Biology and Host Preferences of *Cryptorhynchus melastomae* (Coleoptera: Curculionidae), a Possible Biocontrol Agent for *Miconia calvescens* (Melastomataceae) in Hawaii

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ABSTRACT The introduced plant *Miconia calvescens* (Melastomataceae) poses a grave threat to Hawaii's native ecosystems and biodiversity. One potential candidate for classical biological control is *Cryptorhynchus melastomae* (Coleoptera: Curculionidae: Cryptorhynchinae), a stem-boring weevil from Central and South America. This weevil feeds on *M. calvescens* in its native Costa Rica and has been successfully reared under greenhouse conditions. Comparison of its environmental conditions in Costa Rica with those in the *Miconia* infested areas of Hawaii indicates the latter is a suitable habitat for *C. melastomae*. *C. melastomae* has one or two generations per year. Adults feed on new stems, petioles, leaf buds, veins, and lamina, whereas larvae mine the stem until pupation. Adults appear to prefer saplings for oviposition and feeding. Under greenhouse conditions both adults and larvae can seriously damage and kill small *M. calvescens*. Preliminary host testing indicates that *C. melastomae* may be family specific on Melastomataceae. However, because Hawaii lacks native melastomes and has many other serious melastome weeds, a family specific insect may be suitable as a biocontrol agent in this case.

KEY WORDS biological control, invasive species, Costa Rica, life history, host-specificity

Invading species are an ongoing global dilemma and heavily visited oceanic islands are particularly susceptible to these invasions (Vitousek et al. 1997). Invasive weeds are an especially problematic group, with more than half the flora of some islands made up of introduced plants (Vitousek et al. 1997). The Hawaiian Islands are no exception, of 2,781 plant species included in the 2001–2002 Hawaiian Biological Survey, 1,176 (42.3%) are nonindigenous (Eldredge and Evenhuis 2003).

Currently, the weed of highest priority for control in Hawaii is *Miconia calvescens* DC (Melastomataceae) (Invasive Species Committees of Hawaii and Coordinating Group on Alien Pest Species 2003), a small tree native to tropical Central and South America. Introduced to Hawaii in 1961 as an ornamental (Medeiros et al. 1997, Meyer 1996) it now occurs on the islands of Oahu, Hawaii, Maui, and Kauai. In its introduced range *M. calvescens* forms dense monospecific stands that are believed to displace native vege-

tation, cause erosion and landslides, and alter nutrient and water regimes (Meyer and Florence 1996). Current methods used to manage *M. calvescens* are removal of trees by hand or applying aerial herbicides to those that are inaccessible. These methods are time consuming, expensive and often difficult because of the rough terrain (Loope 1997).

Classical biological control has proven to be an effective way to manage invading weeds (Crawley 1989, Van Driesche 1994), and may be a suitable alternative or addition to current control methods for *M. calvescens*. Weevils (Coleoptera: Curculionidae) are often chosen for release as classical biological control agents and have been effective in many parts of the world (O'Brien 1995). The prospects for finding suitable biocontrol agents for *M. calvescens* are increased by the lack of native melastomes in Hawaii, thus lowering the risk of significant nontarget interactions.

Cryptorhynchus melastomae Champion (Coleoptera: Curculionidae: Cryptorhynchinae) is a stem-boring weevil originally described from Panama, Costa Rica, Nicaragua, and Guatemala (Champion 1906), but now known to be more widely distributed in Central America and northern South America (O'Brien and Wibmer 1982). Although cryptorhynchine weevils are usually associated with dead plant material, some, including *C. melastomae*, feed on live plants. This species feeds on *M. calvescens* in its native Costa Rica and is one of several potential agents being considered for biocon-

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trol (Badenes-Perez et al. 2008). *C. melastomae* is a fairly large robust (8–10 mm long, 3.8–5.3 mm wide) weevil. Its head, prothorax and a triangular section at the base of the elytra are covered in fulvous or reddish-brown scales, while the rest of the elytra are covered in white or brownish-white scales. *C. melastomae* appears to live in low to mid elevations of wet forests and disturbed areas. Many cryptorhynchines are primarily active at night. Over 200 species of Neotropical *Cryptorhynchus* have been identified; however, this genus has become a storehouse for cryptorhynchines with uncertain relationships and many of the species may not be closely related (Anderson 2008). No species have been identified as close relatives of *C. melastomae.*

The purposes of this study were to examine the geographic distribution, habitat requirements, and life history of *C. melastomae;* to conduct a preliminary assessment of its host-specificity; and to develop rearing methods to facilitate its further evaluation as a potential biological control agent for *M. calvescens* in Hawaii.

