**Project Title:**

Novel control methods for California prionus beetle using entomopathogenic fungi

**Executive Summary:**

In the first year of this project, we determined through extensive laboratory testing that entomopathogenic nematodes were not going to be effective biological control agents for the California prionus beetle. Last year, we altered our original plan. We proposed to optimize control of CPB using entomopathogenic fungi (EPF). EPF are pathogens of insects widely found in soil and can contribute to CPB management. California prionus beetle (CPB) is a significant pest of commercial hops throughout the Pacific Northwest. The larvae and adult beetles present significant management challenges for growers when they are present. Historically, CPB management has relied on fumigation of hop yards before planting, pheromone traps for mating disruption and monitoring, and the use of insecticides such as ethoprop (e.g., Mocap EC). Unfortunately, these approaches do not meet current grower needs. EPF are commercially available and are effective against a number of insect pests. We tested several products containing entomopathogenic fungi (EPF) with the intent to deploy these pathogens using an autodissemination plan. Briefly, the adult beetles are attracted to a trap where they are exposed to EPF, infected, and then released. The CPB then succumb to the infection and produce new infective spores, which are environmentally stable and can infect new insects when they come into contact with them. During extensive laboratory testing, we have found several candidate EPF that infect and kill CPB, and within a week produce infective spores on the CPB cadavers. These infective spores, in turn, are highly pathogenic to CPB adults they contact.

**Proposed Duration:**

Year 2 complete, Year 3 proposal

**Project Leader:**

Edwin Lewis, Professor

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**Co-PL or Technical Assistance:**

Glen Stevens, Lab Manager

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**Cooperators:**

Diane and Michelle Gooding

Gooding Farms, Inc.

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Parma, Id 83660

**Amount Requested:**

$22,000

**Other Funding Sources and Support**

Other funding to contribute to the success of the proposal includes support for salary provided by Dr. Lewis to support Dr. Stevens through a number of other funding sources not related to this work.

**Send Funding To:**

HOLLY WATERS, Director
College of Agricultural and Life Sciences
Grant Management Services, Ag Science Room 304 hwaters@uidaho.edu
208-885-5999
875 Perimeter Drive, MS 2337

**Project Title:**

Novel control methods for California prionus beetle using entomopathogenic fungi

**Statement of Problem:**

The California prionus beetle (CPB) is a major pest of hops. Larvae live and feed on the roots of hop plants, resulting in decreased uptake of nutrients and water. This stress can result in wilting and, in severe instances, plant death. Secondary bacterial infections of the roots can also result from feeding damage. Current pest management revolves around monitoring, fumigants, and the use of Mocap EC. Unfortunately, these control methods are often ineffective. As such, new pest management strategies are needed to control problematic populations of CPBs to reduce crop damage. Management of this pest species is vital for the continued success of hop growers in the Pacific Northwest and the United States. We propose to develop improved control methods which utilize entomopathogenic fungi (EPF) for CPB management.

**Justification and Importance of Proposed Research:**

In 2019 Hop production encompassed 56,544 acres in Washington, Idaho and Oregon (NASS, National Hop Report). Integrated pest management (IPM) of CPB is notoriously difficult, with a limited range of chemical management options available. While CPB is generally thought of as a problem in Western Idaho, infested fields can be found throughout the Pacific Northwest. Indeed, CPB adults were seen in Boundary County, ID during the summer of 2021 (personal observation). Current management includes pre-planting fumigation, which costs > $350/ acre (Galinato and Tozer 2016); and can be done only one time in the life of a hopyard and the cost does not include removing the trellis systems. Curative treatment management relies on Mocap EC, a restricted used organophosphate with a 90 day preharvest interval (Barbour 2019). Alternatively, physical management can include removal of infested plants and fields in severe circumstances. Due to the limited options for physical and chemical control, new IPM is needed for the control of problematic populations of CPB. To this end, we proposed to develop novel approaches using entomopathogenic nematodes of the in-soil larval stages of the beetle.

