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DEPARTMENT OF AGRICULTURE
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October 28, 2022

To: Advisory Committee on Plants and Animals

From: Peter Follett, Ph.D.
Agricultural Research Service (ARS)
United States Department of Agriculture (USDA)

Mark Wright, Ph.D.
Department of Plant & Environmental Protection Sciences
University of Hawaii at Manoa (UHM)

Through: Christopher Kishimoto
Entomologist
Plant Quarantine Branch
Hawaii Department of Agriculture (HDOA)

Subject: Request to: (1) Allow the Importation and Possession of Lab-Reared Strains of the Parasitoid Wasp, *Phymastichus coffea* (Hymenoptera: Eulophidae), an Insect on the List of Restricted Animals (Part A), by Permit, for Field Release to Control the Coffee Berry Borer, *Hypothenemus hampei*, by the USDA ARS and UHM;

(2) Establish Permit Conditions for the Importation and Field Release of Lab-Reared Strains of the Parasitoid Wasp, *Phymastichus coffea* (Hymenoptera: Eulophidae), an Insect on the List of Restricted Animals (Part A), to Control the Coffee Berry Borer, *Hypothenemus hampei,* by the USDA ARS and UHM; and

(3) Determine the Probable Impact to the Environment if Lab-Reared Strains of the Parasitoid Wasp, *Phymastichus coffea* (Hymenoptera: Eulophidae), an Insect on the List of Restricted Animals (Part A) Are Accidentally Released.

I. Summary Description of the Request

PQB NOTES**:** *The Plant Quarantine Branch (PQB) submittal for requests for import or possession permits, as revised, distinguishes information provided by the applicants from procedural information and advisory comment and evaluation presented by PQB. With the exception of PQB notes, hereafter "PQB NOTES," the text shown below in section II from page 3 through page 7 of the submittal was taken directly from the applicants', Dr. Follett and Dr. Wright, application and subsequent written communications provided by the applicants. For instance, the statements on pages 5 through 7 regarding effects on the environment are the applicants' statements in response to standard PQB questions and are not PQB's statements. This approach for PQB submittals aims for greater applicant participation in presenting import requests in order to move these requests to the Board of Agriculture (Board) more quickly, while distinguishing applicant provided information from PQB information. The portion of the submittal prepared by PQB, including the environmental assessment and proposed permit conditions, are identified as sections III and IV of the submittal, which start at pages 7 and 8 respectively.*

Commodity: Multiple shipments of lab-reared strains of the parasitoid wasp *Phymastichus coffea* Lasalle (Hymenoptera: Eulophidae).

Shippers: (1) Pablo Benavides
Centro Nacional de Investigaciones de Café (Cenicafé)
Manizales, Colombia
pablo.benavides@cafedecolombia.com.co

(2) Jose Carlos Verle Rodrigues
University of Puerto Rico
1193 Guayacan Street
South Botanical Garden, San Juan, Puerto Rico 00926
jose_carlos@mac.com

Importers: (1) Peter Follett, Ph.D.
USDA ARS
U.S. Pacific Basin Agricultural Research Center
64 Nowelo Street
Hilo, Hawaii 96720
Ph: (808) 959-4303
peter.follett@usda.gov

(2) Mark Wright, Ph.D.
University of Hawaii at Manoa
Department of Plant and Environmental Protection Sciences
College of Tropical Agriculture and Human Resources
Gilmore Hall, Room 511A
3050 Maile Way
Honolulu, Hawaii 96822
Ph: (808) 956-7670
markwrig@hawaii.edu

Category: *Phymastichus coffea* is currently on HDOA's List of Restricted Animals (Part A). Chapter 4-71, Hawaii Administrative Rules (HAR), allows importation of animals on this list to Hawaii for research by universities or government agencies, exhibition in municipal zoos or government-affiliated aquariums, or for other institutions for medical or scientific purposes as determined by the Board.

II. Information Provided by the Applicant in Support of the Application

An application was submitted by the United States Department of Agriculture (USDA) Agricultural Research Service (ARS), Hilo, HI, and University of Hawaii at Manoa Honolulu, HI, to the Hawaii Department Of Agriculture (HDOA) Plant Quarantine Branch (PQB), 1849 Auiki Street, Honolulu, Hawaii 96819, for a permit to introduce *Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae) into the State of Hawaii under the provisions of Hawaii Revised Statutes, Chapter 141, Hawaii Revised Statutes (HRS) and Chapter 150A, HRS, Plant and Non-Domestic Animal Quarantine. *Phymastichus coffea* will be used to control the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae), a serious invasive pest of coffee in Hawaii.

Phymastichus coffea was obtained from Cenicafé in Colombia under USDA Animal Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ), permit number P526P-18-00696 and brought into a fully certified quarantine insect containment facility managed by the USDA Forest Service at Hawaii Volcanoes National Park, Volcano, Hawaii, for host-specificity testing.

Purpose:

This is an application for a permit to import and field release the parasitoid *Phymastichus coffea* (Hymenoptera: Eulophidae) against the coffee berry borer (CBB), *Hypothenemus hampei* (Coleoptera: Curculionidae) in Hawaii coffee. The coffee berry borer is the most serious pest of coffee in most coffee producing countries. In Hawaii, CBB was first reported in 2010 from South Kona and soon spread throughout Hawaii island coffee farms and to the other islands. The coffee berry borer severely affects the yield and quality of

coffee and it is an important constraint on production and development of the crop. The roasted value of Hawaii-grown coffee is estimated to be over $100 million annually. The current crop losses of coffee due to CBB infestation in Hawaii is estimated at $7.7 million. If left uncontrolled coffee berry borer can infest >90% of coffee berries. The primary means of controlling CBB in Hawaii is use of the microbial insecticide *Beauveria bassiana* and sanitation (removal of all coffee berries after harvest). The control of this pest with *Beauveria bassiana* is expensive and has limited success if the borer has reached the endosperm of the seeds. Biological control using parasitoids is a sustainable option to manage the coffee berry borer. *Phymastichus coffea* has proven to be an effective biological control agent of CBB in other coffee growing regions in the world, especially Central and South America. Furthermore, *P. coffea* is the only parasitoid tested thus far that has been shown to reduce yield loss from CBB damage. *P. coffea* has the potential to be an effective biological control agent against the coffee berry borer in Hawaii.

DISCUSSION:

1. Persons Responsible:

Peter Follett, USDA-ARS, 64 Nowelo St., Hilo, HI 96720, (808) 443-8031, peter.follett@usda.gov

Mark Wright, Department of Plant and Environmental Protection Sciences, UH-Manoa, 3050 Maile Way, Honolulu, HI 96822, (808) 956-6737, markwrig@hawaii.edu

2. Safeguard Facility and Practices:

P. coffea will be imported as larvae or pupae infesting Coffee Berry Borer adult beetles. At Cenicafe, parchment coffee used to rear the CBB that will be parasitized will be immersed in fungicides for a period of 12-15 hours to kill any potential fungal pathogens. Adult CBB will be disinfected with a 3% solution of benzalkonium chloride, which acts as a disinfectant, surfactant, bacteriacide, and inhibitor of viral activity. To help separate out the parasitized CBB, the beetles will be exposed to an artificial light source for 15 hours at a temperature of 28°C (82.4°F) at 75%-80% relative humidity so that the live CBB fly to the light while only the parasitized motionless CBB will remain. A portion of the parasitized CBB will also be looked at under a microcope to eliminate any unparasitized dead CBB and other irregularities to ensure quality control. There are no known hyperparasitoids of *P. coffea* in Colombia. No coffee plants or plant material will be shipped to Hawaii.

Shipments will be sent to a quarantine facility at 1) USDA Forest Service at Hawaii Volcanoes National Park or 2) Hawaii Department of Agriculture, 1428 S. King St.

Honolulu, HI initially for positive identification of *Phymastichus coffea* and determination of colony purity. Afterward, colonies will be maintained by Wright and Follett for a minimum of 2 generations before field release, in their laboratories without taking extraordinary precautions to mitigate accidental release. Our objective is to deliberately release *P. coffea* wasps in Hawaii coffee plantations. In the laboratory, *P. coffea* will be reared on adult CBB in plexiglass or fine mesh screen cages with sleeved entry ports.

PQB NOTES**:** *The applicants are currently not allowed to possess P. coffea in HDOA's quarantine facility, any UH facility, USDA's ARS PBARC facility or any other location on State or County lands without completing the State's Office of Planning and Sustainable Development's Environmental Review Program requirements pursuant to Chapter 343, Hawaii Revised Statutes (HRS).*

The applicants are currently going through the State's environmental review process for this application.

3. Method of Disposition:

Any unused material, including packing material, plant material, and host material, including dead insects, etc. will be autoclaved within the quarantine facility. Reared parasitoid adults will be released in coffee plantations throughout the State.

4. Abstract of Organism:

Phymastichus coffea is an idiobiont, gregarious endoparasitoid of adult coffee berry borer, commonly laying two eggs (a male and a female) per host. Both a male and female develop in a single host, the female in the abdomen and the male in the prothorax, although a single female parasitoid is sometimes found living solitarily in the abdomen of the host. This parasitoid develops through four major life stages—egg, larva (three instars lasting ~21 days), pupa (~9 days) and adult. The complete development (egg to adult) occurs over 30-43 days depending on temperature and condition of the CBB host mummies. For example, at 23°C (73.4°F) the life cycle of *P. coffea* is 43 days. The parasitoid emerges by cutting an opening in the host's integument. The average lifespan of the parasitoid is 1-2 days for males and 3-4 days for females. Upon emergence, female parasitoids can have up to 10 eggs in the ovarioles, but more eggs are formed throughout her short lifetime (synovigenic strategy). There is no preoviposition period and the adult female parasitoids can parasitize the coffee berry borer adults immediately after emergence. It has been shown that *H. hampei* is attracted to semiochemicals released from coffee fruits; semiochemicals released during *H. hampei* feeding on fruits have been shown to attract *P. coffea* and may play a significant role in mediating the host specificity of this parasitoid under field conditions.

5. Potential Effects on the Environment and Health:

Phymastichus coffea was chosen as the best candidate parasitoid in Hawaii because of its previously reported high host specificity and ability to significantly reduce and regulate *Hypothenemus hampei* (coffee berry borer) populations in the field. Using the centrifugal phylogenetic method, a widely accepted approach for non-target host screening, host range testing was conducted in quarantine in Hawaii to determine whether *P. coffea* might attack non-target species in addition to coffee berry borer, and thereby pose a risk to the environment (see Yousuf et al. 2020). *H. hampei* is a small bark beetle in the subfamily Scolytinae, so host range testing focused on other scolytine bark beetles, including several other exotic *Hypothenemus* spp. and native species in the genus *Xyleborus*, as well as a variety of other small Curculionidae and other coleopterans. Using no-choice tests, a total of 43 different species of Coleoptera were tested, including 23 scolytines (6 *Hypothenemus* species, 7 native *Xyleborus* species, and 10 others), and 4 additional Curculionidae*. P. coffea* was only able to parasitize the target host *H. hampei* (coffee berry borer) and 4 other adventive species of *Hypothenemus: H. obscurus* (tropical nut borer), *H. seriatus, H. birmanus and H. crudiae*. *Hypothenemus hampei* had the highest parasitism rate and shortest parasitoid development time of the five parasitized *Hypothenemus* spp. Parasitism and parasitoid emergence decreased with decreasing phylogenetic relatedness of the *Hypothenemus* spp. to *H. hampei*, and the most distantly related species tested, *H. eruditus*, was not parasitized. There are no native Hawaiian species in the genus *Hypothenemus*. No species in any of the other genera tested were parasitized, including 7 species of native Hawaiian *Xyleborus*. Our research indicates *Phymastichus coffea* appears to be host-specific at the genus level - only able to survive on species closely related to *H. hampei* and would not parasitize other beetle species released for other biocontrol projects by the Hawaii Department of Agriculture (See Attachment 5).

Additionally, host plant volatiles can be important in host location by parasitoids. It should also be considered that *P. coffea* responds to cues associated with the host plant of *H. hampei* (Rojas et al. 2006), and this interaction is very likely to ensure high host fidelity under field conditions.

Therefore, release of *P. coffea* for control of CBB in Hawaii coffee should cause no harm to the environment. However, no level of host specificity testing can ensure zero risk to non-target organisms when introducing a natural enemy in a new habitat. Release of *P. coffea* will be a permanent, non-reversible action*. P. coffea* is not expected to attack any native Hawaiian species or disrupt native ecosystems given its high host specificity and short life span. Therefore, undesired environmental impacts are not anticipated. (Note: *P. coffea* uses its ovipositor to lay eggs in insect hosts only and does not sting humans.)

References:

Rojas J.C., A. Castillo, and A. Virgen. 2006. Chemical cues used in host location by *Phymastichus coffea*, a parasitoid of coffee berry borer adults, *Hypothenemus hampei*. Biological Control 37(2): 141-147.

Yousuf, F., P. A. Follett, C.P.D.T. Gillett, D. Hornsberger, L. Camorro, M. T. Johnson, M. Giraldo-Jaramillo, P. Benavides-Machado, and M. G. Wright. 2021. Limited host range in the idiobiont parasitoid *Phymastichus coffea* (Hymenoptera: Eulophidae), a prospective biological control agent of the coffee pest *Hypothenemus hampei* in Hawaii. Journal of Pest Science. doi.org/10.1007/s10340-021-01353-8

III. Environmental Assessment (EA)

Pursuant to a May 2008 Hawaii Intermediate Court of Appeals decision (Ohana Pale Ke Ao v. Board of Agriculture, 118 Haw. 247 (Haw. App. 2008), the Department of Agriculture's (Department's) import permit process is subject to the requirements of the Hawaii Environmental Protection Act, chapter 343, Hawaii Revised Statutes (HRS). Under this decision, the requirement for an EA as a condition of the import permit or related authorization applies in those circumstances where the underlying permit activity for the importation initiates a "program or project" and where the use of state or county funds or state or county lands is involved. When those circumstances are present, as they appear to be when a new organism is used in a new program or project located at a facility located at UHM or UHH (state lands), an EA is required to determine whether the proposed project or program is likely to have a significant impact on the environment. However, certain activities may be eligible for "exemption" under provisions established through the Environmental Advisory Council, provided that the project or program is determined to have little or no impact on the environment.

Analysis of Application re EA: Under the above-cited court decision, the EA requirement is triggered under certain circumstances, including when an applicant proposes an action on state or county lands that requires agency approval and is not specifically exempted under Chapter 343, HRS.

This appears to be the case here. The applicants' request in this instance involves importation and possession of *Phymastichus coffea* for field-release as biocontrol of *Hypothenemus hampei* (coffee berry borer) in the environment.

PQB NOTES**:** *Drs. Follett and Wright are currently initiating the environmental review process as a requirement of this project. They have not yet submitted a Draft EA to the*

State of Hawaii's Office of Planning and Sustainable Development's Environmental Review Program. However, they have submitted a Draft EA with an Anticipated Finding of No Significant Impact (Attachment 5), to USDA APHIS for its review.

IV. Proposed Permit Conditions

1. The restricted article(s), lab-reared strains of the parasitoid wasp, *Phymastichus coffea*, including progeny, shall be used for field release to control the coffee berry borer, *Hypothenemus hampei*, a purpose approved by the Board of Agriculture (Board). The restricted article(s), including progeny, shall not be sold, given away, or transferred except as approved by the Board.

2. The permittee(s), Dr. Peter Follett, U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS), 64 Nowelo St., Hilo, HI 96720 and Dr. Mark Wright, Department of Plant and Environmental Protection Sciences, UH-Manoa, 3050 Maile Way, Honolulu, HI 96822, shall be responsible and accountable for all restricted article(s) imported, including progeny, from the time of their arrival until their final disposition.

3. Upon import or while under observation for pest infestation or colony reproduction, the restricted article(s) shall be safeguarded and maintained at the approved site, the USDA approved Insect Containment Facility, USDA Forest Service (FS), Hawaii Volcanoes National Park Quarantine Facility, Kilauea Research Station, Building 34, Volcano, HI 96718, a site inspected and approved by the Plant Quarantine Branch (PQB) prior to importation, by trained or certified personnel designated by the permittee(s).

4. Less than 24 hours prior to field release, the restricted article(s) may be taken out of quarantine and kept at another PQB-inspected and approved facility until their release.

5. The restricted article(s) shall not be reared, bred, stored, or kept in any state or county-owned or operated facility, or maintained or otherwise utilized with state or county funding.

6. Upon request by the PQB, the permittee(s) shall submit samples of the restricted article(s) prior to importation to the PQB.

7. The restricted article(s) shall be screened for other species, predators, parasites, parasitoids or hyperparasitoids for a minimum of two generations in the USDA approved Insect Containment Facility, USDA FS, Hawaii Volcanoes National Park Quarantine Facility, Kilauea Research Station, Building 34, Volcano, HI

96718. A report shall be submitted to PQB detailing the discovery of any organisms besides the restricted article(s).

8. Upon receipt of the restricted article(s) at the approved site:

   a. All packing material shall be sterilized with a minimum 0.5% sodium hypochlorite solution or autoclaving or destroyed by autoclaving or incineration.

   b. All unused or uninfested biological material including plant material, dietary or ovipositional material, and soil shall be destroyed by autoclaving or incineration.

9. In the event the restricted article(s) become parasitized or infected by disease, the permittee(s) shall:

   a. Destroy the entire lot of the restricted article(s) by freezing;

   b. Autoclave all insects, dietary and oviposition media; and

   c. Subject all used cages and equipment to autoclaving or treatment with a bleach solution containing at least 0.5% concentration of sodium hypochlorite.

10. At least 48 hours prior to shipping any parcel containing the restricted article(s), the permittee(s) shall notify the PQB Chief in writing and provide the following information:

   a. Expected arrival date;

   b. Waybill, bill of lading, and/or tracking number;

   c. Name and address of the shipper.

   d. Name and address of the importer or importer's agent in the State of Hawaii;

   e. Number of packages;

   f. Description of contents of each package (including scientific name); and

   g. Port of entry into the State.

11. At least four sides of all parcels containing the restricted article(s) imported into the State shall be clearly and legibly marked: "**This parcel may be opened and delayed for agricultural inspection in Hawaii**" in ½ inch minimum sized font.

12. The restricted article(s) shall be shipped in sturdy PQB-approved containers designed to be escape-proof and leak-proof.

13. Each shipment of the restricted article(s) shall be accompanied by a complete copy of the PQB permit for the restricted article(s) and an invoice, packing list or other similar PQB-approved document listing the scientific and common names of the restricted article(s), the quantity of the restricted article(s), the shipper, and the permittee(s) for the restricted article(s).

14. All parcels containing the restricted article(s) shall be subject to inspection by the PQB prior to entering the State and shall be imported through the port of Honolulu except as designated by the Board. Entry into Hawaii through another port is prohibited unless designated by the Board.

15. The approved site, restricted article(s), progeny, records, and any other document pertaining to the restricted article(s) and progeny under this permit, may be subject to post-entry inspections by the PQB. The permittee(s) shall make the site, restricted article(s), progeny, and records pertaining to the restricted article(s) available for inspection upon request by a PQB inspector.

16. It is the responsibility of the permittee(s) to comply with any applicable requirements of municipal, state, or federal law pertaining to the restricted article(s).

17. The permittee(s) shall submit to the PQB Chief a copy of all valid licenses, permits, certificates or their equivalent required for the restricted article(s) or for their import, possession, movement, or transfer. The permit issued by the PQB Chief may be cancelled upon revocation, suspension, or termination of any of the aforementioned documents.

18. The permittee(s) shall submit an annual report to the PQB by January 31st on results of the post release monitoring programs for the prior calendar year, and shall include the following:

    a. Number of restricted article(s) released and number of releases;

    b. Establishment and current field populations of the restricted article(s);

    c. Effects of the restricted article(s) on native plant and animal species;

d. A summary of any significant changes to the permittee's operation, personnel, and/or procedures regarding the restricted article(s) and progeny; and

e. Any significant events that occurred at the permittee's site regarding the restricted article(s) or progeny.

19. The permittee(s) shall adhere to the use, facility, equipment, procedures, and safeguards described in the permit application, and as approved by the Board and the PQB Chief.

20. The permittee(s) shall have a biosecurity manual available for review and approval by the PQB, at the time of the initial site inspection and any subsequent post-entry inspection(s), which identifies the practices and procedures to be adhered to by the permittee(s) to minimize or eliminate the risk of theft, escape, or accidental release of the restricted article(s), including the risk of introduction and spread of diseases and pests associated with the restricted article(s) to the environment. The permittee(s) shall adhere to all practices and procedures as stated in this biosecurity manual.

21. The permittee(s) shall immediately notify the PQB Chief verbally and in writing under the following circumstances:

a. If any escape, theft, accidental release, parasitoid, hyperparasitoid, or other pest or disease outbreaks involving the restricted article(s) under this permit occurs.

b. If any changes to the approved site, facility, and/or procedures regarding the restricted article(s) or progeny occur or are to be made, the permittee(s) shall obtain written approval from the PQB Chief as soon as practicable (if unplanned) or prior to implementation (if planned). Also, the permittee(s) shall submit a written report documenting the specific changes to the PQB Chief.

c. If a shipment of the restricted article(s) is delivered to the permittee(s) without a PQB "Passed" stamp, tag or label affixed to the article, container, or delivery order that indicates that the shipment has passed inspection and is allowed entry into the State, then the permittee(s) shall not open or tamper with the shipment and shall secure, as evidence, all restricted article(s), shipping container(s), shipping document(s) and packing material(s) for PQB inspection.

d. If the permittee(s) will no longer import or possess the restricted article(s) authorized under this permit.

22. The permittee(s) shall be responsible for all costs, charges, or expenses incident to the inspection, treatment, or destruction of the restricted article(s) under this permit, as provided in Act 173, Session Laws of Hawaii 2010, section 13, including, if applicable, charges for overtime wages, fixed charges for personnel services, and meals.

23. Any violation of the permit conditions may result in citation, permit cancellation, and enforcement of any or all of the penalties set forth in HRS §150A-14.

24. A cancelled permit is invalid and upon written notification from the PQB Chief, all restricted article(s) listed on the permit shall not be imported. In the event of permit cancellation, any restricted article(s) imported and in the permittee(s)' possession under permit may be moved, seized, treated, quarantined, destroyed, or sent out of State at the discretion of the PQB Chief. Any expense or loss in connection therewith shall be borne by the permittee(s).

25. This permit or conditions of this permit are subject to cancellation or amendment at any time due to changes in administrative rules restricting or disallowing import of the restricted article(s) or due to Board action disallowing a previously permitted use of the restricted article(s).

26. These permit conditions are subject to amendment by the PQB Chief in the following circumstances:

    a. To require disease screening, quarantine measures, and/or to place restrictions on the intrastate movement of the restricted article(s), as appropriate, based on scientifically validated risks associated with the restricted article(s), as determined by the PQB Chief, to prevent the introduction or spread of disease(s) and/or pests associated with the restricted article(s).

    b. To conform to more recent Board approved permit conditions for the restricted article(s), as necessary to address scientifically validated risks associated with the restricted article(s).

27. The permittee(s) shall agree in advance to defend and indemnify the State of Hawaii, its officers, agents and employees for any and all claims against the State of Hawaii, its officers, agents, employees, or Board of Agriculture members that may arise from or be attributable to any of the restricted article(s) that are introduced under this permit. This permit condition shall not apply to a permittee that is a federal or State of Hawaii entity or employee, provided that the State of federal employee is a permittee in the employee's official capacity.

V. ADVISORY SUBCOMMITTEE REVIEW: This request was submitted to the Advisory Subcommittee on Entomology for its review and recommendation. Advisory Subcommittee recommendations and comments are as follows:

1. I recommend approval____/____disapproval to allow the importation of lab-reared strains of the parasitoid wasp, *Phymastichus coffea*, an insect on the List of Restricted Animals (Part A) for field release to control the coffee berry borer, *Hypothenemus hampei*, by the USDA ARS and the UHM.

Dr. Daniel Rubinoff: Recommends Approval.

Comments: The background work seems solid and the risk low in terms of non-target impacts.

Dr. Jesse Eiben: Recommends Approval.

Ms. Janis Matsunaga: Recommends Approval.

Comments: "I recommend approval to allow the importation of lab-reared strains of the parasitoid wasp, *Phymastichus coffea*, an insect on the List of Restricted Animals (Part A) for field release to control the coffee berry borer, *Hypothenumus hampei*, by the USDA ARS and the UHM."

Dr. Francis Howarth: Recommends Disapproval.

Comments: "Using no-choice test for host studies is okay, and the methods used as described in Yousef et al. are valid. However, the number of species and specimens of each species tested were limited. The data suggest that the host range is restricted, and that the wasp may pose little risk to non-target species. One lacuna in the report is whether female wasps were allowed to mate before testing. Since they produced female offspring, they probably had mated, but when and how this happened should be stated. Mating condition may affect female behavior, including oviposition and host choice."

Applicants' Comments:" No-choice host tests cover a wide range of taxa in terms of phylogenetic relatedness. The number of species screened is a representative cross-section of the Scolytinae fauna of Hawaii. Also please note that using no-choice tests for host-specificity is more than "okay", it is in fact the most conservative approach to non-target screening, and the method most likely to reveal non-target potential.

Yousuf et al. 2021, Materials and Methods section explained "Emerged male and female parasitoid adults were collected using a manual aspirator into a clean glass container. Parasitoids were held for mating and oocyte

maturation and provided with 50% (w/v) honey (raw organic) solution for ~ 2 h before being used in the experiments (López-Vaamonde and Moore 1998)."

On page 6, paragraph #2, without more information, it is too speculative to say *P. coffea* will rely on volatiles or other cues associated with coffee plants making it very likely to ensure high host fidelity with CBB under field conditions.

Applicants' Comments: "Rojas et al. 2006. Clearly show attraction to CBB damaged coffee and CBB frass. It is not unreasonable to suggest that this same attraction will occur under field conditions. Saying "this interaction is very likely to ensure high host fidelity under field conditions" indeed recognizes that we are not completely certain that this is the case. There is however a reasonable chance that this will be true."

Dr. Howarth Comments: "On page 7 under "Analysis of Application re EA": Legally, it appears that Hawaii EA is **required** for this action! The agent is expected to invade and survive in the natural environment in the state – including on state lands. Also, much of the work will take place in UH labs and involve UH staff and students. Please reconsider the exemption!"

PQB NOTES**:** *The applicants originally did not plan to use State funding, property, lands, or employees for this project. As such, they were not subject to the State's environmental review requirements. The applicants have since decided to use State lands and equipment and are currently going through the State's environmental review requirements. Dr. Howarth's responses and recommendations are partially based off of the applicants' original intent of not using State or county land, funding, and other resources.*

Failure to complete the State's environmental review process will bar the applicants from using State or county land, funding, and other resources for this project, which includes USDA's PBARC facility in Hilo and any UH campus, facility, staff, supplies, vehicles, and equipment. Doing so is a violation of permit condition #4 and the permittees could be subject to the costs and penalties outlined in permit conditions #22 and #23.

Dr. Howarth Comments: "The EA and project description do not provide enough information on the scope or precisely what will be done. It implies that the agents are expected to establish and provide adequate pest control, but it also claims that augmentative releases of high numbers of agents will be released. I recognize the need to be flexible, but if the latter strategy is planned, the methods for this should be appropriately described in the proposal. For example, where and by whom will the agents be mass-reared? What quality control methods will be used?"

Applicants' Response: "*We will initially only release P. coffea reared at the USDA FS quarantine facility in Volcano. We hope to be able to one day import and release mass reared P. coffea from Cenicafe in Colombia. Cenicafe has never found a hyperparasitoid in P. coffea after 30 years of field collections and rearing. However, the possibility that parasitized CBB might carry coffee diseases should be evaluated."*

Dr. Howarth Comments: "Although the environmental risks may be low, they still affect the cost-benefit analysis. The other unknown is how effective the agent will be under Hawaii conditions. Weighing the evidence from both the information given in the proposal and the literature, this project is not worth the cost and risk. Another aspect not mentioned in the proposal is the prospect of the development of safer and more effective methods to control the pest coffee berry borer. For example, see Vega et al. 2017, which reported research conducted in Hawaii.

Vega FE, Simpkins A, Miranda J, Harnly JM, Infante F, Castillo A, Wakarchuk D, Cossé A. A Potential Repellent Against the Coffee Berry Borer (Coleoptera: Curculionidae: Scolytinae). J Insect Sci. 2017 Dec 23;17(6):122. doi: 10.1093/jisesa/iex095. PMCID: PMC5751034."

Applicants' Response: "The reviewer's concern about how effective the agent will be under Hawaii conditions is true for any new biological control agent. Concerning the cost-benefit analysis of this project, *the relative cost of the biocontrol action is certainly lower than perennial applications of Beauveria bassiana. The risks are minimal, as shown by very clear host-preference work. The reviewer's conclusion that this project "is not worth the cost and risk" is based on no actual evidence.*

As for the development of safer and more effective methods to control CBB, we are in the process of examining the use verbenone as a repellent and will seek registration of the product in 2023. We have also considered kaolin applications as CBB repellents. There seems to be no need to mention these approaches in a biological control agent release permit application."

2. I recommend approval____/____disapproval to establish permit conditions for the importation of lab-reared strains of the parasitoid wasp, *Phymastichus coffea,* an insect on the List of Restricted Animals (Part A), for field release to control the coffee berry borer, *Hypothenemus hampei*, by the USDA ARS and the UHM.

Dr. Daniel Rubinoff: Recommends Approval.

Dr. Jesse Eiben: Recommends Approval.

Ms. Janis Matsunaga: Recommends Approval.

Comments: "I recommend approval to establish permit conditions for the importation of lab-reared strains of the parasitoid wasp, *Phymastichus coffea*, an insect on the List of Restricted Animals (Part A), for field release to control the coffee berry borer, *Hypothenemus hampei*, by the USDA ARS and the UHM with the addition of the following permit conditions and the edit to permit condition #17.

ADDITIONAL PERMIT CONDITIONS:

- Plant Pest Control Branch (& Plant Quarantine Branch?) shall be notified at least 1 week prior to first field releases. This should include the proposed location of releases and the number of *P. coffea* to be released into the field.

- Upon release, a series of voucher specimens of the population shall be deposited into the HDOA insect collection. This shall include at least 5 female and 5 male adult specimens pinned and 5 males and 5 females in 70% ethanol.

- And the edit to condition #17
The permittee(s) shall submit an annual report to the PQB by January 31st on results of the post release monitoring programs for the prior calendar year, and shall include the following: date, locations, number of restricted article(s) released, and number of releases;"

Applicants' Response: "We will submit pinned specimens and specimens in 70% alcohol of P. coffea to the Plant Pest Control Branch."

Dr. Francis Howarth:

Comments: "On page 8, "Proposed Permit Conditions**"**: Condition #3: Not true as stated. The animals are to be released into the wild. Rephrase to clarify what is meant."

PQB NOTES**:** *In response to Dr. Howarth's comments, PQB has amended permit condition #3 and created permit condition #4.*

Dr. Howarth Comments: "On page 10, Condition #13: Contradicts Condition #10. Clarify."

PQB NOTES**:** *PQB does not see that there is a contradiction between permit conditions #13 and #10. Condition #10 is for information prior to shipment of the*

restricted article(s). Condition #13 are the requirements for what must accompany the restricted article(s) when entering Hawaii Although there may be slight differences in what is required in both permit conditions both conditions accurately detail the information needed for each shipment of P. coffea to Hawaii.

Dr. Howarth's Comments: "On page 11, Condition #20. I recommend that the advisory committee review this document."

***PQB NOTES*:** The applicants have submitted the biosecurity manual for the USDA FS Volcano quarantine facility as ATTACHMENT 8.

3. If lab-reared strains of the parasitoid wasp, *Phymastichus coffea*, an insect on the List of Restricted Animals (Part A) is accidently released, what is the probable impact on the environment?

___ minimal or no significant effects on the environment.
___ other (if "other", please explain).

Dr. Daniel Rubinoff: Minimal or no significant effects on the environment.

Comments: "The non-target assays seem to indicate a strong preference for CBB and cogeners with no apparent threat to other bark beetles."

Dr. Jesse Eiben: Minimal or no significant effect on the environment.

Ms. Janis Matsunaga: Minimal or no significant effect on the environment.

Dr. Francis Howarth: Other.

Comments: "Please see comments for question #1."

ADVISORY COMMITTEE REVIEW: May we request your recommendation and comments at the next meeting of the Advisory Committee on Plants and Animals.

PQ-7 (01/04)

State of Hawaii
Department of Agriculture
PLANT QUARANTINE BRANCH
1849 Auiki Street, Honolulu, HI 96819-3100
Phone: (808) 832-0566, FAX: (808) 832-0584

For Office Use Only

Fee: $_____ Receipt No. _____

☐ Approve Permit No._____ Date: _____

☐ Disapprove ☐Other _____

Processed by: _____ Date: _____

PERMIT APPLICATION FOR RESTRICTED COMMODITIES INTO HAWAII

Date: *November 24, 2021*

In accordance with the provision of Chapter _____ , Hawaii Administrative Rules of the Division of Plant Industry, Department of Agriculture, a permit is requested for the following commodities:

Please type or print clearly.

Quantity	Commodity	Scientific Name
Multiple	Parasitic wasp	Phymastichus coffea LaSalle (Hymenoptera: Eulophidae)

RECEIVED
JAN 28 2022
PLANT QUARANTINE BRANCH

NO PAYMENT
Date: 1/28/22 Initial: TSY

Name and address of shipper: Multiple: Pablo Benavides, Cenicafe, Manizales, Colombia; Jose Carlo Verle Rodrigues, Univ. Puerto Rico, 1193 Guayacan St. S. Botanical Garden, San Juan, PR 00926; Earl Andress, USDA APHIS, 3545 Chipman Rd., Phoenix, AZ 85040

(Mainland or Foreign address)

Approximate date of arrival: 08/01/2022

Mode of Shipment: ☐ Mail ☐ Air Freight ☐ Boat

Type of Permit:
- --- Import
  - ☐ one time only ☑ multi-shipments
- --- Intrastate shipment
  - ☐ one time only ☑ multi-shipments
- ☐ Possession

Object of importation:
- ☐ Kept caged at all time
- ☐ Used for propagation
- ☐ Imported for exhibition
- ☑ Imported for liberation
- ☐ Other purposes - specify _____

Please type or print clearly.

Applicant's Name Peter Follett

Company Name USDA ARS (if applicable)

Hawaii Mailing Address 64 Nowelo St., Hilo, HI 96720

Telephone number Off: 808-959-4303 Cell: 808-443-8031

Facsimile number _____

Fee Amount Enclosed (cash, check or mail order) $_____

(complete reverse side)

PLEASE COMPLETE THE FOLLOWING INFORMATION (attach extra sheet if necessary)

1. State in detail the reasons for introduction (include use or purpose).

This is an application for a permit to field release the parasitoid Phymastichus coffea (Hymenoptera: Eulophidae) against the coffee berry borer (CBB), Hypothenemus hampei (Coleoptera: Curculionidae: Scolytinae) in Hawaii coffee. Phymastichus coffea has proven to be an effective biological control agent of coffee berry borer in other coffee growing regions in the world, especially Central and South America. P. coffea is the only parasitoid tested thus far that has been shown to reduce yield loss from CBB damage and has potential for effective BC in Hawaii.

2. Person responsible for the organism (include name, address and phone number).

Peter Follett, USDA-ARS, U.S. Pacific Basin Agricultural Research Center, 64 Nowelo St. Hilo, Hawaii 96720, Ph: 808-959-4303, email: peter.follett@usda.gov

Mark Wright, Department of Plant and Environmental Protection Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, 3050 Maile Way, Honolulu, HI 96822, 808-956-6737, markwrig@hawaii.edu

3. Location(s) where the organism will be kept and used (include address, contact and phone number).

Shipments will be sent to a quarantine facility at 1) USDA Forest Service at Hawaii Volcanoes National Park or 2) Hawaii Department of Agriculture, 1428 S. King St. Honolulu, HI initially for positive identification of Phymastichus coffea and determination of colony purity. Afterward, colonies will be maintained in Follett's and/or Wright's labs. Our objective is to deliberately release P. coffea wasps in Hawaii coffee plantations. In the laboratory, P. coffea will be reared on adult CBB in plexiglass or fine mesh screen cages with sleeved entry ports.

4. Method of disposition.

Any unused material during shipment will be autoclaved within the quarantine facility. Reared parasitoid adults will be released in coffee plantations throughout the State.

5. Give an abstract of the organism with particular reference to potential impact on the environment of Hawaii (include impact to plants, animals and humans).

Phymastichus coffea is an short-lived, idiobiont, gregarious endoparasitoid of adult coffee berry borer, commonly laying two eggs (a male and a female) per host. Both a male and female develop in a single host, the female in the abdomen and the male in the prothorax. Host range testing was conducted in quarantine. Using no-choice tests, a total of 43 different species of Coleoptera were tested, including 23 scolytines (6 Hypothenemus species, 7 native Xyleborus species, and 10 others), and 4 additional Curculionidae. P. coffea was only able to parasitize the target host H. hampei (coffee berry borer) and 4 other adventive species of Hypothenemus: H. obscurus (tropical nut borer), H. seriatus, H. birmanus and H. crudiae. P. coffea was host specific at the genus level. Release of P. coffea for control of CBB in Hawaii coffee should not attack any native species or disrupt native ecosystems.

****

I request permission to import the articles as listed on the permit application and further, request that the articles be examined by an authorized agent of the Department of Agriculture upon arrival in Hawaii.

I agree that I, as the importer, will be responsible for all costs, charges or expenses incident to the inspection or treatment of the imported articles.

I further agree that damages or losses incident to the inspection or the fumigation, disinfection, quarantine, or destruction of the articles, by an authorized agent of the Department of Agriculture, shall not be the basis of a claim against the department or the inspectors for the damage or loss incurred.

Signature Peter A Follett _____ **Date** November 24, 2021

(Applicant)

PQ-7 (01/04)

State of Hawaii
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PLANT QUARANTINE BRANCH
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For Office Use Only

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☐ Disapprove ☐ Other _____

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Please type or print clearly.

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Please type or print clearly.

Applicant's Name Mark G Wright

Company Name ~~USDA ARS~~ Univ. Hawaii (if applicable)

Hawaii Mailing Address ~~64 Nowelo St., Hilo, HI 96720~~ 3050 Maile Way Rm 310 Honolulu 96822

Telephone number Off: 808-959-4303 Cell: 808-443-8031 ; 808-271-2037

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(complete reverse side)

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Mark Wright, Department of Plant and Environmental Protection Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, 3050 Maile Way, Honolulu, HI 96822, 808-956-6737, markwrig@hawaii.edu

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I further agree that damages or losses incident to the inspection or the fumigation, disinfection, quarantine, or destruction of the articles, by an authorized agent of the Department of Agriculture, shall not be the basis of a claim against the department or the inspectors for the damage or loss incurred.

Signature _____ **Date** November 24, 2021

(Applicant)

Curriculum Vitae

Peter A. Follett, PhD
Research Entomologist (GS-15)
USDA-ARS, Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center
64 Nowelo St., Hilo, Hawaii 96720 USA
Ph.: (808) 959-4303, Fax: (808) 959-5470
e-mail: peter.follett@ars.usda.gov

Education:
Ph.D., 1993, North Carolina State University, Entomology
M.S., 1984, Oregon State University, Entomology
B.S. (Honors), 1980, University of Vermont, Plant and Soil Science

Research Positions:
1997- present, Research Entomologist (GS-15), USDA-ARS, U.S. Pacific Basin Agricultural Research Center, Hilo, Hawaii
1996-1997, Junior Researcher, Department of Entomology, Univ. of Hawaii at Manoa
1995-1996, Post-Doctoral Fellow, Department of Entomology, Univ. of Hawaii at Manoa
1994-1995, Post-Doctoral Fellow, Center for Conservation Research & Training, Univ. Hawaii at Manoa
1992-1993, Faculty Research Associate, Department of Entomology, Univ. of Maryland

Qualifications:
Dr. Follett has been an active researcher in applied entomology for 25 years and is internationally recognized as an expert in postharvest entomology and quarantine treatment development. He has authored or co-authored 230 scientific publications during his career, including 175 publications in peer-reviewed journals and 20 book chapters and 2 books, covering many important agricultural and quarantine pests in various crops. Dr. Follett currently serves as an associate editor for the Journal of Economic Entomology and Entomologia Experimentalis et Applicata and is a former editor of the Proceedings of the Hawaiian Entomological Society. He has served on expert missions for the International Atomic Energy Agency (IAEA) and USDA Foreign Agriculture Service in Thailand, Turkey, Bangladesh, Guatemala, Mexico, Colombia, Australia, and Argentina to advise on irradiation research and export programs. He was invited to prepare reviews on current trends in quarantine entomology and non-target effects of biological control for the Annual Review of Entomology. He is former president of the Hawaiian Entomological Society and the Pacific Branch of the Entomological Society of America.