Materials and Methods

Distribution and Habitat Requirements. A database of 317 specimen records of *C. melastomae* was compiled using Champion's (1906) original description and the following collections: Instituto Nacional de Biodiversidad, Costa Rica (INBio); Arthropods of La Selva project, Costa Rica (ALAS); University of Costa Rica Insect Museum (UCR), National Museum of Costa Rica (NMCR), Smithsonian Tropical Research Institute, Panama (STRI), Canadian Museum of Nature, Ottawa, Canada (CMN); and the personal collection of Dr. Charles O'Brien. Voucher specimens collected during this project are deposited in the CMN.

A distribution map for *C. melastomae* was plotted using ArcGIS 9.1 (ESRI 2005). Costa Rican records were overlaid onto map layers including elevation, mean annual precipitation and Holdridge life zones of Costa Rica (ATLAS Costa Rica 2000 Project, undated) to determine environmental tolerances of *C. melastomae.* These data were then compared with the environmental conditions of *M. calvescens* in Costa Rica (E. Chacón, unpublished data) and with elevation, mean annual precipitation, and Holdridge life zones (Institute of Pacific Islands Forestry 2006, State of Hawaii Office of Planning 2006) in *M. calvescens* infested areas of Hawaii (Hawaiian Ecosystems at Risk Project 1996–1998).

Field Biology. Because *M. calvescens* is sparsely distributed in its native range, a number of seedlings were out-planted in close proximity to those naturally occurring in Costa Rica. This ensured that there were more *M. calvescens* individuals to work with in areas of suitable environmental conditions, and that natural enemies were easier to locate, identify, and study. Seedlings were transplanted in El Angel, Alajuela (N 10°15′33″, W 84°10′18″) and Turrialba, Cartago (N 09°47′17″, W 83°31′53″) in October 2003 and at San

Ramon, Alajuela (N 10°12′49″, W 84°34′22″) in September 2004 (despite a lack of *M. calvescens* in the area but after finding local evidence of *C. melastomae* attacking *M. theizans* (Bonpl.) Cogn.). All seedlings were grown in the UCRs greenhouses in San Pedro, San José (N 09°56′15″, W 84°02′58″) or transplanted on multiple occasions from other known *M. calvescens* sites. *M. calvescens* at the time of this study ranged from very small seedlings to reproducing trees at these sites, allowing observations on all life stages.

All out-planted *M. calvescens* were inspected for *C. melastomae* weekly at El Angel (≈300 seedlings/trees) and biweekly–monthly at San Ramon and Turrialba (≈50 and 100 seedlings/trees, respectively) in May–July 2005 and January–May 2006. Sites were also checked occasionally during the remaining months of 2005–2006.

M. calvescens (and other Melastomataceae) were individually examined during daylight hours for *C. melastomae* and signs of its feeding damage. Upper and lower surfaces of leaves were checked for resting adults and feeding damage to veins and lamina. Petioles, branches, and stems/trunks were inspected for resting adults, adult feeding damage, eggs, and the occurrence of small breathing/feeding holes indicative of internal feeding by larvae and larger emergence holes suggesting developing pupae inside. Vegetation near the stem base was also checked for concealed adults. Any potential parasitoids of *C. melastomae* were brought back to the lab for rearing and identification. Binoculars were used to search taller trees for individuals and feeding damage.

When located, adults were observed for 2–5 min depending on their behavior and then placed in a plastic vial with a small *M. calvescens* leaf segment and brought back to the lab for sexing using a dissecting microscope. Females were marked with a spot of red nail polish on the left elytron for easy recognition. Eggs were left attached to the leaf or stem segment, placed in a plastic vial and brought back to the lab to be placed in the rearing colony. When a larva was thought to be inside the stem (evident by debris ejected from oviposition sites or breathing holes) or an adult emergence hole was discovered, the stem was cut and brought back to the lab for dissection. Host information (collection site, date and weather, plant height and base diameter, height and diameter at which the individual was found, life stage found, and behavior over the time observed) was recorded for each sighting of *C. melastomae* and/or its damage.