In year 1 of this investigation, we set out to examine whether entomopathogenic nematodes could be used to control CPB by infecting the larval stage of this insect. CPB larvae were collected in the fall of 2020 and 2021 and screened with a suite of different nematode species and rates, and the addition of nematode related pheromones. Through a significant screening endeavor, we were only able to infect prionus larvae with *Steinernema glaseri* and *Steinernema riobrave* at a success rate of approximately 20%. While this adds significant new information to the scientific community, it is well below the needed threshold for an integrated pest management program to control CPB. Working within this same pest species, we pivoted pest management strategies to incorporate the use of entomopathogenic fungi (EPF). These fungi are pathogens of insects and have been previously incorporated into integrated pest management plans for multiple different species of agricultural pests, including aphids, beetles, lygus bugs and multiple other arthropod species. Currently available species of EPF which could be screened against CPB include *Beauveria bassiana*, *Cordyceps fumosorosea*, *Akanthomyces muscarius*, *Metarhizium anisopliae*, *Purpureocillium lilacinum*, and *Trichoderma harzianum*. This pivot resulted in targeting the adult above ground life stage and not the larval stage. Further, after initial infestation, the beetle can be released to spread the fungi to other CPB that they come into contact with before they die. This method is known as autodissemination. This pivot will require no funds beyond our original request for the third year, and we believe we can complete the project within the original timeframe outlined in our proposal.

Autodissemination IPM plans require 5 conditions to be successful: (1) A mobile stage of the target insect (adult CPB are highly mobile), (2) A strong and reliable attractant and trapping system (CPB sex pheromone is effective and commercially available), (3) an insect pathogen that can infect and kill the target insect (our new data supports this, see Figure 1), (4) infective stages of the pathogen must develop within the infected target (our new data also show this), and (5) the infective stages produced by the target cadaver must be able to infect healthy individuals of the same species (our new data show this as well). The pieces are all in place; we will now work on deployment.

**Objectives:**

1. Determine optimal EPN species for CPB control by laboratory and greenhouse tests.
	1. This objective led to the change in strategies to focus on EPF.
2. Screen multiple products/species of EPF for pathogenicity to and reproductive outcomes within CBP.
3. Develop a pheromone based CPB trap that will expose the beetles to lethal doses of EPF and allow their release thereafter.
	1. This is a new objective to be completed before the 2023 beetle flight.
4. Conduct field trials with optimized EPF species and application methods at Gooding Farms.
	1. This objective has changed but will be conducted summer 2023.

5) Develop extension bulletins and guidelines for growers for the use of EPNs to control CPB larvae and other soil dwelling pests of hops.

**Procedures/Methods to accomplish objectives:**



**Objective 2:**

We screened four products, based on either *B*. *bassiana* or *M. anisopleae* against CBP during summer, 2023. As can be seen in Figure 1, the product Botanigard 22WP (green line), based on *B. bassiana* was the most effective. Further, the infective spores of the fungus produced by the CPB cadaver were at least as pathogenic as the product. We will concentrate on this product hereafter. When larvae can be collected, we will conduct similar assays against them in the laboratory. When the adults disseminate the EPF, we need to know what the potential impact on larvae will be. Based on studies with other insect species, when adults are susceptible to infection, the larvae are as well.

**Objective 3:**

We are working currently on a trap design that will lure adult males to the trap, expose them to the pathogen of interest (Botanigard 22WP is the likely candidate) and let them escape after they have been exposed and infected. Several different trap designs have been developed for other pests, and we have reviewed them at some length. The likely design will be something similar to that shown in Figure 2, adapted for an in-ground deployment. We are using 3-D printing technology to fabricate the parts we need.

 **Objective 4:**

Field trials will be conducted in three different hop yards with a history of CPB infestation. The proposed field trials may require destructive sampling at the end of the season to assess CPB larval numbers but monitoring adult numbers will not require this. Specific field locations will be chosen in consultation with growers.

Traps (developed in objective 3) will be deployed when the first males are caught in sentinel traps; this will likely occur in mid-June. The field locations will be divided into two sections each, one with traps that include EPF and the other location will have an equal number of traps without EPF. The traps will be in place for 4 weeks. At the end of this period, the traps with EPF will be cleaned and further trapping will aim to assess populations of CPB. Plant vitality will be assessed throughout the growing season. Hop plants will be allowed to grow and destructively sampled for CPB and damage at the end of the growing season. Roots will be dug at the end of the growing season and rated for damage and recovered CPB will be recorded. Insects will be collected and brought back to the lab to be monitored for the presence of EPF infection. During the experiment, we will also note the presence of black vine weevil and false wireworm, both susceptible to EPF infection.

**Objective 5:**

 To provide guidance for growers on the use of EPF in IPM we will compile extension bulletins through the University of Idaho Extension Department on the use of EPNs in hop pest management. Bulletins will be developed by the PIs and will be provided as open access (free of charge) through University of Idaho Extension. The primary focus of this objective is to make the findings of this study impactful and easily incorporated into hop IPM.