Areas of Expertise:
Dr. Follett has extensive experience in postharvest biology, radiation science, entomology, integrated pest management, ecology and biological control and has worked with >30 species of invasive insect pests in a variety of temperate and tropical crops. The long-term goals of his research program are to develop and protect U.S. export markets for fresh tropical commodities with emphasis on expanding and diversifying agriculture and agricultural exports in Hawaii and other states by providing environmentally sound, economically viable systems, treatments, or processes that control quarantine pests, ensure product quality and food safety, and increase product value while safeguarding the agriculture of other states.

Recent Grant Funding:
USDA APHIS Fam Bill, Systems approach for control of coffee berry borer in Hawaii and Puerto Rico with emphasis on biological control, P. Follett & H. Diaz-Soltero, $1,200,000, 2018-2022.

Professional Honors and Service:
Editor, *Proceedings of the Hawaiian Entomological Society* (1999-2004)
Associate Editor, *Journal of Economic Entomology* (2002-present), *Entomologia Experimentalis et Applicata*, (2006-2021)
President, Hawaiian Entomological Society, 1999-2000
Affiliate Faculty, University of Hawaii at Manoa, University of Hawaii at Hilo, INRS (Univ. Quebec), and Kasetsart University (Thailand)
Entomological Society of America, Distinguished Achievement Award in Horticultural Entomology, 2007
USDA Secretary's Group Honor Award for Excellence, Irradiation and Indian Mango Approval Team, 2007
Federal Laboratory Consortium for Technology Transfer (FLC) Award for Excellence in Technology Transfer, Commercial Adoption of Phytosanitary Irradiation Treatment Protocols for Tropical Fruit, 2010
President, Pacific Branch of the Entomological Society of America, 2016
Certificate of Appreciation, Hawaii Tropical Fruit Growers Assoc., 2016, 2019
Roger I. Vargas Research Award, Hawaii Tropical Fruit Growers Assoc., 2021
USDA ARS Senior Research Scientist of the Year, Pacific West Area, 2022

Recent Publications (past 5 years, 175 total peer-reviewed scientific papers)

Follett, P. A. 2017. Insect-plant interactions: host selection, herbivory, and plant resistance—an introduction. Entomol. Exp. Applic 162 (1): 1-3.

Barkai-Golan, R. and **P. A. Follett**. 2017. *Irradiation for Quality Improvement, Microbial Safety and Phytosanitation of Fresh Produce*. Academic Press, San Diego, CA. 302 pp.

Hossain, F., P. Follett, S. Salmieri, K. D. Vu, M. Jamshidan, and M. Lacroix. 2017. Perspectives on essential oil-loaded nanodelivery packaging technology for controlling stored cereal and grain pests, pp. 487-509, In Green Pesticides Handbook: Essential Oils for Pest Control, L.M.L. Nollet and H.S. Rathore (eds.), CRC Press, Baco Raton, FL.

Dunn, D. and **Follett, P. A.** 2017. The sterile insect technique (SIT)—an introduction. Entomol. Exp. Applic 164: 151-154.

Roberts, P. and **P. A. Follett**. 2017. Food irradiation for phytosanitary and quarantine purposes, p. 169-182, In Food Irradiation Technologies: Concepts, Applications and Outcomes (eds I.C.F.R. Ferreira, A. L. Antonio, S. Cabo Verde). Royal Society of Chemistry, Cambridge, UK. 454 pp.

Wall, M.M. and **P.A. Follett**. 2017. Quality of mixed tropical fruit following irradiation treatment. Acta Hortic. 1178 (18): 99-104.

Follett, P. A. 2017. Insect-plant interactions: host selection, herbivory, and plant resistance—an introduction. Entomol. Exp. Applic 162 (1): 1-3.

Hossain, F., **P. Follett**, S. Salmieri, K. D. Vu, M. Jamshidan, and M. Lacroix. 2017. Perspectives on essential oil-loaded nanodelivery packaging technology for controlling stored cereal and grain pests, pp. 487-509, In Green Pesticides Handbook: Essential Oils for Pest Control, L.M.L. Nollet and H.S. Rathore (eds.), CRC Press, Baco Raton, FL.

Nadel, H., **P. A. Follett,** C. Perry and R. Mack. 2018. Irradiation for quarantine control of the invasive fruit pest *Lobesia botrana* (Lepidoptera: Tortricidae). J. Econ. Entomol. 111 (1): 127-134.

Srimartpirom, M., I. Burikam, W. Limohpasmanee, T. Konggratarporn, T. Thannarin, A. Bunsiri, and **P. A. Follett**. 2018. Low dose irradiation with modified atmosphere packaging for mango against oriental fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 111 (1): 135-140.

Nadel, H., **P. A. Follett,** C. Perry and R. Mack. 2018. Irradiation for quarantine control of the invasive fruit pest *Lobesia botrana* (Lepidoptera: Tortricidae). J. Econ. Entomol. 111 (1): 127-134.

Srimartpirom, M., I. Burikam, W. Limohpasmanee, T. Konggratarporn, T. Thannarin, A. Bunsiri, and **P. A. Follett**. 2018. Low dose irradiation with modified atmosphere packaging for mango against oriental fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 111 (1): 135-140.

Follett, P. A. A. Swedman, and B. Mackey. 2018. Effect of low oxygen created by modified atmosphere packaging on radiation tolerance of *Drosophila suzukii* (Diptera: Drosophilidae) in sweet cherries. J. Econ. Entomol. 111 (1): 141-145.

Myers, R. Y., **P. A. Follett**, C. L. Mello, and K. A. Snook. 2018. Effects of irradiation on reproduction of *Rotylenchulus reniformis*. Nematology doi (online)

Nicholas, A., and **P. A. Follett**. 2018. Postharvest irradiation treatment for quarantine control of western flower thrips. J. Econ. Entomol. 111 (3): 1185-1189

Follett, P. A. 2018. Irradiation for postharvest quarantine control of coffee berry borer (Coleoptera: Curculionidae) and a generic dose for Curculionoidea. J. Econ. Entomol. 111 (4): 1633-1637.

Follett, P. A., N. Manoukis, and B. Mackey. 2018. Comparative cold tolerance in *Ceratitis capitata* and *Zeugodacus cucurbitae* (Diptera: Tephritidae). J. Econ. Entomol. 111 (6): 2632-2636.

Follett, P. A., L. Jamieson, L. Hamilton, and M. M. Wall. 2019. New associations and host status: infestability of kiwifruit by the fruit flies *Bactrocera dorsalis*, *Zeugodacus cucurbitae*, and *Ceratitis capitata* (Diptera: Tephritidae). Crop Protection 115: 113-121.

Avanesyan, A., K. Snook, **P. Follett**, and W. Lamp. 2019. Short-term physiological response of a native Hawaiian plant, *Hibiscus arnottianus*, to injury by the exotic leafhopper, *Sophonia orientalis* (Hemiptera: Cicadellidae). Environ. Entomol. (online)

Hossain, F., **P. A. Follett**, K. D. Vu, M. Harich, S. Salmieri, and M. Lacroix. 2019. Antifungal activity of combined treatments of active methylcellulosic based films containing encapsulated nanoemulsion of essential oils and γ-irradiation: in vitro and in situ evaluations. Cellulose 26 (2): 1335-1354.

Hossain, F., **P. A. Follett**, K. D. Vu, M. Harich, S. Salmieri, and M. Lacroix. 2019. Antifungal activity of combined treatments of irradiation and essential oil encapsulated chitosan nanocomposite films in in vitro and in situ conditions. Inter. J. Food Microbiol. 295-33-40.

Hossain, F., **P. A. Follett**, K. D. Vu, M. Harich, S. Salmieri, and M. Lacroix. 2019. Synergistic effects of nanocomposite films containing essential oil nanoemulsions in combination with ionizing radiation for control of rice weevil *Sitophilus oryzae* in stored grains. J. Food Science 84 (6): 1439-1446.

Follett, P. A., J. Pinero, S. Souder, L. Jamieson, B. Waddell, and M. M. Wall. 2019. Host status of 'Scifresh' apples to the invasive fruit fly species *Bactrocera dorsalis*, *Zeugodacus cucurbitae*, and *Ceratitis capitata* (Diptera: Tephritidae). J. Asia-Pacific Entomol. 22: 458-470.

Calvert, F., R. G. Hollingsworth, **P.A. Follett** and M. M. Wall. 2019. Survey of flowering plants in Hawaii as potential banker plants of anthocorid predators for thrips control. J. Asia-Pacific Entomol. 22 (3): 638-644.

Hamilton, L., R. Hollingsworth, M. Sebado-Halpern, N. C. Manoukis, **P. A. Follett**, and M. A. Johnson. 2019. Coffee berry borer (*Hypothenemus hampei*) (Coleoptera: Curculionidae) development across an elevational gradient on Hawaii Island: applying laboratory degree-day predictions to natural field populations. PLOS One 14(7): e0218321. https://doi.org/10.1371/journal.pone.0218321

Pinero, J. C., B. B. Barratt, G. Bolton and **P. A. Follett**. 2019. β-cyclocitral synergizes the response of adult *Drosophila suzukii* (Diptera: Drosophilidae) to fruit juices and isoamyl acetate in a sex-dependent manner. Scientific Reports 9, article #10574. https://doi.org/10.1038/s41598-019-47081-z

Hall, M., S. P. Redpath, S. Sanxter, M. Wall, **P. A. Follett**, S. Silva, M. Postler, M. Wohlers, L. E. Jamieson, and A. B. Woolf. 2020. Exploring X-ray treatments for disinfesting apples. Acta Hortic. 1275: 93-98.

Follett, P., J. Bruin, and N. Desneux. 2020. Insects in agroecosystems – An introduction. Entomol. Exper. Applic. 168 (1): 3-6.

Follett, P. A. and L. G. Neven. 2020. Phytosanitary irradiation: Does modified atmosphere packaging or controlled atmosphere storage creating a low oxygen environment threaten treatment efficacy? Rad. Phys. Chem. 173: https://doi.org/10.1016/j.radphyschem.2020.108874 (Log # 369913)

Koch, J. B., J. R. Dupuis, M-K. Jardeleza, N. Ouedraogo, S. M. Geib, **P. A. Follett**, and D. K. Price. 2020. Population genomic and phenotype diversity of invasive *Drosophila suzukii* in Hawaii. Biological Invasions. DOI: 10.1007/s10530-020-02217-5

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Hossain, F., **P. A. Follett**, S. Shankar, T. Begum, S. Salmieri, and M. Lacroix. 2021. Radiosensitization of rice weevil *Sitophilus oryzae* using combined treatments of essential oil and ionizing radiation with gamma ray and x-ray at different doses. Radiation Physics and Chemistry DOI: 10.1016/j.radphyschem.2020.109286

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extract against foodborne pathogens and spoilage organisms. J. Food Science http://doi.org/10.1111/1750-3841.15988

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Takeuchi, Y., P. Benavides, M. A. Johnson, **P. A. Follett**, M. K. Hossain, L. Navarro, and M. Giraldo. 2022, Pathway-initiated pest risk assessment: Human-mediated introduction and dispersal of coffee berry borer (*Hypothenemus hampei* Ferrari) in the Hawaiian Islands. Proc. Hawaiian Entomol. Soc. 54:1-20.

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Pulakkatu-thodi, I, J. Dzurisin, **P. A. Follett**. 2022. Evaluation of macadamia felted coccid (Hemiptera: Eriococcidae) damage and cultivar susceptibility using imagery from a small unmanned aerial vehicle (sUAV), combined with ground truthing. Pest Management Science (in review)

CURRICULUM VITAE – MARK GERALD WRIGHT

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EDUCATION:

B.Sc. (1984 -1986): Major subjects: Zoology & Entomology
Institution: University of Stellenbosch, Stellenbosch 7600, South Africa.

B.Sc. (Honours) (1987): Major subject: Zoology (Ecology & environmental physiology). This one-year degree comprises graduate coursework, and an introduction to research.
Institution: University of Stellenbosch, Stellenbosch 7600, South Africa.

M.Sc. (1988-1990): Major subject: Entomology
Institution: University of Stellenbosch, South Africa.
Title of thesis: *The insect communities, herbivory, seed predation and pollination of* Protea magnifica *and* P. laurifolia.

Ph.D. (1991-1996): Subject: Zoology/Entomology
Institution: University of Natal, Pietermaritzburg, South Africa.
Title of dissertation: *Ecological correlates: endophagous insects and plants in Fynbos*.

Post-doctoral Fellow (1999-2001): Department of Entomology, Cornell University.

Other :
Certificate in Environmental Law: (1998): Potchefstroom University (Centre for Regional Development)
Certificate in Environmental Management Systems (ISO 14000): (1998): Potchefstroom University (Centre for Regional Development)
Certificate Programme in Project Management: (1996): University of Stellenbosch (Graduate School of Business)**.**

LANGUAGES

First language: English
Other languages: Afrikaans

EMPLOYMENT HISTORY

August 2012 – present: Promotion to Professor/Extension Specialist (Entomology), Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa. Responsibilities: research and extension on ecology and management of invasive insects on tropical fruit and nut crops, including biological, cultural and chemical control measures. Instruction in insect ecology and biological control. Served as department chair (2013-2016 term). Graduate faculty (and program chair) in Entomology; Graduate faculty in Ecology, Evolution and Conservation Biology program.

August 2007 – July 2012: Tenure and promotion to Associate Professor/Associate Extension Specialist (Entomology), Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa. Responsibilities: research and extension on ecology and management of invasive insects on tropical fruit and nut crops, including biological, cultural and chemical control measures.

October 2001 – July 2007: Assistant Professor/Assistant Extension Specialist (Entomology), Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa. Responsibilities: research and extension on ecology and management of invasive insects on tropical fruit and nut crops, including biological, cultural and chemical control measures. Teaching: pest management / biological control / insect ecology.

July 2000 - September 2001: Research Associate II (non-tenure track faculty), Department of Entomology, Cornell University. Responsible for investigation of inoculative releases of *Trichogramma ostriniae* for biological control of *Ostrinia nubilalis*, and various other aspects of integrated pest management.

April 1999 -July 2000: Post-doctoral Research Associate, Department of Entomology, Cornell University. Investigated aspects of *T. ostriniae e*cology and potential for control of *O. nubilalis*.

April 1992 - March 1999: Agricultural Research Council (South Africa): Vegetable & Ornamental Plant Institute; Research Entomologist. Worked on vegetable pests including the development of: integrated pest management of vegetable pests; design of environmentally compatible pest control measures; investigation of pest control possibilities for use by subsistence farmers and farmer training. Responsibilities included the management of projects on vegetable integrated pest management and on the study and control of pests of ornamental plants as well as subsistence agriculture projects.

January 1988 - April 1992: Employer: Department of Agricultural Development (South Africa); Assistant Agricultural Researcher (Entomology). Responsible for: identification and control of insect pests on fynbos crops; Included basic ecological and biological research on insect-plant interactions, and extension. Responsible for administration and day-to-day management of Plant Protection Section, Fynbos from March 1991.

SERVICE CONTRIBUTIONS:

- Plant-Insect Ecosystems Section, Entomological Society of America: Vice-president, (2016-2017), President (2017-2018), Past-president (2018-2019).
- Pacific Branch, Entomological Society of America: President-elect (2019-2020), President (2020-2021), Past-president (2021-2022).
- Member of Western Governors' Association Invasive Species Data initiative group (2017-2018).
- Council on Study Abroad, UH Manoa (2019-2022).
- Chief-Editor, *Proceedings of the Hawaiian Entomological Society,* current.
- Associate Editor, *Biocontrol Science & Technology*, current.

- Member of Editorial Board, *Biological Control*, current.
- Member of Editorial Board, Entomological Society of America Plant-Insect Ecosystems Section representative, *Environmental Entomology*, 2013-2014 (Chair of editorial board 2014)
- Review manuscripts for journals including *African Entomology*, *Biodiversity and Conservation, Biological Control, Journal of Economic Entomology; Journal of Entomological Science, Crop Science, Environmental Entomology, Diversity and Distributions, Austral Ecology, Journal of Tropical Agriculture, Plant Disease, Entomologia Experimentalis et Applicata, Florida Entomologist, Pacific Science, Biological Invasions, Insects, Journal of Theoretical Biology,*and others.
- Grant proposal panel reviewer for the United States Civilian Research and Development Foundation for the Independent States of the Former Soviet Union (CRDF), Arlington, VA (2002-2008).
- Co-editor of a volume of *Acta Horticulturae*.
- Responsible for the initiation of conservation agriculture projects for ARC-Roodeplaat (1997-1998).

SCIENTIFIC PUBLICATIONS (in peer-reviewed journals/books/invited book reviews)
Book chapters, Reviewed conference proceedings, Book reviews:

1. Kaufman, L.V., **Wright, M.G.** 2022. Erythrina gall wasp successfully controlled by the introduction of a parasitoid wasp in Hawaii. In: contributions of classical biocontrol to the US food security, forestry and biodiversity. Eds van Driesche *et al*. (in review)
2. Cave, R., **Wright, M.G**., Moore, A. 2022. Biological Control of the Cycad Aulacaspis Scale, *Aulacaspis yasumatsui.* In: contributions of classical biocontrol to the US food security, forestry and biodiversity. Eds van Driesche *et al*. (in review)
3. Mafra-Neto, A., **Wright, M**., Fettig, C., Progar, R., Munson, S., Blackford, D., Moan, J., Graham, E., Foote, G., Borges, R., Silva, R., lake, R., Bernardi, C., Saroli, J., Clarke, S., Meeker, J., Nowak, J., Agnello, A., Martini, X., Rivera, M., Stelinski, L. 2022. Repellent semiochemical solutions to mitigate the impacts of global climate change on arthropod pests. In: *Advances in Arthropod Repellents*. Elsevier. pp. 279-322.
4. Day, M., Cock, M., Conant, P., Furlong, M., Paynter, Q., Ramadan, M., **Wright, M.G.** 2021. Biological control success and failures: Oceania region. In*: Biological Control: Global Impacts, Challenges and Future Directions of Pest Management*, Ed. P.G. Mason. CSIRO Publishing, Melbourne. pp. 342-376.
5. **Wright, M.G**. 2017. Assessing host use and population level impacts on non-target species by introduced natural enemies: can host range testing provide insight? *Proceedings of the 5th International Symposium on Biological Control of Arthropods*. Malaysia. P.G. Mason, D.R. Gillespie and C. Vincent (Eds.). CAB International. 50-51.
6. **Wright, M.G**. 2015. Proteas. In: *Insects of Cultivated Plants and Natural Pastures in Southern Africa*. Prinsloo, G.L. & Uys, V.M. (Eds). Entomological Society of Southern Africa, Pretoria: 680-695.
7. **Wright, M.G**. 2014. Biological control of invasive insect pests. In: *Integrated Pest Management – Current concepts and ecological perspective*. Ed. Abrol, D.P. Elsevier Academic Press. pp. 267-281.

8. Kaufman, L.V., **Wright, M.G**., Yalemar, J. & Messing, R.H. 2013. Biological control of the Erythrina gall wasp: protecting a native keystone forest species in Hawaii. *Proceedings of the 4th International Symposium on Biological Control of Arthropods,* Pucón, Chile. Peter G. Mason, David R. Gillespie & Charles Vincent Eds. pp. 72-74. (Invited paper).
9. **Wright, M.G.** 2011. Pineapple insects; ecology and management. *Encyclopedia of Pest Management*. DOI: 10.1081/E-EPM-120042882. Taylor & Francis.
10. Wieczorek, A.M. & **Wright, M.G**. 2011. History of agricultural biotechnology: how crop development has evolved. *Scitable, by Nature Education Knowledge*, Nature Publishing Group. 3(10): 9. http://www.nature.com/scitable/knowledge/library/history-of-agricultural-biotechnology-how-crop-development-25885295.
11. Vorsino, A., Wieczorek, A., **Wright, M.G**., Ramadan, M. & Messing, R. 2009. Using molecular tools to identify and describe ecological and evolutionary processes affecting augmentative biological control. *Proceedings of the Third International Symposium on Biological Control of Arthropods.* P.G. Mason, D.R. Gillespie and C. Vincent (Eds.). pp. 124-133. (Invited paper).
12. **Wright, M.G**. 2005. Biological control in IPM systems in Africa. *American Entomologist* (Book Review) 51: 249.
13. **Wright, M.G**., Kuhar, T.P., Diez, J.M. & Hoffmann, M.P. 2005. Effective augmentative biological control – importance of natural enemy dispersal, host location, and post-release assessment. *International Symposium on Biological Control of Arthropods*, USDA Forest Service, Publication FHTET-2005-08. pp. 495-500. (Invited paper).
14. **Wright, M.G**. 2004. Multivariate analysis of ecological data using CANOCO. *The Quarterly Review of Biology* (Book Review) 79: 222-223.
15. **Wright, M.G**. & Hoffmann, M.P. 2002. Vegetable crop pest management (insects and mites). *Encyclopaedia of Pest Management* Ed: Pimentel, D. Marcel Dekker Inc. pp. 683-686. DOI: 10.1081/E-EPM-120003842.
16. **Wright, M.G**. 1999. Integrated pest management for low-input agriculture in Africa: A review. In John F. Devlin and Ted Zettel, eds., *Ecoagriculture: Initiatives in Eastern and Southern Africa*, University of Guelph & Weaver Press, Harare. pp. 149-154.
17. **Wright, M.G**., Khan, S. & Davison, S. 1999. South African entomopathogens as biological control agents of vegetable pests. In John F. Devlin and Ted Zettel, eds., *Ecoagriculture: Initiatives in Eastern and Southern Africa*, University of Guelph & Weaver Press, Harare. pp. 195-203.
18. Zeier, P. & **Wright, M.G**. 1995. Thrips resistance in *Gladiolus* species: potential for IPM and breeding. In: *Thrips Biology and Management*. Eds: Parker, B.L., Skinner, M. & Lewis, T. Plenum Press, New York. pp 411-416.
19. **Wright, M.G**. 1993. Insect conservation in the Cape Fynbos, with special reference to endophagous insects. In: *Perspectives on Insect Conservation*. (Eds Gaston, K., New, T.R., Samways, M.J.) Applied Biology Series, Intercept Publishers. pp 97-110.

<u>Peer-reviewed scientific journals</u>:

20. Au, M.G. and **Wright, M.G**. *Arcte coerula* (Lepidoptera: Noctuidae): A new invasive pest in Hawai‘i on endemic plants. *Proceedings of the Hawaiian Entomological Society* (Submitted)
21. Ramadan, M.M., Kaufman, L.V., **Wright, M.G.** Recent advances in insect and weed

biocontrol in Hawaii: case studies and trends. *Biological Control* (Submitted)

22. Honsberger, D.N., Huber, J.T. and **Wright, M.G**. 2022. A new *Mymaromma* sp. (Mymarommatoidea, Mymarommatidae) in Hawai‘i and first host record for the superfamily. *Journal of Hymenoptera Research* 89: 73-87. https://doi.org/10.3897/jhr.89.77931
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24. Elliot, C., Gillett, C.P.D.T., Parsons, E., **Wright, M.G**. and Rubinoff, D. 2021. Identifying key threats to a refugial population of an endangered Hawaiian moth. *Insect Conservation and Diversity* doi: 10.1111/icad.12553
25. Gugliuzzo, A., Biedermann, P.H.W., Carrillo, D., Castrillo, L.A., Egonyu, J.P., Gallego, D., Haddi, K., Hulcr, J., Jactel, H., Kajimura, H., Kamata, N., Meurisse, N., Li, Y., Oliver, J.B., Ranger, C.M., Rassati, D., Stelinski, L.L., Sutherland, R., Garzia, G.T., **Wright, M.G**., and Biondi, A. 2021. Recent advances toward the sustainable management of invasive *Xylosandrus* ambrosia beetles. *Journal of Pest Science* https://doi.org/10.1007/s10340-021-01382-3
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27. Yousuf, F., Follett, P.A., Gillett, C.P.D.T., Honsberger, D., Chamorro, L., Johnson, T.M., Jaramillo, M.G., Machado, P.B. & **Wright, M.G.** 2021. Limited host range in the idiobiont parasitoid *Phymastichus coffea*, a prospective biological control agent of the coffee pest *Hypothenemus hampei* in Hawaii. *Journal of Pest Science* https://doi.org/10.1007/s10340-021-01353-8
28. Ali, A.N., & **Wright, M.G**. 2021. Response of *Trichogramma papilionis* to semiochemicals induced by host oviposition on plants. *Biological Control* 154: 104510.
29. Gutierrez-Coarite, R., Cho, A.H., Mollenido, J., Pulakkatu-Thodi, I., & **Wright, M.G**. 2021. Macadamia felted coccid impact on macadamia nut yield in the absence of a specialized natural enemy, and economic injury levels. *Crop Protection* 139: 105378.
30. Rugman-Jones, P.F., Au., M., Ebrahimi, V., Eskalen, A., Gillett, C.P.D.T., Honsberger, D., Husein, D., **Wright, M.G**., Yousuf, F., & Stouthamer, R. 2020. One becomes two: second species of the *Euwallacea fornicatus* complex (Coleoptera: Curculionidae: Scolytinae) species complex is established on two Hawaiian Islands. *PeerJ Life and Environment* 8:e9987 http://doi.org/10.7717/peerj.9987
31. Kaufman, L.V., Zarders, D.R., & **Wright, M.G.** 2020. Susceptibility of endemic *Myoporum* (Naio) species and populations to *Klambothrips myopri* (naio thrips) in Hawaii. *Pacific Science* 74: 309-318.
32. Ali, A.N., & **Wright, M.G.** 2020. Fitness effects of founder female number of *Trichogramma papilionis* reared on *Ephestia kuehniella. Proceedings of the Hawaiian Entomological Society* 52: 25-34.
33. Ali, A.N., & **Wright, M.G.** 2020. Behavioral response of *Trichogramma papilionis* to host eggs, host plants, and induced volatile plant cues. *Biological Control* 149: 104323.
34. Kaufman, L.V., Yalemar, J., & **Wright, M.G**. 2020. Classical biological control of the

erythrina gall wasp, *Quadrastichus erythrinae*, in Hawaii: conserving an endangered habitat. *Biological Control* 142: 104161.
35. **Wright, M.G**. 2019. Cover crops, conservation biocontrol and augmentative releases – can *Trichogramma* impacts be magnified? *Annals of the Entomological Society of America* 112: 295-297.
36. Guitierrez, R., Pulakkatu-thodi, I., & **Wright, M.G**. 2019. Binomial sequential sampling plan for macadamia felted coccid, *Eriococcus ironsidei* (Hemiptera: Eriococcidae) infesting Hawaii macadamia orchards. *Environmental Entomology* 48: 219-226.
37. **Wright, M.G**., Spencer, C.R., Cook, R.M., Henley, M.D., North, W. & Mafra-Neto, A. 2018. African bush elephants response to a honeybee alarm semiochemical blend. *Current Biology* 28: R778-780.
38. Gutierrez-Coarite, R., Mollinedo, J, Cho, A., **Wright, M.G**. 2018. Canopy management of macadamia trees and understory plant diversification to reduce macadamia felted coccid (*Eriococcus ironsidei*) populations. *Crop Protection* 113: 75-83.
39. Pulakkatu-thodi, I., Gutierrez-Coarite, R., & **Wright, M.G**. 2018. Dispersion and sequential sampling plan for coffee berry borer (Coleoptera: Curculionidae) infestations in Hawaii. *Environmental Entomology* 47: 1306-1313.
40. Greco, E., **Wright, M.G**., Burgueno, J., & Jaronski, S. 2018. Efficacy of *Beauveria bassiana* applications on coffee berry borer across an elevation gradient in Hawaii. *Biocontrol Science & Technology* 28: 995-1013.
41. Gutierrez-Coarite, R., Heller, W.P., **Wright, M.G**., Mollinedo, J., Keith, L., Sugiyama, L, & Chun, S. 2018. Entomopathogenic fungi as mortality factors of macadamia felted coccid (*Eriococcus ironsidei*) in Hawaii. *Proceedings of the Hawaiian Entomological Society* 50: 9-16.
42. Gutierrez-Coarite, R., Yoneishi, N., Mollinedo, J., Pulakkattu-thodi, I., **Wright, M.G.**, & Geib, S. 2018. PCR-based gut content analysis to detect predation of *Eriococcus ironsidei* (Hemiptera: Eriococcidae) by Coccinellidae species in macadamia nut orchards in Hawaii. *Journal of Economic Entomology* DOI: https://doi.org/10.1093/jee/toy019.
43. **Wright, M.G**. & Bennett, G.B. 2018. Evolution of biological control agents following introduction to new environments. *BioControl* 63: 105-116. First online July 2017: DOI 10.1007/s10526-017-9830-z (invited.)
44. Leonhardt, K.W. & **Wright**, M.G. 2018. Breeding *Leucospermum* hybrids for potted flower production, landscape uses and for cutflower production in the tropics: genetic factors contributing to plant architecture. *Acta Horticulturae* DOI: 10.17660/ActaHortic.2020.1282.25
45. Manandhar, R., Wang, K.H., Hooks, C.R.R. & **Wright, M.G**. 2017. Effects of strip-tilled cover cropping on the population density of thrips and predatory insects in a cucurbit agroecosystem. *Journal of Asia-Pacific Entomology* 20: 1254-1259.
46. Pulakkatu-thodi, I., Guitierrez, R. & **Wright, M.G**. 2017. Comparison of sampling intensity to estimate infestations of coffee berry borer on Hawaii island. *Proceedings of the Hawaiian Entomological Society* 49: 11-16.
47. Kaufman, L.V. & **Wright, M.G**. 2017. Assessing probabilistic risk assessment approaches for insect biological control introductions. *Insects* 8(3), 67. (Special Issue *Biological Control of Invertebrate Pests*.) doi:10.3390/insects8030067

48. Zarders, D.R. & **Wright, M.G.** 2016. Macadamia felted coccid, *Eriococcus ironsidei*: biology and life cycle in Hawaii. *Proceedings of the Hawaiian Entomological Society* 48: 51-55.
49. Manandhar, R. & **Wright, M.G**. 2016. Within-field spatial distribution of corn planthopper, *Peregrinus maidis* (Hemiptera: Delphacidae), and severity of hopperburn and Maize mosaic virus symptoms as influenced by sunn hemp intercropping. *Entomologia Experimentalis et Applicata* 161: 121-130. DOI: 10.1111/eea.12498.
50. Lu, B., Jin, Q., Wen, H., Bellis, G.A., **Wright, M.G**., Peng, Z., Wan, J., Guo, D. 2016. Biological, physiological and genetic response of *Tetrastichus brontispae* (Ferriere) (Hymenoptera: Eulophidae) following exposure to low temperature. *BioControl* 61: 649-659. DOI 10.1007/s10526-016-9757-9.
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INSTRUCTIONAL RESPONSIBILITIES:

- PEPS 310 "Conservation and Cultivation" (Spring 2005, 2007)
- PEPS 421 "Foundations of Pest Management" (Spring alt yrs, 2002 – 2008; currently team-taught since 2020)
- PEPS 422 "Biocontrol of Invasive Species" (Fall alt yrs, 2001 – 2016; summer online currently)
- PEPS 491 "African ecology and conservation biology" (Study abroad, summer)
- PEPS 671 "Insect Ecology" (Spring, as required)
- PEPS 675 "Biological Control of Pests" (Fall, as required)
- Currently serve as major advisor to four entomology graduate students (M.S. and Ph.D.), and one Tropical Plant & Soil Sciences Ph.D. student.
- Provide financial support to graduate students through grant-funded assistantships.
- Graduate Chair, Entomology Graduate Program, PEPS.

SCIENTIFIC SERVICES PROVIDED and TECHNOLOGY TRANSFER:

At University of Hawaii:

- Extension in tropical fruit and nut crops.
- Presentations at extension meetings.
- Presentations at commodity meetings.
- Development and implementation of a digital distance diagnostics and recommendation system.

At Cornell University:

- Development and editing of an interactive CD on biological control.

At Agricultural Research Council:

- Courses in fynbos pest control;
- Courses in pest management for resource-poor farmers;
- Pesticide registration trials (on crucifers, tomatoes, potatoes and ornamentals);
- Referee for pesticide registrations.
- Diagnostic services and consultation.

PROFESSIONAL ASSOCIATIONS

- Entomological Society of America; Served on editorial board of Environmental Entomology (2013-2014); President, Plant-Insect Ecosystems Section (2018); current past-president.
- Entomological Society of America: President-elect, Pacific Branch.
- Hawaiian Entomological Society; Editor of *Proceedings of the Hawaiian Entomological Society*.
- Registered Professional Natural Scientist with South African Council for Natural Scientists (1992-1999).
- Certified commercial pesticide applicator, NY (1999 – 2001), HI (current).

AWARDS

- S.P. Green Bursary for zoology (1986).
- CSIR Bursary for graduate study (1987).
- International Protea Association Scholarship (1994).
- College of Tropical Agriculture and Human Resources Ka Pouhana (Mentor) Award (2004).
- Hawaii Tropical Fruit Growers Association Award for Service (2015).
- Nominated for Dean's for award for Excellence in Teaching, College of Tropical Agriculture and Human Resources, 2020.
- Invited speaker at international conferences (Protea conference in Zimbabwe, Ecological Agriculture Conference in Zambia, Diversity and ecosystem function, Cape Town; Benefits and Risks of biological control, Denver, CO; International Conference on Biological Control of Arthropods, Davos, Switzerland, Pucon, Chile, Malaysia).

NEW TECHNOLOGY GENERATED:

- Developed a technique for environmental coding of pesticides (world-first for this), and farmer-friendly means of transferring this technology;
- Initiated various aspects of integrated pest management for use on high-value, high quality vegetable crops for export, and tested these under commercial conditions;
- Developed a commercial-scale fumigation system for disinsectation of cut flowers.
- Developed a means of selecting vegetable crops to intercrop as a pest-avoidance strategy, aimed at resource-poor farmers.
- Developed indigenous insect pathogens as biological control agents to the point where commercial trials were conducted in tomato and ornamental crops.
- Inoculative releases of *Trichogramma ostriniae* for biological control of European corn borer.

Last update of CV 28 March 2022.

Draft Environmental Assessment

Field Release of *Phymastichus coffea* (Hymenoptera: Eulophidae) for the Biological Control of Coffee Berry Borer, *Hypothenemus hampei* (Coleoptera: Scolytinae) in Hawaii

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1. Summary

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari), (Coleoptera: Curculionidae: Scolytinae) is the most destructive insect pest of coffee globally. Though endemic to Central Africa, CBB is now found in almost every coffee-producing country in the world. In 2010, it first invaded the island of Hawai'i where high quality coffee is the second largest cash crop, valued at more than $55 million during the 2020-21 season. Coffee berry borer has since invaded coffee on the islands of Oahu, Maui and Kaui. Coffee crop loss due to CBB is estimated at $7.7 million. CBB has had the effect of making coffee farming more intensive and less profitable: damage causes significant losses in yield and alters the flavor profile of salvageable coffee beans. If left unmanaged, CBB can damage >90% of the crop.

Figure 1: CBB gallery inside bean, with visible eggs and larvae

The primary means of control in Hawaii is using the microbial insecticide *Beauveria bassiana* and sanitation (removal of all coffee berries after harvest). Biological control of CBB using parasitoids has been conducted in many countries around the world, especially in Latin America (Mexico south to Brazil) and has potential for Hawaii. One of the most promising agents is a parasitoid wasp, *Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae). *Phymastichus coffea* is a primary, gregarious, idiobiont endoparasitoid of CBB adult females. After being parasitized by *P. coffea*, females stop oviposition and usually die after 4-12 days. Therefore, *P. coffea* was chosen as a potential biological control agent and was brought from Colombia into a quarantine containment facility in Volcano, Hawaii for host range testing to determine whether the parasitoid might attack non-target species and thereby pose a risk to the environment. Using no-choice tests, 43 different species of Coleoptera were tested, including 23 scolytines (6 *Hypothenemus* species, 7 native *Xyleborus* species, and 10 others), and 4 additional Curculionidae*. P. coffea* was only able to parasitize the target host *H. hampei* and 4 other adventive species of *Hypothenemus: H. obscurus, H. seriatus, H. birmanus and H. crudiae*. *Hypothenemus hampei* had the highest parasitism rate and shortest parasitoid development time of the five parasitized *Hypothenemus* spp. Parasitism and parasitoid

emergence decreased with decreasing phylogenetic relatedness of the *Hypothenemus* spp. to *H. hampei*, and the most distantly related species, *H. eruditus*, was not parasitized. There are no native Hawaiian species in the genus *Hypothenemus*. *Phymastichus coffea* appears to be host-specific at the genus level, and only able to survive on species closely related to *H. hampei*. Therefore, release of *P. coffea* for control of CBB in Hawaii coffee should cause no harm to the environment.

This Draft Environmental Assessment (DEA) was prepared by the USDA-ARS (Hilo, Hawaii) for HDOA Plant Quarantine Branch and submitted to the Office of Environmental Quality Control (OEQC), Department of Health, State of Hawaii, to comply with the provisions of Hawaii Revised Statutes, HAR Chapter 11-200.1, Environmental Impact Statements.

I. Proposed Action

An application was submitted by the USDA-ARS, Hilo, Hawaii, to the HDOA Plant Quarantine Branch, 1849 Auiki Street, Honolulu, Hawaii 96819, for a permit to introduce *Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae) into the State of Hawaii under the provisions of Hawaii Revised Statutes, Chapter 141, Department of Agriculture, and Chapter 150A, Plant and Non-Domestic Animal Quarantine. *Phymastichus coffea* will be used to control the coffee berry borer, *Hypothenemus hampei* (Ferrari) (CBB) (Coleoptera: Scolytinae), a serious invasive pest of coffee in Hawaii.

1.1 Purpose of release

The USDA-ARS proposes to introduce the parasitoid wasp, *Phymastichus coffea* from containment into the natural environment of the State of Hawaii as a biological control agent to suppress infestations of the coffee berry borer, *Hypothenemus hampei*. Host specificity studies have been completed in the USDA Forest Service quarantine facility at Hawaii Volcanoes National Park. In addition to its natural host, coffee berry borer, *P coffea* was found to attack four other species in the genus *Hypothenemus*. The parasitoid did not attack any of the native and beneficial beetles tested. It is expected that *P. coffea* will become established as a classical biological control agent, providing sustained population suppression of CBB in Hawaii. If establishment of *P. coffea* is variable or unsuccessful in some areas, additional releases will made, or augmentative releases might be considered in some locations.

1.2 Need for release

The coffee berry borer is the most serious pest of coffee in most coffee producing countries. In Hawaii, coffee berry borer was first reported in 2010 from South Kona and soon spread throughout Hawaii island coffee farms and to the other islands. The coffee berry borer severely affects the yield and quality of the coffee and it is an important constraint on production and development of the crop. The current crop losses of coffee due to the coffee berry borer infestation in Hawaii is estimated at $7.7 million (HDOA 2019). If left uncontrolled coffee berry borer can infest >90% of coffee berries. The control of this pest with pesticides is expensive and has limited success if the borer has reached the endosperm of the seeds (Vega et al., 2015). Biological control is a sustainable option to manage the coffee berry borer. *Phymastichus coffea* has proven to be an effective biological control agent of coffee berry borer in other coffee growing

regions in the world (Escobar-Ramirez et al., 2019). Furthermore, *P. coffea* is the only parasitoid tested thus far that has been shown to reduce yield loss from CBB damage (Infante et al., 2013). *Phymastichus coffea* has the potential to be an effective biological control agent against the coffee berry borer in Hawaii.

Figure 2: Adult CBB as found inside a green berry

1.3 Reasons for choice of entomophagous biological control agent

The parasitoids, *Cepahlonomia stephanoderis* Betrem, *C. hyalinipennis* Ashmead, *Prorops nasuta* Waterston (Hymenoptera:Bethylidae), *Heterospilus coffeicola* Schneideknecht (Hymenoptera:Braconidae), and *Phymastichus coffea* LaSalle (Hymenoptera:Eulophidae), all of African-origin, have been introduced in many coffee producing countries, particularly in Central and South America (Klein-Koch et al. 1988; Barrera et al. 1990; Baker 1999; Jaramillo et al. 2005; Portilla and Grodowitz 2018), but none have been released in Hawaii.

Phymastichus coffea was chosen as the best candidate parasitoid in Hawaii because of its previously reported high host specificity and ability to significantly reduce and regulate *H. hampei* populations in the field (Gutierrez et al. 1998; López-Vaamonde and Moore 1998; Castillo et al. 2004a,b; Rodríguez et al. 2017). In field cage studies in Mexico and Costa Rico, *P. coffea* proved to be the most promising biological control agent against *H. hampei* with parasitism rates as high as 95% (Espinoza et al. 2009; Infante et al. 2013).