Adults were added to rearing cages (125 × 70 × 55 cm, covered by blue mesh) in the UCR greenhouse until they were needed in either the biology study or the host-specificity trial. Eggs were either incubated at room temperature (≈23°C) in petri dishes in the laboratory or transferred to a greenhouse *M. calvescens* sapling (≈20°C). Larvae were transferred to a greenhouse sapling by slicing the new stem to create a flap and then hollowing out a small cavity beneath this flap using a needle. Once placed in the cavity, the flap was replaced and secured with wire. A small notch was cut in the top of the flap to allow the larva to breathe.

Greenhouse Rearing and Biological Observations. To determine duration of each life stage (egg, larva, pupa, and adult) the first adults captured in El Angel and San Ramon in 2005 were brought back to the UCR laboratory, sexed (females were marked) and placed in greenhouse rearing cages according to collection site.

Two to four pairs of adults were placed in each cage containing two *M. calvescens* saplings (average height 78 ± 11 cm, basal diameter 15 ± 2 mm). Plants were watered by hand and checked daily for eggs, which were individually marked with numbered pins. Data recorded for each egg included date found, height on plant, stem diameter, and location (leaf abscission scar, petiole, node, or internode). When saplings had four or more eggs, they were removed and replaced with fresh plants. Plants with eggs were searched daily for evidence of eclosion and larval feeding: a thin trail of chewed materials and frass protruding from the stem. Dissections were made over the following months to track the location and development of larvae and pupae. Saplings containing pupae were placed in a separate rearing cage to ensure emerging adults were caught and identified and the height and diameter of emergence holes were recorded.

Two pairs of newly emerged adults were each placed onto small saplings and each plant was covered with a clear plastic cage constructed of a 4-liter jug with the top and bottom cut off and one end covered with light fabric mesh. Plants were replaced weekly and eggs and larvae found were put into the rearing colony for monitoring. Day and night-time observations were made over a 24-h period on three separate occasions to study feeding and mating behavior within these cages. Insects were observed for the first 20 min of each hour. A red-light was used for night-time observations. The date females began to oviposit, the total number of eggs laid per female per week, and the date of adult death were recorded.

Host Specificity: Adult Feeding and Oviposition. Adult *C. melastomae* pairs, one female and one male, were placed on different species of Melastomataceae and Myrtaceae to test host specificity under no-choice conditions. Because of the small number of adults available, a sequential no-choice design, alternating week-long exposures to a random sequence of test plants with a week on *M. calvescens* controls, was used to permit testing multiple host plants with the same individual weevils. The long life span of *C. melastomae* (adults live several months) makes it particularly amenable to this method. A 1-wk test interval was chosen to avoid fatal starvation of individuals on unacceptable test species.

Test plants included the Hawaiian biotype of *M. calvescens* (grown from seeds at UCR), *M. astroplocama* Donn. Sm., *M. theizans* (Bonpl.) Cogn., *Clidemia hirta* (L.) D. Don., *C. setosa* (Triana) Gleason, *Conostegia xalapensis* (Bonpl.) D. Don., *Ossaea quinquenervia* (Mill.) Cogn. *Tococa platyphylla* Benth. from the Melastomataceae, and *Eugenia truncata* O. Berg and *Psidium guajava* L. from the Myrtaceae. Three replicates were used for each plant species except *M. astroplocama*, with four; 31 plants of the Costa Rican biotype of *M. calvescens* were used as control plants. Most tests were conducted on plants collected from field sites and maintained in pots at the UCR greenhouse; however, tests with Myrtaceae and a few with *Miconia* spp. used larger plants growing on the UCR campus or at field sites. Weevils were exposed to stem ends 20–40 cm in length and 3–15 mm in diameter, including the upper two leaves, inside cylindrical nylon mesh sleeves (45 cm long × 20 cm diameter). Foliage was checked and preexisting damage was noted at the beginning of each test.

After each 7 d exposure on test plants or *M. calvescens* controls, weevil pairs were moved to new plants, and feeding damage and oviposition on the previously occupied plant were evaluated. Area of feeding holes on stems, petioles, and veins was quantified using a clear plastic gauge with predrawn circles of known area. Feeding damage on lamina, which resulted in irregular holes, was measured using WinFolia 2003d Basic (Regent Instruments Inc. 2003) on digital photographs of pressed leaves. Plants were visually examined for eggs, with the aid of a dissecting microscope in the case of some hairy species, and location of each egg on the plant (stem, petiole, vein, leaf abscission scar) was recorded.