**Outcomes:**

The overall outcome of this research is to provide hop growers an effective control method for CPB using autodissemination of EPF. With the optimization experiments outlined above, we expect to develop science-based, publicly available information to this end.

**Outcomes for Objective 1)** Through laboratory testing of EPNs, we have determined that this approach will not be effective.

**Outcome of Objective 2)** We have determined that EPF can be effectively deployed against CPB adults. Further effort will determine this efficacy against CPB larvae.

**Outcome of Objective 3)** An effective trap/release apparatus is under development currently.

**Outcome of Objective 4)** A field trial will allow us to assess the impact of EPF deployment. Follow-up monitoring of these areas will further inform the longer-term impact of EPF.

**Extension and Outreach Activities**:

All information generated during this project will be presented at the Hop Research Council Meetings and entomological conferences, incorporated into University of Idaho Bulletin, and written into a per reviewed academic journal. The overall goal of this research is to develop new management practices that will benefit hop growers in real time and provide hop growers with another tool for pest management in hop yards.

**ATTACHMENTS**

**Time Frame for Objectives:**

FY2022

 **May-September**

1. EPFs were evaluated against CPB adults in the laboratory
2. Several species/strains were effective against the adults
3. Further testing of the infectivity of EPF conidia produced by CPB cadavers against uninfected CPB showed that they were also highly effective killing CPB adults

**September-December**

1. Trap designs are evaluated for deployment
2. Fabrication of traps begins

FY2023

**January- May**

1. Trap design is refined and fabrication of units for field deployment is completed

 **May-September**

1. EPF autodissemination units will be installed to field trial areas upon the first detection of adults in the area
2. CPB populations monitored and bi-weekly plant health assessment for bines will be conducted

**September-October**

1) Hop plants will be dug and beetle numbers and damage to the root system will be examined. Samples will be transported to the University of Idaho main campus for assessment of infection by EPF.

**November-January**

1) All findings will be written into a scientific journal and extension bulletin.

**Project Budget:**

|  |  |
| --- | --- |
| Expenditure | Hop Research FY2023 |
|  |
| Salaries1 | 7,052 |  |
| Employee Benefits | 2,948 |  |
| Temporary or hourly workers | 1,500 |  |
| Travel2 | 3,000 |  |
| USA Hop Convention Registration | 800 |  |
| Grower Field Compensation | 3,000 |  |
| Equipment | 0 |  |
| Lab Supplies (including EPFs) | 2,700 |  |
|  |
| Publication Fee | 1,000 |  |
| Total | 22,000 |  |

1 CO-PD Glen Stevens will commit 0.12 FTE ($7,052/year for FY2023). Dr. Stevens primary function will be to assist with assessment of EPFs to be tested within field locations, data analysis. Benefits for Dr. Stevens are calculated at 41.8% per year ($5,896 total for 2 years)

2 One of the project directors will travel to the annual Hop Research Council Meetings once a year. Extensive travel for one of the project directors back and forth between Moscow and southern Idaho will be more necessary now that Dr. Clements is not part of this project.

**Other Funding Sources and Support:**

Other funding to contribute to the success of the proposal includes support for salary provided by the technician to conduct field work at the Parma Research Station, now supervised by Dr. Lewis.

**Literature Review:**

The production of hops is vital for the continued success of brewing. The hop plant, *Humulus lupulus,* produces flowers (cones) which are harvested for the oils that they contain. The oils are used in the brewing process for their bittering attribute, stability, and preservation of the fermented product. The hops industry in the United States has boomed in recent years, with a 5% increase in production from 2018 to 2019 resulting in revenue of $637 million (NASS, Hops). The production of hops has been adapted to trellis format and is commonly describe as hop yards. The initial financial comment to generate a hop yards is upwards of $14,000 per acre (Michigan State University, Extension) and requires thousands of dollars in pest management control during the life of the yard (which can exceed 10 years).