Figure 3: Phymastichus coffea parasitizing CBB in berry. Photo courtesy of Cenicafé.

To date, *P. coffea* has been released in 12 countries as a classical biological control agent (Bustillo et al. 1998; Damon 2000; Jaramillo et al. 2005; Vega et al. 2015). *P. coffea* is native to Africa and present in most coffee producing countries on that continent. It is a primary, gregarious, idiobiont endoparasitoid of adult *H. hampei* females with a high capacity for host-discrimination (Feldhege 1992; Infante et al. 1994; López-Vaamonde and Moore 1998; Castillo et al. 2004). Two laboratory studies reported that in addition to *H. hampei*, *P. coffea* parasitizes other *Hypothenemus* spp. such as *H. seriatus* and *H. obscurus* (López-Vaamonde and Moore, 1998), and *H. eruditus* Westwood and *H. crudiae* (Panzer) (Castillo et al. 2004a,b). However, parasitism of closely related species in the field has not been reported (Escobar-Ramírez et al. 2019).

1.4 Specific location of rearing/containment facilities and name of qualified personnel operating the facility

Phymastichus coffea was obtained from Cenicafé in Colombia under USDA APHIS PPQ, permit no. P526P-18-00696 and brought into a fully certified quarantine insect containment facility managed by the USDA Forest Service at Hawaii Volcanoes National Park, Volcano, Hawaii, for host-specificity testing. The director and primary user of this facility is Dr. M. Tracy Johnson of the USDA Forest Service, Institute for Pacific Island Forestry.

1.5 Timing of the release as well as factors that affect the timing of release

If *Phymastichus coffea* is approved for release, Cenicafé (Colombia) will supply wasps for the initial releases. Cenicafé is currently mass rearing *P. coffea* on field-collected CBB and can provide *P. coffea* at any time of year. *P. coffea* will be released in coffee on all islands where CBB occurs (Hawaii, Oahu, Maui, Kauai). *Phymastichus coffea* will be released and monitored for establishment in a classical biological control program. In the future, augmentative releases of *P. coffea* from Cenicafé may be possible if documentation and certification of their rearing process and facility demonstrates that the colony is pure and quality control ensures there will be no contamination. Currently, trapping and sampling of infested coffee fruits is conducted to monitor

H. hampei flights and optimize timing of *Beauveria bassiana* applications for control (Aristizabal et al. 2016). After *H. hampei* bores into the coffee berries it is protected and difficult to control with biopesticides or conventional insecticides. To achieve maximum *P. coffea* parasitism in the field, releases should be made at times when *H. hampei* adults are active (e.g. when trap catches are high, or female *H. hampei* are actively boring into fruits) and the coffee crop is at a susceptible stage. Optimal timing of releases may differ for different elevations due to *H. hampei* population dynamics (Hamilton et al. 2019). Studies suggest *P. coffea* may be susceptible to *B. bassiana,* however (Barrera 2005; Castillo et al. 2009; Ruiz et al. 2011), so releases should be timed to avoid *B. bassiana* applications or used in alternation with *B. bassiana* against *H. hampei*. If *P. coffea* is highly effective, then dependence on *B. beauveria* applications could be reduced dramatically.

1.6 Location of planned first release

First releases will be made in the South Kona district of the Big Island of Hawaii in the main coffee growing region as it is close to the USDA ARS laboratory and University of Hawaii experiment station which will facilitate monitoring. Other sites may also be selected depending on the number of parasitoids available.

According to the simulation model output, *P. coffea* is predicted to provide feasible control of coffee berry borers in areas where flowering periods are frequent throughout the year (Rodríguez et al. 2017). In Hawaii, Maui and Oahu due to relatively constant temperatures with abundant rainfall, coffee flowering and harvesting seasons may be irregular. However, Kona is different with more pronounced seasonal conditions. So, depending on the flowering season, releases of *P. coffea* will be made approximately 70 and 170 days after flowering periods (when coffee berries have >20% dry matter content), or at times when CBB adults are active (e.g. trap catches are high) and the crop is at a susceptibility stage.

P. coffea may be sensitive to *Beauveria bassiana*, the fungal biopesticide used against the coffee berry borer and to other insecticides (Castillo et al.2009; Barrera 2005; Gómez et al. 2011). Therefore, it is important to make sure that the parasitoids are not released just before or just after or concurrently with pesticides to prevent any negative effects on survivorship and establishment.

1.7 Methods to be used after agent importation

Newly emerged female *P. coffea* will be collected into plastic containers covered with muslin impregnated with a 50% honey-water solution. The containers will be placed in a cool box and transported to the field. The parasitoids will be released in the center of the coffee field. A ratio of 1 parasitoid per 10 hosts (determined from random field sampling for infested coffee berries) or less would be ideal (Espinoza et al. 2009). Once the parasitoids are released, they will disperse naturally to search for new coffee berry borer hosts to parasitize.

1.8 Methods to be used for disposing of any host material, pathogens, parasities, parasitoids, and hyperparasitoids accompanying an import

Because of its short life span (2-4 days), *P. coffea* will be shipped from Cenicafé as parasitized adult CBB into quarantine containment and reared through a generation to ensure that no hyperparasitoids. A sample of parasitized CBB will be tested for plant pathogens, e.g.

coffee leaf rust, by USDA ARS scientists. Parasitized CBB adults shipped to Hawaii for host range testing exhibited low *P. coffea* emergence (5-20%). Studies are underway with Cenicafé to optimize shipping conditions for improved parasite emergence. No pathogens or hyperparasitoids have been observed at Cenicafé on *P. coffea*-parasitized CBB. *P. coffea* shipments will not contain any plant material, e.g. coffee berries. Parasitized CBB may be shipped on artificial diet, which will be autoclaved after parasitoid emergence.

1.9 Agencies or individuals that will be involved in the release and monitoring

USDA-ARS (Peter Follett, Melissa Johnson), University of Hawaii (Mark Wright, Andrea Kawabata, graduate students), and the Hawaii Department of Agriculture (Mohsen Ramadan, Juliana Yalemar) will be involved in the release and evaluation of *P. coffea* (establishment, dispersal, parasitism rates, behavior, integration with coffee IPM practices, nontarget effects).

2. Target Pest Information

2.1 Taxonomy: scientific name, full classification, synonymy, common name and sufficient characterization to allow unambiguous recognition

Order: Coleoptera
Family: Curculionidae
Subfamily: Scolytinae
Genus: *Hypothenemus*
Species: *H. hampei*
Common name: coffee berry borer (CBB)

Binomial name: *Hypothenemus hampei* (Ferrari, 1867)

Figure 4: CBB. Courtesy HDOA

Synonyms
Cryphalus hampei Ferrari, 1867
Stephanoderes hampei Ferrari, 1871
Stephanoderes coffeae Hagedorn, 1910
Xyleborus coffeivorus Van der Weele, 1910
Xyloborus cofeicola Campos Novaes, 1922
Hypothenemus coffeae (Hagedorn)

The genus *Hypothenemus* is one of the most speciose in the Scolytinae and common in all tropical and subtropical areas. The taxonomic characters useful in identifying *Hypothenemus hampei* and related members of the genus is presented in Vega et al. 2015 (“The Genus *Hypothenemus*, with emphasis on *H. hampei*, the coffee berry borer” pp. 427-494, In *Bark Beetles: Biology and Ecology of Native and Invasive Species* [F. E. Vega and R. W. Hofstetter, Eds.], Academic Press, San Diego). The information below is excerpted from this book chapter.

Most *Hypothenemus* species are very small (<2 mm long), poorly described, and difficult to distinguish. Several species are globally distributed, undoubtedly aided by human activities.

Although the vast majority of *Hypothenemus* species live innocuously in twigs, some have become important pests, most notably the coffee berry borer *Hypothenemus hampei* (Ferrari), which lives inside the coffee berry and consumes the seeds, and the tropical nut borer Hypothenemus obscurus (F.), which attacks a range of seeds and fruits.

The frons of *H. hampei* may have a broad, indistinct frontal groove, or no groove at all. There are usually four marginal asperities. The setae on the pronotum are mixed, with some slightly flattened. The shape of the pronotum, viewed from above, is slightly more narrowly rounded (i.e., more triangular) than the similar *Hypothenemus* species. The elytral declivity of H. hampei is much more broadly rounded than in the similar species, without a distinct transition from the elytral disc. When viewed laterally, the declivity takes up more than half of the length of the elytra, whereas in the similar species, the elytral disc takes up more than half of the length. As with most *Hypothenemus*, the interstrial bristles are prominent and in almost perfectly uniseriate rows. The shape of the interstrial bristles, however, is distinctive, and differentiates the coffee berry borer from most other *Hypothenemus* species. The bristles are long, narrow, and slightly flattened. The tip of each bristle is square, and not much wider than the rest of its length. The bristles on the elytral disc are not much shorter than those on the declivity. Males are smaller with reduced eyes. The interstrial bristles are relatively long, and often not in distinct rows.

Phylogenetically, *H. hampei* is in a clade distantly related to native Hawaiian Scolytinae species, which are all within the Tribe Xyleborini (Johnson et al. 2018). There are other *Hypothenemus* species in Hawaii, all adventive. While there are anecdotal reports of *H. hampei* feeding on plants other than coffee (e.g. *Leucaena leucocephala*), there is no indication that they could complete their life cycle in those hosts. No native Scolytinae are known to utilize those plants.

2.2 Economic impact and benefits of the target pest: *Hypothenemus hampei*

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari), (Coleoptera: Curculionidae: Scolytinae) is the most destructive insect pest of coffee globally, inflicting economical loses of over US$500 million annually. Though endemic to Central Africa, CBB is now found in almost every coffee-producing country in the world. In 2010, it first invaded the island of Hawai'i where high quality coffee is the third largest cash crop, valued at more than $43 million during the 2017-18 season. Coffee berry borer has since invaded coffee on the islands of Oahu and Maui and most recently Kauai. Coffee crop loss due to CBB is estimated at $7.7 million. CBB has had the effect of making coffee farming more intensive and less profitable: damage causes significant losses in yield and alters the flavor profile of salvageable coffee beans. If left unmanaged, CBB can damage >90% of the crop.

CBB has been found on several incidental non-crop host plants in Hawaii such as haole koa (*Leucaena leucocephala*), black wattle (*Acacia decurrens*), and red fruit passionflower or love-in-a-mist (*Passiflora foetida*). However, to date researchers have found only a very low incidence of CBB in any of these other plants, and no signs of CBB reproduction in any of them. Wild (uncultivated) coffee plants are a significant reservoir for CBB populations (Messing 2012).

2.3 Biology and reproductive potential of the target pest

Hypothenemus hampei attacks coffee berries when the dry matter content of the endosperm, which increases with age, exceeds 20% (Jaramillo et al. 2005). After finding a suitable berry host, *H. hampei* bores into the coffee fruit through the central disc and excavates galleries where it lays eggs. The offspring develop inside the seeds and feed on the endosperm tissue (Damon 2000), reducing both coffee yield and quality. *H. hampei* feeding damage can also cause premature fall of berries younger than 80 days (Decazy 1990). *H. hampei* adults boring into the berry may remain in the 'A' position (Jaramillo et al. 2006) with the abdomen half exposed outside the berry potentially for weeks waiting for the dry matter content to reach 20% (Jaramillo et al. 2005). Females are synovigenic and lay eggs in batches of 2-3 eggs beginning three days after penetration into the seed. About 31-119 eggs are laid within a single berry over a period of 3 weeks. Soon after egg laying commences wing muscles of the female degenerate, preventing the colonization of other berries (Ticheler 1963). Multiple generations may occur in the coffee berry under Hawaii conditions. Waterhouse and Norris (1989), suggested that females may leave the berry when all the seed tissue is consumed or deteriorated in some way, or when her progeny begin to emerge, in order to continue egg-laying in another berry. After *H. hampei* bores into the coffee berries it is protected and difficult to control with biopesticides or conventional insecticides.

2.4 Global distribution of the target pest

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari), (Coleoptera: Curculionidae: Scolytinae) is the most destructive insect pest of coffee globally. Though endemic to Central Africa (likely the Ethiopian Highlands), CBB is now found in almost every coffee-producing country in the world. Coffee berry borer was first discovered in 1867 in France in coffee seeds traded from unknown origin (Waterhouse and Norris 1989), and in Africa it was reported in 1901 from Gabon (Le Pelley 1968) and in 1903 from Zaire (Murphy and Moore 1990). The beetle is endemic to central Africa, but the exact origin of the pest is still not clear (Damon 2000).

2.5 Economically, ecologically important (e.g. keystone, endangered) species in North America (introduced and native) that are phylogenetically related or occur in the same habitat as the target pest

We test the hypothesis that *P. coffea* is monophagous, with a physiological host range limited to its natal host, *H. hampei*. There are 11 species of adventive *Hypothenemus* (Tribe Cryphalini) recorded in Hawaii (Nishida 2002). There are no records of native Hawaiian *Hypothenemus* spp. except for a questionable old record (1913) of *H. ruficeps* (Swezey 1954), which has never been collected or reported since and is likely a synonym with *H. eruditus* or *H. crudiae* (C. Gillett, unpublished), which are adventive. There are also no known native species of the Tribe Cryphalini in Hawaii. There are, however, many native species in another scolytine genus, *Xyleborus* (Tribe Xyloborini) (Samuelson 1981; Gillett et al. 2019), which may potentially be impacted by release of an exotic parasitoid against a scolytine pest such as *H. hampei*. The Xyleborini are phylogenetically distant from the adventive Cryphalini species in Hawaii (Johnson et al. 2018). Our host range testing in quarantine included 6 of the 11 species of *Hypothenemus* (all adventive species) and 7 of the 28 species of native *Xyleborus* as well as an additional 10 exotic scolytine species in related genera (*Xyleborinus*, *Xylosandrus*, *Xyleborus*, *Euwallacea*, others).

2.6 Regulatory or pest status of the target pest in the state, provincial or federal law

Hypothenemus hampei is established on all the Hawaiian Islands growing coffee and considered a significant pest that is actively being controlled.

2.7 Knowledge of status of other biological control agents (indigenous or introduced) that attack the pest

No biocontrol agents were previously released in Hawaii against *H. hampei*. Two exotic predatory beetles, *Cathartus quadricollis* and *Leptophloeus* sp., are commonly found in overripe and dried coffee berries naturally predating on the immature stages of *H. hampei* in Hawaii (Follett et al. 2016; Brill et al. 2020). Our host testing in quarantine showed that *P. coffea* will not parasitize these beetles, and that the beetles did not predate on the parasitoids. Also, these predators attack eggs, larvae and pupae of *H. hampei* in overripe and dried berries (left after harvesting), whereas *P. coffea* attacks adult female *H. hampei* primarlily in developing green berries at an earlier stage of crop maturity.

Beauveria bassiana, formulated as BotaniGard®, is sprayed frequently for *H. hampei* control. Repeated applications reduce coffee berry borer damage, but are costly, and efficacy varies depending on local conditions (Greco et al. 2018).

2.8 Life stage of the pest that is vulnerable to the biological control agent

Phymastichus coffea is a primary, gregarious, idiobiont endoparasitoid of adult *H. hampei* females. The beetles are parasitized by *P. coffea* while actively boring into coffee fruits with the abdomen exposed, which can be a prolonged process depending on the ripeness of the fruits. This is unique behavior among Scolytinae, which typically bore into wood.

3. Biological Control Agent Information

3.1 Taxonomy: scientific name (order, family, genus, species, scientific authority

Phymastichus coffea LaSalle (Hymenoptera: Eulophidae). It has no common name. *Phymastichus coffea* was collected in Togo in 1987 and described by LaSalle in 1990. The parasitoid wasp belongs to the family Eulophidae, one of the largest in the Hymenoptera, with nearly 4000 described species. The sub family Tetrastichinae to which the parasitoid belongs has 42 genera and is most widespread of all parasitic groups. Tetrastichinae has an extraordinarily wide host range attacking over 100 families of insects in 10 different orders, as well as mites, spider eggs, and even nematodes (LaSalle 1994). *Phymastichus* can be distinguished from other tetratichinae by the presence of distinctively swollen parastigma and lack the presence of a sensory plaque on the ventral edge of the male scape (LaSalle 1990). There are only two known species in this genus, (i) *Phymastichus coffea* and (ii) *P. xylebori*. Both species have potential value in biological control programs against scolytines. *Phymastichus coffea* attacks mainly adult *H. hampei* (CBB) whereas, *P. xylebori* attacks adults of the highly polyphagous island pinhole borer, *Xyleborus perforans* (Wollaston). A third species, *Phymastichus* sp. nova (D. Honsberger pers. comm.) is currently being described from Hawaii. The latter does not parasitize *H. hampei*.

3.2 Methods used to identify the biological control agent

Phymastichus coffea was imported into Colombia at Cenicafé, where it has been mass reared in pure culture on CBB-infested coffee since its importation.

3.3 Location of reference specimens

Voucher specimens are deposited at Cenicafé (Manizales, Colombia), at the USDA-ARS laboratory in Hilo, Hawaii, and at the University of Hawaii at Manoa.

3.4 Natural geographic range, other areas where introduced, and expected attainable range in Hawaii (also habitat preference and climactic requirements of the biological control agent)

To date, *P. coffea* has been released in 12 countries as a classical biological control agent (Bustillo et al. 1998; Damon 2000; Jaramillo et al. 2005; Vega et al. 2015). *Phymastichus coffea* is native to Africa and present in most coffee producing countries on the continent. According to the CABI Invasive Species Compendium, *P. coffea* occurs in Kenya, Togo, and Mexico. Kenya and Togo are presumably within the native range, whereas it may have established in Mexico after release as a biological control agent against coffee berry borer. Hawaii is characteristically tropical but with moderate temperatures and humidity due to the influence of north and eastern trade winds. The climate at the elevations where coffee is grown should allow survival of *P. coffea* year-round.

3.5 Source of the biological control agent

Centro Nacional de Investigaciones de Café - CENICAFÉ, Manizales, Colombia.

3.6 Host/biological control agent interactions

Phymastichus coffea is an idiobiont, gregarious endoparasitoid of adult coffee berry borer, commonly laying two eggs (a male and a female) per host (Lopez-Vaamonde and Moore 1998). Both male and female develop in a single host, the female in the abdomen and the male in the prothorax (Espinoza et al., 2009), although a single female parasitoid is sometimes found living solitarily in the abdomen of the host. The parasitoid develops through four major life stages—egg, larva (three instars lasting ~21 days), pupa (~9 days) and adult. The complete development (egg to adult) occurs over 30-43 days depending on temperature and condition of the CBB host mummies. For example, at 23°C the life cycle of *P. coffea* is 43 days. The parasitoid emerges by cutting an opening in the host's integument (Feldhege, 1992).

Figure 5: Parasitized CBB with Phymastichus pupa in abdomen.

The average lifespan of the parasitoid is 1-2 days for males and 3-4 days for females (Espinoza et al., 2009). Longevity can be prolonged with 50% honey-water solution as food and if the temperature is decreased (F. Yousuf unpublished). On emergence, female parasitoids can have up to 10 eggs in the ovarioles, but more eggs are formed throughout her lifetime (synovigenic strategy) (Lopez-Vaamonde and Moore, 1998). There is no preoviposition period and the adult female parasitoids can parasitize the coffee berry borer adults immediately after emergence (Infante et al., 1994). It has been shown that *H. hampei* is attracted to semiochemicals released from coffee fruits (Mendesil et al. 2009); semiochemicals released during *H. hampei* feeding on fruits have been shown to attract *P. coffea* (Cruz-Lopez et al. 2016), and may play also a significant role in mediating the host specificity of their parasitoids under field conditions.

3.7 Biology and reproductive potential (including dispersal capability and damage inflicted on the target pest.)

Gravid *P. coffea* females start to search for their hosts immediately after emerging from the adult female host and parasitism occurs within the first hours after emergence (Infante et al. 1994). *Phymastichus coffea* has an extremely short life span as an adult; the longevity of males ranges from 8-48 h and females from 16-72 h (Vergara et al. 2001; Portilla and Grodowitz 2018). *Phymastichus coffea* commonly lays two eggs (a male and a female) (López-Vaamonde and Moore 1998) in an *H. hampei* adult female at the time she is initiating fruit perforation, which causes paralysis and prevents further damage to the coffee berry. Both male and female develop in a single host, the female in the abdomen and the male in the prothorax (Espinoza et al. 2009). The parasitized *H. hampei* usually dies within 4-12 days after parasitism (Infante et al. 1994). The life cycle (egg to adult) of *P. coffea* varies from 30-47 days depending on the environmental conditions (temperature and humidity). Females are ~1 mm long, whereas males are half that size (LaSalle 1990). *P. coffea* can parasitize multiple hosts during its short lifespan. High levels of parasitism have been recorded in previous studies under cage and field conditions.

3.8 Known host range based on the scientific literature, host data from museum specimens, and unpublished records

The parasitoid has been described as a primary, gregarious, endoparasitoid of adult females of coffee berry borer (Feldhege 1992). To the best of our knowledge, no reports of parasitism by *P. coffea* on other hosts under field conditions exist. However, based on the results of no choice laboratory assays, two papers have reported *P. coffea* as oligophagous i.e. attacking other non-target scolytine hosts in addition to its primary host (Table 1) (López-Vaamonde and Moore 1998; Castillo et al. 2004).

Table 1. Previous reports of parasitism of Scolytinae species by *Phymastichus coffea* in no-choice laboratory assays.

Scolytinae species	Parasitism (%)	Parasitoid emergence (%)	Reference
Hypothenemus hampei	67.3, 64	48, 54	López-Vaamonde and Moore 1998, Castillo et al., 2004
Hypothenemus obscurus	83.3	15	López-Vaamonde and Moore 1998
Hypothenemus seriatus	76.6	12	López-Vaamonde and Moore 1998
Hypothenemus eruditus	6	4	Castillo et al., 2004
Hypothenemus crudiae	14	14	Castillo et al., 2004
Hypothenemus plumeriae	0	0	Castillo et al., 2004
Araptus sp.	70	18	López-Vaamonde and Moore 1998
Araptus fossifrons	0	0	Castillo et al., 2004
Scolytodes borealis	0	0	Castillo et al., 2004
Tomicus piniperda	0	0	Castillo et al., 2004
Dendroctonus micans	0	0	López-Vaamonde and Moore 1998

As shown in Table 1, although the parasitoid attacked other scolytines, it was restricted to species belonging to the same genus as its natural host, *Hypothenemus*, mostly. Two *Araptus* species were also tested by López-Vaamonde and Moore (1998), and Castillo et al. (2004) but only one showed positive parasitism. Castillo et al. (2004) report that *P. coffea* did not complete its life cycle in *Araptus*, despite relatively high numbers of parasitism attempts in laboratory exposures, while López-Vaamonde and Moore (1998) reported 70% parasitism, and 10-15% emergence of parasitoids, with high parasitoid mortality. No other records of the parasitoid attacking *Araptus* species are available in the literature.

3.9 History of past use of the biological control agent

The parasitoids, *Cephalonomia stephanoderis* Betrem, *C. hyalinipennis* Ashmead and *Prorops nasuta* Waterston (Hymenoptera:Bethylidae), *Heterospilus coffeicola* Schneideknecht (Hymenoptera:Braconidae) and *Phymastichus coffea* LaSalle (Hymenoptera:Eulophidae), all of African origin, have been introduced in many coffee producing countries, particularly in Central and South America (Klein-Koch et al. 1988; Barrera et al. 1990; Baker 1999; Jaramillo et al. 2005; Portilla and Grodowitz 2018), but none have been released in Hawaii. To date, *P. coffea* has been released in 12 countries as a classical biological control agent (Bustillo et al. 1998; Damon 2000; Jaramillo et al. 2005; Vega et al. 2015). Cenicafé (Colombia) recently released

~800,000 *P. coffea* (Feb-Jun 2021) in 40 ha of coffee to examine parasitism rates and the potential for inundative releases of mass reared parasitoids for *H. hampei* control (P. Benevides, pers. comm.).

3.10 Pathogens, parasites, parasitoids and hyperparasitoids (order, family, genus, species, scientific authority) of the agent and how they will be eliminated from the imported culture of the agent.

Imported *P. coffea* will be reared for a generation in quarantine before release to inspect for hyperparasitoids or other insect contaminants. A sample of *P. coffea*-parasitized CBB will be tested for the presence of plant pathogens, e.g. coffee leaf rust, by USDA ARS scientists.

3.11 Procedures stating how the biological control agent will be handled in containment (e.g. scaling up for release)

Phymastichus coffea will be obtained from an established stock maintained at the National Coffee Research Center-Cenicafé, Manizales (Caldas) Colombia, which was started from *P. coffea* collected in Kenya and shipped to Colombia in 1996 and has been maintained in colony in large numbers since that time (Orozco and Aristizábal 1996). *Phymastichus coffea* has been mass reared by Cenicafé on wild-caught CBB for field releases on multiple occasions and the colony receives frequent infusions of field collected material. For nontarget testing, *Phymastichus coffea* was shipped from Cenicafé in its larval stage in parasitized *H. hampei* hosts under USDA APHIS PPQ, permit no. P526P-18-00696 to a certified quarantine insect containment facility managed by the USDA Forest Service at Hawaii Volcanoes National Park, Volcano, Hawaii. Parasitized *H. hampei* were incubated in controlled climate chambers at 25° ± 1°C, 75 ± 10% relative humidity, and 8:16 h light:dark photocycle at the quarantine containment facility. In the future, we hope that USDA APHIS and HDOA will allow the shipment of *P. coffea* from Cenicafé to Hawaii for release directly in the field without containment. Cenicafé is developing a new rearing system on diet rather than infested coffee beans to improve quality control and reduce the risk of contaminants.

3.12 Closely related genera, sibling species, cryptic species and ecologically similar species of the biological control agent in Hawaii, when they occur

The eulophid genus *Phymastichus* contains two described species: *P. coffea* and *P. xylebori*. The candidate biological control agent *Phymastichus coffea* is not known to occur in Hawaii. *Phymastichus xylebori* is adventive in Hawaii and has been found on the Big Island parasitizing *Xyleborus perforans*; *P. xylebori* has not been found in coffee parasitizing *H. hampei* in Hawaii.

4. Host Specificity Testing

4.1 Selection of nontarget test arthropods

The selection of non-target hosts in Hawaii was based on phylogenetic relatedness to the target host (Johnson et al. 2018), sympatry of target- and non-target species, and size. Coleoptera species commonly occurring in the coffee landscape and species in culture at USDA-ARS in Hilo,

Hawaii were also tested; these were species not phylogenetically close to the target host but could provide insights into unexpected host use. There are 21 native and 38 non-native scolytine species in Hawaii (Samuelson 1981; Nishida 2002; Cognato and Rubinoff 2008). Because of the relatively large native scolytine fauna in Hawaii, and their remote or poorly studied habitats, only a subset of these species could be tested for their suitability as hosts to *P. coffea*. Exotic and native scolytine species were collected from coffee and macadamia farms and their surrounding habitats, and extensive searches from native forests from different Hawaiian Islands (Hawaii Island, Oahu, Maui, Molokai, Lanai and Kauai) (Gillett et al. 2020a). We investigated the host selection and parasitism response of *P. coffea* adult females to 43 different species of Coleoptera, including 23 Scolytinae (six *Hypothenemus* species and 17 others), and four additional Curculionidae (Yousuf et al. 2021). The list included Hawaiian endemic species (several Scolytinae in the genus *Xyleborus* and *Nesotocus giffardi,* a curculionid weevil), exotic pest species (e.g. the scolytines *Hypothenemus obscurus* [tropical nut borer] and *Xylosandrus compactus* [black twig borer], and the curculionids *Sitophilus oryzae* [rice weevil] and *Cylas formicarius* [sweetpotato weevil]), and beneficial species (e.g. a weed biocontrol agent *Uroplata girardi* from lantana, several coccinellids, and two flat bark beetle predators of *H. hampei*, *Cathartus quadricollis* and *Leptophloeus* sp.) (Tables 2-5) (Appendix A: Yousuf et al. 2021). All beetles used in host specificity tests were collected live and later preserved in 75% alcohol or pinned for identification by taxonomists with expertise in the respective taxa. The body size of the collected species ranged from 1-7 mm but the majority of species were similar in size to *H. hampei* which is 1.5-2.0 mm in length. Beetles were collected using Lindgren funnels or bucket or Broca traps baited with denatured ethanol only or ethanol + methanol + ethylene glycol lures, or collected directly from infested plant material (fruits, pods, stems, bark and seeds), or reared from infested wood in the laboratory (Gillett et al. 2020b). All non-target testing was conducted at the USDA Forest Service quarantine containment facility at Hawaii Volcanoes National Park, Volcano, Hawaii.

4.2 Laboratory tests

No-choice tests

We used no-choice tests because these would reflect physiological host range and the most conservative assessment of potential for parasitism in the field, rather than choice tests (Van Driesche and Murray 2004). Choice tests that include the target host may mask the acceptability of lower ranked hosts, thereby producing false negative results (Withers and Mansfield 2005). Twenty individuals of each test species were placed in a sterilized glass Petri dish (80 mm in diameter) lined with filter paper and immediately afterwards four *P. coffea* females (<12h old) that had not been exposed to adult hosts prior to the experiments were introduced. Therefore, when ample hosts were available, each replicate consisted of 20 hosts and four parasitoids for a 5:1 host:parasitoid ratio. However, due to difficulties in finding certain species live in adequate numbers, e.g. native scolytine bark beetles, and difficulties synchronizing parasitoid emergence with field collection or emergence from wood of live beetles, the host:parasitoid ratio and numbers of replicates were adjusted as needed. For example, if only 10 non-target beetles were available for screening, then two replicates each with 5 beetles and 1 parasitoid (maintaining the 5:1 host:parasitoid ratio) were performed. In all non-target host screening tests, *H. hampei* was

included as a positive control to confirm parasitoid viability. The host:parasitoid ratio of the *H. hampei* controls was adjusted to match the nontarget species in the test, whether it was 5:1 or otherwise. The generalized behavioral response of the parasitoids towards target and non-target hosts was also determined for a subset of parasitoids by visual observation and video recording of parasitoid behavior, e.g. any contact with the host by landing on the host or antennation, and/or walking on the host. Host acceptance was noted when the parasitoid adopted a characteristic oviposition position on top the elytra of the host (Lopez-Vaamonde and Moore 1998).

After *P. coffea* exposure, *H. hampei* and all other non-target species were incubated at 25 ± 1°C, 75 ± 10% RH and 24:0 (L:D) photoperiod for 72h. After 72h, parasitoids and filter paper linings were removed and the beetles were provided with a small cube (2 x 2 x 2 cm) of general beetle diet (F. Yousuf, unpublished). The beetles were again incubated at the same environmental conditions but now at 0:24 (L:D). After 10 days all the remaining diet and frass was removed (without disturbing the parasitized beetles) to avoid fungal contamination. Parasitized beetles typically became paralyzed and eventually died within 4-12 days after parasitoid oviposition. Beetles were held for a total of ~5-6 weeks for parasitoid emergence. Beginning after 25 days incubation, *H. hampei* mummies were inspected daily for adult wasp emergence. Parasitism was assessed based on observation of emergence of parasitoid progeny (F1 adult wasps) from the parasitized beetles, by inspection for exit holes on cadavers, or by dissection. Beetles with no exit holes were dissected (by separating the thorax from the abdomen) under a stereomicroscope using fine forceps and entomological pins at 20-100X magnification for evidence of parasitism, i.e., presence of *P. coffea* immature life stages (eggs, larvae or pupae), or unemerged adults. The number of unemerged life stages was recorded for each dissected beetle. After 5-6 weeks of incubation, dead beetle specimens sometimes became very dry and searching for the presence of eggs and early instar larvae was difficult. In such cases, beetles were dissected and examined under a compound microscope at 200X to seek unemerged *P. coffea*. The sex of emerged adult *P. coffea* offspring was determined by examination using a stereomicroscope. In most cases, two parasitoids (one male and one female) emerged per beetle host. To confirm this the sum of the emerged male and female parasitoids in each replicate was divided by two and compared to the number of parasitized hosts with exit holes. The sex of unemerged parasitoids was not determined. For data on parasitism, life stages, sex ratio, and development time, averages were calculated for each replicate (per Petri dish) for each species and used in statistical analysis. Grand means of all the replicates for each of the five *Hypothenemus* species are presented in figures and tables.

Statistical analysis

Parasitism rate was calculated by dividing the number of parasitized hosts by the total number of hosts exposed to the parasitoids in each replicate. Parasitism included both emerged and unemerged wasps. Emergence rate was calculated by dividing the number of beetles with exit holes by the total number of parasitized hosts (emerged plus unemerged wasps). The sex ratio of the parasitoid progeny was calculated by dividing the number of emerged female parasitoids (F) by the total number of emerged male (M) and female (F) parasitoids [F/ (F+M) x 100]. The Shapiro–Wilk test (Shapiro and Wilk 1965; Razali and Wah 2011), numerical approaches (skewness and kurtosis indices), and the normal Q-Q plot-based graphical method were used to check the distribution of the data and showed that the data were not normally distributed. Generalized linear models (GLM) were therefore used to analyze the data, with

appropriate distribution function links. Parasitism and emergence rates of the parasitoids, and the percentage of different life stages (larvae, pupae and adults) in parasitized beetles with unemerged parasitoids were analyzed using GLM with a binary logistic function and sex ratio with a gamma log link function. Developmental time of the F1 offspring (egg to adult) was analyzed using GLM with a negative binomial log link function because data were overdispersed (i.e. variance > mean). Wald χ^2 approximations are reported. All analyses were performed using IBM SPSS statistics software.

Results

Out of 43 total coleopteran species tested, including 23 scolytines, *P. coffea* oviposited and completed developed only in the target *Hypothenemus hampei* and four other species of *Hypothenemus*: *H. obscurus*, *H. seriatus, H. birmanus* and *H. crudiae* (Tables 2-5). Mean percentages of parasitism and emergence for the *Hypothenemus* spp. tested are shown in Figure 6. Parasitism (χ^2 = 65.13, df = 4, p = 0.0001) and emergence (χ^2 = 23.20, df = 4, p = 0.0001) were significantly higher in *H. hampei* than all other *Hypothenemus* species. *Hypothenemus hampei* had the highest percentage emergence of *P. coffea* at 70.4%, whereas *H. crudiae* had the lowest at 16.7% (Figure 6). In *H. crudiae*, out of five parasitized hosts only one had emergence. Although *P. coffea* only parasitized *Hypothenemus* spp., it did inspect three other non-target scolytine hosts, *Hypothenemus eruditus*, *Xyleborus kauaiensis* and *Xyleborus ferrugineus,* but left hosts without initiating oviposition (i.e. no parasitism found). The phylogenetic relationship of five *Hypothenemus* species included in our tests, extracted from Johnson et al. (2018), is also shown in Figure 6; *H. crudiae* is not included in the phylogeny because it was not included in Johnson et al (2018). Both parasitism and emergence in our tests decreased across *Hypothenemus* species with decreasing phylogenetic relatedness to *H. hampei*. *Hypothenemus eruditus*, the most distantly related species tested from *H. hampei* according to Johnson et al. (2018) was not parasitized (Figure 6).

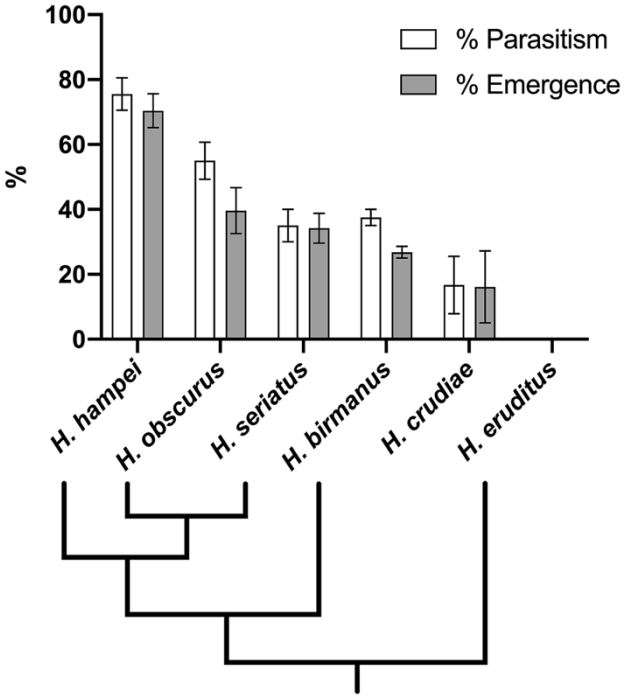

Figure 6: Percentage parasitism and emergence (mean ± SE) of adult Phymastichus coffea parasitoids from Hypothenemus spp. Inferred from Johnson et al (2018).

Parasitoid development time among the three different *Hypothenemus* spp. did not differ significantly compared with *H. hampei* (χ^2 = 0.17, df = 4, p = 0.997), but did differ with *H. crudiae* (Table 2). The mean development time of *P. coffea* from oviposition to adult emergence was

shortest in *H. hampei* (32.2 ± 0.5 days, mean ± SE), longest in *H. crudiae* (41.0 ± 0.0 days) and intermediate in the other three *Hypothenemus* spp. (Table 2), which generally agrees with the phylogenetic pattern observed for parasitism and emergence (Figure 1). The percentage of female versus male *P. coffea* emerging from parasitized *H. hampei* was 50.8% ± 0.4 (mean ± SE), which was significantly different (χ^2 = 27.3, df = 4, p = 0.0001) from *H. seriatus* and *H. birmanus* (Table 2). *Hypothenemus eruditus* was not parasitized by *P. coffea* and hence was not included in any statistical analyses.

Table 2. Development time and sex ratio of *Phymastichus coffea* in no-choice in vitro non-target host selection screening of *Hypothenemus* species, including *H. hampei* as a control species.

Species	**Insect status**	**Total beetles exposed**	**Development time (days ± SE)**	**Sex ratio (mean % females ± SE)**
Hypothenemus hampei (control)	Exotic/Pest	170	32.2 ± 0.5	50.8 ± 0.4
Hypothenemus obscurus	Exotic/Pest	80	35.0 ± 0.9	54.8 ± 1.6*
Hypothenemus seriatus	Exotic	60	38.0 ± 1.0	51.1 ± 1.1
Hypothenemus birmanus	Exotic	40	37.0 ± 1.0	57.7 ± 3.8*
Hypothenemus crudiae	Exotic	30	41.0 ± 0.0*	50.0
Hypothenemus eruditus	Exotic	80	-	-

* significantly different from *Hypothenemus hampei* (control), $p < 0.05$.

Parasitized *H. hampei* had the lowest percentage of unemerged parasitoids compared to the other four *Hypothenemus* species (Figure 7), indicating that *H hampei* is a superior host for *P. coffea* development. For each parasitized host beetle with unemerged parasitoids, invariably two parasitoids were present, and the parasitoids were of the same life stage (larva, pupa, or adult). The frequency of the different life stages for parasitized hosts with unemerged parasitoids differed among *Hypothenemus* species (Figure 7). Parasitized *H. hampei* had a significantly lower percentage of larval (χ^2 = 15.10, df= 3, p= 0.001) and higher percentage of adult parasitoids that were unemerged (χ^2 = 18.36, df= 3, p= 0.0001) compared to the other *Hypothenemus* species. The higher percentage of unemerged parasitoids developing to the adult stage again indicates that *H. hampei* is a superior developmental host than the other *Hypothenemus* spp. The percentage of unemerged pupae found in parasitized *H. hampei* was not significantly different from *H. obscurus*, *H. seriatus* and *H. birmanus*, but *H. crudiae* had a significantly higher percentage of pupae than *H. hampei* (χ^2 = 95.40, df= 4, p= 0.0001) (Figure 7). No eggs were found in any of the parasitized *Hypothenemus* hosts.