Tests were conducted with a total of 14 *C. melastomae* pairs from 1 July to 5 August 2005, 17 February to 21 July 2006, and 6 November to 4 December 2006. Total feeding damage was evaluated using a mixed model analysis of variance (ANOVA) with planned contrasts (SAS Institute Inc. 2007) between *M. calvescens* biotypes, *Miconia* species, melastome species, and between melastomes and nonmelastomes. Feeding damage per plant structure and total eggs laid per structure were evaluated using the Kruskal-Wallis function of Minitab Student 12 (Minitab Inc. 1998) to determine preferences in feeding and oviposition sites on *M. calvescens.*

Results

Distribution and Habitat Requirements. The original description (Champion 1906) was based on individuals from Panama, Costa Rica, Nicaragua, and Guatemala. This study increased the number of records and localities of *C. melastomae* from these countries, and extended the known distribution north to central Mexico and south to southern Ecuador (Fig. 1).

C. melastomae occurs at sites with elevations between 50–1500 m, which receive 2,000–5,500 mm of precipitation, and are within 11 of the Holdridge life zones (Table 1). In Costa Rica, *C. melastomae* habitat completely overlaps that of *M. calvescens* and continues into lower, drier areas in another seven life zones (Table 1).

Comparison of the environmental conditions of *C. melastomae* in Costa Rica to those present in the *Miconia*-infested areas of Hawaii, Maui, Kauai, and Oahu suggested that *C. melastomae* is adapted to Hawaiian ranges of elevation, precipitation, and life zones (Table 2).
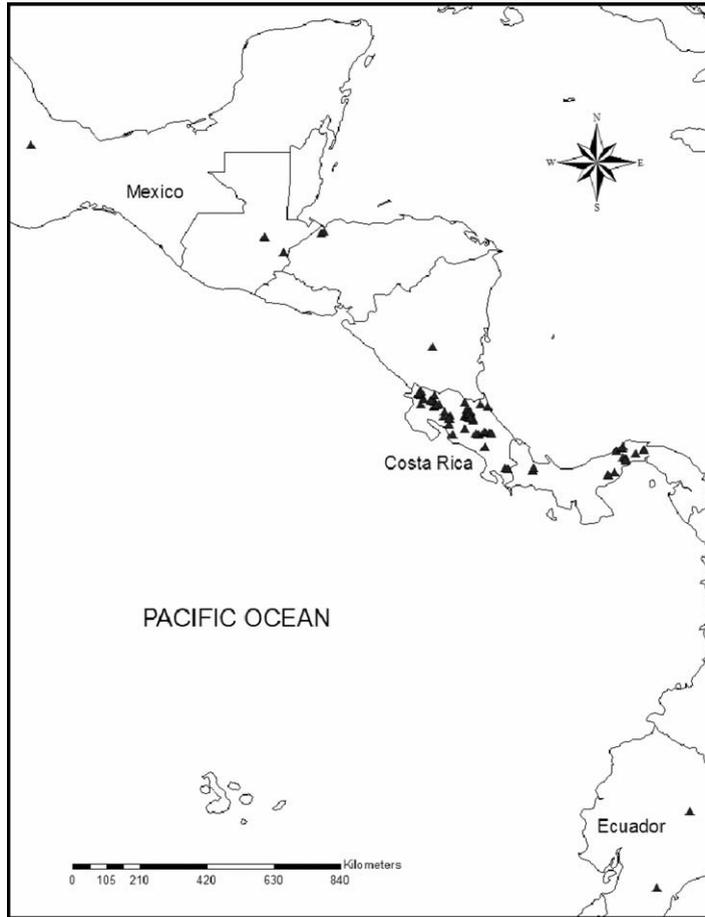
Fig. 1. Known distribution (triangles) of *C. melastomae* Champion based on 317 specimens examined.