 There are multiple pest species that grower’s mange in order to provide a clean product to consumers, including plant pathogens and arthropod pests. Arthropod pests of hops include mites and insects. Some common pests are two spotted spider mite, black vine weevil, aphids, California prionus beetle and others. One of the major pest species that hop growers mange is the California prionus beetle (*Prionus californicus*) (CPB). This species is one of the largest beetles found in the United States. While adult CPB can be present in high numbers during the growing season, the adult life stage does not feed upon hop plants. Instead, the damage to the hop plant is the result of the feeding of the larval stage on roots (Barbour et al. 2006). Larvae take multiple years to mature within the hop fields, and during development they continually feed on the root system and burrow into root tissue (Field guide for Integrated Pest Management of Hops, 2017). Damage from larval feeding results in tissue death, loss of nutrient and water movement through the hop plant, and secondary infections from pathogens (Barbour 2011). Fiscal cost of damage from feeding larvae can be hard to quantify because pest management activities range from removal of infected plants to removal of infected fields (Beetles, Hop Integrated Pest Management, 2017). Current chemical management relies on the use of pre-planting fumigants, monitoring of adult male beetles through pheromone traps, and the use of Mocap EC (Barbour 2011).

The use of EPF in IPM has been established as a biological control method for multiple pest species (Skinner et al. 2014). The EPF life cycle is ideal for deployment in autodissemination systems when certain conditions are met. These conditions have been described elsewhere in the document. Successful deployment of EPF has been achieved against Japanese beetles (Klein and Lacey 2010), tsetse flies (Maniania and Ekesi 2013), black vine weevil (Pope et al. 2018) and many other insect species. To use this technique against CPB successfully requires the optimization steps outlined in this proposed work.

 The current lack of registered chemicals and the development of chemical resistance in multiple pest species of hops warrant the investigation of new approaches for pest management. Through revisiting and revising current pest management approaches, science-based extension recommendations can provide guidance to growers for sustained crop production, including developing new biorational pest controls to mitigate off target effects, prevent insecticide resistance, and provide a lasting improvement on environmental impact. With proper management, hop production in the Pacific Northwest and the United States will continue to flourish.

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**Curriculum Vitae**

**Edwin E. Lewis**

**Education**

Ph.D. Entomology. 1991. Auburn University, Auburn, AL.

M.S. Entomology. 1987. University of Missouri, Columbia, MO.

B.S. Natural Resources. 1980. Cornell University, Ithaca, NY.

A.A.S. Science and Mathematics. Cayuga County Community College, Auburn, NY.

**Professional Experience**

Current Professor, Dept. Entomology, Plant Pathology and Nematology, University of Idaho

2014-2017 Associate Dean, College of Agricultural and Environmental Sciences, University of California, Davis

2008-2017 Professor, Dept. Nematology / Entomology, University of California, Davis

2004-2008 Associate Professor, Depts. Nematology / Entomology, University of California, Davis

2004 Associate Professor/Cooperative Extension Specialist, Dept. of Entomology, Virginia Tech

1998-2004 Assistant Professor/Cooperative Extension Specialist, Dept. of Entomology, Virginia Tech

1995-98 Research Assoc., Dept. of Entomology, University of Maryland.

1994-95 Assistant Research Professor, Dept. of Entomology, Rutgers University.

1991-94 Post-doctoral Research Assoc., Dept. of Entomology, Rutgers University.

**Publication Summary**

Refereed Papers: 124

Book Chapters: 12

Edited Books: 1

Patents: 2

**Professional Societies**

Entomological Society of America

Society of Invertebrate Pathology

**leadership and Professional Service**

Current

* Co-Director, Institute for Health in the Human Ecosystem, University of Idaho
* Editor-in-Chief, Biological Control (Published by Elsevier)

**Teaching**

* Entomology 135: Biological Control – Principles of biological control of arthropod pests and weeds. Biology of pathogens, entomopathogenic nematodes, parasitoids, and predators. Implementation in classical and augmentative biological control. Role of biological control in pest management. (20 – 30 students)
* Entomology 104: Behavioral Ecology of Insects – Basic principles and mechanisms of insect behavior and ecology. An evolutionary approach to understanding behavioral ecology of insects. (275 – 300 students)

**Publication Record (last 4 years)**

Refereed Publications

Erdogan, H., Stevens, G., Stevens, A., Shapiro-Ilan, D., Kaplan, F., Alborn, H., Lewis, E.E. 2021. Infected host responses across entomopathogenic nematode phylogeny. Journal of Nematology. DOI: 10.21307/jofnem-2021-105

Nikoukar, A., Ensafi, P., Lewis, E.E., Crowder, D.W., Rashed, A. 2021. Efficacy of naturally occurring and commercial entomopathogenic nematodes against sugar beet wireworm (Coleoptera: Elateridae). Journal of Economic Entomology. 114: 2241-2244.

