic fungus, *Metarhizium anisopliae* Strain 2575, to conduct infectivity trails and subsequent survivorship analysis on a species of insect, the eastern subterranean termite, *Reticulitermes flavipes*.

The fungus and the insect are both found locally in Ontario and thus are not exotic or imported.

The purpose of these trails it to establish the fungal spore concentration at which small groups of termites become immune-challenged. Once established, the effective concentration will be used to induce the up-regulation of immune genes. These candidate immune genes will be the basis for further study at the molecular level.

This work is part of Ms Catherine Goa's graduate research.

Dr Graham Thompson
X 86570

From: ImportZoopath <ImportZoopath@inspection.gc.ca>
Subject: **Re: CFIA containment question**
Date: January 12, 2009 12:07:46 PM GMT-05:00
To: Graham Thompson <graham.thompson@uwo.ca>
1 Attachment, 0.2 KB

Dear Ms Thompson,

Our organism database di not have this organism listed so I did a quick research to see how it would be classified. I only found one article detailing this organism infecting a cat. Knowing this an organism research/used intensively as a biocontrol agent, this only case does not reflect an high pathogenicity.

I have decided to classify this organism as a level 1 organism, and therefore if you decide to import this organism, you will not need an import permit from my group.

Have a nice day,

Cinthia Labrie

Office of Biohazard Containment & Safety, CFIA I Bureau du confinement des biorisques et de la sécurité, ACIA
Government of Canada I Gouvernement du Canada
59 Camelot, Ottawa ON K1A0Y9
Phone/Tél.: (613) 221-7068
Fax/ Téléc.: (613) 228-6129
ImportZoopath@inspection.gc.ca

Please visit our website at: http://www.inspection.gc.ca/english/sci/bio/bioe.shtml
Veuillez visiter notre site internet au: http://www.inspection.gc.ca/francais/sci/bio/biof.shtml

> > > Graham Thompson <graham.thompson@uwo.ca> 2009-01-12 11:07:44 >>>
Dear CFIA

I was hoping to learn whether a species of entomopathogenic fungus, Metarhizium anisopliae, is listed as an organism of interest with CFIA of Health Canada, and if so, what Level of Containment (1, 2, 3 or 4) should it be listed under.

Is there are database that I can search for this information?

Many thanks for any advice,

Graham

Graham Thompson
Assistant Professor
Department of Biology
University of Western Ontario
1151 Richmond Street North
London, Ontario N6A 5B7
CANADA

519 661 2111 (ext 86570)
519 615 6066 (cell)
graham.thompson@uwo.ca
http://www.uwo.ca/biology/Faculty/thompson

ImportZoopath.vcf (0.2 KB)

Metarhizium anisopliae

From Wikipedia, the free encyclopedia
(Redirected from Metarhizium)

Metarhizium anisopliae, formerly known as *Entomophthora anisopliae* (basionym), is a fungus that grows naturally in soils throughout the world and causes disease in various insects by acting as a parasite; it thus belongs to the entomopathogenic fungi[1]. It is known to infect over 200 insect species, including termites. It is currently being used as a biological insecticide to control a number of pests such as grasshoppers, termites, thrips, etc. and its use in the control of malaria-transmitting mosquitos is under investigation[2].

Red locusts killed by *M. anisopliae* var. *acridum* during a biological control campaign. Notice the green coat of spores on the insects that develops under humid conditions.

The disease caused by the fungus is called **green muscardine disease** because of the green colour of its spores. When these mitotic (asexual) spores (called conidia) of the fungus come into contact with the body of an insect host, they germinate and the hyphae that emerge penetrate the cuticle. The fungus then develops inside the body eventually killing the insect after a few days; this lethal effect is very likely aided by the production of insecticidal cyclic peptides (destruxins). The cuticle of the cadaver often becomes red. If the ambient humidity is high enough, a white mould then grows on the cadaver that soon turns green as spores are produced. Most insects living near the soil have evolved natural defenses against entomopathogenic fungi like *M. anisopliae*. This fungus is therefore locked in an evolutionary battle to overcome these defenses, which has led to a large number of isolates (or strains) that are adapted to certain groups of insects[3].

Metarhizium anisopliae

Tsetse flies killed by *M. anisopliae*

Scientific classification

Kingdom:	Fungi
Subkingdom:	Dikarya
Phylum:	Ascomycota
Class:	Sordariomycetes
Order:	Hypocreales
Family:	Clavicipitaceae
Genus:	*Metarhizium*
Species:	***M. anisopliae***

Binomial name

Metarhizium anisopliae
(Metchnikoff) Sorokin

Some isolates are so specific that they have attained variety status, like *Metarhizium anisopliae* var. *acridum*[4], which almost exclusively infects grasshoppers in the suborder Caelifera of the Orthoptera. Various research groups, including the international LUBILOSA Programme (http://www.lubilosa.org/) ,

have identified key technical challenges in the development of mycoinsecticide products including: isolate selection, mass production and delivery systems (formulation and application)[5]. In other words, insect control (mortality) depends on factors like the number of spores applied against the insect host, the formulation[6] and weather conditions[7]. Oil-based formulations allow the application of fungal spores under dry conditions, and is compatible with existing Ultra-Low Volume (ULV) application techniques for locust control.