Field Biology. All collections were made during the morning to early afternoon in both clear and rainy conditions. Fresh adult feeding damage was found on the new stem, petiole, leaf bud, vein, and lamina tissue of trees no taller than 2–3 m and 1–8 cm in diameter. Taller trees had old brown feeding marks on lower older stems, petioles and leaves. Evidence of larvae (small holes in the stem for breathing and ejecting

Table 1. Elevation range, mean annual precipitation, and Holdridge life zones for areas occupied by *Miconia calvescens* and *Cryptorhynchus melastomae* in Costa Rica

Parameter	*Miconia calvescens*[a]	*Cryptorhynchus melastomae*
Elevation (m)	550–1,350	50–1,500
Precipitation (mm)	3,000–5,000	2,000–5,500
Life zones[b]	Premontane[c] wet forest	Premontane wet forest
	Lower montane rain forest	Lower montane rain forest
	Wet tropical forest	Wet tropical forest
	Premontane rain forest	Premontane rain forest
		Tropical wet forest, Premontane transition
		Tropical wet forest
		Tropical moist forest, Premontane transition
		Premontane moist forest
		Premontane wet forest, basal belt transition
		Tropical moist forest, Premontane transition
		Premontane wet forest, rain transition
		Premontane moist forest, basal belt transition

[a] E. Chacôn (unpublished data).
[b] (ATLAS Costa Rica 2000 Project, undated).
[c] "Premontane" in this life zone classification is equivalent to "Subtropical basal" in Table 2.

Table 2. Elevation range, mean annual precipitation, and Holdridge life zones present in *Miconia calvescens* infested areas of Hawaii

Parameter	Hawaii	Maui	Kauai	Oahu
Elevation (m)	0–900	0–600	0–300	0–450
Precipitation (mm)	3,000–6,000	750–6,000	1,500–2,000	1,500–4,000
Life zones[a]				
Subtropical basal dry forest		X		
Subtropical basal moist forest	X	X	X	X
Subtropical basal wet forest	X	X		X
Subtropical wet basal rain forest	X	X		

[a] (Institute of Pacific Islands Forestry 2006).

frass) was found in trees both shorter and taller than 2 m; however, in trees taller than 2 m these holes were in side branches and not the trunk.

Collections at the three main field sites yielded a total of 84 adults (45 females and 39 males), five eggs and 22 larvae or pupae over nearly 2 yr of collecting. All adults found were stationary, apparently inactive during the day. Adults were only found on trees of 2.5 m or less in height. Solitary males and females were found resting between young apical *M. calvescens* leaves, at petiole/stem junctions, on or under leaves of varying sizes and within folded leaves and debris on the plant. In a few cases male-female pairs were found, never copulating, but rather resting male on top of female.

All adults from the El Angel and Turrialba field sites were found on *M. calvescens* whereas all adults from the San Ramon field site were found on *M. theizans* and *Conostegia micrantha* Cogn. (Melastomataceae). In each case only one individual was found per plant, except where a male–female pair was discovered. All individuals were out of direct sunlight.

Larvae were found mining the stems of *M. calvescens* at all field sites, as well as *M. theizans* at the San Ramon field site. Adult weevils from all sites were confirmed as *C. melastomae* through examination of external morphology and male genitalia.

In two instances large exit holes were found in the base of two *M. calvescens* sapling stems. After laboratory dissection, one healthy pupa and one healthy adult were found. Three other stems of *M. theizans* from San Ramon containing large emergence holes were also dissected. Each of these stems included a parasitized late instar larva. These three individuals were placed in small glass vials plugged with moist cotton. Solitary adult parasitoids emerged from two larvae, and another died after partial emergence from the third larva. These parasitoids were identified as an undescribed species of *Capitonius* Brullé (Hymenoptera: Braconidae).

Eggs were found on *M. calvescens* at El Angel and *M. theizans* at San Ramon. However, none of the five eggs brought back to the lab eclosed, and none showed evidence of parasitism.

Greenhouse Rearing and Biological Observations. From 17 May 2005 through 15 December 2005 the greenhouse rearing colony produced 229 eggs, 45 larvae, 18 pupae, and 8 adults. The average duration of each life stage was 27 ± 1.34 d as an egg ($n = 42$), 136 ± 10.96 d as a larva ($n = 6$), 18 ± 3.26 d as a pupa ($n =$

6) and 217 d as an adult ($n = 1$). Longevity of additional reared adults was not determined, because the study ended before they died.

Eggs were yellowish-white and oval (Fig. 2a). Eggs from the El Angel colony (1.16 ± 0.01 mm long; 0.88 ± 0.01 mm wide, $n = 63$) were noticeably smaller than those from the larger adults of the San Ramon colony (1.27 ± 0.11 mm long; 1.01 ± 0.09 mm wide, $n = 10$). No eggs were recovered from the Turrialba colony.

