UVU COLLEGE OF SCIENCE

Scholarly Activities Committee (SAC)

STUDENT PROPOSAL FOR FACULTY MENTORED RESEARCH

Students working with a faculty or staff mentor may submit this proposal to obtain funds for research supplies or travel to conduct research. The completed form will be submitted electronically as a Word document to the Department Chair or Department SAC representative of the faculty/staff mentor's department. Upon review and approval, the signed form should be emailed by the Chair or Department SAC representative to the COS Associated Dean SAC representative for final approval.

Criteria for submission:
The proposal and budget must be written and developed by the student(s), with guidance from the mentor. Students must be directly involved in the research in a significant way and demonstrate a thorough knowledge of the research project and budget. See last page for Guidelines and Limitations. *

1) **Title of project**: Effects of Ethanol Vapor on the Germination of *Baudoinia compniacensis*
2) **Lead student information**:
    i) Name: Nicole Paulette
    ii) Email: Nikkihpaulette@gmail.com
    iii) UVID: 10839535
    iv) # of UVU credit hours earned: 116
    v) Anticipated date of graduation: May 2023
    vi) Major: Biology
    vii) Phone #: 801-616-6350
3) **Faculty/staff mentor**:
    Geoffrey Zahn
4) **Start/Stop dates of project (a project summary will be due by the stop date of the project.)**:
    9/1/22- 12/17/22
5) **Name, UVID, and email address of other students to be involved in the project (if any). Also include # of UVU credit hours earned and anticipated date of graduation for each student:**
    Caleb Sawyer
    UVID: 10781573
    Email: 10781573@uvu.edu
    UVU Credit Hours Earned: 112
    Anticipated Graduation: August 2023

    Christine Nakonechnyy
    UVID: 10844630
    Email: 10844630@uvu.edu
    UVU Credit Hours Earned: 164
    Anticipated Graduation: May 2023

    Denise Jimenez
    UVID: 10807655
    Email: 10807655@my.uvu.edu
    UVU Credit Hours Earned: 99

Anticipated Graduation: August 2023

6) Have any students involved in this project received funding prior SAC funding for this or any other project? If so, list name(s) of students, titles of previously funded project(s), date(s) of funding, and amount(s) of funding received for each student.

Name: Nicole Paulette

Title: The use of hoarding cages to conduct imidacloprid feeding experiments on honey bees.

Date: September 2021-May 2022

Amount of funding received: $1,495.00

7) If this a continuation of a project that was previously funded by SAC, please describe what work related to the project has been completed and what are the results of that work. *(Please attach any papers, abstracts, etc.)*

N/A

8) List any other UVU or off-campus funding sources you have applied to for this project (e.g., OEL, Department funds, NSF, NIH, etc.):

N/A

9) List other sources of funding, including amount, already received for this project, if any *(Please note that priority is given to projects that seek funding from sources other than SAC).*

N/A

10) Do you require funding from <u>both</u> SAC <u>and</u> other source(s) in order for your proposed project to proceed? ~~Yes~~ or **<u>No</u>**

11) Is any part of this proposal redundant with the proposal submitted to any other funding source (e.g., are you seeking funds for the same supplies from both SAC and the other source)? ~~Yes~~ or **<u>No</u>**
(If yes, and if you are successful in obtaining funds from the other source, the SAC award may be reduced.)

12) Description of the proposed work/project (1-2 pages). Read evaluation criteria listed in the proposal writing guidelines at the end of this form for guidance:

Introduction

Baudoinia compniacensis (Whiskey Fungus) is mentioned as early as the 1881 French Mycology Review Journal, describing a black fungus growing on the walls of Cognac, (Roumeguere 1881) where the famous brandy is distilled (Scott, 2016). This leads to Whiskey Fungus being known for its trademark dry, raised, sooty black texture and its rapid growth where ethanol vapors are abundant. The enduring nature of *B. compniacensis* allows for it to grow on a variety of surfaces, both indoors and outside (Scott, 2007). *B. compniacensis* stains nearby buildings from the bottom up, decreasing property value significantly; leading to lawsuits being filed against Daigeo, a large American distillery in the case of *Merrick v. Daigeo* and Heaven Hill Distilleries, Incorporated in the case *Brown-Forman Corp. v. Miller*. These cases were used to set a precedent to determine whether large-scale distilleries should be held responsible for the diminishing property values caused by *B. compniacensis.* Since its discovery, there has been minimal research done regarding Whiskey Fungus germination. The experimental results used in the *Merrick v. Diageo* lawsuit were extracted from figure 4 of "Ethanol physiology in the warehouse-staining fungus, *Baudoinia compniacensis."*(Ewaze, 2008). This experiment proved problematic in terms of statistical significance regarding the lack of replications in the experiment, invalidating the significance of their results. The objective of this experiment is to provide statistically significant data about the effects of ethanol vapors on germination of *B. compniacensis* by emulating their methods with replications, while testing other variables such as carbon source, ethanol concentration, and fungus strain.

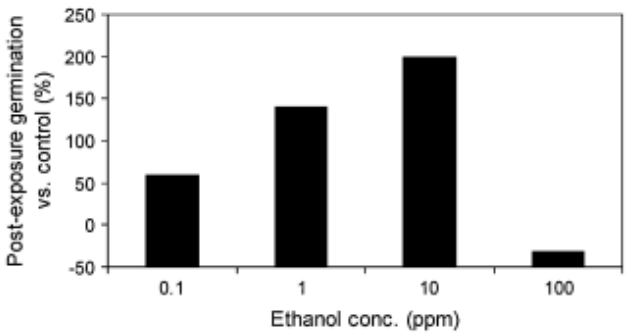

Fig 4 – Ethanol vapour stimulation or inhibition of colony formation by dormant inoculum of UAMH 10762. Zero on the ordinate was set by the germination level obtained under control conditions (no exposure to ethanol).

The figure shown above is figure 4 from "Ethanol physiology in the warehouse-staining fungus, *Baudoinia compniacensis,"* (Ewaze 2008) with its original caption. This same figure was used against Diageo in the case of Merrick vs. Diageo. This figure is supported only by one set of data, with no replications.

Methodology

Growing Cultures and Sample Preparation

Small amounts of a grown stock culture of *B. compniacensis* were taken from the classroom lab to produce new cultures. Four cultures were inoculated on Potato Dextrose Agar (PDA). The cultures were grown in an incubator for 14 days at 20°C (room temperature). To extract the propagules from the culture, garnet beads will be used to break open the propagules in 500uL distilled water. Colony forming units (CFU) will be counted using a hemocytometer and the solution will be adjusted to a concentration of 300 CFU's per 10uL. Using Henry's law, the concentration of ethanol vapor will be calculated for 0.1 - 100 ppm, and appropriate volumes of ethanol will be added in our bottles to reflect the needed headspace concentration.

Ethanol Vapor Experiment

The treatments in this experiment will be four concentrations of ethanol in distilled water ranging from 0.1- 100 ppm and a control with only distilled water. Each treatment will have five replications to assure statistical significance. The experimental setup of this experiment consists of 500 mL bottles that will be filled with the treatments and the control. 300 CFU's of *B. compniacensis* will be used to inoculate a filter membrane that will be placed within these bottles. The filter membrane will be affixed to the cap of the bottle from the inside and sealed with parafilm. The bottles will be left in an incubator for 14 days at 20°C to allow for germination. The filter paper will then be removed for analysis.

The illustration above depicts the experimental set up for the ethanol vapor experiment. The blue represents the solution of ethanol and distilled water, the red disc is the membrane that will house the propagules from *B. compniacensis* and the dark grey is the cap sealed with parafilm.

Results

Filter membranes will be removed and placed on slides to view under a microscope. Evidence of germination based on the number of visible colonies versus the number initially inoculated will be recorded. The results will be measured in a ratio of germination of treatments compared to the control. The experiment will use a 2-way ANOVA to determine whether the increase or decrease of germination is statistically significant in the presence of ethanol.