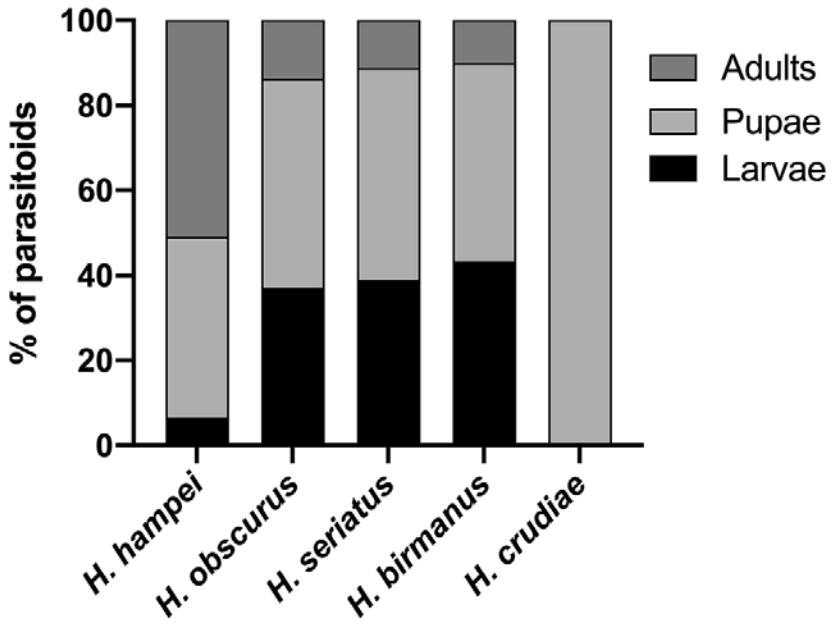

Figure 7: Fate of unemerged Phymastichus coffea parasitoids from parasitized Hypothenemus spp. in no-choice in vitro non-targeted selection screening. Parasitized Hypothenemus beetles with unemerged parasitoids were dissected to identify life stages (larva, pupa, adult)

Summary of laboratory tests in quarantine

The candidate biological control agent *Phymastichus coffea* was brought from Colombia into a Hawaii quarantine containment facility for host range testing to determine whether the parasitoid might attack non-target species in addition to the target host *H. hampei* and thereby pose a risk to Hawaiian endemic species. Using no-choice tests, 43 different species of Coleoptera were exposed to *P. coffea in vitro*, including 23 scolytines (six natives, 17 non-native species including *H. hampei* as seen in Table 3), six beneficial species (Table 4) and 12 other species including one native weevil (*N. giffardi)* (Table 5). Only five species from the genus *Hypothenemus* were parasitized by *P. coffea,* including the two pest species *H. hampei* (coffee berry borer) and *H. obscurus* (tropical nut borer, a macadamia nut pest), and three other exotic species *H. seriatus*, *H. birmanus*, and *H. crudiae* (Figure 6). Thus, *P. coffea* appears to be host specific at the genus level and should pose no harm to endemic species if released in Hawaii coffee for classical biological control of *H. hampei*. Nevertheless, no level of host specificity testing can ensure zero risk to non-target organisms when introducing a natural enemy in a new habitat (Louda et al. 2003).

Table 3. Parasitism and parasitoid emergence rates in no-choice in vitro non-target host acceptance screening of *Phymastichus coffea* exposed to various Scolytinae (Hawaii native and non-native) species.

Family	Species	Insect status	Total beetles exposed	Parasitism (%) (Mean ± SE)	Parasitoid emergence (%) (Mean ± SE)
Curculionidae: Scolytinae	*Xylosandrus compactus*	Exotic/Pest	80	0	0
	Xylosandrus crassiusculus	Exotic	80	0	0
	Xyleborinus saxeseni	Exotic	80	0	0
	Xyleborinus andrewesi	Exotic	60	0	0
	Xyleborus ferrugineus	Exotic	60	0	0
	Euwallacea fornicatus	Exotic	60	0	0
	Euwallacea interjectus	Exotic	60	0	0
	Hypochryphalus sp.	Exotic	60	0	0
	Chryphalus sp.	Exotic	80	0	0
	Ptilopodius pacificus	Exotic	80	0	0
	Xyleborus molokaiensis	Native	30	0	0
	Xyleborus mauiensis	Native	15	0	0
	Xyleborus simillimus	Native	18	0	0
	Xyleborus hawaiiensis	Native	9	0	0
	Xyleborus lanaiensis	Native	19	0	0
	Xyleborus obliquus	Native	3	0	0
	Xyleborus kauaiensis	Native	35	0	0

Table 4. Parasitism and parasitoid emergence rates in no-choice in vitro non-target host acceptance screening of Phymastichus coffea on beneficial Coleoptera species.

Family	Species	Insect status	Total beetles exposed	Parasitism (%)	Parasitoid emergence (%)
Chrysomelidae: Cassidinae	*Uroplata girardi*	Exotic	60	0	0
Coccinellidae	*Scymnodes lividigaster*	Exotic	40	0	0
Coccinellidae	*Rhyzobius forestieri*	Exotic	60	0	0
Coccinellidae	*Halmus chalybeus*	Exotic	40	0	0
Laemophloeidae	*Leptophloeus* sp.	Unknown	60	0	0
Silvanidae	*Cathartus quadricollis*	Exotic	80	0	0

Table 5. Parasitism and parasitoid emergence rates in no-choice in vitro non-target host acceptance screening of *Phymastichus coffea* on Hawaiian native and introduced coleopteran species from families and subfamilies other than Curculionidae:Scolytinae.

Family	Species	Insect status	Total beetles exposed	Parasitism (%)	Parasitoid emergence (%)
Anthribidae	*Araecerus simulatus* or *A. levipennis*	Unknown	6	0	0
Anthribidae	*Araecerus* sp. near *varians*	Unknown	15	0	0
Brentidae:Brentinae	*Cylas formicarius*	Exotic/Pest	80	0	0
Chrysomelidae:Bruchinae	*Acanthoscelides macrophthalmus*	Unknown	10	0	0
Curculionidae:Cossoninae	*Phloeophagosoma tenuis*	Unknown	8	0	0
Curculionidae:Cossoninae	*Nesotocus giffardi*	Native	12	0	0
Curculionidae:Curculioninae	*Sigastus* sp.	Exotic/Pest	6	0	0
Curculionidae:Platypodinae	*Crossotarsus externedentatus*	Exotic	60	0	0
Dryophthoridae:Dryophthorinae	*Sitophilus oryzae*	Exotic/Pest	60	0	0
Dryophthoridae:Dryophthorinae	*Sitophilus linearis*	Exotic	40	0	0
Nitidulidae:Carpophilinae	*Carpophilus dimidiatus*	Exotic	10	0	0
Nitidulidae:Carpophilinae	*Carpophilus zeaphilus*	Exotic	60	0	0
Tenebrionidae	*Tribolium castaneum*	Exotic/Pest	21	0	0
Tenebrionidae	*Hypophloeus maehleri*	Exotic	60	0	0

4.3. Information on the biological control agent from the area of origin based of field surveys or experimental field manipulation

In field cage studies in Mexico and Costa Rico, and also in Colombia (P. Benevides, pers. comm.), parasitism by introduced *P. coffea* was as high as 95% (Espinoza et al. 2009; Infante et al. 2013).

5. Environmental and Economic Impacts of the Proposed Release

5.1 Known impact of the biological control agent on humans and vertebrates

None.

5.2. Expected benefits of releasing this biological control agent

Phymastichus coffea is a potentially effective biological control agent for *H. hampei* and could be incorporated into existing IPM programs in Hawaii. To achieve maximum *P. coffea* parasitism in the field, releases should be made at times when *H. hampei* adults are active (e.g., when trap catches are high or female *H. hampei* are actively boring into fruits) and the coffee crop is at a susceptible stage. Studies suggest *P. coffea* may be susceptible to *B. bassiana,* however (Barrera 2005; Castillo et al. 2009; Ruiz et al. 2011), so releases should be timed to avoid *B. bassiana* applications or used in alternation with *B. bassiana* against *H. hampei*. If *P.*

coffea is highly effective, then dependence on *B. beauveria* applications could be reduced dramatically.

5.3 Direct impact of the biological control agent on target and non-target species.

Phymastichus coffea is expected to help suppress *H. hampei* populations in coffee and may also provide a level of suppression of *H. obscurus* in macadamia nut farms which are relatively close to coffee growing areas or interspersed with coffee farms in some cases. Using a no-choice laboratory bioassay, we demonstrated that *P. coffea* was only able to parasitize the target host *H. hampei* and four other adventive species of *Hypothenemus*: *H. obscurus, H. seriatus, H. birmanus* and *H. crudiae* (Figure 6; Yousuf et al. 2021). *Hypothenemus hampei* had the highest parasitism rate and shortest parasitoid development time of the five parasitized *Hypothenemus* spp. Parasitism and parasitoid emergence decreased with decreasing phylogenetic relatedness of the *Hypothenemus* spp. to *H. hampei*, and the most distantly related species included in the trials, *H. eruditus*, was not parasitized. No species in any of the other genera tested were parasitized. These results suggest that the risk of harmful non-target impacts is minimal because there are no native species of *Hypothenemus* in Hawaii, and *P. coffea* could be safely introduced for classical biological control of *H. hampei* in Hawaii. Furthermore, as *P. coffea* is attracted to semiochemicals released from coffee fruit damaged by *H. hampei*, it is likely that under field conditions they will not be attracted to non-target species on different host plants lacking those cues.

5.4 Indirect impacts

Potentially, *P. coffea* might interfere with two resident predators, *Cathartus quadricollis* and *Leptophloeus* sp., that naturally occur in coffee and attack CBB, or vice versa. However, these predators are mainly found in overripe and dried coffee berries naturally predating on the immature stages of *H. hampei* in Hawaii (Follett et al. 2016; Brill et al. 2020). Our host testing in quarantine showed that *P. coffea* will not parasitize these beetles, and that the beetles did not predate on the parasitoids. Also, these predators attack eggs, larvae and pupae of *H. hampei* in overripe and dried berries (left after harvesting), whereas *P. coffea* attacks adult female *H. hampei* primarlily in developing green berries at an earlier stage of crop maturity. The biopesticide *Beauveria bassiana* also has the potential to interfere with *P. coffea* parasitism of CBB and survival. Indeed, studies suggest *P. coffea* may be susceptible to *B. bassiana* (Barrera 2005; Castillo et al. 2009; Ruiz et al. 2011). Therefore, releases of *P. coffea* should be timed to avoid *B. bassiana* applications or used in alternation with *B. bassiana* against *H. hampei*. If *P. coffea* is highly effective, then dependence on *B. bassiana* applications could be reduced dramatically.

5.5 Possible direct or indirect impact on threatened or endangered species in Hawaii

Only five species from the genus *Hypothenemus* were parasitized by *P. coffea,* including the two pest species *H. hampei* (coffee berry borer) and *H. obscurus* (tropical nut borer, a macadamia nut pest), and three other exotic species *H. seriatus*, *H. birmanus*, and *H. crudiae* (Figure 1). Thus, *P. coffea* appears to be host specific at the genus level, on beetles relatively closely related to *H. hampei*, and, as there are no native Hawaiian species of *Hypothenemus*, should pose no harm to endemic species if released in Hawaii coffee for classical biological

control of *H. hampei*. However, no level of host specificity testing can ensure zero risk to non-target organisms when introducing a natural enemy in a new habitat (Louda et al. 2003).

5.6 Impact of biological control agent on physical environment

None anticipated (see attached cultural impact assessment)

5.7 Proposed contingency plan to mitigate undesired environmental impacts

Release of *P. coffea* will be a permanent, non-reversible action. *P. coffea* is not expected to attack any native Hawaiian species or disrupt native ecosytsems given its high host specificity and short life span. Therefore, undesired environmental impacts are not anticipated.

6. Post-release Monitoring

6.1 Biological control agent establishment and spread

Once permits for release of *P. coffea* are obtained, field releases will begin on commercial coffee farms. In selected locations, data will be taken on establishment, dispersal from release points, parasitism rates, coffee berry infestation rates, and crop damage. Non-release sites will be used as controls initially to determine spread. Establishment is not certain and repeated releases may be required. *P. coffea* could not be found 8-12 months after release in Mexico and it also did not establish in coffee in Colombia after several years of mass releases. In Colombia and Mexico, coffee growers can effectively clean-pick their plantations. This may result in a dearth of hosts for the parasitoids, impacting their ability to establish. In Hawaii, there are widespread feral coffee stands, unmanaged coffee farms, and clear picking is seldom a viable option for various reasons. The year-round presence of hosts is expected to facilitate establishment of *P. coffea*. After release in Hawaii, regular surveys will be conducted to recover *P. coffea* in release areas. Adult *H. hampei* will be collected from fruit and returned to the laboratory for to determine whether they are parasitized. Diapause has not been investigated previously in *P. coffea* but it has been suggested that diapause may be the survival mechanism for the parasitoids between for the period when hosts are rare (McClay 1993). Overripe and drying coffee berries will be collected from release sites during the of-season and held to determine whether parasitoids emerge over time, possibly from a diapause state.

6.2 Biological control agent and target pest densities and distribution over time

Coffee berry borer densities in Hawaii coffee are variable from year to year depending on climactic conditions and control measures (sanitation, *Beauveria bassiana* applications). *P. coffea* releases will be made on farms where USDA-ARS maintains CBB population monitoring and crop loss assessment activities as part of a long-term area-wide program. Data will be taken on percentage parasitism 1 week after *P. coffea* release and adult CBB will be held for parasitoid emergence. Coffee is a 7-month crop from the time of flowering to harvest. *P. coffea* releases will be made when trapping indicates peak flights of adult CBB and field sampling shows CBB adults boring into coffee berries, the time at which adult CBB are most susceptible to parasitism. Samples will be collected over a range of distances from release sites to assess dispersal of the parasitoids within and among coffee plantations over time. After harvest, samples will be collected

from residual fruits on coffee trees and from fallen fruits that lie beneath plants and sustain *H. hampei* reservoirs. The abundance of adult *H. hampei* available as hosts to *P. coffea* will decline during the months between harvest and the fruit set, a period of 4-5 months depending on location. We will investigate the potential for *P. coffea* to enter diapause during this period, allowing them to survive within *H. hampei* in desiccating fruit on trees or on the ground. Possible diapause will be detected by collecting desiccated fruits form the ground and overripe fruit remaining on trees, andholding them to determine if parasitoids emerge over a prolonged period. Laboratory trials will be conducted to assess whether diapause can be induced in *P. coffea* under controlled conditions.

The above studies will measure dispersal of *P. coffea*, as well as inter-seasonal survival of the wasps, thus whether wide-spread establishment occurred. We will simultaneously commence measuring the intergenerational impact of *P. coffea* on *H. hampei* populations. Cohorts of *H. hampei* will be monitored commencing when newly developed coffee fruit become susceptible in the field. Using life table analyses, the contribution of *P. coffea* to *H. hampei* generational mortality will be quantified and compared with other mortality factors that may be acting on the beetle population. These analyses will provide an accurate assessment of the impact of the biological control agent on the target pest densities over time since introduction of the natural enemy.

6.3 Impact on selected non-target species for which potential impacts are identified

Preliminary data will be collected on semiochemical attraction of *Phymastichus coffea* to different *Hypothenemus* and other Scolytinae spp. *in vitro*., to investigate the potential for developing methods to screen parasitoids for non-target effects based on responses to semiochemical diversity. We will compare *P. coffea* responses to chemical signals from Scolyitinae species of varying host-specificity and compare this with two other *Phymastichus* species in Hawaii, *Phymastichus xylebori* LaSalle and *Phymastichus* sp. nova. *P. xylebori* parasitizes *Xyleborus perforans*, while *Phymastichus* sp. nova has been recorded from at least five host beetles (D. Honsberger pers. comm.). These comparisons will provide insights into the cues used by *Phymastichus* to locate hosts, and potentially the extent to which host specificity is mediated by parasitoid-host chemical interactions.

Various scolytines in the vicinity of release sites will be sampled periodically to determine whether any non-target parasitism occurs. While no non-target host use is predicted in Hawaii, this will serve as a test of the quarantine host-range testing predictions. This information will contribute to our overall understanding of and ability to prediction zero impact on nontarget species.

7. Pre-release compliance

7.1 Reference specimens

Phymastichus coffea specimens in vials with alcohol have been deposited at multiple locations including Cenicafé, USDA ARS in Hilo, Hawaii, and the University of Hawaii at Manoa. Hundreds of specimens are available for DNA extraction. All specimens were reared at Cenicafé

in Colombia and shipped to Hawaii during host specificity testing in quarantine. A smaller number of pinned specimens is also available.

7.2 Planned location and timing of first release

The planned site for the first release is Greenwell Farms (Kealakekua, HI) in Kona, Big Island. The owner, Tom Greenwell, is a long-time cooperator with one of the largest coffee farms on the island. Interest is high across the coffee industry and among individual growers to have *P. coffea* releases. The number and timing of releases will be partly dictated by the number of *P. coffea* available. A letter confirming the release dates and locations will be submitted to USDA APHIS within 3 months after release.

8. List of Agencies and Persons Consulted

Dr. Tracy Johnson, Research Entomologist, U.S. Forest Service, Institute of Pacific Islands Forestry, and director of the Hawaii Volcanoes National Park Quarantine Facility, Volcano, Hawaii.

Dr. Pablo Benavides Machado, Scientific Investigator III, Entomology, National Coffee Research Center-Cenicafé, Manizales (Caldas) Colombia. Provided *Phymastichus coffea* for testing.

Dr. Marisol Giraldo Jaramillo, Scientific Investigator I, Entomology, National Coffee Research Center-Cenicafé, Manizales (Caldas) Colombia. Provided *Phymastichus coffea* for testing.

Dr. Maribel Portilla, Research Entomologist, USDA-ARS Southern Insect Management Research Unit, Stoneville, Mississippi. Provided training on *Phymastichus coffea* rearing.

Dr. Conrad P.D.T. Gillett, Postdoctoral Research Fellow, University of Hawai‘i Insect Museum Department of Plant and Environmental Protection Sciences, Entomology, College of Tropical Agriculture and Human Resources, University of Hawai‘i at Mānoa, Honolulu, Hawaii. Confirmed identification of Scolytinae.

Dr. Lourdes Chamorro, Research Entomologist/Curator of Curculionoidea, Systematic Entomology Laboratory - ARS, USDA, c/o Smithsonian Institution - National Museum of Natural History. Provided identification of Curculionidae other than Scolytinae.

Tabetha Block, HETF Resource Associate, Forest Service Contractor, Institute of Pacific Islands Forestry, Hilo. HETF permit issuer.

Jay Hatayama, Forest Management Supervisor II, State of Hawaii, Division of Forestry and Wildlife, Hilo, Hawaii. DNLR permit issuer.

Other: Various coffee and macadamia growers on Hawaii Island.

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Appendix A

Yousuf, F., Follett, P.A., Gillett, C.P.D.T., Honsberger, D., Chamorro, L., Johnson, T.M., Jaramillo, M.G., Machado, P.B. & Wright, M.G. 2021. Limited host range in the idiobiont parasitoid *Phymastichus coffea*, a prospective biological control agent of the coffee pest *Hypothenemus hampei* in Hawaii. *Journal of Pest Science* https://doi.org/10.1007/s10340-021-01353-8

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ORIGINAL PAPER

Limited host range in the idiobiont parasitoid *Phymasticus coffea*, a prospective biological control agent of the coffee pest *Hypothenemus hampei* in Hawaii

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Abstract
Phymastichus coffea LaSalle (Hymenoptera:Eulophidae) is an adult endoparasitoid of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera:Curculionidae:Scolytinae), which has been introduced in many coffee producing countries as a biological control agent. To determine the effectiveness of *P. coffea* against *H. hampei* and environmental safety for release in Hawaii, we investigated the host selection and parasitism response of adult females to 43 different species of Coleoptera, including 23 Scolytinae (six *Hypothenemus* species and 17 others), and four additional Curculionidae. Non-target testing included Hawaiian endemic, exotic and beneficial coleopteran species. Using a no-choice laboratory bioassay, we demonstrated that *P. coffea* was only able to parasitize the target host *H. hampei* and four other adventive species of *Hypothenemus*: *H. obscurus, H. seriatus, H. birmanus* and *H. crudiae. Hypothenemus hampei* had the highest parasitism rate and shortest parasitoid development time of the five parasitized *Hypothenemus* spp. Parasitism and parasitoid emergence decreased with decreasing phylogenetic relatedness of the *Hypothenemus* spp. to *H. hampei*, and the most distantly related species, *H. eruditus*, was not parasitized. These results suggest that the risk of harmful non-target impacts is low because there are no native species of *Hypothenemus* in Hawaii, and *P. coffea* could be safely introduced for classical biological control of *H. hampei* in Hawaii.

Keywords Coffee berry borer · Host specificity testing · Non-target · Biocontrol · Endoparasitoid · Scolytinae

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Key message

- *Phymastichus coffea* is an idiobiont adult parasitoid of the coffee pest *Hypothenemus hampei*.
- In host range testing, *P. coffea* parasitized only five *Hypothenemus* spp.
- The parasitism rate was highest and parasitoid development time was shortest in *H. hampei.*
- No Hawaiian native species was parasitized by the parasitoid.
- *Phymasticus coffea* can be introduced safely for biocontrol of coffee berry borer in Hawaii.

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Introduction

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera:Curculionidae:Scolytinae), native to Central Africa, is the most damaging insect pest of coffee worldwide, inflicting economical losses of over US $500 million dollars annually (Vega et al. 2015). In Hawaii, *H. hampei* was first recorded in Kona, Hawaii island, in 2010 (Burbano et al. 2011) and is now widespread throughout all the coffee-growing areas of Hawaii. Coffee is the third largest cash crop in the state of Hawaii, valued at more than $43 million (USDA-NASS 2018). *Hypothenemus hampei* has had the effect of making coffee farming more intensive and less profitable, which is a major economic challenge to small-scale coffee production like that in Hawaii (Johnson et al. 2020). If left unmanaged, *H. hampei* can damage > 90% of the crop.

Hypothenemus hampei attacks coffee berries when the dry matter content of the endosperm, which increases with age, exceeds 20% (Jaramillo et al. 2005). After finding a suitable berry host, *H. hampei* bores into the coffee fruit through the central disk and excavates galleries where it lays eggs. The offspring develop inside the seeds and feed on the endosperm tissue of the berries (Damon 2000), reducing both coffee yield and quality. *Hypothenemus hampei* feeding damage can also cause premature fall of berries younger than 80 days (Decazy 1990). *Hypothenemus hampei* adults boring into the berry may remain in the 'A' position (Jaramillo et al. 2006) with the abdomen half exposed outside the berry potentially for weeks waiting for the dry matter content to reach 20% (Jaramillo et al. 2005).

Strategies to control *H. hampei* include mechanical, chemical and biological controls (Infante 2018). Sanitation and biological control (using parasitoids, predators and entomopathogenic microorganisms) are the most sustainable, environmentally friendly and widely used non-chemical control methods. The parasitoids, *Cephalonomia stephanoderis* Betrem, *C. hyalinipennis* Ashmead and *Prorops nasuta* Waterston (Hymenoptera:Bethylidae), *Heterospilus coffeicola* Schneideknecht (Hymenoptera:Braconidae) and *Phymastichus coffea* LaSalle (Hymenoptera:Eulophidae), all of African origin, have been introduced in many coffee producing countries, particularly in Central and South America (Klein-Koch et al. 1988; Barrera et al. 1990; Baker 1999; Jaramillo et al. 2005; Portilla and Grodowitz 2018), but none have been released in Hawaii. In Hawaii, the primary methods for controlling *H. hampei* are sanitation (frequent harvests and removal of all left over coffee berries after harvest) and applications of the biopesticide *Beauveria bassiana* (Ascomicota:Hypocreales), an entomopathogenic fungus (Aristizábal et al. 2016). Two generalist predators, *Leptophloeus* sp. and *Cathartus quadricollis* (Coleoptera:Laemophloeidae and Silvanidae, respectively), occur naturally in Hawaii coffee and have been shown to feed on immature stages of *H. hampei* in overripe and dried berries (Follett et al. 2016; Brill et al. 2020), but are not very efficient in preventing damage in the first place.

Most of the studies on biological control of *H. hampei* have been conducted outside Hawaii, but in similar coffee production systems. In field-cage studies conducted in Mexico and Costa Rica, *P. coffea* proved to be the most promising biological control agent against *H. hampei* with parasitism rates as high as 95% (Espinoza et al. 2009; Infante et al. 2013). To date, *P. coffea* has been released in 12 countries as a classical biological control agent (Bustillo et al. 1998; Damon 2000; Jaramillo et al. 2005; Vega et al. 2015). *Phymastichus coffea* is native to Africa and present in most coffee producing countries on that continent. It is a primary, gregarious, idiobiont endoparasitoid of adult *H. hampei* females with a high capacity for host discrimination (Feldhege 1992; Infante et al. 1994; López-Vaamonde and Moore 1998; Castillo et al. 2004). Two laboratory studies reported that in addition to *H. hampei*, *P. coffea* parasitizes other *Hypothenemus* spp. such as *H. seriatus* and *H. obscurus* (López-Vaamonde and Moore 1998), and *H. eruditus* Westwood and *H. crudiae* (Panzer) (Castillo et al. 2004). However, parasitism of closely related species in the field has not been reported (Escobar-Ramírez et al. 2019). Gravid *P. coffea* females start to search for their hosts immediately after emerging from the adult female host and parasitism occurs within the first hours after emergence (Infante et al. 1994). *Phymasticus coffea* has an extremely short life span as an adult; the longevity of males ranges from 8 to 48 h and females from 16 to 72 h (Vergara et al. 2001; Portilla and Grodowitz 2018). *Phymastichus coffea* generally lays two eggs (into the abdomen, thorax, or between the thorax and abdomen) in an *H. hampei* adult female at the time she is initiating fruit perforation, which causes paralysis and prevents further damage to the coffee berry. The parasitized *H. hampei* usually dies within 4–12 days after parasitism (Infante et al. 1994). The life cycle (egg to adult) of *P. coffea* varies from 30 to 47 days depending on the environmental conditions (temperature and humidity). Females are ~ 1 mm long, whereas males are half that size (LaSalle 1990).

Earlier studies have shown the high host specificity of *P. coffea* and its ability to significantly reduce and regulate *H. hampei* populations (Gutierrez et al. 1998; López-Vaamonde and Moore 1998; Castillo et al. 2004; Rodríguez et al. 2017). Therefore, we decided to consider *P. coffea* as a biological control agent of *H. hampei* in Hawaii. A critical step was to determine its host specificity and assess possible risks to the Hawaii environment though impacts on endemic and other non-target species (Follett and Duan 1999; Messing and Wright 2006). Greatest non-target species impacts from

introduced biological control agents are likely to occur on species closely related to the target pest species (Van Driesche and Murray 2004), but not always (Messing 2001), and thus, phylogenetically closely and distantly related species should be included in non-target screening efforts. This is an important element of biological control, particularly in Hawaii, where classical biological control may have had significant negative impacts on native species in the past (e.g., Howarth 1991; Henneman and Memmott 2001). While some studies have suggested that this is true (see references in Messing and Wright 2006), a number of carefully crafted field studies of population level impacts on non-target species have suggested that introduced parasitoids have had minimal, or sometimes moderate, impacts on endemic species (Johnson et al. 2005; Kaufman and Wright 2009). Where higher impacts have been detected, they are typically from accidentally introduced parasitoid species, and host insects in disturbed habitats are most susceptible to these impacts (Kaufman and Wright 2011). However, the potential for non-target impacts must be carefully considered, and outcomes of exposures of unintended hosts to prospective biological control agents can provide insights into host range patterns and determinants.

In this paper, we present new insights into the host specificity of *P. coffea*, a prospective biological control agent of *H. hampei* in Hawaii, by testing it against 43 different species of Coleoptera. Non-target testing included Hawaiian endemic, exotic and beneficial coleopteran species. There are currently no records of native Hawaiian *Hypothenemus* spp. except for an old record (1913) of *H. ruficeps* (Swezey 1954), which has never been collected or reported since and is possibly a synonym with the adventive species *H. eruditus* or *H. crudiae* (C. Gillett, unpublished). There are, however, many native species in another scolytine genus, *Xyleborus* (Samuelson 1981; Gillett et al. 2019), which may potentially be impacted by release of an exotic parasitoid against a scolytine pest such as *H. hampei*. We test the hypothesis that *P. coffea* is host specific and will not attack native Hawaiian Scolytinae species.

Materials and methods

Parasitoid, Phymastichus coffea

Phymastichus coffea used in this study were obtained from an established stock maintained at the National Coffee Research Center-Cenicafé, Manizales (Caldas) Colombia, which was started from *P. coffea* collected in Kenya and shipped to Colombia in 1996 and has been maintained in colony in large numbers since that time (Orozco-Hoyas and Aristizábal 1996). *Phymastichus coffea* has been mass reared by Cenicafé for field releases on multiple occasions and the colony receives frequent infusions of field-collected material. *Phymastichus coffea* was shipped from Cenicafé in its larval stage in parasitized *H. hampei* hosts under USDA APHIS PPQ, permit no. P526P-18-00,696 to a certified quarantine insect containment facility managed by the USDA Forest Service at Hawaii Volcanoes National Park, Volcano, Hawaii. Parasitized *H. hampei* were incubated in controlled climate chambers at 25° ± 1 °C, 75 ± 10% relative humidity and 8:16 h light:dark photocycle at the quarantine containment facility.

Emerged male and female parasitoid adults were collected using a manual aspirator into a clean glass container. Parasitoids were held for mating and oocyte maturation and provided with 50% (w/v) honey (raw organic) solution for ~ 2 h before being used in the experiments (López-Vaamonde and Moore 1998). Infante et al. (1994) reported that *P. coffea* does not go through a preoviposition period and exhibits facultative arrhenotokous-type parthenogenesis, where the female parasitizes its host before or after copulation, producing haploid males (Portilla and Grodowitz 2018). Feldhege (1992) reported a preoviposition period of between 5 min and 4 h. The adult parasitoids are very short-lived: males (~ 8–48 h) and females (~ 16–72 h) (Vergara et al. 2001; Rojas et al. 2006; Espinoza et al. 2009; Portilla and Grodowitz 2018). The ability to parasitize hosts decreases with age, so it was important to use freshly emerged parasitoids (< 12 h old) in all experiments.

Coffee berry borer, Hypothenemus hampei

Field-collected *H. hampei* were used in all no-choice host specificity experiments. *Hypothenemus hampei*-infested coffee berries were collected from coffee trees (*Coffea arabica*) at OK Coffee Farm in Hilo, Hawaii (19.727583, − 155.111186, elevation 156 m). These collections were transported in cold boxes to the USDA-ARS laboratory and placed in a custom-made extraction unit lined with tissue paper (Tech wipes 1709/7052, Horizon) to absorb condensation and prevent mold growth. Adult *H. hampei* were collected directly from the infested coffee berries by dissecting the berries or from the extraction unit using an aspirator. All the collected *H. hampei* were provided with artificial diet (modified from Brun et al. 1993) until use in the experiments.

Collection of non-target coleopteran species

The selection of non-target hosts was based on phylogenetic relatedness to the target host, sympatry of target and non-target species, and size. Species commonly occurring in the coffee landscape and species in culture at USDA-ARS in Hilo, Hawaii, were also tested. There are 21 native and 38 non-native scolytine species in Hawaii (Samuelson 1981;

Nishida 2002; Cognato and Rubinoff 2008). Because of the relatively large native scolytine fauna in Hawaii, and their remote or poorly studied habitats, only a subset of these species could be tested for their suitability as hosts to *P. coffea*. Exotic and native scolytine species were collected from coffee and macadamia farms and their surrounding habitats, and from native forests from different islands (Hawaii Island, Oahu, Maui, Molokai and Kauai) in Hawaii (Gillett et al. 2020a). Host specificity tests were conducted with a total of 43 species from seven different coleopteran families including Hawaiian endemic species (several Scolytinae in the genus *Xyleborus* and *Nesotocus giffardi,* a curculionid weevil), exotic pest species (e.g., the scolytines *Hypothenemus obscurus* [tropical nut borer] and *Xylosandrus compactus* [black twig borer], and the curculionids *Sitophilus oryzae* [rice weevil] and *Cylas formicarius* [sweetpotato weevil]), and beneficial species (e.g., a weed biocontrol agent *Uroplata girardi* from lantana, several coccinellids, and two flat bark beetle predators of *H. hampei*, *Catharus quadricollis* and *Leptophloeus* sp.) (Tables 1, 2, 3, 4). All beetles used in host specificity tests were collected live and later preserved in 75% alcohol or pinned for identification by taxonomists with expertise in the respective taxa. The body size of the collected species ranged from 1 to 7 mm, but the majority of species were similar in size to *H. hampei* which is 1.5–2.0 mm in length. Beetles were collected using Lindgren funnels or bucket or Broca traps baited with denatured ethanol only or ethanol + methanol + ethylene glycol lures or collected directly from infested plant material (fruits, pods, stems, bark and seeds) or reared from infested wood in the laboratory (Gillett et al. 2020b). All non-target testing was conducted at the USDA Forest Service quarantine containment facility at Hawaii Volcanoes National Park, Volcano, Hawaii.

Table 1 Development time and sex ratio of *Phymasticus coffea* in no-choice in vitro non-target host selection screening of *Hypothenemus* species, including *H. hampei* as a control species

Species	Insect status	Total beetles exposed	Development time (days ± SE)	Sex ratio (mean % females ± SE)
Hypothenemus hampei (control)	Exotic/pest	170	32.2 ± 0.5	50.8 ± 0.4
Hypothenemus obscurus	Exotic/pest	80	35.0 ± 0.9	54.8 ± 1.6*
Hypothenemus seriatus	Exotic	60	38.0 ± 1.0	51.1 ± 1.1
Hypothenemus birmanus	Exotic	40	37.0 ± 1.0	57.7 ± 3.8*
Hypothenemus crudiae	Exotic	30	41.0 ± 0.0*	50.0
Hypothenemus eruditus	Exotic	80	–	–

*significantly different from *Hypothenemus hampei* (control), $p < 0.05$

Table 2 Parasitism and parasitoid emergence rates in no-choice in vitro non-target host acceptance screening of *Phymastichus coffea* exposed to various Scolytinae (Hawaii native and non-native) species

Family	Species	Insect status	Total beetles exposed	Parasitism (%) (Mean ± SE)	Parasitoid emergence (%) (Mean ± SE)
Curculionidae:Scolytinae	*Xylosandrus compactus*	Exotic/pest	80	0	0
	Xylosandrus crassiusculus	Exotic	80	0	0
	Xyleborinus saxeseni	Exotic	80	0	0
	Xyleborinus andrewesi	Exotic	60	0	0
	Xyleborus ferrugineus	Exotic	60	0	0
	Euwallacea fornicatus	Exotic	60	0	0
	Euwallacea interjectus	Exotic	60	0	0
	Hypochryphalus sp.	Exotic	60	0	0
	Chryphalus sp.	Exotic	80	0	0
	Ptilopodius pacificus	Exotic	80	0	0
	Xyleborus molokaiensis	Native	30	0	0
	Xyleborus mauiensis	Native	15	0	0
	Xyleborus simillimus	Native	18	0	0
	Xyleborus hawaiiensis	Native	9	0	0
	Xyleborus lanaiensis	Native	19	0	0
	Xyleborus obliquus	Native	3	0	0
	Xyleborus kauaiensis	Native	35	0	0

Table 3 Parasitism and parasitoid emergence rates in no-choice in vitro non-target host acceptance screening of *Phymastichus coffea* on beneficial Coleoptera species

Family	Species	Insect status	Total beetles exposed	Parasitism (%)	Parasitoid emergence (%)
Chrysomelidae:Cassidinae	*Uroplata girardi*	Exotic	60	0	0
Coccinellidae	*Scymnodes lividigaster*	Exotic	40	0	0
Coccinellidae	*Rhyzobius forestieri*	Exotic	60	0	0
Coccinellidae	*Halmus chalybeus*	Exotic	40	0	0
Laemophloeidae	*Leptophloeus* sp.	Unknown	60	0	0
Silvanidae	*Cathartus quadricollis*	Exotic	80	0	0

Table 4 Parasitism and parasitoid emergence rates in no-choice in vitro non-target host acceptance screening of *Phymastichus coffea* on Hawaiian native and introduced coleopteran species from families and subfamilies other than Curculionidae:Scolytinae

Family	Species	Insect status	Total beetles exposed	Parasitism (%)	Parasitoid emergence (%)
Anthribidae	*Araecerus simulatus* or *A. levipennis*	Unknown	6	0	0
Anthribidae	*Araecerus* sp. near *varians*	Unknown	15	0	0
Brentidae:Brentinae	*Cylas formicarius*	Exotic/Pest	80	0	0
Chrysomelidae:Bruchinae	*Acanthoscelides macrophthalmus*	Unknown	10	0	0
Curculionidae:Cossoninae	*Phloeophagosoma tenuis*	Unknown	8	0	0
Curculionidae:Cossoninae	*Nesotocus giffardi*	Native	12	0	0
Curculionidae:Curculioninae	*Sigastus* sp.	Exotic/Pest	6	0	0
Curculionidae:Platypodinae	*Crossotarsus externedentatus*	Exotic	60	0	0
Dryophthoridae:Dryophthorinae	*Sitophilus oryzae*	Exotic/Pest	60	0	0
Dryophthoridae:Dryophthorinae	*Sitophilus linearis*	Exotic	40	0	0
Nitidulidae:Carpophilinae	*Carpophilus dimidiatus*	Exotic	10	0	0
Nitidulidae:Carpophilinae	*Carpophilus zeaphilus*	Exotic	60	0	0
Tenebrionidae	*Tribolium castaneum*	Exotic/Pest	21	0	0
Tenebrionidae	*Hypophloeus maehleri*	Exotic	60	0	0

No-choice tests

In this study, we used no-choice tests because these would reflect physiological host range and the potential for parasitism in the field more accurately than choice tests (Van Driesche and Murray 2004). Choice tests that include the target host may mask the acceptability of lower ranked hosts, thereby producing false negative results (Withers and Mansfield 2005). Twenty individuals of each test species were placed in a sterilized glass Petri dish (80 mm in diameter) lined with filter paper and immediately afterward four *P. coffea* females (< 12 h old) that had not been exposed to adult hosts prior to the experiments were introduced. Therefore, when ample hosts were available, each replicate consisted of 20 hosts and four parasitoids for a 5:1 host–parasitoid ratio. However, due to difficulties in finding certain species live in adequate numbers, e.g., native scolytine bark beetles, and difficulties synchronizing parasitoid emergence with field collection or emergence from wood of live beetles, the host–parasitoid ratio and numbers of replicates were adjusted as needed. For example, if only 10 non-target beetles were available for screening, then two replicates each with 5 beetles and 1 parasitoid (maintaining the 5:1 host–parasitoid ratio) were performed. In all non-target host screening tests, *H. hampei* was included as a positive control to confirm parasitoid viability. The host–parasitoid ratio of the *H. hampei* controls was adjusted to match the non-target species in the test, whether it was 5:1 or otherwise. The generalized response of the parasitoids toward target and non-target hosts was also determined for a subset of parasitoids by visual observation and video recording of parasitoid behavior, e.g., any contact with the host by landing on the host or antennation, and/or walking on the host. Host acceptance was noted when the parasitoid adopted a characteristic oviposition position on top the elytra of the host (Lopez-Vaamonde and Moore 1998).

After *P. coffea* exposure, *H. hampei* and all other non-target species were incubated at 25 ± 1 °C, 75 ± 10% RH and 24:0 (L–D) photoperiod for 72 h. After 72 h, parasitoids and filter paper linings were removed and the beetles were

provided with a small cube (2 × 2 × 2 cm) of general beetle diet (FY, unpublished). The beetles were again incubated at the same environmental conditions, but now at 0:24 (L–D). After 10 days, all the remaining diet and frass was removed (without disturbing the parasitized beetles) to avoid fungal contamination. Parasitized beetles typically become paralyzed and eventually die within 4–12 days after parasitoid oviposition. Beetles were held for a total of ~ 5–6 weeks for parasitoid emergence. Beginning after 25-day incubation, *H. hampei* mummies were inspected daily for adult wasp emergence. Parasitism was assessed based on observation of emergence of parasitoid progeny (F1 adult wasps) from the parasitized beetle, by inspection for exit holes on cadavers or by dissection. Beetles with no exit holes were dissected (by separating the thorax from the abdomen) under a stereomicroscope using fine forceps and entomological pins at 20-100X magnification for evidence of parasitism, i.e., presence of *P. coffea* immature life stages (eggs, larvae or pupae), or unemerged adults. The number of unemerged life stages was recorded for each dissected beetle. After 5–6 weeks of incubation, dead beetle specimens sometimes became very dry and searching for the presence of eggs and early instar larvae was difficult. In such cases, beetles were dissected and examined under a compound microscope at 200X to seek unemerged *P. coffea*. The sex of emerged adult *P. coffea* offspring was determined by examination using a stereomicroscope. In most cases, two parasitoids (one male and one female) emerged per beetle host. To confirm this, the sum of the emerged male and female parasitoids in each replicate was divided by two and compared to the number of parasitized hosts with exit holes. The sex of unemerged parasitoids was not determined. For data on parasitism, life stages, sex ratio and development time, averages were calculated for each replicate (per Petri dish) for each species and used in statistical analysis. Grand means of all the replicates for each of the five *Hypothenemus* species are presented in figures and tables.