Ilya I. Mechnikov named *Metarhizium anisopliae* after the insect species it was originally isolated from, the beetle *Anisoplia austriaca*. It is a mitosporic fungus with asexual reproduction, which was formerly classified in the form class Hyphomycetes of the form phylum Deuteromycota (also often called Fungi Imperfecti). The Deuteromycota were used to bring together all fungi for which no sexual stage (teleomorph) was known. It is therefore not a real taxon in the classical sense. With the advent of genetic profiling, it has now become possible to place these fungi in proper taxa. Most turn out to be the asexual forms (anamorphs) of fungi in the phylum Ascomycota. The teleomorphs of *Metarhizium* species appear to be members of the genus *Metacordyceps*[8]. *Metacordyceps taii* (as *Cordyceps taii*) has been described as the teleomorph of *Metarhizium taii*[9], but the latter was later synonymised with *Metarhizium anisopliae* var. *anisopliae*[10]. This means that *Metacordyceps taii* can now be considered the teleomorph of *M. a. anisopliae*. It is not yet clear whether the other varieties of *M. anisopliae* have their own teleomorphs. It is, however, possible that some, if not most, strains of *M. anisopliae* have lost the capability of reproducing sexually.

M. anisopliae does not appear to infect humans or other animals and is considered safe as an insecticide. The microscopic spores are typically sprayed on affected areas. A possible technique for malaria control is to coat mosquito nets or cotton sheets attached to the wall with them.

In August 2007, a team of scientists at the Indian Institute of Chemical Technology discovered a more efficient way of producing biodiesel which uses lipase, an enzyme produced in significant quantities by *Metarhizium anisopliae*; as opposed to other reactions which use enzymes that require heat in order to become active, the reaction that uses lipase runs at room temperature. The fungus is now a candidate for mass production of the enzyme.

See also

- Beauveria bassiana, the fungus that causes *white muscardine disease* in various insects
- Biological insecticides

References

1. ^ Cloyd, Raymond A. (1999). "The Entomopathogenic Fungus Metarhizium anisopliae". *Midwest Biological Control News* **VI** (7). http://www.entomology.wisc.edu/mbcn/kyf607.html.
2. ^ McNeil, Donald G. Jr. (10 June 2005). "Fungus Fatal to Mosquito May Aid Global War on Malaria". *The New York Times* **104**: 135-151. http://www.nytimes.com/2005/06/10/science/10mosquito.html.
3. ^ Freimoser, F. M., Screen, S., Bagga, S., Hu, G. and St. Leger, R.J. (2003). "EST analysis of two subspecies

of *Metarhizium anisopliae* reveals a plethora of secreted proteins with potential activity in insect hosts". *Microbiology* **149**: 239-247.
4. ^ Driver, F., Milner, R.J. and Trueman, W.H.A. (2000). "A Taxonomic revision of *Metarhizium* based on sequence analysis of ribosomal DNA". *Mycological Research* **104**: 135-151.
5. ^ Lomer, C.J., Bateman, R.P., Johnson, D.L., Langwald, J. and Thomas, M. (2001). "Biological Control of Locusts and Grasshoppers". *Annual Review of Entomology* **46**: 667-702.
6. ^ Burges, H.D. (ed.) (1998). *Formulation of Microbial Biopesticides, beneficial microorganisms, nematodes and seed treatments*. Dordrecht, Netherlands: Kluwer Academic. p. 412 pp..
7. ^ Thomas, M.H. and Blanford, S. (2003). "Thermal biology in insect-parasite interactions". *Trends in Ecology and Evolution* **18**: 344-350.
8. ^ Sung, G.-H., Hywel-Jones, N.L., Sung, J.-M., Luangsa-ard, J.J., Shrestha, B. and Spatafora1, J.W. (2007). "Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi". *Studies in Mycology* **57**: 5–59.
9. ^ Liang, Z.-Q., Liu, A.-Y., Liu, J.-L. (1991). "A new species of the genus *Cordyceps* and its *Metarhizium* anamorph". *Acta Mycologica Sinica* **10**: 257-262.
10. ^ Huang B., Li C., Humber R.A., Hodge K.T., Fan M. and Li Z. (2005). "Molecular evidence for the taxonomic status of *Metarhizium taii* and its teleomorph, *Cordyceps taii* (Hypocreales, Clavicipitaceae)". *Mycotaxon* **94**: 137-147.

External links

- Index Fungorum record (http://www.indexfungorum.org/Names /namesrecord.asp?RecordID=199430) , links to a list of synonyms
- LUBILOSA Programme (http://www.lubilosa.org) , website of the programme that developed *Metarhizium* for locust control
- [1] (http://blog.wired.com/wiredscience/2007/08/fungi-make-biod.html) Fungi Make Biodiesel Efficiently at Room Temperature

Retrieved from "http://en.wikipedia.org/wiki/Metarhizium_anisopliae"
Categories: Hypocreales | Parasitic fungi