Conclusion

Based on the results of the experiment, we will be able to determine whether the research done by Ewaze (2008) is true, provide it statistical significance and contribute to the evidence used in the case of *Merrick vs. Diageo.* In addition, this experiment will expand the knowledge we have of *B. compniacensis*. If time and results permit, we will replicate the experiment measuring germination with other conditions, including more precisely defining significant ethanol concentrations based on significant data, evaluating other carbon sources such as acetone in place of ethanol, and alternative strains' propensity towards ethanol germination in the same genus, such as *B. panamericana*.

References

Ewaze, J. O., Summerbell, R. C., & Scott, J. A. (2008). Ethanol physiology in the warehouse-staining fungus, *Baudoinia compniacensis*. *Mycological Research*, *112*(Pt 11), 1373–1380. https://doi.org/10.1016/j.mycres.2008.05.003

Scott, J.A., Summerbell, R.C. (2016). Biology of the Whiskey Fungus. In: Li, DW. (eds) Biology of Microfungi. Fungal Biology. Springer, Cham. https://doi.org/10.1007/978-3-319-29137-6_16

Merrick v. Diageo Americas Supply, Inc., 5 F. Supp. 3d 865 - Dist. Court, WD Kentucky 2014

Brown-Forman Corp. v. Miller, 528 SW 3d 886 - Ky: Supreme Court 2017

1) Is the proposed research of sufficient quality and significance?
2) How will the proposed work benefit the student(s)/faculty/UVU?
3) Is the proposal written by the student demonstrating a clear understanding of the purpose and scope of the project? (Note: The proposal should not be written at the level of a faculty member who is an expert in the field.)
4) Has the proposal taken into account existing department resources that may be used for the project?
5) How complete is the budget narrative?

13) Describe planned outcomes, including dissemination of this work. Outcomes might include presentations by students at professional meetings or department seminars, senior theses, papers for peer-reviewed journals, other types of papers, etc. Please provide approximate dates of planned outcomes. Note: Travel funds to conferences for dissemination are available through separate URSCA dissemination and SAC dissemination applications.
    a) We are planning to replicate the research done by Juliet O. Ewaze in her paper Ethanol physiology in the warehouse-staining fungus, *Baudoinia compniacensis.* Her findings stated that the ethanol vapors from emitted whiskey led to the germination of whiskey fungus. We plan to do this by using five replicates per treatment to provide clear, more precise, and accurate results. We want to substantiate the claims of that paper and prove the significance of their findings as well.
    b) Currently, Diageo Americas Supply, Inc. is being sued by homeowners that claim that they are experiencing diminishing property values and quality of life due to whiskey fungus growing on everything outside of their homes. Brown-Forman Corporation and Heaven Hill Distilleries, Inc. are also

being sued by a homeowner for similar claims as the whiskey fungus has taken over their entire outdoor surfaces. Our findings would provide insight that could impact the outcome of these lawsuits along with future lawsuits that rely on the data from this report.

c) In December 2022, we plan to present our findings at Utah Valley University's end of semester poster presentation in the Science Building atrium.
d) Nicole Paulette: As a pre-med student planning on applying for medical school this application cycle, research is essential in being a strong candidate. Presenting our research at the end of the semester will provide a research presentation experience, which I have not yet had the opportunity to undergo.
e) Caleb Sawyer: I'm looking towards graduate school to study genomics, with an emphasis in fungal and virus pathology. This project creates multiple opportunities to present our findings locally, such as the 2023 Student Showcase. These findings can also lay a foundation for publication, leading to a heavy focal point for graduate school applications. There are also several experiment designs that can directly branch off from this one, allowing abundant research opportunities to pursue this upcoming year, through myself or otherwise.
f) Christine Nakonechnyy: I am a microbiology major with plans to pursue a PhD in microbiology. Being able to have this unique experience of working with fungi will aid me in expanding my range within microbiology. This experiment will provide me with opportunities to hone my skills in research, grant proposals, presentation and writing reports.
g) Denise Jimenez: I am planning on pursuing a PhD. I love research and this will give me another amazing opportunity to complete another research project that can help me prepare to complete even more research in the future. This experimental design can help me apply and practice materials taught in many courses such as statistics, chemistry, and biology.
h) We also consulted with statisticians' students Emmi Cottam and Mason Barnes.
i) Dr. Shurtleff was consulted on using Henrys Law and Raoults law.

