Final Report on FY2015 HISC Project:

Technical support of miconia biocontrol research in Volcano, Hawaii

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The development of biocontrol of *Miconia* was progressed with the completion of host specificity testing of *Euselasia chrysippe*, leaf-eating gregarious caterpillars from Costa Rica. Results of feeding tests with 73 different plant species were consistent with field observations in the native range: these caterpillars fed on several species within the plant family Melastomataceae but did not feed on other plants within the Order Myrtales or beyond (see attached poster). No feeding damage was observed on any native Hawaiian plants in these highly conservative no-choice host specificity tests. Based on these results we are preparing publications and the petition for release of this promising agent. Larval feeding by *Euselasia* is expected to reduce miconia's growth rate and spread in Hawaiian ecosystems.

In addition to feeding tests on a wide range of plants, long term larval survival of *Euselasia* was examined on eight melastomes. Caterpillars were found to complete development only on miconia and its closest relatives (within the tribe Miconieae). Larvae did not survive well on *Clidemia hirta*, however, which is a close relative of miconia but has leaves protected by dense trichomes. These results indicate that the actual host range of *E. chrysippe* would be restricted to *M. calvescens* and *Tetrazygia bicolor* in Hawaii. Our Pacific partners are concerned about the safety of this potential agent since they have native *Melastoma* species, but the long term no-choice tests indicate that representatives of this genus were not acceptable to the caterpillars.

Our final challenge to the successful development of *Euselasia* for biological control in Hawaii has been the sustained rearing of multiple generations in the quarantine laboratory. In the last two years we have had some limited success with mating and oviposition of this difficult butterfly. In our first multiple-choice oviposition tests, adult females laid eggs a number of times exclusively on *Miconia*. Unfortunately the number of fertile eggs has not been sufficient to build a laboratory colony. We continue to work on this problem with new adults grown from field collected larvae collected in Costa Rica. In spite of obstacles to rearing the butterfly, we propose to move ahead with a proposal to release this agent based on its narrow specificity using a release protocol that would not require a full life cycle in quarantine.

Other miconia agents targeted for development and evaluated in our Volcano quarantine during the last two years include a stem weevil (*Cryptorhynchus melastomae*) and a fruit gall wasp (*Allorhogas* sp.). We are continuing to rear the stem weevil as we analyze data on its specificity. It appears to feed broadly within the melastome family and on rare occasions lays eggs indiscriminately – a normal occurrence in lab studies, but one that requires careful evaluation.

The gall wasp *Allorhogas* has been successfully imported but not successfully reared on miconia owing to the difficulty of maintaining flowering and fruiting trees in quarantine. Our discovery of a related gall wasp on *Clidemia hirta* in Brazil in 2015 has presented the opportunity to more easily rear and evaluate an *Allorhogas* species, progress which should advance our work with the miconia wasp in the long run, in addition to supplying an exciting new agent for clidemia. Our collaborator in Brazil has successfully transferred and reared the miconia gall wasp on field planted miconia at his facility in southern Brazil,

further supporting optimism over this agent. Both wasps on miconia and clidemia are being examined by a specialist for possible species descriptions.

Galled fruits of *Miconia calvescens* in Brazil.

Allorhogas wasp on *Clidemia hirta* in quarantine. Wasps lay eggs in developing flowers, which subsequently develop into fruit that are greatly enlarged and do not ripen normally (two large green fruit shown here contain wasp galls instead of viable seeds).

Euselasia chrysippe as a potential biocontrol for *Miconia calvescens* in Hawai'i

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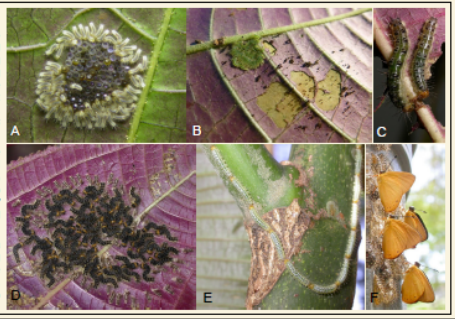
Biology and Rearing

***Euselasia chrysippe* (Lepidoptera: Riodinidae)** is a small butterfly whose caterpillars feed on several *Miconia* species in its native Costa Rica. Larvae hatch from large egg masses (up to 115 eggs) (A), feed (B,C) and molt (D) in unison, moving between feeding sites in single-file processions (E). This gregarious behavior is thought to improve feeding on tough leaves, optimize foraging, and deter enemies. After 6 instars, larvae move off the plant to pupate in smaller groups. Development from egg to adult is completed in about 2 months, and adult females emerge one day prior to males from the same cohort (F). Butterflies live up to 2 months along forest edges and gaps where *Miconia calvescens* occurs.