Statistical analysis

Parasitism rate was calculated by dividing the number of parasitized hosts by the total number of hosts exposed to the parasitoids. Parasitism included both emerged and unemerged wasps. Emergence rate was calculated by dividing the number of beetles with exit holes by the total number of parasitized hosts (emerged plus unemerged wasps). The sex ratio of the parasitoid progeny was calculated by dividing the number of emerged female parasitoids (F) by the total number of emerged male (M) and female (F) parasitoids [F/(F + M) × 100]. The Shapiro–Wilk test (Shapiro and Wilk 1965; Razali and Wah 2011), numerical approaches (skewness and kurtosis indices) and the normal Q–Q plot-based graphical method were used to check the distribution of the data and showed that the data were not normally distributed. Generalized linear models (GLM) were therefore used to analyze the data, with appropriate distribution function links. Parasitism and emergence rates of the parasitoids, and the percentage of different life stages (larvae, pupae and adults) in parasitized beetles with unemerged parasitoids were analyzed using GLM with a binary logistic function and sex ratio with a gamma log link function. Developmental time of the F1 offspring (egg to adult) was analyzed using GLM with a negative binomial log link function because data were overdispersed (i.e., variance > mean). Wald Chi-squared approximations are reported. All analyses were performed using IBM SPSS statistics software.

Results

Out of 43 total coleopteran species tested, including 23 scolytines, *P. coffea* oviposited and completed developed only in the target *Hypothenemus hampei* and four other species of *Hypothenemus*: *H. obscurus*, *H. seriatus, H. birmanus* and *H. crudiae*. Mean percentages of parasitism and emergence for the *Hypothenemus* spp. tested are shown in Fig. 1. Parasitism ($\chi^2 = 65.13$, df = 4, $p = 0.0001$) and emergence ($\chi^2 = 23.20$, df = 4, $p = 0.0001$) were significantly higher in *H. hampei* than all other *Hypothenemus*

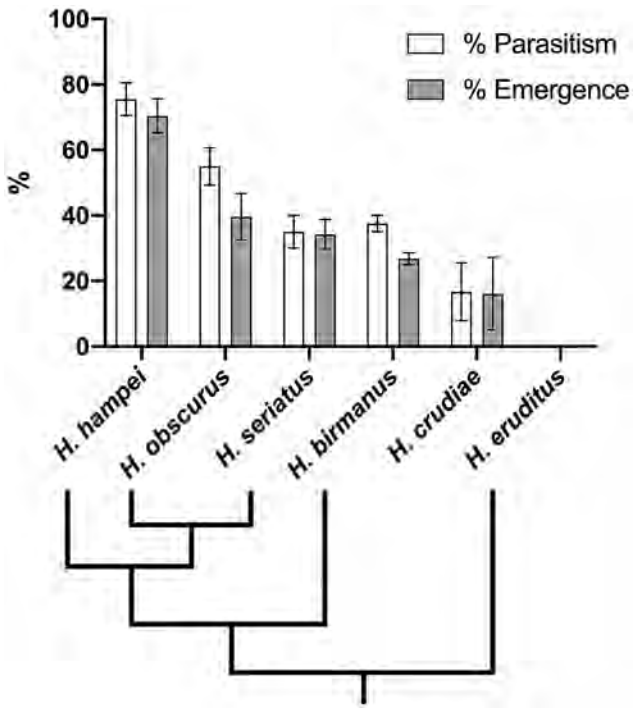

Fig. 1 Percentage parasitism and emergence (mean ± SE) of adult *Phymastichus coffea* parasitoids from *Hypothenemus* spp. The phylogeny below the graph for the species included in the study (except *H. crudiae*) was inferred from Johnson et al. (2018)

species. *Hypothenemus hampei* had the highest percentage emergence of *P. coffea* at 70.4%, whereas *H. crudiae* had the lowest at 16.7% (Fig. 1). In *H. crudiae*, out of five parasitized hosts only one had emergence. Although *P. coffea* only parasitized *Hypothenemus* spp., it did inspect three other non-target scolytine hosts, *Hypothenemus eruditus*, *Xyleborus kauaiensis* and *Xyleborus ferrugineus,* but left hosts without initiating oviposition (i.e., no parasitism found). The phylogenetic relationship of five *Hypothenemus* species included in our tests, extracted from Johnson et al. (2018), is also shown in Fig. 1; *H. crudiae* is not included in the phylogeny because it was not included in Johnson et al (2018). Both parasitism and emergence in our tests decreased across *Hypothenemus* species with decreasing phylogenetic relatedness to *H. hampei*. *Hypothenemus eruditus*, the most distantly related species from *H. hampei* according to Johnson et al. (2018), was not parasitized (Fig. 1).

Parasitoid development time among the three different *Hypothenemus* spp. did not differ significantly compared with *H. hampei* ($\chi^2 = 0.17$, df = 4, $p = 0.997$), but did differ with *H. crudiae* (Table 1). The mean development time of *P. coffea* from oviposition to adult emergence was shortest in *H. hampei* (32.2 ± 0.5 days, mean ± SE), longest in *H. crudiae* (41.0 ± 0.0 days) and intermediate in the other three *Hypothenemus* spp. (Table 1), which generally agrees with the phylogenetic pattern observed for parasitism and emergence (Fig. 1). The percentage of female versus male *P. coffea* emerging from parasitized *H. hampei* was 50.8% ± 0.4 (mean ± SE), which was significantly different ($\chi^2 = 27.3$, df = 4, $p = 0.0001$) from *H. seriatus* and *H. birmanus* (Table 1). *Hypothenemus eruditus* was not parasitized by *P. coffea* and hence was not included in any statistical analyses.

Parasitized *H. hampei* had the lowest percentage of unemerged parasitoids compared to the other four *Hypothenemus* species (Fig. 1), indicating that *H hampei* is a superior host for *P. coffea* development. For each parasitized host beetle with unemerged parasitoids, invariably two parasitoids were present, and the parasitoids were of the same life stage (larva, pupa or adult). The frequency of the different life stages for parasitized hosts with unemerged parasitoids differed among *Hypothenemus* species (Fig. 2). Parasitized *H. hampei* had a significantly lower percentage of larval ($\chi^2 = 15.10$, df = 3, $p = 0.001$), and higher percentage of adult parasitoids that were unemerged ($\chi^2 = 18.36$, df = 3, $p = 0.0001$) compared to the other *Hypothenemus* species. The higher percentage of unemerged parasitoids developing to the adult stage again indicates that *H. hampei* is a superior developmental host than the other *Hypothenemus* spp. The percentage of unemerged pupae found in parasitized *H. hampei* was not significantly different from *H. obscurus*, *H. seriatus* and *H. birmanus*, but *H. crudiae* had a significantly higher percentage of pupae than *H. hampei* ($\chi^2 = 95.40$, df = 4, $p = 0.0001$) (Fig. 2). No eggs were found in any of the parasitized *Hypothenemus* hosts.

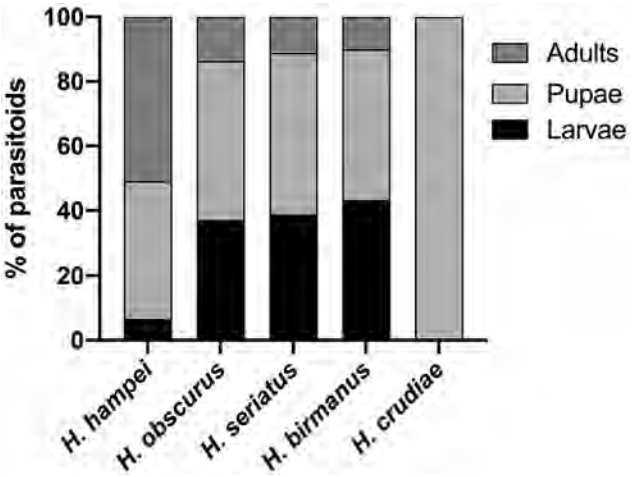

Fig. 2 Fate of unemerged *Phymastichus coffea* parasitoids from parasitized *Hypothenemus* spp. in no-choice in vitro non-target host selection screening. Parasitized *Hypothenemus* beetles with unemerged parasitoids were dissected to identify life stages (larva, pupa, adult)

Discussion

Phymastichus coffea is a potential biological control agent of *H. hampei* and was brought from Columbia into a quarantine containment facility in Hawaii for host range testing to determine whether the parasitoid might attack non-target species and therefore pose a risk to Hawaiian endemic species. Using no-choice tests, 43 different species of Coleoptera were exposed to *P. coffea* in vitro, including 23 scolytines (six natives, 17 non-native species including *H. hampei*), six beneficial species and 12 other species including one native weevil (*N. giffardi).* Only five species from the genus *Hypothenemus* were parasitized by *P. coffea,* including the two pest species *H. hampei* (coffee berry borer) and *H. obscurus* (tropical nut borer, a macadamia nut pest), and three other exotic species *H. seriatus*, *H. birmanus* and *H. crudiae* (Fig. 1). Thus, *P. coffea* appears to be host specific at the genus level and should pose no harm to endemic species if released in Hawaii coffee for classical biological control of *H. hampei*. Nevertheless, no level of host specificity testing can ensure zero risk to non-target organisms when introducing a natural enemy in a new habitat (Louda et al. 2003).

We observed that once the host and parasitoids were exposed in the Petri dish arena that *P. coffea* inspected *H. hampei* and other *Hypothenemus* spp. hosts by antennation before proceeding to oviposition or rejection. *Phymastichus coffea* did not show any oviposition response to other non-target hosts. This could be dependent on several factors

because parasitoids may search and decide host suitability by using a broad spectrum of different stimuli such as plant–host complex volatiles, host feces volatiles, host sex pheromones, and tactile and visual cues (Chiu-Alvarado and Rojas 2008; Yang et al. 2008). Host habitat and host diet may influence the volatile composition emitted by the potential host insect, which can either deter or attract parasitoids from a distance. To minimize the effect of diet, we provided a general beetle diet to all the field-collected coleopteran hosts during the experiments. Parasitism of non-target hosts in the field may not be the same as our in vitro test results because of various factors related to the host's natural habitat. Most of the coleopteran species tested in our study are normally found tunneling in seeds, decomposing wood (under the bark and/or in sapwood) or decaying fruits. This cryptic behavior would likely provide protection from *P. coffea* which is accustomed to searching for *H. hampei* adult females, while they are exposed on the surface of coffee berries.

Phymastichus coffea was attracted to and parasitized only four species of *Hypothenemus* in addition to its target host *H. hampei*. This is consistent with studies reported by López-Vaamonde and Moore (1998), and Castillo et al. (2004). Combining information from our study and previous studies, seven species of beetles are now known to be able to serve as hosts in captive exposure studies for *P. coffea*: *H. hampei, H. obscurus*, *H. seriatus*, *Araptus* sp. (Lopez-Vaamonde and Moore 1998), *H. crudiae* and *H. eruditus* (Castillo et al. 2004), in addition to *H. birmanus* (this study). Parasitism of the scolytine *Araptus* sp. seems to be an outlier, but this genus does not occur in Hawaii. Aside from *Araptus*, *P. coffea* appears to be genus specific attacking closely related, but not all *Hypothenemus* species, given that species from closely related genera were not parasitized under no-choice test conditions. In our study, *P. coffea* did not attack *H. eruditus*. We believe that *H. eruditus* may not be a suitable host for the parasitoid because of its small size (≤ 1 mm)*; Phymastichus coffea* usually lays two eggs per host (1 male and 1 female), and in such a small host, successful development would be unlikely due to the limited availability of resources within the host. Host size is an important variable on which the survival and growth of parasitoid progeny depends. Females of most parasitoids preferentially lay eggs on larger hosts (Fox and Mousseau 1995). Also, *H. eruditus* is phylogenetically distant from *H. hampei* (Fig. 1) which is addressed below.

Our results also showed that *H. hampei* had the lowest numbers of unemerged parasitoids when compared with the other four *Hypothenemus* species (Fig. 2). The number of larvae and pupae were lower, and adults were higher in parasitized *H. hampei* with unemerged parasitoids. Similarly, in other three *Hypothenemus* spp. (*H. obscurus*, *H. seriatus* and *H. birmanus*) many unemerged parasitoids could not complete their development and died in their larval or pupal stage with only a few reaching to the adult stage. In parasitized *H. crudiae* with unemerged parasitoids, most apparently could not reach the adult stage. Although the rate of completing the life cycle differed among *Hypothenemus* species, eggs did hatch in all parasitized species. Many factors can be responsible for suitability of the host for parasitoid development (Pennacchio and Strand 2006). Factors such as host physiology (e.g., presence of endosymbiotic bacteria), behavior (e.g., feeding habitat-sequestering secondary metabolites) and ecology (e.g., spatial/temporal overlap) may influence host acceptance by parasitoids and successful development (Desneux et al. 2009). All the non-target species used in the experiments were freshly collected from the field and may have carried toxins (accumulated from plant feeding) that may have interfered with the successful development of immature parasitoids within the hosts due to the ingestion of unsuitable food (e.g., see Desneux et al. 2009).

Phymastichus coffea also did not successfully parasitize any of the non-*Hypothenemus* species tested, including both native (*Xyleborus*) and exotic (*Xyleborinus*, *Xylosandrus, Xyloborus, Euwallacea,* others) Scolytinae, and other curculionid species from subfamilies other than Scolytinae, including the native weevil, *N. giffardi*. We did not find any *P. coffea* life stages (eggs, larvae, pupae, adults) after dissection in any of the non-*Hypothenemus* non-target species tested (Tables 2, 3, 4). Host specialization is relatively common in parasitic Hymenoptera and can be related to phylogeny, ecology and life histories (Price 1980; Stireman et al. 2006). It appears that at least host phylogeny was an important factor in host selection for *P. coffea* under our laboratory conditions.

Host range of idiobiont parasitoids is typically broader than koinobiont species (Askew and Shaw 1986; Hawkins et al. 1992), and it would hypothetically be reasonable to expect that *P. coffea* would follow this pattern. However, our results show that *P. coffea* was unable to successfully parasitize any species outside of the genus *Hypothenemus* and, even within the genus, was only moderately successful on species even closely related to *H. hampei*. While parasitism of *H. hampei* and subsequent parasitoid emergence was relatively high, both were significantly lower in *H. obscurus* and *H. seriatus*, sister species to *H. hampei*; *H. eruditus*, in a sister clade to the other species (Johnson et al. 2018), had zero parasitism. This demonstrates decreasing susceptibility to *P. coffea* with increasing phylogenetic distance among the *Hypothenemus* spp. exposed to the parasitoids in this study. Among the *Hypothenemus* spp. included in the phylogenetic reconstruction published by Johnson et al. (2018), *H. hampei* is the only species that has undergone a reversal in host range breadth, to become monophagous on coffee, while the other *Hypothenemus* spp. have retained a host generalist biology. *Hypothenemus hampei* has developed a unique association with *Pseudomonas* bacterial endosymbionts to

facilitate detoxification of caffeine, permitting it to exploit *Coffea arabica* seeds as their host (Ceja-Navarro et al. 2015), and potentially other physiological adaptations to its unique host, possibly providing adaptive challenges to parasitoids, and mediating host specificity of *P. coffea*. Messing (2001) questioned the practicality of applying centrifugal phylogeny approaches to selecting species to examine in non-target studies of potential biological control agents, particularly parasitoids. Our results support the predictions of the latter approach, with more distantly related *Hypothenemus* species less susceptible to *P. coffea* attack and more distantly related genera (e.g., *Xyleborus* spp.) not attacked at all. However, Messing (2001) emphasized the fact that interactions between the host insect and its host plant may override host phylogenetic patterns, by providing the stimuli for parasitoids to attack hosts, a consideration which may play a role in this study system. If this is the case, it is possible that *P. coffea* will produce even higher levels of parasitism than recorded in the artificial environment we used in our study, when attacking wild *H. hampei* boring into coffee fruits, producing the full range of cues stimulating parasitism, and lower field parasitism of the non-target *Hypothenemus* spp. included here.

Among all the parasitized *Hypothenemus* species, *H. hampei* had the highest rate of parasitoid emergence. The total developmental time (from egg to adult) of *P. coffea* was shortest in *H. hampei* (32 days); parasitism of *H. crudiae* resulted in the longest developmental time (41 days). Another study reported a similar development time of the *P. coffea* in *H. hampei*, 38–42 days at 23 °C and 66% RH (Rafael et al. 2000). Castillo et al. (2004) reported a *P. coffea* development time of 42.6 days for *H. hampei* and 40 days for *H. crudiae* at 26 ± 2 °C and 70–80% RH. Total developmental time is directly related to the temperature. For example, the total development period of *Diglyphus isaea* (Hymenoptera:Eulophidae) decreased with increasing temperature between 15 and 35 °C and no development was found at 10 and 40 °C (Haghani et al. 2007). Temperature is a critical abiotic factor influencing the physiology and dynamics of insects. Therefore, in this study we selected a temperature for our no-choice assays which reflects the ambient field temperature the insects are expected to experience. In addition to temperature, age of the parasitoids and host play an important role in the subsequent development of parasitoid offspring (Pizzol et al. 2012). Hence, we used uniformly aged parasitoids and hosts throughout our experiments to minimize any impact on host parasitism and parasitoid development.

Phymastichus coffea commonly lays two eggs (a male and a female) per host (López-Vaamonde and Moore 1998). Both male and female develop in a single host, the female in the abdomen and the male in the prothorax (Espinoza et al. 2009). In this study, slightly fewer male parasitoids emerged as compared to females from parasitized hosts. The proportion of females emerging from *H. hampei* was 50.8% which is consistent with the results obtained by López-Vaamonde and Moore (1998) and Rafael et al. (2000). Likewise, sex ratios of *P. coffea* emerging from *H. obscurus* 54.8%, *H. seriatus* 51.1% and *H. crudiae* 50.0% were consistent with the sex ratio results reported by (López-Vaamonde and Moore 1998; Castillo et al. 2004) of 1.25:1, 1:1 and 1:1 (female–male), respectively, for these species. In our study, the proportion of females emerging from parasitized *H. birmanus* 57.7%, was the highest among all other *Hypothenemus* species tested. The slightly fewer males produced per host in our study could be due to either to some parasitoid's preference to lay one egg per host (Feldhege 1992) or the lower survivorship of male eggs or larvae. Preference to lay female eggs over male can be dependent on several factors such as host quality, host age, immune response, genetic factors, photoperiod and relative humidity, host density or host-related volatile composition (King 1987).

All the above tests were conducted in a quarantine laboratory with no field studies. We conducted no-choice tests because they may provide more accurate and conservative information on host preferences and physiological host range than choice tests because of lower levels of interference due to unexpected responses to multiple host cues (Van Driesche and Murray 2004). Sands (1997) showed that laboratory studies often overestimate the host range of the parasitoid and realized ranges under field conditions may be substantially less than predicted from no-choice tests, but they are necessary to give a worst-case prediction of the number of hosts at risk of being attacked in the field (Avilla et al. 2016). *Phymastichus coffea* attacked other non-target *Hypothenemus* species in our no-choice trials, but this does not necessarily mean that those species will be attacked in the field. For example, an idiobiont braconid wasp, *Bracon hebetor* is reported to parasitize a wide variety of moths within and outside in Phycitinae (Lepidoptera:Noctuidae) in the laboratory, but in the field it is restricted to only larvae of *Plodia interpunctella* (Lepidoptera:Noctuidae) (Antolin et al. 1995). This is because in the field, parasitoids use a spectrum of long- and short-range cues (chemical, visual, vibrational and tactile signals) to locate hosts (Strand and Pech 1995). Chemical cues (infochemicals) can play an important role in host location. A study conducted by Rojas et al. (2006) showed that *P. coffea* can distinguish between *H. hampei*-infested and uninfested coffee berries, and were highly attracted to the dust/frass originating from *H. hampei* infested berries, but showed no response to the dust/frass originated from the closely related non-target host, *H. crudiae*. This behavior depending on plant and host cues suggests that it is very unlikely that *P. coffea* will have any

negative effects on non-target scolytids, or any other beetles, under field conditions.

No biocontrol agents were previously released in Hawaii against *H. hampei*. Two exotic predatory beetles, *Cathartus quadricollis* and *Leptophloeus* sp., are commonly found in overripe and dried coffee berries predating on the immature stages of *H. hampei* (Follett et al. 2016; Brill et al. 2020). Our host testing in quarantine showed that *P. coffea* will not parasitize these beetles and that the beetles did not predate on the parasitoids. Also, these predators attack eggs, larvae and pupae of *H. hampei* in overripe and dried berries (left after harvesting), whereas *P. coffea* only attacks adult female *H. hampei* at an earlier stage of crop maturity. The other four *Hypothenemus* species that were attacked by *P. coffea* have very different field habitats, but might serve as useful transitory hosts for *P. coffea* at times when, or in areas where, *H. hampei* populations are at low densities, such as between coffee seasons. For example, macadamia nut farms are often located close to coffee farms in Hawaii and may provide a year-round source of *H. obscurus*, a pest of macadamia nut. Feral coffee in Hawaii could also serve as a continuous source of *H. hampei* throughout the year.

Phymastichus coffea is a potentially effective biological control agent for *H. hampei* and could be incorporated into existing IPM programs in Hawaii. *Phymastichus coffea* may be simply released and monitored for establishment in a classical biological control program, or it may be mass reared for inundative releases. Currently, trapping and sampling of infested coffee fruits is conducted to monitor *H. hampei* flights and optimize timing of *Beauveria bassiana* applications for control (Aristizabal et al. 2016). After *H. hampei* bores into the coffee berries, it is protected and difficult to control with biopesticides or conventional insecticides. To achieve maximum *P. coffea* parasitism in the field, inundative releases should be made at times when *H. hampei* adults are active (e.g., when trap catches are high or female *H. hampei* are actively boring into fruits) and the coffee crop is at a susceptible stage. Optimal timing of inundative releases may differ for different elevations due to *H. hampei* population dynamics (Hamilton et al. 2019). Studies suggest *P. coffea* may be susceptible to *B. bassiana,* however (Barrera 2005; Castillo et al. 2009; Ruiz et al. 2011), so inundative releases should be timed to avoid *B. bassiana* applications or used in alternation with *B. bassiana* against *H. hampei*. If *P. coffea* is highly effective, then dependence on *B. beauveria* applications could be reduced dramatically.

Author contributions

FY designed methodology, conducted the experiments and wrote the manuscript; PF designed the experiments; PF and MW provided overall project management and manuscript editing; FY, CG and DH conducted field surveys and collected live beetles for testing. FY, CG and LC identified beetle species; MGJ and PBM reared and supplied *Phymastichus coffea*. All authors read and gave final approval for publication.

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Declarations

Conflict of interest The authors have declared that no conflict of interest exists.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Appendix B

Cultural Impact Assessment for Proposed Statewide Release of *Phymastichus Coffea* to Control Coffee Berry Borer

Cultural Impact Assessment for Proposed Statewide Release of *Phymastichus Coffea* to Control Coffee Berry Borer

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Introduction

At the request of the University of Hawai‘i and the United States Department of Agriculture Agricultural Research Service, the Synergistic Hawai‘i Agriculture Council (SHAC) conducted a Cultural Impact Assessment (CIA) for the proposed statewide release of *Phymastichus coffea*. Used as a biocontrol in coffee, *P. coffea* is a tiny wasp that targets and parasitizes the coffee berry borer beetle (CBB) *Hypothenemus hampei* (Coleoptera: Curculionidae).

This CIA and its interviews were designed to identify any utilization of coffee for cultural practices or community concerns about environmental impacts from the release of *P. coffea*. It is a companion document to an Environmental Assessment drafted by USDA and was prepared in adherence with the Office of Environmental Quality Control (OEQC) *Guidelines for Assessing Cultural Impact*, adopted by the Environmental Council, State of Hawai‘i, on November 19, 1997 and pursuant to Chapter 343 of the Hawaii Revised Statutes as well as the 2019 revisions to HAR Chapter 11-200.1.

Proposed Action

Biological control (biocontrol) is a component of an integrated pest management strategy. It is defined as the reduction of pest populations by natural enemies and typically involves an active human role (Flint, 1998). Classical biocontrol is the selection and introduction of a natural enemy of an invasive plant or insect pest, and then “reuniting” of this natural enemy with the invasive pest to provide long-term, cost-effective, and sustainable pest management. Both State and Federal agencies have been cooperating on biocontrol activities to minimize the threat of invasive pests in Hawaii’s natural environment. Selection of a biocontrol for potential release undergoes a multi-step regulatory process to ensure native plants, insects, or traditional and customary practices are not impacted by the introduction.

Coffea arabica and *Phymastichus coffea*

Coffee (*Coffea arabica*) is an introduced plant to Hawai‘i , and familiar to most people. Thought to be native to Ethiopia, the intensive cultivation of coffee in Northern Africa (and beyond) began as early as the 16th century. Thriving in subtropical climate zones, there is now a “coffee belt” between the Tropics of Cancer and Capricorn, where some 70 countries grow and export the bean. Early traders noticed Hawai‘i ’s place on the belt, and began to import seeds in the 19th century.

Coffea favors a tropical climate with a distinct wet and dry season. Despite this preference, the well-draining cinder soils of Hawai‘i can support the plant even in extremely wet locations.

Feral coffee is readily found in Maui's ʻIao Valley and in the gulches of Hilo. The plant does not tolerate extreme heat, nor frost, and is typically grown commercially between 400 ft (122 m) and 2,400 ft (732) in elevation (Bittenbender, 1999). At the time of its arrival in Hawaiʻi , the *Coffea* plant had been hybridized by commercial breeders. The first varietal to take root commercially, and still grown extensively in Kona, was Typica. Subsequent introductions included the hybrid Caturra, Catuai and Mokka varietals, each suited to a slightly different climate.

Figure 1: Typica tree in flower, Haiku, Maui

In 1842, the Kingdom of Hawaiʻi recognized the potential importance of Hawaiian coffee and taxed any foreign coffees brought into the islands. Coffee continues to be an economically important crop, with a farmgate green bean value of $102.9 million, likely top in the state when considering roasted valuations (NASS, 2021). It is also a familiar plant found growing wild in every county statewide. While it is not a traditional Hawaiian crop, coffee has contributed

greatly to the post-contact agricultural history of the State. There are almost 1,500 coffee farmers in the state. The majority of commercial growers are smallhold, operating less than five acres of land, and are considered socially-disadvantaged by the USDA (NASS, 2017).

Figure 2: Commercial coffee field in Pahala, Ka'u District, Island of Hawai'i

CBB is the most devastating invasive insect pest in coffee plantations and is estimated to cause more than $500 million in damage around the world (Vega 2020). The *Hypothenemus* is a genus of over 200 described oriental bark beetles within the Curculionidea family (Johnson et al., 2020). CBB was first reported in coffee plantations during an 1897 survey of the West African nation of Liberia (Hopkins, 1915). The pest is notably distinguished from all 850 other insect species that can feed on parts of the coffee plant in that it is the only one able to feed and complete its life cycle in the coffee bean itself. The female beetle bores a small hole into the developing fruit and lays up to 100 eggs in the bean (Jaramillo, 1997). Larvae subsequently feed on the bean, and create cavities, greatly reducing quality and impacting market value. Because the lifecycle occurs largely within the protection of the bean, once the insect penetrates the bean, she and her progeny are relatively protected from insecticides or other conventional control measures. The insect rapidly propagates in Hawai'i , with a mean life cycle of approximately 51 days, totaling more than 7 generations per year (Hamilton 1999).

Figure 3: Bore holes indicate CBB activity in ripening coffee fruit

The arrival of the invasive CBB pest created a significant challenge for growers. First reported in the South Kona region of the Big Island in 2010, the beetle quickly spread to Ka'u and on to the neighbor islands of O'ahu (2014), Maui (2016), Kaua'i (2020), and Lanai (2020), causing widespread damage and economic loss. The negative consequences of this invasion continue to be felt by growers, processors, buyers, and consumers. The estimated economy-wide impact of CBB for the crop years 2011/12 and 2012/13 was a $12.7M loss in crop value, a $25.7M loss in sales, a $7.6M loss in household earnings, and a loss of more than 380 jobs (Lueng ,2013). The added production costs of CBB are significant, and have the potential to drive small farms out of business (Woodill, 2014). CBB found in unmanaged and feral coffee trees can be a source of ongoing infestations for neighbor farms (Johnson et al., 2020).

Mitigation and containment are possible, whereas eradication has not proven feasible. Current control methods are laborious and costly, involving hand removal of beans as well as repeated

applications of chemical insecticides. In the years following, detection, multiple programs, and resources were directed at the problem of CBB in Hawai‘i , including pest subsidies, grower education programs and a relaxation of the Hawai‘i Department of Agriculture quality standards (Johnson et al., 2020). Despite these efforts, CBB remains an intractable issue for growers due to high labor costs and the unsuitability of control through chemical pesticides. Thus, management strategies that limit human labor, such as biocontrol, are identified by farmers as a major need.

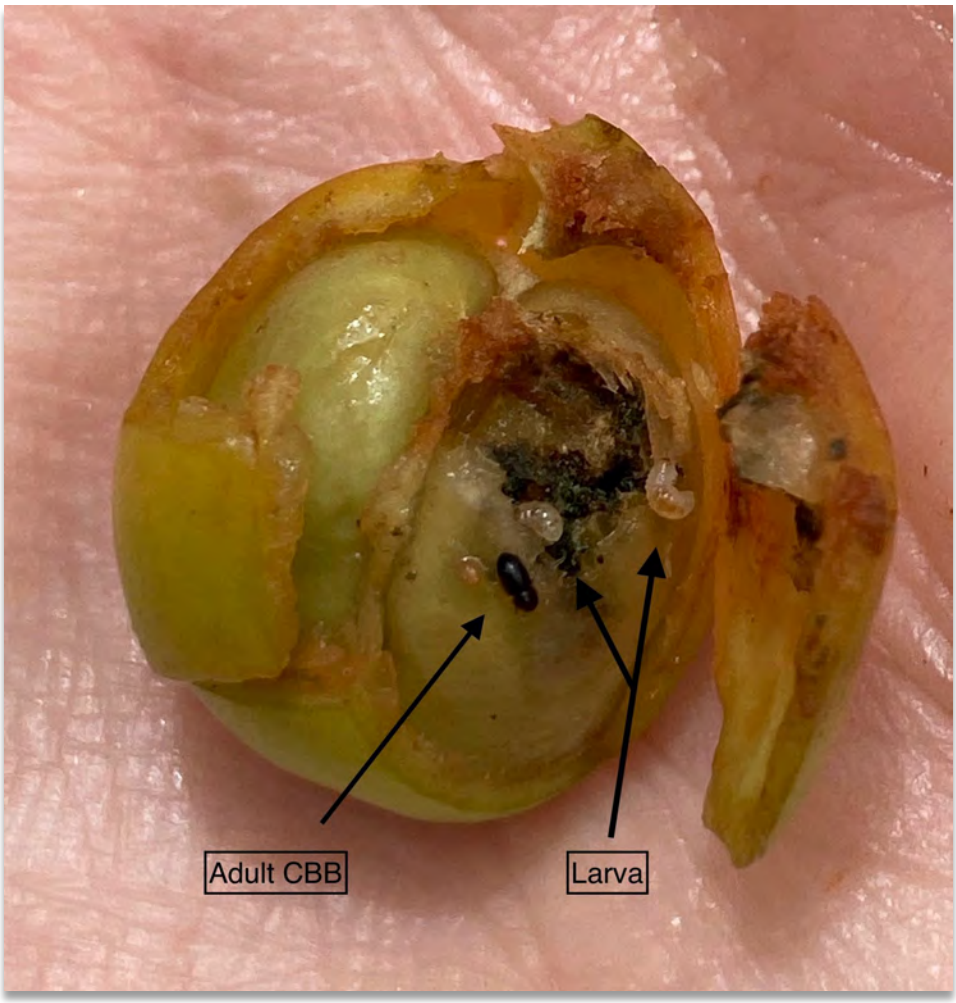

Figure 4: Live beetle and damage inside the bean. Adult CBB is approximately 1.8 mm long

There are three natural enemies to CBB that are indigenous to Africa. One of these, *Phymastichus coffea* is an endoparasitoid that attacks CBB adults and is found widespread in African coffee regions. Females, under 1mm in length, oviposit in the abdomen of the CBB adults, laying a single male and a single female egg, which hatch and feed on the internal tissues of the host. Host CBB that are parasitized by *P. coffea* die within 15 days (Espinoza, 2009). This species of parasitoid is considered ideal for use as a CBB biocontrol agent because

of its highly discriminatory nature and its ability to enter the bean itself. It has been released in at least 12 countries to date. Although it is short-lived (2–3 days), it can be released any time after fruit colonization, with studies showing successful parasitization up to seven days after CBB have initiated berry entry.

The Espinoza (2009) study results demonstrate that using *P. coffea* at a density of 1 parasitoid per 10 hosts results in a 3- to 5.6-fold decrease in CBB damage to the coffee beans when compared to the control. This is due to the fact that individuals parasitized by *P. coffea* drastically changed their behavior, stopped reproducing and died before they damaged the coffee bean.

Unlike other CBB-management methods, release of *P. coffea* does not require trespassing on private land to treat unmanaged coffee trees or application of chemicals to feral coffee in public areas as the wasp can fly up to 70 meters from its release site. Using *P. coffea* to control CBB is an opportunity to reduce the collateral impacts of areawide control activities.

Hawai‘i – Historical and Cultural Background

PRE-SETTLEMENT

GEOGRAPHICAL

The Hawaiian Islands lie in the middle of the vast Pacific Ocean located approximately 2,500 miles from the nearest continent on the Earth. Islands rose individually to the surface as the Pacific Plate drifted north-northwest over a lava hot spot creating these new land masses. The youngest and most southern island in the chain, Hawai‘i, is thought to be about 400,000 years old. Ni‘ihau and Kauai to the northwest end of the main Hawaiian islands are aproximated to be about 3-5 million years old. The newest formation south of Hawai‘i island, Loihi, will most likely reach the surface in 50,000 years. Kure Atoll to the far northwest of the archipelago is one of the atolls still above water, close to 30 million years old (Olson, 2004). The islands are host to many diverse climate zones and the largest mountain on the planet, Mauna Kea on Hawai‘i island, standing at 39,000 feet (14,000 metres) if taken from measurement at its sub-surface base to its summit (Wylie, 2015).

The main and most populated islands in the Hawaiian-Emperor Chain are Hawai‘i, Maui, Kaho‘olawe, Lāna‘i, Moloka‘i, O‘ahu, Kauai and Ni‘ihau. The Papahānaumokuākea Marine National Monument, established in 2006, extends from Nihoa northwest to Kure Atoll. Stretching over 1,350 miles and covering 582,578 square miles, it is one of the largest marine conservation areas on Earth, offering both environmental and Native Hawaiian cultural protections (Papahānaumokuākea Marine National Monument).

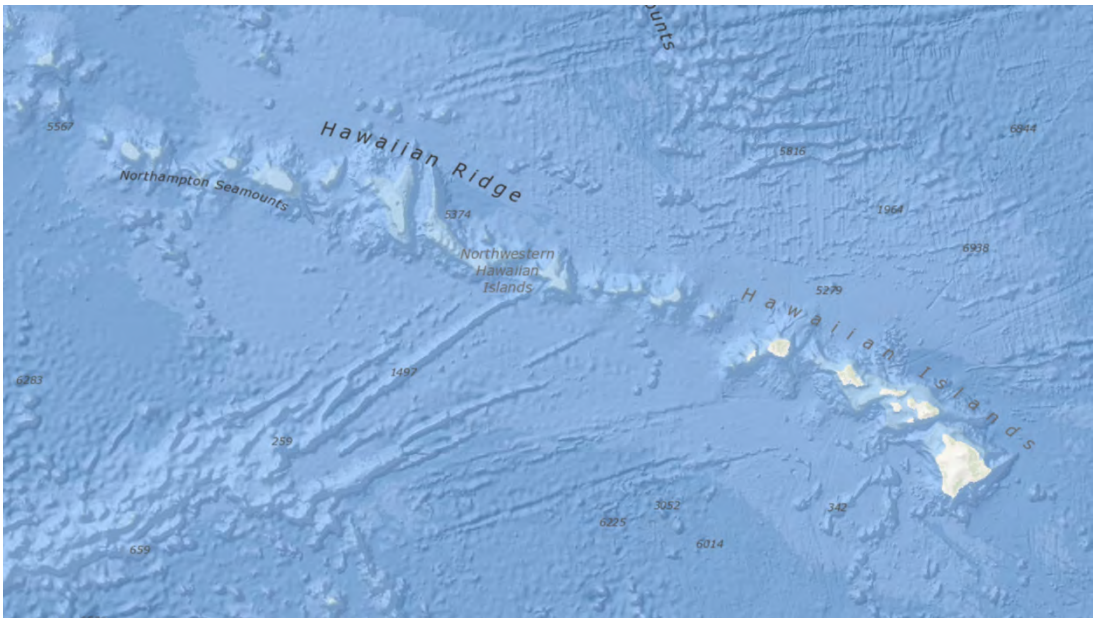

Figure 5: Map of the Hawaiian Archipelago
NOAA

PLANTS AND ANIMALS

The position of these islands on the planet created space in which flora and fauna developed unimpeded and unchallenged. Various birds, trees, plants, and creatures of the sea and land made their way by air or water here to thrive on the shores and slopes of this volcanic chain, creating an abundance of life (Olson, 2004). This life would eventually come to support the Polynesians who made their way across the Pacific to the many island groupings in one of the most rapid settlement excursions known to humans.

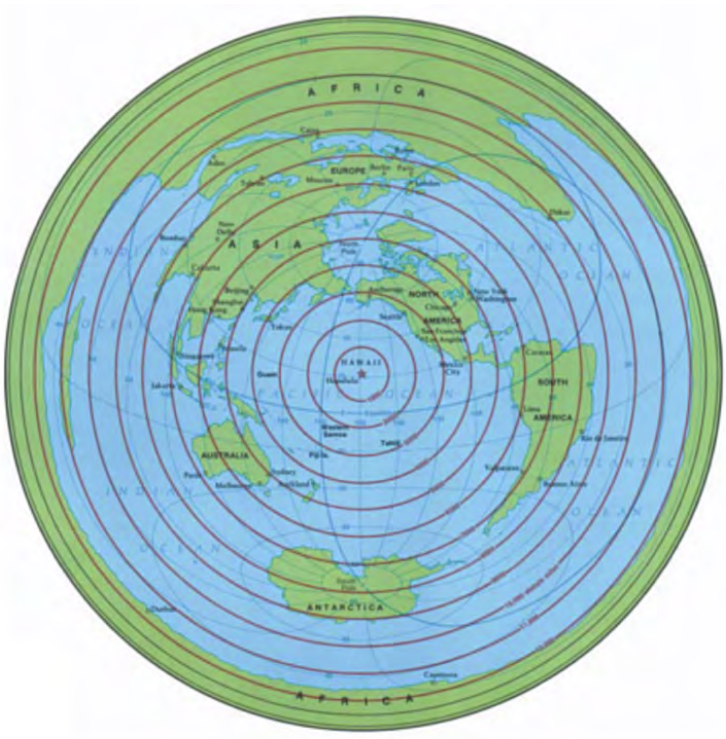

Figure 6: Azimuthal equidistant projection map Hawaii (Armstrong, 1983)

Prior to the arrival of the Polynesians, Hawaii lay untouched except for the natural forces of tsunami, earthquakes, hurricanes, drought and even blizzards atop the peaks of its highest mountains. The plants, animals and insects that made their way here established themselves and became some of the most unique species on the planet. Although similarities can be seen with their counterparts on the continents, many developed interesting new characteristics. Typical protective defense systems in place in these organisms on the continents were lost over time as there were no predators nor competitors to challenge them (Olson, 2004). Stinging nettles on the mainland of North America, for example, has a relative here in Hawai‘i known as māmaki (*Pipturus albidus*). Māmaki has lost the stinging leaf its mainland relative is known for, however still carries the same usages in medicinal remedies (Bishop Museum, 2021).

Early examples of these pre-settlement species include fern spores, koa, pōhuehue (beach morning glory), snails, and insects most likely from North America. Tradewinds that prevail from the Northeast and storms from the South most likely helped propel them to the Hawaiian islands (Dunford et. al, 2013). Once here, as mentioned above, many lost their natural defenses due to lack of predation and continually diversified, adapting to the wetlands and drylands of the islands.

Plants existing pre-settlement:

Koa, pūkiawe, māmaki, ʻaʻaliʻi, olonā, ʻukiʻuki, kauila, ʻōlapa, ʻākala, maile, māmane, ʻōhelo, ʻūlei, hāpuʻu, ʻilima, alaheʻe, alani, ʻōhiʻa lehua, mokihana and wiliwili (Dunford, et. al. 2013).