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Rodriguez A.M., Hambly, M.G., Jandu, S., Simão-Gurge, R., Lowder, C., Lewis, E.E., Riffell, J.A., Luckhart, S. 2021. Histamine ingestion by Anopheles stephensi alters important vector transmission behaviors and infection success with diverse Plasmodium species. Biomolecules. 11(5). doi: 10.3390/biom11050719.

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Oliveria-Hofman, C., F. Kaplan, G. Stevens, E.E. Lewis, S.H. Wu, H.T. Alborn, A. Perret-Gentil, and D. Shapiro-Ilan. 2019. Pheromone extracts act as boosters for entomopathogenic nematode efficacy. Journal of Invertebrate Pathology. 164: 38-42.

Li, C., X. Zhou, E.E. Lewis, Y. Yu and C. Wang. 2019. Study on host-seeking behavior and chemotaxis of entomopathogenic nematodes using Pluronic F-127 gel. Journal of Invertebrate Pathology. 161: 54-60.

Gulcu, B., A. Hodson, V. Omaleki, A.B. Ross and E.E. Lewis. 2019. A biological control approach to reducing Naupactus godmani (Curculionidae) populations in citrus using entomopathogenic nematodes. Crop Protection. 115: 99-103.

Wu, S.H., F. Kaplan, E.E. Lewis, H.T. Alborn, D.I. Shapiro-Ilan. 2018. Infected host macerate enhances entomopathogenic nematode movement towards hosts and infectivity in a soil profile. Journal of Invertebrate Pathology. 159: 141-144.

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Parrella, M.P. and E.E. Lewis. 2017. Biological control in greenhouse and nursery production: Present status and future directions. American Entomologist. 63: 237–250.

**Biographical Sketch for Glen Stevens**

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**Education**

Ferrum College, B.S., Environmental Science, 1997

Virginia Tech, M.S. Biology, 2001

Virginia Tech, PhD Biology, 2005

**Academic Employment**

**2018 – present:** Lab Manager, Department of Entomology, Plant Pathology and Nematology, University of Idaho

**2014 – 2018:** Associate Professor of Biology and Environmental Science, Ferrum College

**2008 – 2013:** Assistant Professor of Biology and Environmental Science. Ferrum College

**2007 – 2014:** Research Scientist.Conservation Management Institute, Virginia Tech

**2005 – 2007:** Postdoctoral Research Fellow. Department of Nematology, University of California, Davis

**2001 – 2005:** Maly Research Fellow. Department of Biology, Virginia Polytechnic Institute and State University

**1998 – 2001:** Graduate Research Assistant. Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA

**1996:** Field and lab assistant, soil ecology. Institute of Ecology, University of Georgia, Athens, Georgia

**Teaching Experience**

General Biology I and II (lecture and laboratory), Entomology (lecture and laboratory), Intro Environmental Science (lecture and laboratory), Environmental Methods and Statistics (l & l), Fundamentals of Ecology (l & l), Conservation Biology (lecture), Introductory Soil Science (lecture and laboratory), Professional Preparation (lecture), Senior Seminar (lecture), and Internship (experiential).

**Relevant Professional Experience**

Lead PI on a multi-year USDA Conservation Innovation Grant, leading efforts at field data collection on multiple private- and publicly-owned sites in the Chesapeake Bay region of Virginia.

Co-Principal Investigator at a site-intensive assessment of environmental impacts of land conversion efforts. Research included comprehensive assessment of impacts of restoring native grasses; my efforts focused on soil assessments and insect community responses.

Several grants to public and private organizations to assess impacts of manipulations on beneficial and plant parasitic nematodes.

For 10 years, worked with undergraduates to search for and obtain required internship experiences. This required networking between students, professional contacts, and off-campus site visits.

Led an active undergraduate lab focused on responses of entomopathogens to manipulations; this involved coordination between industry and students. Six students trained, including several who went on to graduate school and/or positions at USDA-ARS.

**Research Publications (since 2010)**

Erdogan, H., Cruzado-Gutierrez, K., Stevens, G., Shapiro-Ilan, D., Kaplan, F., Alborn, H., & Lewis, E. 2021. Nematodes Follow a Leader. Frontiers in Ecology and Evolution, 786.

Erdogan, H., Stevens, G., Stevens, A., Shapiro-Ilan, D., Kaplan, F., Alborn, H. and Lewis, E.E. 2021. Infected host responses across entomopathogenic nematode phylogeny. Journal of Nematology, vol.53, no.1, pp.1-9.

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