14) Budget:

a) Budget Table (If the project spans fiscal years, indicate the proposed expenditures in each fiscal year. Use a table for each fiscal year. See SAC Guidelines below for more information.)

Materials/Supplies (add additional rows as needed)[1]	**Cost**
Tips (1000uL)	$0*
Tips (200uL)	$438.05
Tips (20mL)	$0*
Gloves (L)	$0*
Gloves (M)	$0*
Plates (Regular)	$0*
Plates (Small)	$0*
Bacto Agar	$0*
Malt Extract	$0*
500mL Bottles	$124.88
Filter Disks	$48.93
Ethanol (Pure)	$64.32
Ethanol (Denatured)	$66.52
Acetone	$25.79
Water	$0*
Original Culture UMAH 01808	$0**
Parafilm	$0*
Shipping costs:	$0*
Material/Supplies /Shipping Total:	**$768.49**

Table 1: Budget of individual items used for the project.

* Indicates an item that will be used, whose cost is shared/covered by another group that we will share materials with.
** We are using an original sample of *Baudoinia compniacensis* (UMAH01808) that Dr. Zahn provided.

Research-Related Travel Expenses	**Cost**
[2]Transportation	None needed
[3]Lodging fees	None needed
Other[4]	None needed
Travel Total:	$0

Total requested budget (material/supply + research travel):	$768.49

[1]Material/Supplies must be purchased by June 1 of the current fiscal year.
[2]Transportation: If you are requesting funds for transportation (airfare or UVU Fleet Operations), please provide destination, dates of travel, representative airfare estimates and number of people flying **or** UVU Fleet Operations Vehicle estimate cost. (https://www.uvu.edu/fleetops/). Students will not be reimbursed for private vehicle use.
[3]Lodging fees: If you are requesting funds for lodging, please provide the dates, city/cities, names and cost per night for required rooms in for modestly-priced hotels in appropriate areas.
[4]Other: If per diem is requested, there is a $30/day limit to align with the Office of Engaged Learning. Departments may supplement per diem. Work with department chair.

b) Budget Narrative: Briefly describe how the requested Materials/Supplies will be used in the research.

Gloves and tips will be used uniformly throughout the project, as well as denatured ethanol used for sterilization.

Plates, parafilm, tips, water, bacto agar, malt extract, and gloves will be used in culturing usable working cultures of Whiskey Fungus (original culture of UMAH 1808 provided by Dr. Zahn) for the procedural inoculations of the filter membranes.

The 500mL bottles, Ethanol (Pure), Acetone, parafilm, gloves and tips will be used in the procedure of our experiment, taking place inside the bottles. Pure ethanol and acetone will be used to create the appropriate headspace gaseous concentrations as described above. The filter disks will be suspended from the lids of the bottles, inoculated with approximately 300 cfu from our stock plates to test germination in the differing headspaces, with parafilm used to seal the jars.
We have no travel expenses, as the students involved will be commuting to campus and performing the experiment around their normally scheduled classes.

c) Travel Narrative: Briefly describe the travel needed to complete field work or travel to research sites. Travel is subject to UVU travel restrictions and policies.
There is no outside travel required for our project. Our research will be conducted inside the classroom.