SETTLEMENT & PRE-EUROPEAN CONTACT

There is dispute as to the actual dates of arrival of the Polynesians who settled the Hawaiian islands. Current archaeological carbon dating points to 1000 CE as the approximate date of first settlement in the islands although ranges from 800-1200 CE are possible (Kirch, 2011 and Cordy 2000). Two possible sources for the voyagers who made their way to Hawaiʻi are the Marquesas (Nuʻuhiwa) c. 900 CE and Tahiti (Kahiki) c. 1200 CE (Dunford et. al. 2013).

Polynesian settlers sailed with many plants and animals on their waʻa (canoes). The history of settlement is also the history of agriculture, and of species introduction. During the pre-contact era up to about 1450 CE, when migration seems to have slowed perhaps due to the Little Ice Age (Dunford, et al. 2013), several species were introduced.

Species introduced by Polynesians:

puaʻa (pig), moa (chicken), ʻīlio (dog), ʻiole (rat)

kō (sugar cane), 'ohe (bamboo), niu (coconut palm), kalo (taro), kī (ti), pia (Polynesian arrowroot,), uhi (yam) Pi'a (Five-Leafed yam), mai'a (banana), 'ōlena (turmeric) 'awapuhi (wild ginger), 'awa (kava), 'ulu (breadfruit) wauke (paper mulberry), pa'ihi (nasturtium), auhuhu (Fish Poison plant), kukui (candlenut tree), hau (hibiscus), milo (Portiatree) kamani (Alexandrian laurel), 'ōhi'a 'ai (mountain apple) ʻuala (sweet potato), kou (Cordia wood), noni (Indian mulberry) ipu (Bottle gourd) (Dunford, et. al. 2013 and St. John et. al 1980).

The introduction of these new species provided great sustenance for the kanaka maoli (Hawaiians) (Dunford et. al. 2013). These species, however, also began to encroach upon the endemic pre-settlement species. Puaʻa dug up rooted vegetables and “the main source of destruction of the native forests was the introduction of the Polynesian rat, *Rattus exulans*” (Athens et. al, 2002). Prehistoric avian species also suffered from the rat but also from human settlement as initially forests where the birds resided were burned and cleared for agricultural development by the settlers.

LAND DIVISIONS AND SOCIETAL STRUCTURE

"Hawaiian integrated farming systems evolved and proliferated within a unique socio-cultural context" (Costa-Pierce, 1987).

AHUPUA'A

Islands in the Hawaiian language ('ōlelo Hawai'i) were called mokupuni. Mokupuni were divided into moku (districts) and within these moku were created smaller areas called ahupua'a (Williams, 1997). In some ahupua'a there were even smaller areas: 'ili kūpono and 'ili 'aīna (Dunford et.al, 2013 and Cordy, 2000). Most important, however, were the ahupua'a.

Ahupua'a usually ran from mauka to makai (mountain to ocean) with possible smaller ones that didn't have this feature. Residents worked and gathered within their ahupua'a which were designed to provide resources for them from upland crops to ocean provisions (William,1997).

There were three distinct areas within these ahupua'a: uka, which included mountain and upland areas; kula, the flat and sloping plains and fields; and kai, the seashore and sea environment sometimes up to a mile offshore (Williams, 1987). Frequently the uka and kula zones would be terraced cross-slope to retain soil and prevent erosion. However, this pattern was notably different in the dry Kona region, where kua'iwi, or stone ridges, ran mauka-makai in a diverse matrix of crops (Lincoln, 2014).

The Kona Field System was considered a marvel by early European visitors, and was indicative of the intensive agricultural activity and horticultural expertise of Hawaiian farmers. Archibald Menzies, a botanist who traveled with Captain George Vancouver, wrote in 1794:

> "On leaving this station, we soon lost sight of the vessels, and entered their breadfruit plantations…The size of the trees, the luxuriance of their crops and foliage, sufficiently show they thrive equally well…The space between the trees did not lay idle. It was chiefly planted with sweet potatoes and rows of cloth plant (wauke). As we advanced beyond the breadfruit plantations, the country became more and more fertile, being in a high state of cultivation…In clearing the ground, the stones were heaped up in ridges between the little

Figure 7: Kua'iwi mauka-makai wall in a Hōnaunau field. Height is 2 ft, width is 12 feet.

fields and planted on each side, either with a row of sugar cane or the sweet root of these island (ti)…so that even these stony uncultivated banks are by this means made useful to the proprietors, as well as ornamental to the fields they intersect. The product of these plantations, besides the above mentioned, are the cloth plant, taro, and sweet potatoes…The whole field is generally covered with a thick layer of hay, made from the long coarse grass or the tops of sugar cane, which continually preserves a certain degree of moisture in the soil that would otherwise be parched by the scorching heat of the solar rays…Their fields in general are productive of good crops that far exceed in point of perfection the produce of any civilized country within the tropics."

The kua‘iwi system is still evident today, and forms the backbone of land in use for agriculture and coffee in South Kona.

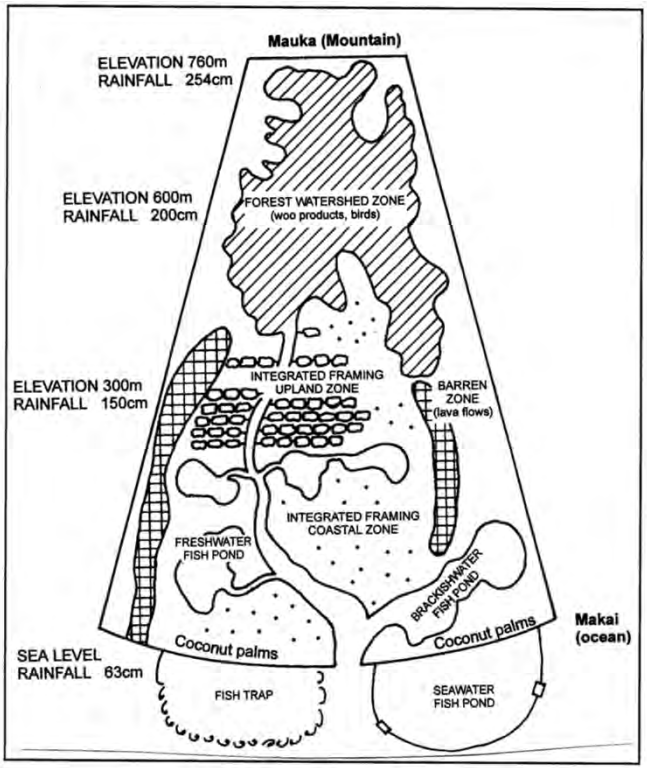

Figure 8: Example of individual ahuapu‘a configuration (Davidson-Hunt, 2021) Adapted from Costa-Pierce (1987)

Within each ahupua‘a area, crops were cultivated for specific microclimate zones. Uka provided trees and plants used for canoe-building, weaponry, tools, cloth (kapa), cordage, lei and feathers for ali‘i clothing collected from the native birds in these upland forests. The kula plains grew most of the food plants including mai‘a (at the fringes of uka), kalo, ‘ulu, ‘uala and uhi. Kukui for oil, ipu for gourds, kī for capes and pili grass for thatched roofing were also grown in the kula areas. Finally, kai was where Hawaiians resourced fish (i‘a), salt (pa‘akai),

limu (seaweed), coconut, hau, hala and noni. The kai sections, especially in leeward areas where the water was calm and shallow, sometimes were host to the loko iʻa (fish pond). These loko iʻa housed Hawaiian fish farms which are being revitalized even today (Dunford et. al, 2013 and Williams, 1997).

Governance of these ahupuaʻa followed a distinct chain of command. Mokupuni were led by an aliʻi nui (high chief). Each moku, or district, within the mokupuni was governed by an aliʻi ʻai moku (lesser chief). Ahupuaʻa divisions within a moku were controlled by the aliʻi ʻai ahupuaʻa who in turn had konohiki (headmen) to oversee the people (makaʻāinana) farming and caretaking the lands. Sometimes the aliʻi ʻai ahupuaʻa and konohiki were the same person (Dunford et. al, 2013).

LAND TENURESHIP

Most of the population chose to live in small villages on non-agricultural land near the shore or clustered around bays where the air was warm and dry (Dixon, 1789). Hawaiian settlements developed around not just the environmental landscape, but also in accordance to societal organization of the aliʻi, konohiki and makaʻāinana (Kirch, 2011). Farming was usually done by a family unit known as an ʻohana. These family relationships were core to the pre-contact farming practices and of great significance to the Hawaiians (Costa-Pierce, 1987). ʻOhana created and maintained complex agricultural systems "that connected agricultural watersheds to oceanic environments" (Costa-Pierce, 1987).

The traditional management system for the early Hawaiians was based on strict kapu, laws meant to preserve societal order. These kapu pertained to aspects of daily life which included practices in religion, ways of eating, areas one was allowed to enter and times of harvest and gathering to name just a few. Some of these kapu were so strict they carried the penalty of death (Dunford et. al, 2013). In general practice, the ʻauhau (taxes) were gathered during the Makahiki (gathering time for collecting taxes with focus on more celebratory aspects of life versus war) (Iʻi , 1959).

The concept of land ownership viewed through Western culture is far different from the Hawaiian socio-cultural understanding of ownership. The makaʻāinana worked the land for the aliʻi ʻai moku who oversaw the district in turn for the aliʻi nui. In essence, it was a system of feudal tenureship with freedom to move within the ahapuaʻa and with the responsibility to pay your taxes in the form of food and animals once a year to the aliʻi (Handy & Pukui, 1998).

This idea and practice of tenureship is what would help contribute to the downfall of the Hawaiian farming practices. It would also provide the opening for Westerners, post-contact, to permanently change the landscape and traditional lifestyle and welfare of the Hawaiian people.

EUROPEAN CONTACT & THE HISTORIC PERIOD

"With the general demise of native Hawaiian society, the majority of Hawaiian integrated farming systems fell into disuse and disrepair" (Costa-Pierce, 1987).

The arrival of Captain James Cook to the islands in 1778 CE heralded immense change for the Hawaiian people who had lived for approximately a millenia without contact except from other occasional Polynesian voyagers (Kirch, 1998).

The next most significant person in the initial contact years was Captain George Vancouver who had served as an officer to Cook. Returning in 1791 leading the second British expedition, he made several trips to the islands bringing cattle (pipi), goats, geese, sheep and oranges (Speakman & Hackler 1989 and Hawai'i Dept. Of Agriculture). Eventually, mangoes, papaya, plumeria, coffee and lychee would also be introduced in the early nineteenth century (Dunford et. al, 2013).

After Cook's arrival to Hawai'i, the islands become a stopping point and eventual base for Western political and economical expansion into the Pacific and Asia. Landscape and cultural changes sailed in with the explorers, New England whaling industry and the missionaries who arrived in its wake. Over time, the raising of the new crops and animals they introduced to Hawai'i would contribute to the undermining of the traditional farming practices (Lâm, 1989). Development of imported agricultural in the Hawaiian islands increased rapidly during the early nineteenth century. The increase in the foreign population and creation of whaler ports on several of the islands produced a new supply and demand chain that would forever alter the islands.

'Iliahi (sandalwood) became a major commodity in 1810 heralding the increased economic investment by foreigners. Eventually when the sandalwood trade waned, the damage to the traditional subsistence economy had been done. The whaling industry as well now had a foothold in the islands and the ali'i had incurred massive debt to the foreign investors. By 1826, the first gunboat incidence occurred when the U.S. Navy moored in Honolulu harbor attempting to forcefully collect on these ali'i debts.

The whaling industry impacted traditional Hawaiian lifestyles in many areas. The cash economy began to supplant the previous subsistence economy. Hawaiians began to relocate to the now town and city centers for work, with many men signing on to the whaling ships. Agriculutre turned to growing crops to be sold to the peoples inhabiting these areas and to provision all the trade and merchant vessels at port. Disruption of the agriculutral farming systems that had served Hawai'i for a millenia seriously impacted the traditional socio-cultural basis for the kanaka maoli. It would pave the way for the end of land tenureship and the evolution of private property rights especially to be held by foreign entities (Kent, 1993).

THE GREAT MAHELE OF 1848 and THE KULEANA ACT OF 1850

Foreign economic disruption of the traditional subsistence trade practices led to a cultural clash related to the concept of land ownership. Hawaiians' utilization of a method of tenureship approach to the land was in opposition to and undermined the Western cultures' idea of right to privately own land which placed great value both economically and politically on this type of usage.

Between 1839 and 1845, major shifts occurred within the Hawaiian political system in response to decades of foreign influence. Hawai'i was recognized as a consitutional monarchy by France, Belgium and Great Britain; the Bill of Rights was drawn up, and a constitution was signed in 1840 (Kamakakau, 1992). Several other pieces of legislation followed which would lead to the privatization of land ownership. The Act to Quiet Lands Titles was the first in 1844, initiating ten years of land ownership transformation. The Act created a Board of Commissioners to oversee the process of the division of lands between the king and his subjects. It also opened up the potential, perhaps not intentionally, for foreign buyers to gain a foothold into land ownership in Hawai'i.

The Great Māhele spanned the years of 1845 to 1855 culminating in The Great Māhele Act of 1848 and the Kuleana Act of 1850. The 1848 act relocated one third of the lands to the king, which would be known as crown lands, another one third to the konohiki or chiefs and the last third to the maka'āinana. Importantly, the initial Māhele did not change the tenureship concept for the maka'āinana (Lâm,1989).

The Resident Alien Land Ownership Act of July, 1850 and the Kuleana Act of August, 1850 would effectively be the instruments to commit the final severance. The Resident Alien Act gave foreigners the right to own land privately. The Kuleana Act gave Hawaiians two years to pay for and complete surveys on land that they were currently using but only up to 0.25 acre. Most Hawaiians did not understand nor took advantage of, nor perhaps weren't financially able, to take advantage of this process. At the end of the two years only 8,200 kuleana parcels were recognized and awarded which amounted to less than 1% of the lands (Lâm 1989). Combined, these two acts, whether good-intentioned or not, effectively ended traditional land use in the Hawaiian islands.

The rise of the plantation in coordinance with the sugar trade was a direct result of these processes. Labor and land were restructured to maximize profits in the hands of the owners of these plantations. These owners would eventually play a large part in the overthrow of the Hawaiian kingdom in the late nineteenth century (Kent 35-6).

IMPACT OF INTRODUCED DISEASES

Prior to outside contact, Hawaiians had already suffered greatly from warfare, famine and infant mortalities. However, the economic and socio-cultural changes brought upon Hawai'i

were only part of the process of the change in society. For a long period of time, Hawai‘i enjoyed the separation from the outside world and along with that, freedom from newly transmitted diseases. That changed with the arrival of Cook in 1778 and led to a steep decrease in Hawaiian population over the next century (Bushnell,1993).

Sailors on the voyaging ships introduced several venereal diseases, followed by tuberculosis in 1786. By 1804, Hawai‘i saw its first large epidemic of what was most likely typhoid fever. Leprosy made its way to the islands by 1823 (Kamakau, 1992). There were continual outbreaks from 1826-57 derived from insect-borne disease, venereal disease and epidemics from inbound ships. An American warship brought in measles to Hilo in 1848 killing off 1/3 of the population. Several outbreaks of colds and flus occurred and by 1853 smallpox had arrived.

Figure 9: Map showing population decrease
(Office of Hawaiian Affairs, 2017)

Decimation of the native Hawaiian population in the nineteenth century along with changes in the laws governing land ownership, created a space into which foreign investment and eventual political policy would lay the foundations for the modern era in Hawai‘i.

AGRICULTURE IN THE POST-CONTACT ERA

The rise of foreign influences and trading ports saw a divergence in the agricultural production of each island.

O‘ahu, Maui and Kauai followed similar paths during the period from the late 1790‘s through the 1850‘s. Whaling ports were the main drivers for change on these three islands and Honolulu, Lahaina and Kōloa Harbors became major resupply points for ships.

O‘AHU

The first half of the nineteenth century saw a diversification of imported food crops, supplanting the traditional crops that had been grown by the Native Hawaiians. As was similar on the other islands, imported crops were grown to resupply the visiting ships and cater to changing tastes in a rapidly diversifying population. The rise in a cash economy supplanted the traditional subsistence and ‘ohana-based structure.

The sugar industry was king during the mid-1800‘s but as the twentieth century fast approached, sugar began to wane economically. Other potential crops were explored for both local use and exportation. Specific to O‘ahu, the plains of Wahiawa had developed an irrigation system and American homesteaders experimented with several crops. These included banana, papaya, fig, olive, orange, mango, pineapple and also coffee and vegetable oils, with pineapple and coffee eventually becoming the focal crops (evols.library).

Modern agriculture on O‘ahu includes more than 40 different crops including pineapple, tropical flowers, coffee, melons, papayas, pumpkins, and bananas. O‘ahu is also home to University of Hawai‘i and the College of Tropical Agriculture and Human Resources (CTAHR). CTAHR is engaged in the study of and promulgation of agriculture throughout the Hawaiian islands.

KAUA‘I

Waimea was the first point of contact on Kaua‘i for Captain James Cook in January of 1778. The south shore of the island would eventually host the whaling and sugar industries for the better part of the nineteenth century. Kōloa Village and Landing were the main point for distribution of products like sugar, molasses, beef and sweet potatoes to the ships (kauai.gov). Commercial pineapple as an industry navigated from O‘ahu to the neighbor islands, especially Kaua‘i and Maui as the previously-established sugar plantation farming methodology supported the growing and harvesting infrastructure for pineapples (Bartholomew).

Modern crops include papaya, tropical flowers, large kalo (taro) lo‘i or ponded fields, and GMO biotech seed crops. GMO corn research fields were implemented on Kaua‘i as early as the late 1960‘s and remain in rotation. The largest coffee plantation in the state is located in Kalaheo.

MAUI

Like Kaua‘i, Maui‘s agricultural history followed the whaling industry‘s needs from the 1820‘s to the 1850‘s. Crops shifted from traditional Hawaiian foods to those desired for the ships‘ stores. Lahaina on the west side was the main harbor used for the export of goods. Towards the

advent of the twentieth century, pineapple became a staple crop and eventually canneries were started on the island (Bartholomew et al., 2002).

Modern agriculture now includes a thriving coffee industry, cattle, pineapple, onions, papayas, tropical flowers, raw sugar, and the GMO biotech seed industry (mauicounty.gov).

LĀNAʻI

Lānaʻi has a uniquely different history of agricultural development than the other islands. The population had been decimated by wars within the Hawaiian kingdomʻs expansion under Kamehameha I and remained sparsely populated with subsistence farmers and fishermen. It wasnʻt until Walter Gibson arrived in the 1860ʻs and acquired private land that agriculture shifted to more modern crops. Gibson brought ranching to the island which was followed by sugar from 1899-1921. The first pineapples were grown during the latter period of that time, and in 1921, James Dole acquired the island under private ownership. Soon Lānaʻi became known as the pineapple island (lanaichc.org). Pineapple was phased out of production by 1992, due to high labor and land costs. Today, with 91% of the island in private ownership, the focus is increasingly on tourism and resort development instead of major agricultural crops (Land Use Baseline).

MOLOKAʻI

Aquaculture and ranching were mainstays of the transitional agricultural landscape on Molokaʻi. When the Hawaiian Homes Act of 1920 was estabished, many homesteads were created on the north shore in Hoʻolehua. Initially, land was leased out for pineapple production but moved into diversified crops as did the other islands, just at a later rate of change (hdoa.hawaii.gov).

Molokaʻiʻs strong winds and lack of water prevented the larger crop systems from maintaining economic sustainability. Pineapple companies left in the 1970ʻs, as did a large portion of the population dependent on their income. Today, Molokaʻi is predominantly Hawaiian by population and the residents do not cater highly to tourism. In the homestead area, foodcrops such as banana, papaya, taro, sweet potatoes and onions are grown (molokai.org). There is a large commercial coffee farm in the Kualapuʻu village area.

The GMO biotech seed companies comprise more than 50% of the crop production on the island and as with other islands, has become a controversial land use issue (molokai.org). The only true port on the island is Kaunakakai on the south shore.

HAWAIʻI

Hawaiʻi island has a rich history in agricultural development, both pre- and post-contact. A variety of ethnographic materials exist for West Hawai'i, primarily because it was the ancestral seat of a powerful line of hereditary chiefs, including Kamehameha. The early European visitors

paying their respects to the ruling power in the islands left behind journals and logs as they investigated the Kona and Kohala districts (Greene 1993).

As the largest of the Hawaiian islands, it also is home to an abundance of climate zones and can sustain a wide diversity of crops. About half of the state's commercial farms are located here (NASS Census, 2017).

Hawai'i Island's forests were host to the majority of the 'iliahi (sandalwood) growth. Kamehameha I controlled much of the trade, but on his death, the trade (and subsistence agriculture as a whole) began to fall apart for the Hawaiians. His kapu on felling young trees collapsed, and the mountains were eventually stripped of most of these trees. His son, Kamehameha II sank into debt as the crop declined and the industry had collapsed by 1830 (hawaiihistory.org). Kamehameha III banned the collection of sandalwood in 1839. This rare and expensive crop is still propagated and harvested on the upland slopes of the west side, albeit in very small quantities (nativeplants.hawaii.edu).

Many varieties of crops were introduced to the island, concurrent with other islands (nativeplants.hawaii.edu). This included oranges and cattle in the 1790's followed by pineapple and coffee by 1810. Commercial crops of mango, rice, eucalyptus and macadamia nuts were all introduced before the turn of the century. Sugar was primarily farmed in the south and east sides of Hilo, Hamakua and Puna until its economic collapse on the island in the 1990's.

On the Kona side, coffee production moved to the forefront during the mid-1800's. The ease of exporting the raw bean by sea trade allowed the crop to rise in prominence. The districts of North and South Kona were granted special labeling rules by the Department of Agriculture. With the closure of the last sugar mills in Hamakua (1994) Puna (1995) and Pahala (1996), a nascent coffee industry began to grow in these regions. Ka'u coffee (Pahala) rapidly grew in size and reputation.

There are many individual small farms focused on a large variety of crops including chocolate, honey, avocado, tropical fruits and flowers, sweet potato, and kalo. The GMO biotech seed crops also have a presence, mostly on the east side. Parker Ranch, in Kamuela, is one of the largest cattle ranches in the United States. Large macadamia nut farms are located in the Hilo and Ka'u districts.

MODERN ERA

The Hawai'i of today is a far cry from what it was pre-contact. There are no illusions that life pre-contact was a perfect utopia. However, a Hawaiian such as Kamehameha I might be hard-pressed to see any familiarities of his time in the current era.

At this moment, the islands face many challenges. Hawai'i is deeply dependent on a tourist-based economy, which proved fragile during COVID-19 quarantines. Home ownership is

virtually impossible for many Kānaka Maoli as housing prices have risen well beyond what is affordable to many residents in a service-based economy. Even the neighbor islands of Hawai‘i, Maui, Moloka‘i, Lāna‘i and Kauai have seen housing prices rise close to equal of those on O‘ahu. This has led to an exodus of Hawaiians to the mainland United States in search of better jobs and housing opportunities.

On the upside, there is a nascent effort in smaller communities to restructure the economy. The focus is on industries that serve and benefit the community especially in the areas of economic, social and mental welfare. Agriculture is one of the industries that could help alleviate the reliance on tourism. Coffee, avocados, kalo, bananas, papayas, mangoes and pineapples are just a few of these crops that are produced locally. Perhaps with strong support to these farming endeavors, Hawai‘i can reclaim its inherent agricultural proficiency in order to support a healthier economic base for its social and cultural communities.

Community Interviews

To gain deeper understanding of the project area, a variety of stakeholders was interviewed for their knowledge of cultural practices within the coffee-growing Hawaiian islands: Oahu, Maui, Molokai, and Hawai‘i Island. In keeping with the *Guidelines for Assessing Cultural Impacts* from the State's Department of Health - Office of Environmental Quality Control, interviews concerned not just coffee on these islands, but larger areas and cultural practices that could be affected by the release of *Phymastichus coffea.*

SHAC staff contacted eight community members for these interviews via telephone and email. Two declined, while six others agreed to be interviewed in May 2021. Each person contacted fits into one or more of the following categories: 1) Native Hawaiian cultural practitioner, 2) coffee farmer in Hawai‘i, or 3) conservationist managing lands planted with Hawaiian coffee. To solicit additional feedback from members of the public who fit these criteria, a public notice was published on June 1 in Ka Wai Ola, the Office of Hawaiian Affairs newspaper and on their website at https://kawaiola.news/hoolahalehulehu/public-notice-june-2021/. No responses were received.

Following is the list of interviewees and the method of each interview:

Name of Interviewee	(Island) Title, Organization	Method of Interview
Shalan Crysdale	(Kaʻu District, Hawaiʻi Island; Molokai) Hawaiʻi Island Program Director, The Nature Conservancy	Zoom
Hiʻilani Shibata	(Oahu) Co-owner, Ka Mahina Project; Lead Cultural Trainer of the Native Hawaiian Hospitality Association	Zoom
Kimokeo Kapahulehua	(Maui) President, Kimokeo Foundation	Facetime
Bryce Nakamura	(Kona District, Hawaiʻi Island) Third generation Kona coffee farmer	Zoom
Chuck Leslie	(Kona District, Hawaiʻi Island) Third-generation Nāpōʻopoʻo fisherman	Zoom
Wally Young	(Kaʻu District, Hawaiʻi Island) Kaʻu coffee farmer	In person

Each interview started with a short introduction to *P. coffea,* including photos of the parasitoid wasp laying eggs in a coffee berry borer (CBB) beetle as it entered a coffee cherry. All interviewees already were aware of CBB and its threat to Hawaiʻi-grown coffee. Points emphasized included the following:

- *P. coffea* originally is from Africa, and it has been introduced to coffee producing countries, such as Colombia, Guatemala, Honduras, Jamaica, El Salvador, Ecuador, India, Brazil, and Mexico for biological control purposes.
- *P. coffea* was brought from Colombia into a quarantine containment facility in Volcano for 1.5 years of tests. PBARC scientist Peter Follett aimed to determine whether the wasp might attack other beetle species and thereby pose a risk to the environment.
- During the USDA's tests, the wasp did not impact any native species. Only 5 insect species were parasitized by *P. coffea,* including CBB and Tropical Nut Borer, a macadamia nut pest. *Phymasticus coffea* appears to be very specific in what it attacks and should cause no harm to the environment if released for CBB control.

- The wasp cannot sting humans or animals.

Subsequent questions focused on four areas: 1) each individual's background and cultural practices, as well as experiences with pests and plant diseases that impact their cultural practices; 2) their knowledge about coffee production and Hawaiian agriculture; 3) their views about proper methods of pest control; and 4) any additional comments and concerns. SHAC staff prepared draft summaries of participants' interviews for them to review and add revisions. Below are the approved summaries of each interview:

Shalan Crysdale, The Nature Conservancy

Since 2009, Crysdale has been working on Hawai'i Island for The Nature Conservancy (TNC). He began his tenure with TNC as the field coordinator for the Ka'u Preserve, was promoted to natural resource manager, and is now the Hawai'i Island forest program director. As such, he is directly responsible for three units of TNC-owned lands: Ka'u Preserve, Kona Hema in South Kona, and Kamehame in Ka'u District.

Of these three, Kona Hema has a few patches of naturalized planted coffee. Situated on old terraces, these thick patches of coffee may date to the turn of the 20th century, Crysdale says. In addition, Kona Hema has an experimental, high-elevation strand of macadamia nuts planted by longtime agribusiness developer Sally Rice, who currently co-owns consultancies Agricon Hawaii and Agro Resources Hawaii.

Another TNC-owned unit, Pelekunu on North Molokai, is the site of a long-gone village that once grew coffee. Some coffee trees still exist there, Crysdale says.

Like others interviewed for this CIA, Crysdale doesn't know of any traditional Hawaiian cultural practices utilizing the coffee plant, fruit or seeds. Instead, coffee was a cash crop that many Japanese families depended on at the turn of the 20th century. Crysdale recalls hearing stories about agricultural workers who declined to renew their contracts as sugarcane workers, choosing instead to grow coffee on the Kona side of Hawai'i Island. For decades, those farms have provided harvesting jobs for new arrivals to the island. Coffee picking, Crysdale says, "is an entry point to Hawai'i living."

One hundred years ago, the farmers had limited themselves to the best areas for growing coffee. But as the popularity of Kona coffee grew, Crysdale increasingly saw native forests and more marginal lands converted to coffee farms. With the addition of more farms came an increased reliance on herbicide.

"In the long run, that's a negative," he says. We don't want to see that show up in our water table."

Crysdale himself has some experience with tending coffee. This past season, from trees surrounding his home, Crysdale's family had a small harvest that resulted in about 25 pounds of roasted coffee. While this is a small amount, it was enough for Crysdale to see firsthand the unfortunate impacts of CBB on his own crop.

For Crysdale, protecting agriculture and ecosystems from introduced pests is "very top priority. What we're dealing with in Hawai'i is a rate of extinction that's unparalleled anywhere else in the world. It was like a flatline of species lost until these last 200 years."

He pointed out a few pests that have impacted his conservation work: 1) Rats are the number one pest in the forest, especially for forest birds with low-lying nests. 2) Mosquitoes carry avian malaria. 3) Invasive plants, such as strawberry guava and Christmas berry, grow prolifically and crowd out native plants.

There have been advancements in controlling these pests, Crysdale says. Automatic rat traps reset themselves and release just a little non-toxic bait, preventing the accidental poisoning of native birds. Sterilized male mosquitoes mate with female mosquitoes and leave them barren. *Tectococcus ovatus* is a biological control for strawberry guava. But to Crysdale, the best cure is prevention. He would like to see the State invest in more robust inspections and severe penalties.

Crysdale generally is supportive of insect biocontrols because of the success of *Eurytoma erythrinae*, a parasitoid wasp of the Erythina Gall Wasp (*Quadrastichus erythrinae)*. Before the release of *E. erythrinae* as a biocontrol, the Erythina Gall Wasp was unchecked in laying its eggs in the leaves and stems of *wiliwili* trees, a dryland forest species native to Hawai'i. Crysdale recalls *wiliwili* trees with gnarled new growth. Severe infestations resulted in defoliation, or even death.

The release of *E. erythrinae* had an "instantaneous" effect, Crysdale says. The difference was like "night and day." New growth looked normal again, and *wiliwili* trees started growing at South Point on Hawai'i Island for the first time in years.

He hopes *P. coffea* would have a similar effect on CBB, for the sake of coffee and other host plants. Crysdale wondered whether CBB also infests any native plants. If so, *P. coffea* would benefit those as well.

"The idea that we are going to fence and spray ourselves out of these [pest] problems is too hard," Crysdale says. "Biocontrol is better. Let nature be a solution to nature. This is a very effective tool."

Hi'ilani Shibata, Ka Mahina Project and Native Hawaiian Hospitality Association

Originally from Hilo and now living on Oahu, Shibata is a longtime educator of Native Hawaiian cultural practices and history. She is co-owner of the Ka Mahina Project, which promotes a healthier life through traditions that honor Hina, the Hawaiian moon goddess. Shibata also is lead cultural trainer for the Native Hawaiian Hospitality Association. Previously, she spent 14 years as education manager at the Bishop Museum in Honolulu. Shibata's own cultural practices include *lomilomi* and traditional *ho'oponopono*.

She also has conducted farmer education, based on her own family's experience with small-scale agriculture. Her husband had a two-acre farm that grew crops such as taro, *'ulu*, sugarcane and bananas -- just enough to feed family and friends. (They are looking for another plot of land to resume farming.) Over the years, she has seen growth in the number of Hawai'i's small and large farms. She hopes to see the establishment of more small ones.

Shibata's family doesn't grow coffee, but she has participated in coffee harvesting and processing at the Hawai'i Agricultural Research Center. She has noticed a difference in flavor between coffee produced on Hawai'i Island, versus coffee grown on the other Hawaiian islands. It's a variance she attributes to Hawai'i Island's younger volcanic soils.

Kona coffee has contributed much to the history of Hawai'i, especially since it's known globally, Shibata says. As coffee is not a traditional Hawaiian plant, she doesn't know of Native Hawaiian cultural practices that incorporate it. "It's not like they rejected it," she says of the Polynesian pioneers to Hawai'i. "I just don't think it's something they had."

Shibata has seen invasive pests affect both agriculture and plants important to Native Hawaiian culture. *'Uala* and taro are targeted by sweet potato weevil and apple snails, respectively. On her husband's farm, they noticed longneck turtles, poisonous dark frogs, and Japanese eels -- all non-native species, Shibata says. *Wiliwili* trees have been harmed by the Erythrina Gall Wasp. And the leaves of the *hala* tree, used by lauhala weavers, suffer from hala scale.

"Any time a native plant is affected negatively, it will have multiple effects on our culture," Shibata says. Since shipping introduces invasive species, she hopes more local agriculture would reduce imports. Shibata also would like to see more inspectors looking for invasive pests: "Protecting agriculture and ecosystems, it's really important. And it's really hard, because there's very little money."

When it comes to controlling pests, Shibata prefers physical and biological controls. She has participated in removing invasive miconia trees. And she's in favor of parasitoids -- as long as they are researched as extensively as the one that saved the *wiliwili* trees from the Erythrina Gall Wasp. "I'm not into chemicals because they go into our water systems," she says.

Kimokeo Kapahulehua, Kimokeo Foundation

Born in Lihue, Kauai and now living in Kihei, Maui, 73-year-old Kapahulehua is a cultural educator with a long history of spreading Native Hawaiian traditions. The organization he founded, the Kimokeo Foundation, describes Kapahulehua's work this way: "His accomplishments are vast, spanning from being heavily involved in Hawaiian outrigger canoe paddling and voyaging, to the preservation of Native Hawaiian forests, to the revitalization of an ancient Hawaiian fishpond to educating thousands of youth about the Hawaiian culture and its practices, to raising money for cancer survivors."

For all of his life, Kapahulehua's cultural practices have involved the sea. He grew up in a fishing family that used both nets and spears to catch their prey. By the time he was about eight years old, he was paddling the outrigger canoes called *waʻa*.

Kapahulehua eventually became Maui Island's Gray Line tour manager. As an adult, his most ambitious project was to travel by *waʻa* along the length of the entire Hawaiian island chain. Starting with Hawaiʻi Island and ending with the Kure Atoll, the voyage took his team six years and spanned 1,750 miles.

Kapahulehua also has led teams in harvesting logs to make *waʻa*. In 2000, his team harvested a log for a canoe at Haleakalā, Maui. It was the first time in 64 years that a log was cut down for this purpose. In 2010, his team went to Mauna Kea and harvested eight logs to make canoes. Each time, they followed protocols established by their ancestors.

Kapahulehua points out that Native Hawaiians originally didn't have coffee, so there were no traditional cultural practices with it. "When I was brought up, there was only one place to get coffee," he says. "That was in Kona." He recalls seeing family members in Kona harvesting red coffee cherries, as well as coffee beans drying on platforms known as hoshidanas.

As coffee cultivation spread to areas such as Kaʻu, Molokai and Maui, it became "a significant way of life for our people," Kapahulehua says. Coffee farming supported a lot of families and is now as integral to Hawaiʻi's agricultural history as sugarcane, pineapple, papaya, banana, ti leaves, anthuriums, bird of paradise and other tropical flowers, he adds.

For all of the above reasons, he considers it very important to control coffee berry borer (CBB) and other pests. "We're the number one state in growing coffee," he says. "That's a greater concern -- for us to maintain that industry."

Kapahulehua has seen pests damage other industries and ecosystems. The *taʻape*, a yellow-skinned snapper introduced to Hawaiʻi by what was known as the Division of Fish and Game in the 1950s and 1960s, eats the eggs of native fish, he says. Mongolian seaweed, an invasive species, has overtaken native Hawaiian seaweed in some ocean areas. The US Fish & Wildlife Service now requires inspections of canoe hulls and boat hulls to prevent the spread of this invasive species.

To protect both farms and natural ecosystems, Kapahulehua prefers biocontrol methods over chemical sprays as long as they are tested properly. Such a process would involve scientists studying flora and fauna, in addition to entomologists, he says. With regards to *P. coffea*, the parasitoid wasp that would kill CBB, Kapahulehua questions what percentage of native insect species in Hawai‘i were tested against it. His concern is adequate testing to ensure the protection of Hawai‘i’s endangered insects -- such as moths, as well as of Hawai‘i’s native plants and fruits.

Bryce Nakamura, Kona coffee farmer

Nakamura, 67, is a third-generation Kona coffee farmer. He is descended from Japanese immigrant laborers for Hawai‘i’s sugar industry. His great-grandfather established the family farm on 30 acres of Bishop Estate (now Kamehameha Schools) land overlooking Kealakekua Bay. The family’s first crop was tobacco, followed by coffee.

Coffee’s importance to his family is economic. Before tourism grew, agriculture was the main industry in Hawai‘i. And back then, anyone who leased Bishop land was required to improve it with agriculture, Nakamura says. In subsequent decades, the Kona coffee brand helped build more farms.

Watching his father work so hard on the farm convinced Nakamura to become a pharmacist. He spent 29 years working at Kona Community Hospital before retiring. “I went to school to run away from coffee,” he says. But now that Nakamura’s father has died and his mother is in her 90s, the responsibility for tending the fields rests on him.

Granted, the acreage isn’t as much as it used to be. Nakamura’s father sold off most of the farm in the early 2000s, leaving 5.5 acres of Bishop Estate land under the family’s control. Two acres are planted with interspersed macadamia nut trees and coffee trees. A separate 1-acre plot is planted with only coffee.

When asked if he knew of Native Hawaiian cultural practices that involve coffee, Nakamura couldn’t think of any. His family’s own Japanese cultural practices consisted of pounding mochi with a rock his great-grandfather found in Waipio Valley and crafted into a mochi pounding bowl, as well as going to Obon dances. None of these activities have been affected by pests, but his farm certainly has been.

Nakamura knows firsthand what it’s like to battle coffee berry borer (CBB). It’s recommended that farmers spray *Beauveria bassiana*, the fungus that dessicates the beetles upon contact, every three weeks in his area. But the CBB population is high in nearby wild coffee stands and poorly-tended neighboring farms -- which means Nakamura must spray every two weeks to control the beetles on his own farm. He sees a difference between the CBB populations in his two fields: On the one acre planted only with coffee, the CBB infestation stays under 5%. But in

the field that is macadamia nut trees interspersed with coffee trees, the CBB infestation stubbornly stays at about 15%. If he didn't spray *B. bassiana* at all, the infection rate in both fields would shoot up to 70%-80%, he says.

In addition to CBB in his coffee trees, Nakamura has a pest problem on his macadamia nut farm. Beetles are boring into the trunks of his macadamia nut trees, which releases resin and allows a fungus to enter. So far, he says he has lost 50%-60% of his trees to this fungus. Last season, his farm's nut production decreased by 70%. Instead of replanting macadamia nut trees, Nakamura slowly is letting the coffee take over that section of the farm.

Based on these experiences, Nakamura says it's a good idea to protect agriculture from pests. He considers both sprays and insect biocontrols to be important in this goal. Subsidies for farmers -- such as the federal and state programs that reimbursed coffee farmers for *B. bassiana*, are the best way to motivate growers to use these pest-control methods. Critical to these programs' success, however, is ease of applying and record keeping. He personally found the federal reimbursement program for *B. bassiana* easier to use.

As for the parasitoid wasp *P. coffea*, Nakamura was heartened to hear that it didn't attack humans or animals. As long as *P. coffea* doesn't harm native species or populations of beneficial insects, "I have no reservations here," he says. One concern is whether he can maintain his schedule of spraying *B. bassiana* every two weeks without hurting the parasitoid wasp.

In general, Nakamura would like to see additional biocontrols similar to *P. coffea*. "Anytime you can get nature to work with you, it's better," he says.

Chuck Leslie, Third-generation Nāpōʻopoʻo fisherman

Charles "Chuck" Kealoha Leslie, 80, is one of the few remaining Native Hawaiian fishermen of *ʻopelu*, a type of mackerel. He grew up along Hawaiʻi Island's Kealakekua Bay, where his father started teaching him how to hand weave fishing nets at the age of five. Now, Leslie is the last in his lineage still fishing at Kealakekua Bay with traditional nets. The ones he makes are particularly good at catching larger volumes of fish. Leslie is training a younger generation in net weaving, before the art is lost.

Leslie's family also has a long history in Kona coffee. His great-grandfather, John Gaspar, built Hawaiʻi's first coffee mill in 1880. Leslie himself spent four seasons working in the Captain Cook Coffee Mill. He recalls days of hauling 1,600-1,800 heavy bags of fresh coffee cherries, loading them into a pulper to strip off their skins, then spreading their seeds to dry on covered platforms called *hoshidanas* or in mechanical dryers.