Euselasia eggs from Costa Rica were hatched and larvae reared on *Miconia* plants in the Hawai'i Volcanoes National Park Quarantine facility. Pupae were held in sleeve cages until adult emergence. Sexed adults were released in a large walk-in cage (approx. 3x3x4m, G) with overhead mist irrigation and potted *Miconia* plants and shade cloth along one side to simulate the edge of a rainforest gap. Butterflies were offered nutrients in the form of watermelon, banana, *Nektar+* hummingbird food, wet clay soil from Mauna Kea, bird droppings and canned tuna, which were refreshed every 2-3 days.

Adults generally survived only 1-2 weeks – hand feeding with *Nektar+* and mashed banana appeared to prolong survival. Although males were seen performing morning spiral mating flights, copulation was not observed. Three sets of caged adults laid egg masses on the undersides of leaves of caged *Miconia*: In total 200+ eggs in May/14 (in a 2x2x2m cage), 181 eggs in Dec/14, and 60+ eggs in Feb/15. Only 2 egg masses from Dec/14 appeared to be fertile, with 66 larvae hatching – the first successfully lab-reared *Euselasia chrysippe*.



Miconia Biocontrol

Biological control is considered a critical tool for long term management of *Miconia calvescens*, a neotropical tree that is a major threat to Hawaiian forest ecosystems. Explorations in Costa Rica and Brazil yielded several promising natural enemies which are being evaluated now for host specificity. Our strategy is to develop a suite of biocontrol agents attacking stems, leaves and fruits of miconia.

Euselasia is our most promising leaf-feeding enemy of miconia, because of its gregarious habit and potential to avoid the parasitoids that commonly suppress other lepidopteran weed biocontrol agents in Hawaii. Results of our specificity testing indicate that *E. chrysippe* is suitable for introduction to our state. Mass rearing this species in containment remains a significant challenge, in spite of our recent breakthroughs.

Future work with *Euselasia chrysippe* may involve exploring additional adult foods to improve butterfly survival, and limited testing of adults for ovipositional specificity.

Host-specificity

Methods: No-choice feeding test

- 73 plant species from Hawaii and Costa Rica
- Whole or cut leaves in 90mm Petri dishes (H)
- 4+ replicates per species, *M. calvescens* as control.
- 10 early or 5 late instar larvae per dish exposed for 3 days
- Feeding assessed from 0 (no damage) to 5 (severe) (I)

Results (Figure 1)

- Varying levels of feeding on many Melastomataceae. (No melastomes are native in Hawaii).
- *M. calvescens* and *Tetrazygia bicolor* most damaged of melastomes occurring in Hawaii.
- No sustained feeding outside Melastomataceae. Limited "tasting" (damage rating 1) of some Myrtales.

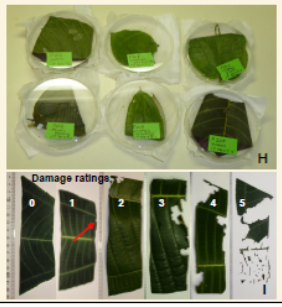
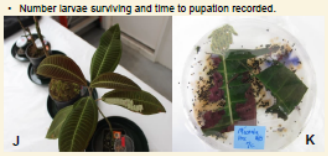


Figure 1, below. *Euselasia chrysippe* average feeding damage from no-choice tests conducted for 3 days in 90mm Petri dishes. Replicate numbers along top axis. Plant species arranged phylogenetically. *Miconia* species in dark green are Costa Rican hosts. * Native Hawaiian plants.

Methods: No-choice survival test

- 8 species of Melastomataceae from Hawaii.
- 10 first instars in 90mm Petri dishes or 13 first instars on potted plants (J), leaves replaced as necessary
- 3+ replicates per species, *M. calvescens* as controls (K).
- Observed every 2-4 days until pupation or larval mortality.
- Number larvae surviving and time to pupation recorded.



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Results (Figure 2)

- Survival to pupation only on *M. calvescens* and *Tetrazygia bicolor* in whole plant experiments.
- No-choice survival to pupation restricted primarily to tribe Miconieae.
- One larva (out of 40) survived to pupate on *Heterocentron subtriplinervium* and needed 10 additional days to develop.

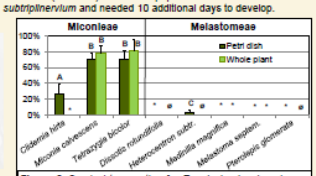

Figure 2. Survival to pupation for *Euselasia chrysippe* larvae on leaves in Petri dishes versus potted plants. Shared letters not significantly different. Species arranged by tribe. (*) No pupation found. (ø) Not tested on potted plants.