Signature Page

Typed signatures are acceptable

1) Title of project: Effects of Ethanol Vapor on Germination of *Baudoinia compniacensis*

2) Lead Student Name and UVID: Nicole Paulette, UVID: 10839535

3) Faculty/Staff Mentor: Geoffrey Zahn

4) Start/stop dates of project: 9/1/22- 12/17/22

5) Names and UVIDs of other students involved in the project (if any):

Caleb Sawyer
UVID: 10781573

Christine Nakonechnyy
UVID: 10844630

Denise Jimenez
UVID: 10807655

By signing this form, we agree that:

1. This proposal was written by the student(s), with guidance from the faculty mentor.
2. A summary of the results of the project will be submitted to the faculty mentor and then COS Associate Dean SAC representative by the date indicated in the award notification. Failure to provide a summary may result in suspension of further funding for the student(s) and faculty mentor.
3. If awarded, all stipulations in the award notification will be followed.
4. The SAC Guidelines** applicable for student participation in faculty mentored research have been read and understood.

_Nicole Paulette_____ September 13th, 2022
Lead Student Applicant Date

Geoffrey Zahn _____ September 13th, 2022
Faculty Mentor Date

Chair or Department SAC representative:

Please meet with the student(s) on the proposal to review and discuss the application thoroughly. Ensure they understand the project and that the request makes appropriate use of existing department/college resources and funds.

I have met with the students. I have reviewed and support the proposal. The proposed research makes appropriate use of existing department and/or college resources and is appropriate in scope.

__Michael C. Rotter____ __9.13.2022_

Department Chair/Dept. SAC Representative Date

This signed form should be forwarded via email from the Department Chair or Department SAC Representative to the Associate Dean SAC Representative.

*Proposal Writing Guidelines and Limitations:

Each proposal submitted will be evaluated according to the following criteria. Keep in mind that the proposal should be understood by people who are scientifically literate, though not necessarily experts in your field.
Evaluation criteria

6) Is the proposed research of sufficient quality and significance?
7) How will the proposed work benefit the student(s)/faculty/UVU?
8) Is the proposal written by the student demonstrating a clear understanding of the purpose and scope of the project? (Note: The proposal should not be written at the level of a faculty member who is an expert in the field.)
9) Has the proposal taken into account existing department resources that may be used for the project?
10) How complete is the budget narrative?

SAC **may** provide funds for:

- Supplies, small equipment, and travel to research sites

SAC **will not** provide funds for:

- Major equipment, student wages, faculty per diem

****The following is an excerpt from the SAC Guidelines, Part 1.b.:**

Students who desire to participate in faculty-mentored research projects may apply at any time for an award to purchase supplies, small equipment, and travel to conduct research.

i. Individual students are limited to an award total of $3,000 toward research projects in their UVU career ($1,500 max per request).***
ii. Teams of students may submit one proposal, but the award will be limited to $1,500 per student with a maximum award of $5,000 per proposal. An equally divided award amount will be credited toward each student's career maximum.
iii. Student(s) must write the proposal and develop the budget, with guidance from their mentor. Student(s) must be directly involved in the research in a significant way and demonstrate a thorough knowledge of the research project and budget. Mentors should not be the primary authors of student submitted proposals.
iv. Student proposals may be submitted via email at any time to the Department Chair and/or Department SAC representative (check with Department for submission guidelines).
v. Proposals that span fiscal years need to specify what portion of the proposal will be used by June 1 of the current fiscal year and what portion will be used after July 1 of the next fiscal year. (Purchases must be made by June 1 to allow for shipping/receiving. Research related travel can be completed in June, but all documentation and submission of travel expenses must be completed before June 30.) The award may be split between the fiscal years.
vi. Department Chair and/or Department SAC representative will meet with the students(s) and faculty mentor to and review the proposal to:
    1. Ensure the request is completed by student(s) and they understand the nature and scope of the project (Department may choose to meet only with lead student and faculty mentor.)
    2. Ensure the request is for necessary supplies that makes appropriate use of SAC funds and existing department resources.
    3. Meets department guidelines for faculty-mentored research
vii. The Department Chair and/or Department SAC representative will review, sign, and forward the proposal via email to the Associate Dean SAC representative.