While growing up, Leslie harvested coffee with his siblings and parents. The farmers they worked for included Bryce Nakamura's father. (Nakamura also was interviewed for this Cultural

Impact Assessment.) Even though coffee was much cheaper when Leslie was a child compared to now, there was still money to be made, he says.

Like the other interviewees, Leslie doesn't recall any Native Hawaiian cultural traditions around coffee. Interestingly, peak *ʻopelu* fishing season coincides with peak ripeness of the coffee harvest in Kona -- a parallel that has remained true over the decades despite variations in harvest season from year to year, he says.

Leslie is familiar with coffee berry borer (CBB) and its damaging impact on Hawaiʻi's coffee industry. He believes in quickly protecting agriculture and ecosystems from invasive species. "If you know it's gonna be a pest, get rid of it as soon as possible," he says.

To Leslie, past introductions of non-native species offer cautionary tales. Roi, a type of grouper that was brought from French Polynesia to Hawaiʻi in the 1950s, has since spread to coral reefs throughout the State. In addition to eating native fish, roi can harbor the toxin that causes ciguatera fish poisoning. Leslie says a number of his friends have fallen ill with ciguatera -- sometimes from roi, and sometimes from other species. Like Kimokeo Kapahulehua (see interview above), Leslie points out that the yellow-skinned snapper called *taʻape* eats the eggs of native fish. It was introduced to Hawaiʻi by the Division of Fish and Game in the 1950s and 1960s. *Toʻau*, an invasive blacktail snapper, hurts coral reefs. And gorilla ogo, a seaweed that was introduced to Hawaiʻi with the aim of producing agar, spreads quickly and overruns fishponds.

Some of these fish and seaweed species were introduced in an uncontrolled manner. After seeing their effects on oceans surrounding Hawaiʻi, Leslie is glad that potentially beneficial species are far more rigorously tested than they were before. Regarding the USDA assessment for *P. coffea*, he wonders what would happen if the parasitoid wasp is successful in eliminating CBB in Hawaiʻi. Would *P. coffea* also eliminate the other three species that the USDA identified as parasite targets? Or are there other environmental factors that would prevent their elimination?

He stressed the importance of using biocontrols such as *P. coffea* instead of chemical sprays, provided the proper research is conducted. Says Leslie: "If the wasp can control the CBB, that's better."

Wally Young Sr., Kaʻu Coffee Farmer

Wally Young, 79, was born in the Kaʻu District's town of Waiohinu and has lived there almost his entire life. His father moved the family from Kona to Kaʻu to join the sugarcane industry. At the time, there were two separate sugar mills in Kaʻu: Hawaiian Agricultural Company in Pahala and Hutchinson Plantation in Naʻalehu.

Young is one of 10 brothers and sisters who learned Native Hawaiian traditions from their mother. Her flower garden produced blossoms for lei making. She also gathered leaves from hala trees to weave hats, mats and lauhala baskets for harvesting coffee. “In Kona, that’s how they made money,” he said.

Young’s family has an agricultural background. In the 1950s, his uncle ran the AC Young Farm in Kiola Kaʻa, about two miles away from Waiohinu. The farm grew vegetables such as taro, cabbage, tomatoes and cucumbers. Young’s father helped out, and also used some of the field to grow vegetables for his own family.

Cooking by Young’s mother upheld Native Hawaiian traditions. She pounded poi -- both from the taro her husband grew and *ʻulu* from a tree in their yard. They would gather kukui nuts to make *ʻinamona*, a condiment of pounded nuts, Hawaiian sea salt and chili pepper. Young’s parents also taught him how to cook kahlua pig in an imu, a skill he has passed onto his children.

Young remembers five Kaʻu coffee farmers in the 1940s and 1950s -- three in Waiohinu and two in Pahala. As a child, he used to pick for one farmer and would sometimes help in the wet mill. At the time, the Kaʻu farmers used to sell their coffee as cherry or parchment to Kona operations.

Native Hawaiians didn’t have traditional cultural uses for coffee or the coffee plant. Instead, the crop was important because it economically supported people, Young said. Japanese growers owned the early Kona coffee farms, and they hired the Native Hawaiians to harvest for them.

After serving in the Army for three winters in Germany, Young returned to Waiohinu and worked in construction. Eventually, the two sugar mills merged into a single company, called Kaʻu Agriculture. Then he worked in Kaʻu’s sugar industry for 33 years, a job that allowed him to purchase property in Waiohinu. “When they brought down the last load of the sugar cane, it was sad,” he said.

After the closure of Kaʻu Agriculture in 1996, the sugarcane plantations were transformed into coffee fields by the former sugar-mill employees. Young was one of the first to start his coffee farm in 1997, with five acres of land. With the launch of the Kaʻu coffee industry, Native Hawaiians, Portuguse and Filipinos started growing coffee in larger numbers, he said.

In the early years, making money from Kaʻu coffee was difficult, Young says. His farm initially was a side business, while his main income was as an auto mechanic. As sales of coffee improved, Young was able to sell his mechanic business to his son and expand his farm to 16 acres.

Coffee has positively contributed to Hawai‘i’s larger cultural history, Young says. It’s now one of the top agricultural industries in Hawai‘i. The biggest negatives are the arrival of CBB, and now coffee leaf rust.

Young believes in attacking pests as soon as they arrive in Hawai‘i. As a conventional farmer, he sees the benefits of both chemical and biocontrol methods for pests. “If it’s invasive, I think you should control it right away,” he said.

Young had no specific recommendations for testing the effectiveness of new pest controls. He trusts that the process is more stringent than in the 1950s, when the *ta‘ape*, an invasive fish, was released into Hawai‘i. “Now, they check ‘em out real good and make sure it doesn’t screw up the environment,” Young said.

He welcomes *P. coffea* as an additional tool in the fight against CBB. But he wonders about its resiliency against various sprays on the farm: the fungus *B. bassiana*, a biocontrol for CBB; anti-fungal copper sprays for coffee leaf rust; herbicides and pesticides. Just *B. bassiana* alone requires spraying every two weeks. At the time of this writing, one local vendor was selling a gallon of this fungus for $198.00, with a price increase expected soon. “It’s really expensive,” Young said.

He worries about killing *P. coffea*, but he can’t reduce spraying to accommodate the wasp -- unless *P. coffea* demonstrates a strong ability to kill the CBB on its own.

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Appendix A: Public Notice

Classified ads only $12.50 - Type or clearly write your ad of no more than 175 characters (including spaces and punctuation) and mail, along with a check for $12.50, to: ***Ka Wai Ola* Classifieds, Office of Hawaiian Affairs, 560 N. Nimitz Hwy., Suite 200, Honolulu, HI 96817**. Make check payable to OHA. (We cannot accept credit cards.) Ads and payment must be received by the 15th for the next month's edition of *Ka Wai Ola*. Send your information by mail, or e-mail **kwo@oha.org** with the subject "Makeke/Classified." OHA reserves the right to refuse any advertisement, for any reason, at our discretion.

GOT MEDICARE? With Medicare you have options. We compare those options for you! No Cost! No Obligations! Call Kamaka Jingao 808.286.0022, or visit www.kamakajingao.com. Hi Lic #433187

HAWAIIAN MEMORIAL PARK CEMETERY Kaneohe, Garden-Devotion. Lot #106, Section-D. Price $6,000 or B/O. Great Feng Shui plot located on a hill facing ocean. Contact #808-885-4501 landline or 808-345-7154 cell

HOMES WITH ALOHA - Hot Hot Market! Thinking of making a move? Relocating or life changes, Hawaiian Homes Lands, Fee Simple, Neighbor islands properties, we can help you through the process from beginning to end and into your replacement property. Contact the expert,Charmaine I. Quilit Poki(R) (RB-15998) Keller Williams Honolulu (RB-21303) (808) 295-4474.

HOMES WITH ALOHA - Kula/Maui 43,429 sq.ft. res lot with a 600 sq.ft. structure $390,000. This is a Leasehold property- Charmaine I. Quilit Poki(R) (RB-15998) Keller Williams Honolulu (RB-21303) (808) 295-4474.

HOMES WITH ALOHA - Waianae 3 bedroom, 2 bath, Great potential! $219,000 This is a Leasehold property- Charmaine I. Quilit Poki(R) (RB-15998) Keller Williams Honolulu (RB-21303) (808)295-4474.

KEOKEA-KULA, MAUI/DHHL HOME OWNERS! Are you looking to sell your 1,2,3 or more bedroom home in the near future? **LET'S TALK!** I'm approved for AG & Pastoral with DHHL on Maui. Please call Marcus Ku-760-310-5645, Mahalo!

NEED TO BUY OR SELL A HOME? Are you relocating, moving, or downsizing? I'm here to assist your real estate needs! Chansonette F. Koa (R) (808) 685-0070 w/ HomeSmart Island Homes LIC: #RB-22929 | LIC: #RB-22805 call, email, or checkout my online info at: www.chansonettekoa.com

THINKING OF BUYING OR SELLING A HOME? Call Charmaine I. Quilit Poki (R) 295- 4474 RB-15998. Keller Williams Honolulu RB-21303. To view current listings, go to my website HomeswithAloha.com. Call or email me at Charmaine.QuilitPoki@kw.com to learn more about homeownership. Mahalo nui! Specialize in Fee Simple & Homestead Properties for over 30 years.

VALLEY OF THE TEMPLES MEMORIAL PARK. Kaneohe, Oahu. Memory Slope Map 1, Lot 114, Site 4. Includes concrete urn and bronze marker. Valued at $10,500, selling at $9,500. Text or call (808) 987-9201. ■

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HOʻOLAHA LEHULEHU
PUBLIC NOTICE

CULTURAL IMPACT ASSESMENT: INVASIVE COFFEE BERRY BORER BEETLE

At the request of the University of Hawaii, the Synergistic Hawaii Agriculture Council is preparing a Cultural Impact Assessment for the statewide release of a wasp (Phymastichus coffeea) to control the invasive Coffee Berry Borer beetle. The wasp is harmless to humans. Please contact Suzanne Shriner at 808-365-9041 or suzanne@shachawaii.org to share your mana'o about any cultural or historical resources relating to the lands now in use for coffee growing or any other information you feel is relevant. This could include mo'olelo, history, or knowledge of traditional and customary practices (both past and present). Letters can be sent to 190 Keawe St, Suite 25, Hilo, 96720. ■

Response to HDoA PPC comments provided in email on 8 February 2022:

COMMENT:

Needs supplemental studies to safeguard several biological control agents established in Hawaii for controlling invasive weed populations around the State, all of them in the same size range as the target pest CBB. None of them are tested, some are of African origin as the target pest and the proposed parasitoid.

- Acythopeus coccinea [correct spelling: *cocciniae*] O'Brean & Pakaluk biocontrol agent of ivy gourd, Coccinia grandis, native to Kenya
- Microlarinus lareynii (Jacquelin du Val) biocontrol agent of Puncturevine, Tribulus terrestris.
- Microlarinus lypriformis (Wollaston), biocontrol agent of Puncturevine, Tribulus terrestris.
- Apion scutellare Kirby, biocontrol agent of Gorse, Ulex europaeus.
- Exaprion ulicis (Forster) biocontrol agent of Gorse seeds, Ulex europaeus.
- Perapion antiquum (Gyllenhal), biocontrol agent of devil's-thorn, Emex spinosa, native to Africa.
- Perapion violaceum (Kirby), similar in size, no vouchers at HDOA

RESPONSE: The non-target host range testing (Yousuf et al. 2021) included a diversity of Curculionidae from the Subfamilies Anthribinae, Brentinae, Patypodinae, Curculioninae, Cossoninae, and Scolytinae. Emphasis was placed on native endemic species in the Scolytinae. This represents a broad sample of taxa from the family Curculionidae, see attached phylogeny from Shin et al. (2018).

The Subfamily designation of the species listed in the comment above is as follows:

Acythopeus cocciniae: Baridinae = Conoredinae
Microlarinus lareynii: : Lixinae = Molytinae
Microlarinus lypriformis: Lixinae = Molytinae
Apion scutellare: Apioninae
Exaprion ulicis: Apioninae
Perapion antiquum: Apioninae
Perapion violaceum: Apioninae

The phylogenetic placement of these subfamilies (and other Curculionidae), including estimates of the time since their shared ancestors existed, is shown in the attached annotated figure 1 from Shin et al. (2018). Subfamilies included in the comment above are underlined in red.

Annotated on the Shin et al. (2018) Fig. 1 are also the Subfamilies included in the non-target screening reported by Yousuf et al. (2021) and presented in the DEA, with TNP indicating tested never parasitized. The phylogenetic relationships among the only non-target hosts parasitized in the non-target screening study (all non-native *Hypothenemus* spp., phylogeny from Johnson et al. 2018), are shown to the right of the appended Curculionidae phylogeny, indicated by the green arrow. This shows that even among closely related Scolytinae in the same genus, *Hypothenemus*, *Phymastichus coffea* was unable to parasitize the species most phylogenetically distant (*H. eruditus*) from CBB. No other Scolytinae were parasitized, including species from the exotic genera *Xylosandrus*, *Xyloborinus*, *Euwallacea*, *Hypochryphalus*, *Chryphalus*, and *Ptilopodius*,

and the native genus *Xyloborus* (Yousuf et al. 2021), showing distinct lack of ability of the parasitoid to utilize even relatively closely related beetles, with relatively recent common ancestors.

Other beetles tested within the Cossininae and Curculioninae (closest relatives to Scolytinae on the Shin et al. 2018 phylogeny), were not parasitized by *P. coffea*.

The phylogenetically closest species (subfamilies Conoderinae and Molytinae) listed above in the comments from HDoA all fall into the clade that shared a common ancestor with Scolytinae approximately 75 million years ago. This is a long time in evolutionary terms. The Apioniae and Scoytinae on the HDoA list shared a common ancestor approximately 150 million years ago.

The "centrifugal phylogenetic testing" method, proposed by Wapshere (1974) for weed biological control agent non-target host screening, and expanded to insect species by Kuhlmann et al. (2006), provides a means of selecting appropriate non-target species. Species more closely related to the target may be within a prospective biological control agents host range, and those distantly related will be less susceptible or not susceptible to parasitism by host-specific agents. The results we present show that *P. coffea* is able to attack and complete development on a small subset of very closely related species in the genus *Hypothenemus*, but even relatively closely related species in the same genus are not suitable hosts (see inset on Shin et al. (2018), from Yousuf et al. (2021)).

Also of interest is the fact that *Acythopeus cocciniae* is native to Kenya, as highlighted in the above comments from HDoA, the country of origin of *P. coffea*. There are no records of parasitism of this beetle by the wasp in Kenya.

The proposed list of additional species to consider all belong to taxa distantly related to the target insect and therefore highly unlikely to be parasitized by *P. coffea*. We therefore suggest that such tests are not required.

Additionally, host plant volatiles can be important in host location by parasitoids. It should also be considered the *P. coffea* responds to cues associated with the host plant of *H. hampei* (Rojas et al. 2006), and this interaction is very likely to ensure high host fidelity under field conditions.

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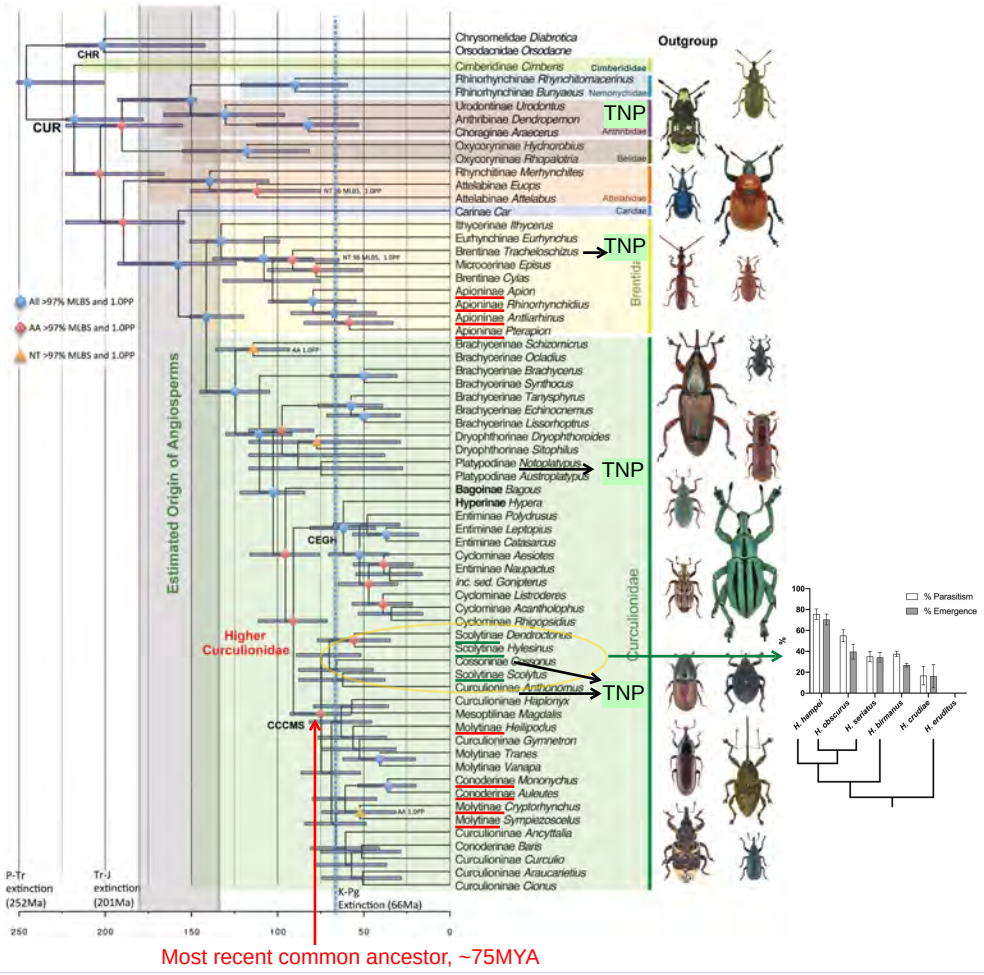
Fig. 1. Chronogram for weevils (superfamily Curculionoidea) showing RelTime estimates of lineage divergence times.



Mol Biol Evol, Volume 35, Issue 4, April 2018, Pages 823–836, https://doi.org/10.1093/molbev/msx324

Other comments are:
Throughout this Draft Environmental Assessment, authors argued that Phymastichus coffea is one of the most promising agents of CBB, citing conflicting information on its status in Latin America (i.e., the degree of parasitism by P. coffea was more than 95%).

- Authors did not make it clear that this result came from sleeve experiment and cage tests where branches of coffee plant with non-infested fruits with entomological sleeve placed on the branch, then branch was infested with CBB adults and exposed to the parasitoids.

RESPONSE: In Colombia both cage and field studies have been conducted with *Phymastichus coffea*. In a recent field study carried out by Cenicafe, early season release of the larval-pupal parasitoid *Proprops nasuta* followed by multiple releases of *P. coffea* during CBB flights resulted in a 70-83% reduction in CBB populations compared to no-release fields, demonstrating the potential for augmentative biological control using this parasitoid. Also, *P. coffea* parasitism of CBB was estimated at 15% at 4 months after the last release, suggesting it has the ability to persist when CBB are available.

- Field parasitism in Mexico was reported shortly after a mass release. Further surveys in Mexico and other countries after the release showed no permanent establishment of parasitoids.

RESPONSE: In Colombia and Mexico, coffee berries are only available part of the year because of clear-picking during harvest and wide area stumping after harvest. In Hawaii, some coffee growing areas e.g. Kau, Kona, have infested coffee berries on the tree year-round which may facilitate establishment and persistence of *P. coffea.*

COMMENT: Aside from its susceptibility to the fungus, Beauveria bassiana, life cycle of P. coffea is too long for a good biocontrol agent, varies from 30-47 days. Adult female parasitoid lives for 2–3 days only and does not enter the fruit searching for CBB for parasitism as in Bethylid and Braconid parasitoids. There is a very short window of opportunity of 8 hours (time of CBB adult to enter the fruit and escape parasitism) for female P. coffea to encounter the host CBB.

RESPONSE: Thanks for your opinions. *P. coffea* is clearly well adapted to survive on its sole known natural host in the wild in its native Africa. It is possible that asynchronous/overlapping generations will occur, increasing likelihood of adult wasps encountering hosts in variable windows of opportunity. The window of opportunity for *P. coffea* parasitism is often much longer than 8 hours, depending on the age of the fruit: during times of adult CBB activity, beetles may wait in the exposed 'A' position for days or even weeks in young berries. Also, we have observed *P. coffea* entering the fruit searching for CBB in the laboratory. How effective *P. coffea* will be in Hawaii is hard to predict. Cenicafe in Colombia first released *P. coffea* 30 years ago and although it did not become established, *P. coffea* is still the main focus of their CBB biocontrol research program.

With regard to the statement that *Beauveria bassiana* poses risk of impact to *P. coffea*: Like integrating biocontrol with pesticides, one would time the *B. bassiana* applications with care, or hopefully avoid them to a large extent. That would be a benefit of biocontrol with a parasitoid. If the wasp is effective, farmers can reduce their dependance on *B. bassiana*, potentially quite significantly. This would result in considerable economic benefits to growers.

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ORIGINAL PAPER

Limited host range in the idiobiont parasitoid *Phymastichus coffea*, a prospective biological control agent of the coffee pest *Hypothenemus hampei* in Hawaii

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Abstract
Phymastichus coffea LaSalle (Hymenoptera:Eulophidae) is an adult endoparasitoid of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera:Curculionidae:Scolytinae), which has been introduced in many coffee producing countries as a biological control agent. To determine the effectiveness of *P. coffea* against *H. hampei* and environmental safety for release in Hawaii, we investigated the host selection and parasitism response of adult females to 43 different species of Coleoptera, including 23 Scolytinae (six *Hypothenemus* species and 17 others), and four additional Curculionidae. Non-target testing included Hawaiian endemic, exotic and beneficial coleopteran species. Using a no-choice laboratory bioassay, we demonstrated that *P. coffea* was only able to parasitize the target host *H. hampei* and four other adventive species of *Hypothenemus*: *H. obscurus, H. seriatus, H. birmanus* and *H. crudiae. Hypothenemus hampei* had the highest parasitism rate and shortest parasitoid development time of the five parasitized *Hypothenemus* spp. Parasitism and parasitoid emergence decreased with decreasing phylogenetic relatedness of the *Hypothenemus* spp. to *H. hampei*, and the most distantly related species, *H. eruditus*, was not parasitized. These results suggest that the risk of harmful non-target impacts is low because there are no native species of *Hypothenemus* in Hawaii, and *P. coffea* could be safely introduced for classical biological control of *H. hampei* in Hawaii.

Keywords Coffee berry borer · Host specificity testing · Non-target · Biocontrol · Endoparasitoid · Scolytinae

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Key message

- *Phymastichus coffea* is an idiobiont adult parasitoid of the coffee pest *Hypothenemus hampei*.
- In host range testing, *P. coffea* parasitized only five *Hypothenemus* spp.
- The parasitism rate was highest and parasitoid development time was shortest in *H. hampei.*
- No Hawaiian native species was parasitized by the parasitoid.
- *Phymasticus coffea* can be introduced safely for biocontrol of coffee berry borer in Hawaii.

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Introduction

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera:Curculionidae:Scolytinae), native to Central Africa, is the most damaging insect pest of coffee worldwide, inflicting economical losses of over US $500 million dollars annually (Vega et al. 2015). In Hawaii, *H. hampei* was first recorded in Kona, Hawaii island, in 2010 (Burbano et al. 2011) and is now widespread throughout all the coffee-growing areas of Hawaii. Coffee is the third largest cash crop in the state of Hawaii, valued at more than $43 million (USDA-NASS 2018). *Hypothenemus hampei* has had the effect of making coffee farming more intensive and less profitable, which is a major economic challenge to small-scale coffee production like that in Hawaii (Johnson et al. 2020). If left unmanaged, *H. hampei* can damage > 90% of the crop.

Hypothenemus hampei attacks coffee berries when the dry matter content of the endosperm, which increases with age, exceeds 20% (Jaramillo et al. 2005). After finding a suitable berry host, *H. hampei* bores into the coffee fruit through the central disk and excavates galleries where it lays eggs. The offspring develop inside the seeds and feed on the endosperm tissue of the berries (Damon 2000), reducing both coffee yield and quality. *Hypothenemus hampei* feeding damage can also cause premature fall of berries younger than 80 days (Decazy 1990). *Hypothenemus hampei* adults boring into the berry may remain in the 'A' position (Jaramillo et al. 2006) with the abdomen half exposed outside the berry potentially for weeks waiting for the dry matter content to reach 20% (Jaramillo et al. 2005).

Strategies to control *H. hampei* include mechanical, chemical and biological controls (Infante 2018). Sanitation and biological control (using parasitoids, predators and entomopathogenic microorganisms) are the most sustainable, environmentally friendly and widely used non-chemical control methods. The parasitoids, *Cephalonomia stephanoderis* Betrem, *C. hyalinipennis* Ashmead and *Prorops nasuta* Waterston (Hymenoptera:Bethylidae), *Heterospilus coffeicola* Schneideknecht (Hymenoptera:Braconidae) and *Phymastichus coffea* LaSalle (Hymenoptera:Eulophidae), all of African origin, have been introduced in many coffee producing countries, particularly in Central and South America (Klein-Koch et al. 1988; Barrera et al. 1990; Baker 1999; Jaramillo et al. 2005; Portilla and Grodowitz 2018), but none have been released in Hawaii. In Hawaii, the primary methods for controlling *H. hampei* are sanitation (frequent harvests and removal of all left over coffee berries after harvest) and applications of the biopesticide *Beauveria bassiana* (Ascomicota:Hypocreales), an entomopathogenic fungus (Aristizábal et al. 2016). Two generalist predators, *Leptophloeus* sp. and *Cathartus quadricollis* (Coleoptera:Laemophloeidae and Silvanidae, respectively), occur naturally in Hawaii coffee and have been shown to feed on immature stages of *H. hampei* in overripe and dried berries (Follett et al. 2016; Brill et al. 2020), but are not very efficient in preventing damage in the first place.

Most of the studies on biological control of *H. hampei* have been conducted outside Hawaii, but in similar coffee production systems. In field-cage studies conducted in Mexico and Costa Rica, *P. coffea* proved to be the most promising biological control agent against *H. hampei* with parasitism rates as high as 95% (Espinoza et al. 2009; Infante et al. 2013). To date, *P. coffea* has been released in 12 countries as a classical biological control agent (Bustillo et al. 1998; Damon 2000; Jaramillo et al. 2005; Vega et al. 2015). *Phymastichus coffea* is native to Africa and present in most coffee producing countries on that continent. It is a primary, gregarious, idiobiont endoparasitoid of adult *H. hampei* females with a high capacity for host discrimination (Feldhege 1992; Infante et al. 1994; López-Vaamonde and Moore 1998; Castillo et al. 2004). Two laboratory studies reported that in addition to *H. hampei*, *P. coffea* parasitizes other *Hypothenemus* spp. such as *H. seriatus* and *H. obscurus* (López-Vaamonde and Moore 1998), and *H. eruditus* Westwood and *H. crudiae* (Panzer) (Castillo et al. 2004). However, parasitism of closely related species in the field has not been reported (Escobar-Ramírez et al. 2019). Gravid *P. coffea* females start to search for their hosts immediately after emerging from the adult female host and parasitism occurs within the first hours after emergence (Infante et al. 1994). *Phymasticus coffea* has an extremely short life span as an adult; the longevity of males ranges from 8 to 48 h and females from 16 to 72 h (Vergara et al. 2001; Portilla and Grodowitz 2018). *Phymastichus coffea* generally lays two eggs (into the abdomen, thorax, or between the thorax and abdomen) in an *H. hampei* adult female at the time she is initiating fruit perforation, which causes paralysis and prevents further damage to the coffee berry. The parasitized *H. hampei* usually dies within 4–12 days after parasitism (Infante et al. 1994). The life cycle (egg to adult) of *P. coffea* varies from 30 to 47 days depending on the environmental conditions (temperature and humidity). Females are ~ 1 mm long, whereas males are half that size (LaSalle 1990).

Earlier studies have shown the high host specificity of *P. coffea* and its ability to significantly reduce and regulate *H. hampei* populations (Gutierrez et al. 1998; López-Vaamonde and Moore 1998; Castillo et al. 2004; Rodríguez et al. 2017). Therefore, we decided to consider *P. coffea* as a biological control agent of *H. hampei* in Hawaii. A critical step was to determine its host specificity and assess possible risks to the Hawaii environment though impacts on endemic and other non-target species (Follett and Duan 1999; Messing and Wright 2006). Greatest non-target species impacts from

introduced biological control agents are likely to occur on species closely related to the target pest species (Van Driesche and Murray 2004), but not always (Messing 2001), and thus, phylogenetically closely and distantly related species should be included in non-target screening efforts. This is an important element of biological control, particularly in Hawaii, where classical biological control may have had significant negative impacts on native species in the past (e.g., Howarth 1991; Henneman and Memmott 2001). While some studies have suggested that this is true (see references in Messing and Wright 2006), a number of carefully crafted field studies of population level impacts on non-target species have suggested that introduced parasitoids have had minimal, or sometimes moderate, impacts on endemic species (Johnson et al. 2005; Kaufman and Wright 2009). Where higher impacts have been detected, they are typically from accidentally introduced parasitoid species, and host insects in disturbed habitats are most susceptible to these impacts (Kaufman and Wright 2011). However, the potential for non-target impacts must be carefully considered, and outcomes of exposures of unintended hosts to prospective biological control agents can provide insights into host range patterns and determinants.

In this paper, we present new insights into the host specificity of *P. coffea*, a prospective biological control agent of *H. hampei* in Hawaii, by testing it against 43 different species of Coleoptera. Non-target testing included Hawaiian endemic, exotic and beneficial coleopteran species. There are currently no records of native Hawaiian *Hypothenemus* spp. except for an old record (1913) of *H. ruficeps* (Swezey 1954), which has never been collected or reported since and is possibly a synonym with the adventive species *H. eruditus* or *H. crudiae* (C. Gillett, unpublished). There are, however, many native species in another scolytine genus, *Xyleborus* (Samuelson 1981; Gillett et al. 2019), which may potentially be impacted by release of an exotic parasitoid against a scolytine pest such as *H. hampei*. We test the hypothesis that *P. coffea* is host specific and will not attack native Hawaiian Scolytinae species.

Materials and methods

Parasitoid, Phymastichus coffea

Phymastichus coffea used in this study were obtained from an established stock maintained at the National Coffee Research Center-Cenicafé, Manizales (Caldas) Colombia, which was started from *P. coffea* collected in Kenya and shipped to Colombia in 1996 and has been maintained in colony in large numbers since that time (Orozco-Hoyas and Aristizábal 1996). *Phymastichus coffea* has been mass reared by Cenicafé for field releases on multiple occasions and the colony receives frequent infusions of field-collected material. *Phymastichus coffea* was shipped from Cenicafé in its larval stage in parasitized *H. hampei* hosts under USDA APHIS PPQ, permit no. P526P-18-00,696 to a certified quarantine insect containment facility managed by the USDA Forest Service at Hawaii Volcanoes National Park, Volcano, Hawaii. Parasitized *H. hampei* were incubated in controlled climate chambers at 25° ± 1 °C, 75 ± 10% relative humidity and 8:16 h light:dark photocycle at the quarantine containment facility.

Emerged male and female parasitoid adults were collected using a manual aspirator into a clean glass container. Parasitoids were held for mating and oocyte maturation and provided with 50% (w/v) honey (raw organic) solution for ~ 2 h before being used in the experiments (López-Vaamonde and Moore 1998). Infante et al. (1994) reported that *P. coffea* does not go through a preoviposition period and exhibits facultative arrhenotokous-type parthenogenesis, where the female parasitizes its host before or after copulation, producing haploid males (Portilla and Grodowitz 2018). Feldhege (1992) reported a preoviposition period of between 5 min and 4 h. The adult parasitoids are very short-lived: males (~ 8–48 h) and females (~ 16–72 h) (Vergara et al. 2001; Rojas et al. 2006; Espinoza et al. 2009; Portilla and Grodowitz 2018). The ability to parasitize hosts decreases with age, so it was important to use freshly emerged parasitoids (< 12 h old) in all experiments.

Coffee berry borer, Hypothenemus hampei

Field-collected *H. hampei* were used in all no-choice host specificity experiments. *Hypothenemus hampei*-infested coffee berries were collected from coffee trees (*Coffea arabica*) at OK Coffee Farm in Hilo, Hawaii (19.727583, − 155.111186, elevation 156 m). These collections were transported in cold boxes to the USDA-ARS laboratory and placed in a custom-made extraction unit lined with tissue paper (Tech wipes 1709/7052, Horizon) to absorb condensation and prevent mold growth. Adult *H. hampei* were collected directly from the infested coffee berries by dissecting the berries or from the extraction unit using an aspirator. All the collected *H. hampei* were provided with artificial diet (modified from Brun et al. 1993) until use in the experiments.

Collection of non-target coleopteran species

The selection of non-target hosts was based on phylogenetic relatedness to the target host, sympatry of target and non-target species, and size. Species commonly occurring in the coffee landscape and species in culture at USDA-ARS in Hilo, Hawaii, were also tested. There are 21 native and 38 non-native scolytine species in Hawaii (Samuelson 1981;

Nishida 2002; Cognato and Rubinoff 2008). Because of the relatively large native scolytine fauna in Hawaii, and their remote or poorly studied habitats, only a subset of these species could be tested for their suitability as hosts to *P. coffea*. Exotic and native scolytine species were collected from coffee and macadamia farms and their surrounding habitats, and from native forests from different islands (Hawaii Island, Oahu, Maui, Molokai and Kauai) in Hawaii (Gillett et al. 2020a). Host specificity tests were conducted with a total of 43 species from seven different coleopteran families including Hawaiian endemic species (several Scolytinae in the genus *Xyleborus* and *Nesotocus giffardi,* a curculionid weevil), exotic pest species (e.g., the scolytines *Hypothenemus obscurus* [tropical nut borer] and *Xylosandrus compactus* [black twig borer], and the curculionids *Sitophilus oryzae* [rice weevil] and *Cylas formicarius* [sweetpotato weevil]), and beneficial species (e.g., a weed biocontrol agent *Uroplata girardi* from lantana, several coccinellids, and two flat bark beetle predators of *H. hampei*, *Catharus quadricollis* and *Leptophloeus* sp.) (Tables 1, 2, 3, 4). All beetles used in host specificity tests were collected live and later preserved in 75% alcohol or pinned for identification by taxonomists with expertise in the respective taxa. The body size of the collected species ranged from 1 to 7 mm, but the majority of species were similar in size to *H. hampei* which is 1.5–2.0 mm in length. Beetles were collected using Lindgren funnels or bucket or Broca traps baited with denatured ethanol only or ethanol + methanol + ethylene glycol lures or collected directly from infested plant material (fruits, pods, stems, bark and seeds) or reared from infested wood in the laboratory (Gillett et al. 2020b). All non-target testing was conducted at the USDA Forest Service quarantine containment facility at Hawaii Volcanoes National Park, Volcano, Hawaii.

Table 1 Development time and sex ratio of *Phymasticus coffea* in no-choice in vitro non-target host selection screening of *Hypothenemus* species, including *H. hampei* as a control species

Species	Insect status	Total beetles exposed	Development time (days ± SE)	Sex ratio (mean % females ± SE)
Hypothenemus hampei (control)	Exotic/pest	170	32.2 ± 0.5	50.8 ± 0.4
Hypothenemus obscurus	Exotic/pest	80	35.0 ± 0.9	54.8 ± 1.6*
Hypothenemus seriatus	Exotic	60	38.0 ± 1.0	51.1 ± 1.1
Hypothenemus birmanus	Exotic	40	37.0 ± 1.0	57.7 ± 3.8*
Hypothenemus crudiae	Exotic	30	41.0 ± 0.0*	50.0
Hypothenemus eruditus	Exotic	80	–	–

*significantly different from *Hypothenemus hampei* (control), $p < 0.05$

Table 2 Parasitism and parasitoid emergence rates in no-choice in vitro non-target host acceptance screening of *Phymastichus coffea* exposed to various Scolytinae (Hawaii native and non-native) species

Family	Species	Insect status	Total beetles exposed	Parasitism (%) (Mean ± SE)	Parasitoid emergence (%) (Mean ± SE)
Curculionidae:Scolytinae	*Xylosandrus compactus*	Exotic/pest	80	0	0
	Xylosandrus crassiusculus	Exotic	80	0	0
	Xyleborinus saxeseni	Exotic	80	0	0
	Xyleborinus andrewesi	Exotic	60	0	0
	Xyleborus ferrugineus	Exotic	60	0	0
	Euwallacea fornicatus	Exotic	60	0	0
	Euwallacea interjectus	Exotic	60	0	0
	Hypochryphalus sp.	Exotic	60	0	0
	Chryphalus sp.	Exotic	80	0	0
	Ptilopodius pacificus	Exotic	80	0	0
	Xyleborus molokaiensis	Native	30	0	0
	Xyleborus mauiensis	Native	15	0	0
	Xyleborus simillimus	Native	18	0	0
	Xyleborus hawaiiensis	Native	9	0	0
	Xyleborus lanaiensis	Native	19	0	0
	Xyleborus obliquus	Native	3	0	0
	Xyleborus kauaiensis	Native	35	0	0

Table 3 Parasitism and parasitoid emergence rates in no-choice in vitro non-target host acceptance screening of *Phymastichus coffea* on beneficial Coleoptera species

Family	Species	Insect status	Total beetles exposed	Parasitism (%)	Parasitoid emergence (%)
Chrysomelidae:Cassidinae	*Uroplata girardi*	Exotic	60	0	0
Coccinellidae	*Scymnodes lividigaster*	Exotic	40	0	0
Coccinellidae	*Rhyzobius forestieri*	Exotic	60	0	0
Coccinellidae	*Halmus chalybeus*	Exotic	40	0	0
Laemophloeidae	*Leptophloeus* sp.	Unknown	60	0	0
Silvanidae	*Cathartus quadricollis*	Exotic	80	0	0

Table 4 Parasitism and parasitoid emergence rates in no-choice in vitro non-target host acceptance screening of *Phymastichus coffea* on Hawaiian native and introduced coleopteran species from families and subfamilies other than Curculionidae:Scolytinae

Family	Species	Insect status	Total beetles exposed	Parasitism (%)	Parasitoid emergence (%)
Anthribidae	*Araecerus simulatus* or *A. levipennis*	Unknown	6	0	0
Anthribidae	*Araecerus* sp. near *varians*	Unknown	15	0	0
Brentidae:Brentinae	*Cylas formicarius*	Exotic/Pest	80	0	0
Chrysomelidae:Bruchinae	*Acanthoscelides macrophthalmus*	Unknown	10	0	0
Curculionidae:Cossoninae	*Phloeophagosoma tenuis*	Unknown	8	0	0
Curculionidae:Cossoninae	*Nesotocus giffardi*	Native	12	0	0
Curculionidae:Curculioninae	*Sigastus* sp.	Exotic/Pest	6	0	0
Curculionidae:Platypodinae	*Crossotarsus externedentatus*	Exotic	60	0	0
Dryophthoridae:Dryophthorinae	*Sitophilus oryzae*	Exotic/Pest	60	0	0
Dryophthoridae:Dryophthorinae	*Sitophilus linearis*	Exotic	40	0	0
Nitidulidae:Carpophilinae	*Carpophilus dimidiatus*	Exotic	10	0	0
Nitidulidae:Carpophilinae	*Carpophilus zeaphilus*	Exotic	60	0	0
Tenebrionidae	*Tribolium castaneum*	Exotic/Pest	21	0	0
Tenebrionidae	*Hypophloeus maehleri*	Exotic	60	0	0

No-choice tests

In this study, we used no-choice tests because these would reflect physiological host range and the potential for parasitism in the field more accurately than choice tests (Van Driesche and Murray 2004). Choice tests that include the target host may mask the acceptability of lower ranked hosts, thereby producing false negative results (Withers and Mansfield 2005). Twenty individuals of each test species were placed in a sterilized glass Petri dish (80 mm in diameter) lined with filter paper and immediately afterward four *P. coffea* females (< 12 h old) that had not been exposed to adult hosts prior to the experiments were introduced. Therefore, when ample hosts were available, each replicate consisted of 20 hosts and four parasitoids for a 5:1 host–parasitoid ratio. However, due to difficulties in finding certain species live in adequate numbers, e.g., native scolytine bark beetles, and difficulties synchronizing parasitoid emergence with field collection or emergence from wood of live beetles, the host–parasitoid ratio and numbers of replicates were adjusted as needed. For example, if only 10 non-target beetles were available for screening, then two replicates each with 5 beetles and 1 parasitoid (maintaining the 5:1 host–parasitoid ratio) were performed. In all non-target host screening tests, *H. hampei* was included as a positive control to confirm parasitoid viability. The host–parasitoid ratio of the *H. hampei* controls was adjusted to match the non-target species in the test, whether it was 5:1 or otherwise. The generalized response of the parasitoids toward target and non-target hosts was also determined for a subset of parasitoids by visual observation and video recording of parasitoid behavior, e.g., any contact with the host by landing on the host or antennation, and/or walking on the host. Host acceptance was noted when the parasitoid adopted a characteristic oviposition position on top the elytra of the host (Lopez-Vaamonde and Moore 1998).

After *P. coffea* exposure, *H. hampei* and all other non-target species were incubated at 25 ± 1 °C, 75 ± 10% RH and 24:0 (L–D) photoperiod for 72 h. After 72 h, parasitoids and filter paper linings were removed and the beetles were

provided with a small cube (2 × 2 × 2 cm) of general beetle diet (FY, unpublished). The beetles were again incubated at the same environmental conditions, but now at 0:24 (L–D). After 10 days, all the remaining diet and frass was removed (without disturbing the parasitized beetles) to avoid fungal contamination. Parasitized beetles typically become paralyzed and eventually die within 4–12 days after parasitoid oviposition. Beetles were held for a total of ~ 5–6 weeks for parasitoid emergence. Beginning after 25-day incubation, *H. hampei* mummies were inspected daily for adult wasp emergence. Parasitism was assessed based on observation of emergence of parasitoid progeny (F1 adult wasps) from the parasitized beetle, by inspection for exit holes on cadavers or by dissection. Beetles with no exit holes were dissected (by separating the thorax from the abdomen) under a stereomicroscope using fine forceps and entomological pins at 20-100X magnification for evidence of parasitism, i.e., presence of *P. coffea* immature life stages (eggs, larvae or pupae), or unemerged adults. The number of unemerged life stages was recorded for each dissected beetle. After 5–6 weeks of incubation, dead beetle specimens sometimes became very dry and searching for the presence of eggs and early instar larvae was difficult. In such cases, beetles were dissected and examined under a compound microscope at 200X to seek unemerged *P. coffea*. The sex of emerged adult *P. coffea* offspring was determined by examination using a stereomicroscope. In most cases, two parasitoids (one male and one female) emerged per beetle host. To confirm this, the sum of the emerged male and female parasitoids in each replicate was divided by two and compared to the number of parasitized hosts with exit holes. The sex of unemerged parasitoids was not determined. For data on parasitism, life stages, sex ratio and development time, averages were calculated for each replicate (per Petri dish) for each species and used in statistical analysis. Grand means of all the replicates for each of the five *Hypothenemus* species are presented in figures and tables.

Statistical analysis

Parasitism rate was calculated by dividing the number of parasitized hosts by the total number of hosts exposed to the parasitoids. Parasitism included both emerged and unemerged wasps. Emergence rate was calculated by dividing the number of beetles with exit holes by the total number of parasitized hosts (emerged plus unemerged wasps). The sex ratio of the parasitoid progeny was calculated by dividing the number of emerged female parasitoids (F) by the total number of emerged male (M) and female (F) parasitoids [F/(F + M) × 100]. The Shapiro–Wilk test (Shapiro and Wilk 1965; Razali and Wah 2011), numerical approaches (skewness and kurtosis indices) and the normal Q–Q plot-based graphical method were used to check the distribution of the data and showed that the data were not normally distributed. Generalized linear models (GLM) were therefore used to analyze the data, with appropriate distribution function links. Parasitism and emergence rates of the parasitoids, and the percentage of different life stages (larvae, pupae and adults) in parasitized beetles with unemerged parasitoids were analyzed using GLM with a binary logistic function and sex ratio with a gamma log link function. Developmental time of the F1 offspring (egg to adult) was analyzed using GLM with a negative binomial log link function because data were overdispersed (i.e., variance > mean). Wald Chi-squared approximations are reported. All analyses were performed using IBM SPSS statistics software.

Results

Out of 43 total coleopteran species tested, including 23 scolytines, *P. coffea* oviposited and completed developed only in the target *Hypothenemus hampei* and four other species of *Hypothenemus*: *H. obscurus*, *H. seriatus, H. birmanus* and *H. crudiae*. Mean percentages of parasitism and emergence for the *Hypothenemus* spp. tested are shown in Fig. 1. Parasitism ($\chi^2 = 65.13$, df = 4, $p = 0.0001$) and emergence ($\chi^2 = 23.20$, df = 4, $p = 0.0001$) were significantly higher in *H. hampei* than all other *Hypothenemus*

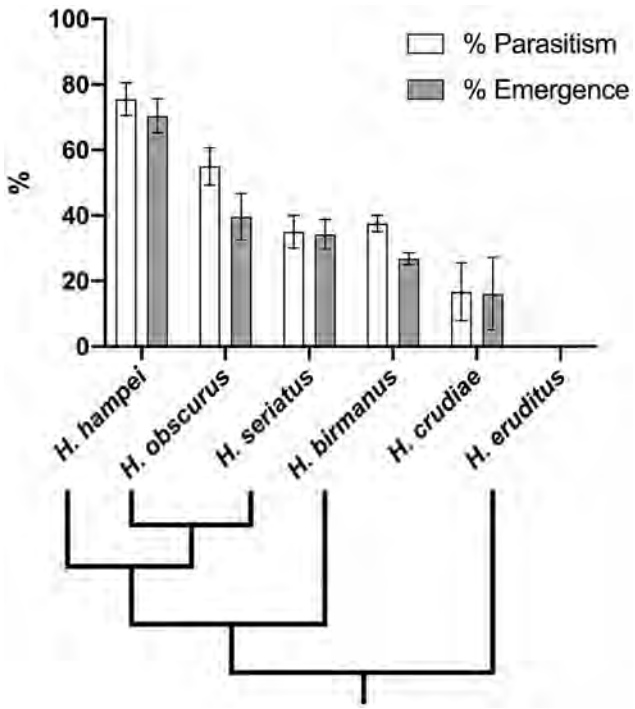

Fig. 1 Percentage parasitism and emergence (mean ± SE) of adult *Phymastichus coffea* parasitoids from *Hypothenemus* spp. The phylogeny below the graph for the species included in the study (except *H. crudiae*) was inferred from Johnson et al. (2018)

species. *Hypothenemus hampei* had the highest percentage emergence of *P. coffea* at 70.4%, whereas *H. crudiae* had the lowest at 16.7% (Fig. 1). In *H. crudiae*, out of five parasitized hosts only one had emergence. Although *P. coffea* only parasitized *Hypothenemus* spp., it did inspect three other non-target scolytine hosts, *Hypothenemus eruditus*, *Xyleborus kauaiensis* and *Xyleborus ferrugineus,* but left hosts without initiating oviposition (i.e., no parasitism found). The phylogenetic relationship of five *Hypothenemus* species included in our tests, extracted from Johnson et al. (2018), is also shown in Fig. 1; *H. crudiae* is not included in the phylogeny because it was not included in Johnson et al (2018). Both parasitism and emergence in our tests decreased across *Hypothenemus* species with decreasing phylogenetic relatedness to *H. hampei*. *Hypothenemus eruditus*, the most distantly related species from *H. hampei* according to Johnson et al. (2018), was not parasitized (Fig. 1).

Parasitoid development time among the three different *Hypothenemus* spp. did not differ significantly compared with *H. hampei* ($\chi^2 = 0.17$, df = 4, $p = 0.997$), but did differ with *H. crudiae* (Table 1). The mean development time of *P. coffea* from oviposition to adult emergence was shortest in *H. hampei* (32.2 ± 0.5 days, mean ± SE), longest in *H. crudiae* (41.0 ± 0.0 days) and intermediate in the other three *Hypothenemus* spp. (Table 1), which generally agrees with the phylogenetic pattern observed for parasitism and emergence (Fig. 1). The percentage of female versus male *P. coffea* emerging from parasitized *H. hampei* was 50.8% ± 0.4 (mean ± SE), which was significantly different ($\chi^2 = 27.3$, df = 4, $p = 0.0001$) from *H. seriatus* and *H. birmanus* (Table 1). *Hypothenemus eruditus* was not parasitized by *P. coffea* and hence was not included in any statistical analyses.

Parasitized *H. hampei* had the lowest percentage of unemerged parasitoids compared to the other four *Hypothenemus* species (Fig. 1), indicating that *H hampei* is a superior host for *P. coffea* development. For each parasitized host beetle with unemerged parasitoids, invariably two parasitoids were present, and the parasitoids were of the same life stage (larva, pupa or adult). The frequency of the different life stages for parasitized hosts with unemerged parasitoids differed among *Hypothenemus* species (Fig. 2). Parasitized *H. hampei* had a significantly lower percentage of larval ($\chi^2 = 15.10$, df = 3, $p = 0.001$), and higher percentage of adult parasitoids that were unemerged ($\chi^2 = 18.36$, df = 3, $p = 0.0001$) compared to the other *Hypothenemus* species. The higher percentage of unemerged parasitoids developing to the adult stage again indicates that *H. hampei* is a superior developmental host than the other *Hypothenemus* spp. The percentage of unemerged pupae found in parasitized *H. hampei* was not significantly different from *H. obscurus*, *H. seriatus* and *H. birmanus*, but *H. crudiae* had a significantly higher percentage of pupae than *H. hampei* ($\chi^2 = 95.40$, df = 4, $p = 0.0001$) (Fig. 2). No eggs were found in any of the parasitized *Hypothenemus* hosts.

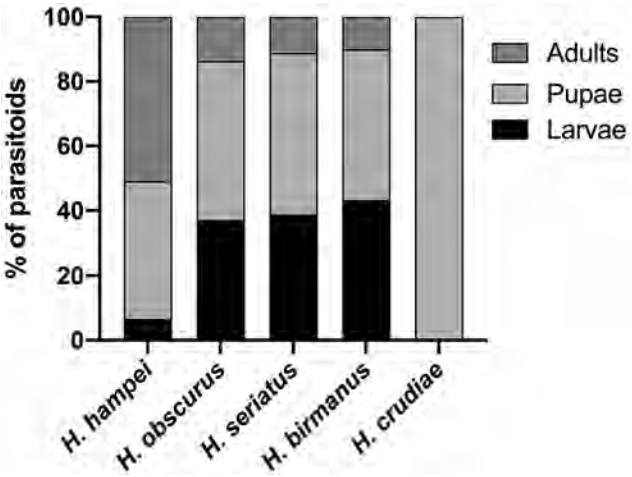

Fig. 2 Fate of unemerged *Phymastichus coffea* parasitoids from parasitized *Hypothenemus* spp. in no-choice in vitro non-target host selection screening. Parasitized *Hypothenemus* beetles with unemerged parasitoids were dissected to identify life stages (larva, pupa, adult)

Discussion

Phymastichus coffea is a potential biological control agent of *H. hampei* and was brought from Columbia into a quarantine containment facility in Hawaii for host range testing to determine whether the parasitoid might attack non-target species and therefore pose a risk to Hawaiian endemic species. Using no-choice tests, 43 different species of Coleoptera were exposed to *P. coffea* in vitro, including 23 scolytines (six natives, 17 non-native species including *H. hampei*), six beneficial species and 12 other species including one native weevil (*N. giffardi).* Only five species from the genus *Hypothenemus* were parasitized by *P. coffea,* including the two pest species *H. hampei* (coffee berry borer) and *H. obscurus* (tropical nut borer, a macadamia nut pest), and three other exotic species *H. seriatus*, *H. birmanus* and *H. crudiae* (Fig. 1). Thus, *P. coffea* appears to be host specific at the genus level and should pose no harm to endemic species if released in Hawaii coffee for classical biological control of *H. hampei*. Nevertheless, no level of host specificity testing can ensure zero risk to non-target organisms when introducing a natural enemy in a new habitat (Louda et al. 2003).

We observed that once the host and parasitoids were exposed in the Petri dish arena that *P. coffea* inspected *H. hampei* and other *Hypothenemus* spp. hosts by antennation before proceeding to oviposition or rejection. *Phymastichus coffea* did not show any oviposition response to other non-target hosts. This could be dependent on several factors

because parasitoids may search and decide host suitability by using a broad spectrum of different stimuli such as plant–host complex volatiles, host feces volatiles, host sex pheromones, and tactile and visual cues (Chiu-Alvarado and Rojas 2008; Yang et al. 2008). Host habitat and host diet may influence the volatile composition emitted by the potential host insect, which can either deter or attract parasitoids from a distance. To minimize the effect of diet, we provided a general beetle diet to all the field-collected coleopteran hosts during the experiments. Parasitism of non-target hosts in the field may not be the same as our in vitro test results because of various factors related to the host's natural habitat. Most of the coleopteran species tested in our study are normally found tunneling in seeds, decomposing wood (under the bark and/or in sapwood) or decaying fruits. This cryptic behavior would likely provide protection from *P. coffea* which is accustomed to searching for *H. hampei* adult females, while they are exposed on the surface of coffee berries.

Phymastichus coffea was attracted to and parasitized only four species of *Hypothenemus* in addition to its target host *H. hampei*. This is consistent with studies reported by López-Vaamonde and Moore (1998), and Castillo et al. (2004). Combining information from our study and previous studies, seven species of beetles are now known to be able to serve as hosts in captive exposure studies for *P. coffea*: *H. hampei, H. obscurus*, *H. seriatus*, *Araptus* sp. (Lopez-Vaamonde and Moore 1998), *H. crudiae* and *H. eruditus* (Castillo et al. 2004), in addition to *H. birmanus* (this study). Parasitism of the scolytine *Araptus* sp. seems to be an outlier, but this genus does not occur in Hawaii. Aside from *Araptus*, *P. coffea* appears to be genus specific attacking closely related, but not all *Hypothenemus* species, given that species from closely related genera were not parasitized under no-choice test conditions. In our study, *P. coffea* did not attack *H. eruditus*. We believe that *H. eruditus* may not be a suitable host for the parasitoid because of its small size (≤ 1 mm)*; Phymastichus coffea* usually lays two eggs per host (1 male and 1 female), and in such a small host, successful development would be unlikely due to the limited availability of resources within the host. Host size is an important variable on which the survival and growth of parasitoid progeny depends. Females of most parasitoids preferentially lay eggs on larger hosts (Fox and Mousseau 1995). Also, *H. eruditus* is phylogenetically distant from *H. hampei* (Fig. 1) which is addressed below.

Our results also showed that *H. hampei* had the lowest numbers of unemerged parasitoids when compared with the other four *Hypothenemus* species (Fig. 2). The number of larvae and pupae were lower, and adults were higher in parasitized *H. hampei* with unemerged parasitoids. Similarly, in other three *Hypothenemus* spp. (*H. obscurus*, *H. seriatus* and *H. birmanus*) many unemerged parasitoids could not complete their development and died in their larval or pupal stage with only a few reaching to the adult stage. In parasitized *H. crudiae* with unemerged parasitoids, most apparently could not reach the adult stage. Although the rate of completing the life cycle differed among *Hypothenemus* species, eggs did hatch in all parasitized species. Many factors can be responsible for suitability of the host for parasitoid development (Pennacchio and Strand 2006). Factors such as host physiology (e.g., presence of endosymbiotic bacteria), behavior (e.g., feeding habitat-sequestering secondary metabolites) and ecology (e.g., spatial/temporal overlap) may influence host acceptance by parasitoids and successful development (Desneux et al. 2009). All the non-target species used in the experiments were freshly collected from the field and may have carried toxins (accumulated from plant feeding) that may have interfered with the successful development of immature parasitoids within the hosts due to the ingestion of unsuitable food (e.g., see Desneux et al. 2009).

Phymastichus coffea also did not successfully parasitize any of the non-*Hypothenemus* species tested, including both native (*Xyleborus*) and exotic (*Xyleborinus*, *Xylosandrus, Xyloborus, Euwallacea,* others) Scolytinae, and other curculionid species from subfamilies other than Scolytinae, including the native weevil, *N. giffardi*. We did not find any *P. coffea* life stages (eggs, larvae, pupae, adults) after dissection in any of the non-*Hypothenemus* non-target species tested (Tables 2, 3, 4). Host specialization is relatively common in parasitic Hymenoptera and can be related to phylogeny, ecology and life histories (Price 1980; Stireman et al. 2006). It appears that at least host phylogeny was an important factor in host selection for *P. coffea* under our laboratory conditions.

Host range of idiobiont parasitoids is typically broader than koinobiont species (Askew and Shaw 1986; Hawkins et al. 1992), and it would hypothetically be reasonable to expect that *P. coffea* would follow this pattern. However, our results show that *P. coffea* was unable to successfully parasitize any species outside of the genus *Hypothenemus* and, even within the genus, was only moderately successful on species even closely related to *H. hampei*. While parasitism of *H. hampei* and subsequent parasitoid emergence was relatively high, both were significantly lower in *H. obscurus* and *H. seriatus*, sister species to *H. hampei*; *H. eruditus*, in a sister clade to the other species (Johnson et al. 2018), had zero parasitism. This demonstrates decreasing susceptibility to *P. coffea* with increasing phylogenetic distance among the *Hypothenemus* spp. exposed to the parasitoids in this study. Among the *Hypothenemus* spp. included in the phylogenetic reconstruction published by Johnson et al. (2018), *H. hampei* is the only species that has undergone a reversal in host range breadth, to become monophagous on coffee, while the other *Hypothenemus* spp. have retained a host generalist biology. *Hypothenemus hampei* has developed a unique association with *Pseudomonas* bacterial endosymbionts to

facilitate detoxification of caffeine, permitting it to exploit *Coffea arabica* seeds as their host (Ceja-Navarro et al. 2015), and potentially other physiological adaptations to its unique host, possibly providing adaptive challenges to parasitoids, and mediating host specificity of *P. coffea*. Messing (2001) questioned the practicality of applying centrifugal phylogeny approaches to selecting species to examine in non-target studies of potential biological control agents, particularly parasitoids. Our results support the predictions of the latter approach, with more distantly related *Hypothenemus* species less susceptible to *P. coffea* attack and more distantly related genera (e.g., *Xyleborus* spp.) not attacked at all. However, Messing (2001) emphasized the fact that interactions between the host insect and its host plant may override host phylogenetic patterns, by providing the stimuli for parasitoids to attack hosts, a consideration which may play a role in this study system. If this is the case, it is possible that *P. coffea* will produce even higher levels of parasitism than recorded in the artificial environment we used in our study, when attacking wild *H. hampei* boring into coffee fruits, producing the full range of cues stimulating parasitism, and lower field parasitism of the non-target *Hypothenemus* spp. included here.

Among all the parasitized *Hypothenemus* species, *H. hampei* had the highest rate of parasitoid emergence. The total developmental time (from egg to adult) of *P. coffea* was shortest in *H. hampei* (32 days); parasitism of *H. crudiae* resulted in the longest developmental time (41 days). Another study reported a similar development time of the *P. coffea* in *H. hampei*, 38–42 days at 23 °C and 66% RH (Rafael et al. 2000). Castillo et al. (2004) reported a *P. coffea* development time of 42.6 days for *H. hampei* and 40 days for *H. crudiae* at 26 ± 2 °C and 70–80% RH. Total developmental time is directly related to the temperature. For example, the total development period of *Diglyphus isaea* (Hymenoptera:Eulophidae) decreased with increasing temperature between 15 and 35 °C and no development was found at 10 and 40 °C (Haghani et al. 2007). Temperature is a critical abiotic factor influencing the physiology and dynamics of insects. Therefore, in this study we selected a temperature for our no-choice assays which reflects the ambient field temperature the insects are expected to experience. In addition to temperature, age of the parasitoids and host play an important role in the subsequent development of parasitoid offspring (Pizzol et al. 2012). Hence, we used uniformly aged parasitoids and hosts throughout our experiments to minimize any impact on host parasitism and parasitoid development.

Phymastichus coffea commonly lays two eggs (a male and a female) per host (López-Vaamonde and Moore 1998). Both male and female develop in a single host, the female in the abdomen and the male in the prothorax (Espinoza et al. 2009). In this study, slightly fewer male parasitoids emerged as compared to females from parasitized hosts. The proportion of females emerging from *H. hampei* was 50.8% which is consistent with the results obtained by López-Vaamonde and Moore (1998) and Rafael et al. (2000). Likewise, sex ratios of *P. coffea* emerging from *H. obscurus* 54.8%, *H. seriatus* 51.1% and *H. crudiae* 50.0% were consistent with the sex ratio results reported by (López-Vaamonde and Moore 1998; Castillo et al. 2004) of 1.25:1, 1:1 and 1:1 (female–male), respectively, for these species. In our study, the proportion of females emerging from parasitized *H. birmanus* 57.7%, was the highest among all other *Hypothenemus* species tested. The slightly fewer males produced per host in our study could be due to either to some parasitoid's preference to lay one egg per host (Feldhege 1992) or the lower survivorship of male eggs or larvae. Preference to lay female eggs over male can be dependent on several factors such as host quality, host age, immune response, genetic factors, photoperiod and relative humidity, host density or host-related volatile composition (King 1987).

All the above tests were conducted in a quarantine laboratory with no field studies. We conducted no-choice tests because they may provide more accurate and conservative information on host preferences and physiological host range than choice tests because of lower levels of interference due to unexpected responses to multiple host cues (Van Driesche and Murray 2004). Sands (1997) showed that laboratory studies often overestimate the host range of the parasitoid and realized ranges under field conditions may be substantially less than predicted from no-choice tests, but they are necessary to give a worst-case prediction of the number of hosts at risk of being attacked in the field (Avilla et al. 2016). *Phymastichus coffea* attacked other non-target *Hypothenemus* species in our no-choice trials, but this does not necessarily mean that those species will be attacked in the field. For example, an idiobiont braconid wasp, *Bracon hebetor* is reported to parasitize a wide variety of moths within and outside in Phycitinae (Lepidoptera:Noctuidae) in the laboratory, but in the field it is restricted to only larvae of *Plodia interpunctella* (Lepidoptera:Noctuidae) (Antolin et al. 1995). This is because in the field, parasitoids use a spectrum of long- and short-range cues (chemical, visual, vibrational and tactile signals) to locate hosts (Strand and Pech 1995). Chemical cues (infochemicals) can play an important role in host location. A study conducted by Rojas et al. (2006) showed that *P. coffea* can distinguish between *H. hampei*-infested and uninfested coffee berries, and were highly attracted to the dust/frass originating from *H. hampei* infested berries, but showed no response to the dust/frass originated from the closely related non-target host, *H. crudiae*. This behavior depending on plant and host cues suggests that it is very unlikely that *P. coffea* will have any

negative effects on non-target scolytids, or any other beetles, under field conditions.

No biocontrol agents were previously released in Hawaii against *H. hampei*. Two exotic predatory beetles, *Cathartus quadricollis* and *Leptophloeus* sp., are commonly found in overripe and dried coffee berries predating on the immature stages of *H. hampei* (Follett et al. 2016; Brill et al. 2020). Our host testing in quarantine showed that *P. coffea* will not parasitize these beetles and that the beetles did not predate on the parasitoids. Also, these predators attack eggs, larvae and pupae of *H. hampei* in overripe and dried berries (left after harvesting), whereas *P. coffea* only attacks adult female *H. hampei* at an earlier stage of crop maturity. The other four *Hypothenemus* species that were attacked by *P. coffea* have very different field habitats, but might serve as useful transitory hosts for *P. coffea* at times when, or in areas where, *H. hampei* populations are at low densities, such as between coffee seasons. For example, macadamia nut farms are often located close to coffee farms in Hawaii and may provide a year-round source of *H. obscurus*, a pest of macadamia nut. Feral coffee in Hawaii could also serve as a continuous source of *H. hampei* throughout the year.

Phymastichus coffea is a potentially effective biological control agent for *H. hampei* and could be incorporated into existing IPM programs in Hawaii. *Phymastichus coffea* may be simply released and monitored for establishment in a classical biological control program, or it may be mass reared for inundative releases. Currently, trapping and sampling of infested coffee fruits is conducted to monitor *H. hampei* flights and optimize timing of *Beauveria bassiana* applications for control (Aristizabal et al. 2016). After *H. hampei* bores into the coffee berries, it is protected and difficult to control with biopesticides or conventional insecticides. To achieve maximum *P. coffea* parasitism in the field, inundative releases should be made at times when *H. hampei* adults are active (e.g., when trap catches are high or female *H. hampei* are actively boring into fruits) and the coffee crop is at a susceptible stage. Optimal timing of inundative releases may differ for different elevations due to *H. hampei* population dynamics (Hamilton et al. 2019). Studies suggest *P. coffea* may be susceptible to *B. bassiana,* however (Barrera 2005; Castillo et al. 2009; Ruiz et al. 2011), so inundative releases should be timed to avoid *B. bassiana* applications or used in alternation with *B. bassiana* against *H. hampei*. If *P. coffea* is highly effective, then dependence on *B. beauveria* applications could be reduced dramatically.

Author contributions

FY designed methodology, conducted the experiments and wrote the manuscript; PF designed the experiments; PF and MW provided overall project management and manuscript editing; FY, CG and DH conducted field surveys and collected live beetles for testing. FY, CG and LC identified beetle species; MGJ and PBM reared and supplied *Phymastichus coffea*. All authors read and gave final approval for publication.

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Declarations

Conflict of interest The authors have declared that no conflict of interest exists.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Hawaii Volcanoes National Park Quarantine Facility
Standard Operating Procedures

INTRODUCTION

The Hawaii Volcanoes National Park Quarantine Facility is the result of a cooperative agreement among federal and state of Hawaii agencies to implement a biological control program against alien weeds in Hawaii's forests. Participants in the cooperative agreement are: National Park Service; Biological Resources Division of the U.S. Geological Survey; U.S.D.A. Forest Service; Hawaii Department of Land and Natural Resources; Hawaii Department of Agriculture; and University of Hawaii. The Quarantine Facility was completed in March 1984 and certified in November 1984. The original 600 sq. ft. structure was modified in 1996 with addition of 600 sq. ft. of space for a workroom. The entire structure was modified with the addition of a metal roof and skylights in 2003. The facility is located at 3,800 feet elevation in Hawaii Volcanoes National Park, approximately 1 mile southeast of the park headquarters and visitor center.

This facility is designed to contain arthropod agents under evaluation for biological control of noxious forest weeds. Until approved for release by federal and state authorities, agents are considered restricted plant pests. They must be handled with appropriate caution to prevent escape because their establishment could have irreversible negative consequences for the environment. Containment of agents requires a secure, properly maintained building and strict adherence to precautionary protocols by all personnel. These Standard Operating Procedures (SOP) are meant to inform personnel on management policies and procedures to correctly and safely perform duties while working in this specialized structure.

PHYSICAL CONTAINMENT STANDARDS

Description of the facility and safeguards

(Refer to attached map and floor plan.)

The Quarantine Facility (Building #338) consists of two anterooms (A and B), the quarantine greenhouse (with walls and skylights made of Lexan polycarbonate), autoclave room, workroom (room 1 of the quarantine addition), temperature cabinet room (room 2), and handling room (room 3). A storage room is located at the external opening of the autoclave.

Entry and exit from the Quarantine Building is through the two anterooms enclosed by three airtight doors. The anterooms are completely dark except for insect light traps formed by windows looking into the Quarantine Greenhouse. There is an Emergency Exit Door in the workroom.

A. Walls, ceiling, and floors

The Quarantine Facility and adjacent glass greenhouse are surrounded by a water moat to prevent entry of unwanted organisms. Walls are double-walled plywood or Lexan mounted with flexible gaskets. Floors are cement painted grey. Drains in floors are covered with 100-mesh stainless steel screen. Ceilings are sealed wood with insulation. Skylights are sealed, double-

pane Lexan polycarbonate. All plumbing and electrical systems located in the walls are sealed with caulking.

B. Windows

Double-paned, Lexan windows are present in each room of the quarantine addition. The window in the Autoclave Room is single-paned Lexan, as part of an original design as an emergency exit.

C. Exterior doors

The entry and emergency exit doors are steel-plated. The three entry doors through Anterooms A and B are equipped with sensors that indicate via red and green lights when all doors are closed (green) or any door is open (red). The emergency exit is equipped with a sensor that sounds an alarm if the door is opened.

D. Ventilation

Air temperature inside quarantine is regulated by two fans that draw outside air through double, 250-mesh stainless steel screens located in the gable over the entrance and exhaust it through double, 250-mesh stainless steel screens located at the opposite end of the Quarantine Greenhouse. Stainless steel screens are protected from clogging dust by standard air filters. Temperature is adjusted by increasing fan rotational speed to increase cooling or decreasing fan speed to allow warming. The fan control box is located near the anteroom entry/exit door.

Hot air generated by temperature cabinets is exhausted from the temperature cabinet room into the adjoining greenhouse by a fan mounted near the ceiling.

E. Negative pressure

Negative air pressure within quarantine is maintained by the fans and is indicated by the manometer in the autoclave room. The purpose of negative pressure is to decrease the chance of flying or airborne insects from being sucked out when a door is opened.

F. Electrical system

All outlets are on ground-fault interrupt (GFI) circuits. The circuit breaker panel for all electrical sources is located in the Storage Room accessible from outside quarantine. A propane emergency generator is located in the Storage Room. Instructions for operating the generator are located in the Storage Room. Use of earplugs is recommended when working in the immediate area while the generator is in operation. The emergency generator will supply all normal quarantine electrical requirements, except for operation of the autoclave.

G. Communication system

The Quarantine Facility is supplied with a telephone located in the Autoclave Room (line 808-967-7122 which also serves the Forest Service office in Magma House).

H. Waste disposal

Waste materials that can be safely pressure-heated are sterilized in the autoclave before exiting quarantine via the Storage Room. Autoclave doors seal and lock automatically so that only one door can be opened at a time. The external door can only be opened after a sterilizing cycle is completed.

Liquid waste such as dishwashing water is eliminated through the plumbing system that feeds into a closed, covered cesspool. Floor drains covered with stainless steel mesh also are tied to the cesspool, which serves only the quarantine building.

I. Fire and chemical safety

Smoke detectors are installed on ceilings of the quarantine greenhouse, workroom, autoclave room, temperature cabinet room, and storage room. Fire extinguishers are hung in the quarantine greenhouse, quarantine workroom, outside Building 342 (plant containment building), outside the HAVO nursery office, and outside the Magma House office.

Emergency exits from quarantine include the main entrance doors and an emergency door in the workroom. An emergency eye-wash and shower are installed in the handling room. A first aid kit is located in the autoclave room. Ear plugs, full-face shields, and gloves are available on site. Chemicals are inventoried and their locations listed within the Chemical Safety Plan, together with material safety data sheets (MSDS). Inventories and MSDS are stored just inside entrances of the office, quarantine, and Building 342.

OPERATIONAL STANDARDS

1. Designation of Quarantine Officer and Assistant Quarantine Officer

Quarantine Officer: Dr. Tracy Johnson, Research Entomologist
Mailing Address: USDA Forest Service, P.O. Box 236, Volcano, HI 96785
Work tel: 808-967-7122; Fax: 808-967-7158; Cell: 808-938-7818

Assistant Quarantine Officer: Nancy Chaney, Biological Science Technician
Work tel: 808-967-7122; Home tel: 808-967-8581; Cell: 808-333-0433

2. Authorized personnel

Access to the Quarantine Facility is restricted to individuals authorized by the Quarantine Officer. Access is generally limited to individuals involved in biological control research. Visiting guests must be accompanied by the Quarantine Officer or his designee.

3. Signs

A. A sign permitting entry to authorized personnel only is posted at the entry to the Quarantine Building.

B. A sign with emergency contact information is posted at the entry to the Quarantine Building.

C. A sign indicating the emergency exit door is posted upon the door.

4. Access to the facility

A. Before entering quarantine

Plan your work in advance so that only required materials and equipment are taken into the quarantine facility. All plants, plant materials, supplies and equipment brought into quarantine

shall be free of insects and arthropods. This may be accomplished by visual inspection and removal, treatment with appropriate pesticides, or other approved methods.

B. Entering the facility

Wipe off your shoes outside the first entry door. Before entering, check the red and green lights to the left of the entrance door. Enter only if the green light is on. A red light indicates that someone has opened one of the interior doors. In this case you should wait until they have finished entering or exiting. Open only one door at a time after observing that the indicator lights are green.

Upon entry to Anteroom A, place any personal belongings (e.g. hat, coat) on the shelf and/or hooks. Items not needed in quarantine should not be taken further into the facility.

After passing into Anteroom B, put on a laboratory coat. Additional coats are available for guests if needed.

Upon entering the quarantine greenhouse, record your name, the date, time and any materials you have brought with you on the Sign-in Sheet attached to the door.

C. Exiting the facility

Wash your hands and take a few moments to visually inspect your clothing for insects. Sign out before exiting and note any items you are removing from the facility. **Materials exiting the facility shall be treated as described under Sanitation** (see below). Remove your laboratory coat inside the quarantine, shake it out, and repeat visual inspection. Check for the green light before opening the door.

Enter Anteroom B and close the door. Hang up laboratory clothing. Brush yourself off starting high and working down. Stamp your feet to dislodge materials on shoes. If at any time during this procedure you observe insects, they should be captured or killed and you should return to quarantine to verify that no others can escape.

If inspection and brushing reveal no insects, proceed to Anteroom A. Shake out personal clothing left in this room before putting it back on. Wipe off your feet on the mat again before exiting.

5. Sanitation

A. Autoclave

The quarantine autoclave is a Steris double-doored, gravity/laboratory sterilizer with a single-input power supply. The autoclave features microcomputer controls that monitor all cycle phases. It provides both audible and visual notification of progress sterilization.

All items that are small enough and can withstand 120^{o}C and 20 Atm pressure for 30 minutes should be autoclaved before exiting quarantine. For new or unusually bulky items, place Sterilizer Indicator Strips at the center of the item and check to verify that sterilization was sufficient. Check each load to verify that heat was sufficient to partially melt plastic bag containers.

All soil and plant matter brought into quarantine shall be autoclaved before its removal.

All trash, including residues from wastebaskets and floor sweepings, shall be autoclaved.

Removal of living insects, plants, or plant materials from the quarantine building is prohibited without specific authorization from the Quarantine Officer and regulatory agencies.

B. Removal of objects from the facility

Small items (pieces of paper, cameras) that can be thoroughly inspected can be wiped clean and removed.

Items such as plastic pots should be immersed in 5% bleach and held in Anteroom B for several hours, overnight if possible, before being removed.

Lab coats shall be periodically removed by the Quarantine Officer for washing in bleach and hot water. Coats must be shaken clean, visually inspected inside quarantine, and then sealed in a plastic bag for removal.

Equipment that cannot be autoclaved (microscopes, electrical equipment, etc.) shall be decontaminated with alcohol or placed in a plastic bag and fumigated with an appropriate insecticide within Anteroom A. If it is necessary to fumigate an object, this should be done at the end of the day after everyone has left in order to minimize pesticide exposure. Any use of pesticides must be performed in consultation with the Quarantine Officer and follow required safety procedures found in the Chemical Safety Plan.

Larger pieces of equipment (such as refrigerators and temperature cabinets) should be cleaned thoroughly, wrapped in plastic, and fumigated with insecticide for 24 hours. If it is possible to treat all surfaces, spraying with 95% alcohol can be used as an alternative to fumigation.

6. Facility maintenance and repairs

Maintenance personnel are authorized for entry only after notifying the Quarantine Officer or Assistant Quarantine Officer and receiving a pre-work briefing.

The quarantine facility will be inspected by the Quarantine Officer or Assistant Quarantine Officer once a month. Items to be checked include: 1) caulking around windows and greenhouse panels; 2) seals around all doors to be sure that latches close properly and that no light can be seen when they are closed; 3) caulking around the autoclave unit, and plumbing and electric lines entering the facility; 4) filters covering air intake and exhaust vents.

A complete inspection of all parts of the facility will be conducted annually. Based on the results of this inspection, maintenance needs will be identified and submitted in writing to the Chief of Resources Management of HAVO.

7. Emergencies and contingency plans

Before working in quarantine, familiarize yourself with the location and operation of smoke detectors, fire extinguishers, emergency exits, emergency eye-wash and shower, first aid kit, personal protective equipment and chemicals. Access to emergency equipment must remain unobstructed.

Earthquakes/Hurricanes

During an earthquake, leave the greenhouse, autoclave room, or temperature cabinet room immediately. Take cover under a metal desk in the workroom or evacuate the building.

Immediately following a minor earthquake (five and lower on the Richter scale) or major storm, the facility should be checked for breaches to quarantine. Areas to be checked include all seals checked in the course of monthly maintenance inspections (see above).

A major earthquake is one in which damage to the physical building could occur and is strong enough to shake loose items off benches. The first and primary goal of the quarantine staff after a major earthquake is human safety.

If quarantine can be entered safely, check all containers with quarantine insects. If breakage has occurred, steps must be taken immediately to prevent insects from escaping. Cracks should be sealed with tape or plastic. Items that have broken or spilled should be sealed in plastic bags.

After verifying that all sleeve cages and other containers are sealed, the entire quarantine facility should be closely inspected to determine if breaks occurred. If breaks are small, quarantine personnel shall repair them immediately. Damage requiring extensive repair will be referred to the National Park Service.

If an approaching major storm poses a threat to the quarantine, all insect colonies shall be packaged in airtight containers and placed inside refrigerators or temperature cabinets with the doors closed and secured.

Major structural damage

If it is judged that the physical facility has been damaged to the point that it is no longer operational and may be incapable of containing escaped insects, the following steps shall be taken: 1) If cages have broken open, insects have been freed and escape is possibly occurring, the entire facility will be immediately fumigated. 2) If cages are intact and no insects have escaped, insect colonies shall be packaged in airtight plastic bags, held in the refrigerator or temperature cabinets, and then the quarantine facility fumigated. Later, the colonies shall be packaged and transferred to an alternate quarantine facility for temporary holding.

Shutdown of quarantine during a park closure due to elevated SO_2 levels

Shut off the quarantine ventilation system at main controls. This will prevent circulation of air in or out of the building. Turn off all appliances except one refrigerator and internal fan. This will serve to reduce heat buildup inside quarantine so that temperature remains moderate and nonfatal to insects. After turning off power to temperature cabinets, leave doors open to allow some air circulation and prevent excessive humidity to insects left inside.

If it is safe to enter the park during a closure (as authorized by park safety officers), quarantine colonies will be inspected and maintained every few days (2-3 times per week if possible). Authorized personnel may minimize exposure to SO_2 through use of park-approved respirators and by keeping the time in and out of the park to 1 hr or less. Any access must be controlled with check-in and check-out contacts with both HAVO and Forest Service points of contact. When HAVO officials reopen the park, quarantine will be ventilated and inspected for security and functionality.

SPECIAL PROCEDURES FOR HANDLING ORGANISMS UNDER PERMIT

The Hawaii Volcanoes National Park Quarantine Facility is certified for containment of arthropods only. Pathogenic organisms are not tested in this quarantine facility unless they are found in the natural environment in Hawaii.

Only shipments of agents approved for introduction into the quarantine facility by the State of Hawaii and APHIS will be accepted. Approved shipments will be covered by a PPQ Form 526 signed by the appropriate officers for the State of Hawaii and APHIS/PPQ.

Under no conditions or circumstances will a package be opened before it enters the quarantine facility. State or Federal Inspection personnel may accompany the package to the quarantine facility and be present when it is opened. All arriving shipments shall be unpacked inside the handling room with the door completely closed. Each layer of wrapping as it is encountered will be individually examined for agents. Plant materials and packaging in which insects were received from a foreign country shall be bagged immediately and autoclaved. All instruments and work surfaces shall be sterilized with 95% alcohol.

Live agents shall be removed from the handling room in sealed containers and transferred to sleeve cages in the main quarantine area for rearing and study. Dead or weakened agents shall be checked for pathogens or parasitoids and then preserved or reared for inspection or for voucher specimens. All extraneous species shall be killed and saved.

After receiving and inspecting a shipment, the Hawaii Department of Agriculture and APHIS shall be notified in writing of the shipment, the type and exact number of insects it contained, and their condition. A log will be kept of each shipment received in the quarantine facility and contain all information pertaining to the shipment (i.e., original shipper, dates, number and condition of insects found (both the desired species and extraneous species.

Before transporting live insects to another quarantine facility, approval will be obtained in writing from the State of Hawaii or the APHIS/PPQ. Dead specimens may be removed from the quarantine to be used for voucher or other scientific purposes, but only in consultation with the Quarantine Officer and following appropriate treatment (e.g., alcohol, fumigant, freezing). The release of insects into the field in Hawaii requires state and federal approval. Voucher specimens will be prepared and submitted to both the Hawaii Department of Agriculture and the USDA ARS at Beltsville, Maryland.

Quarantine Floorplan