Larvae began mining the stem after eclosion. Larvae traveled in both directions inside the stem continuously hollowing out its center until pupation (Fig. 2b). In many small saplings, mining led to leaf drop and eventually plant death. Larvae ranged from 2 to 11 mm in length. As larvae grew, the diameter of the feeding tunnel inside the stem grew proportionally. An attempt to determine number of instars from head capsule measurements was unsuccessful because larvae tended to suffer high mortality after handling.

Pupation occurred inside the stem (Fig. 2c). A large exit hole (5.3 ± 0.2 mm high and 4.8 ± 0.2 mm wide, $n = 10$) was partially (leaving only the thin cuticle) or fully chewed in the stem by the larva (Fig. 2d) in preparation for pupation and eventual adult emergence (Fig. 2e). The larva created a pupation chamber (33.6 ± 4.1 mm above the exit hole, $n = 10$) from material shredded from inside the stem.

Newly emerged females ($n = 2$) fed on young *M. calvescens* leaves within hours of emergence and first oviposition occurred after 32 d. Adults fed in the same manner as in the field, consuming new stems, petioles, leaf veins, and lamina. Adults also caused leaf drop and even plant death in smaller saplings (Fig. 3).

Adults were primarily nocturnal, active between the hours of sunset and sunrise (1800–0600 h). In a few instances individuals were seen feeding and even copulating during the day, however these instances were rare and lasted only a very short period of time. From dusk until dawn adults were observed roaming, feeding, copulating, and ovipositing. Observed feeding events lasted between 2–38 min, with an average of 14.5 ± 2.8 min for females ($n = 14$) and 13.7 ± 2.4 min for males ($n = 9$). Individuals fed on previously undamaged areas on the leaf/vein/stem; however, in a few instances individuals began feeding on the edges of previous feeding holes. Females often fed with the male resting on her back.

Copulation was seen, and in a few instances heard, at night. Copulation began with the male climbing onto the back of the female and hooking his hind tibia
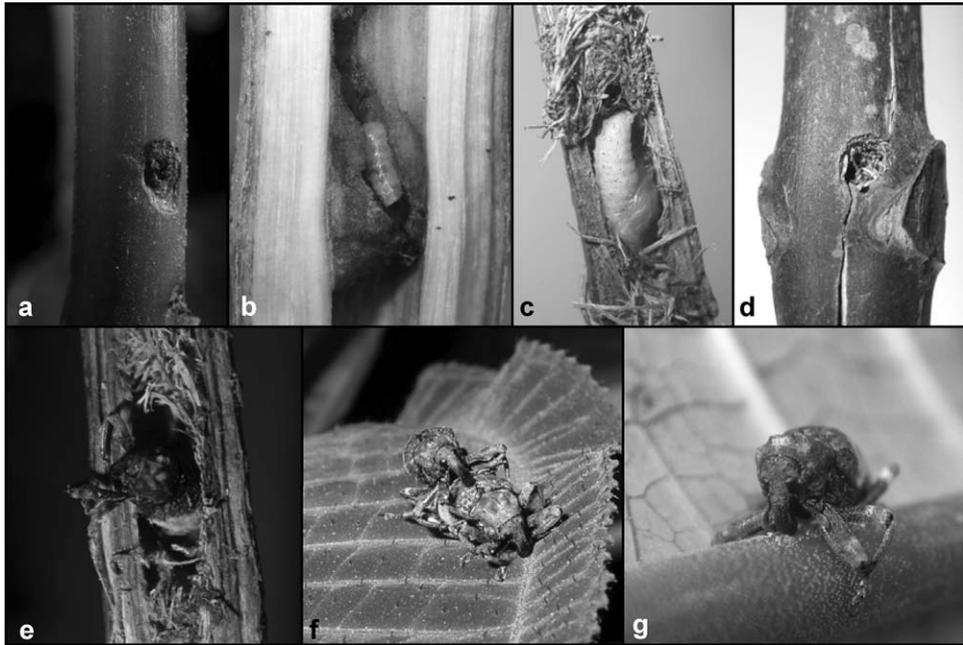
Fig. 2.   Life cycle of *C. melastomae:* (a) egg in stem and covered in frass and exudates, (b) larva mining the stem, (c) pupa in stem chamber, (d) emergence hole after adult exit, (e) adult in dissected pupal chamber, (f) copulation, and (g) excavating oviposition cavity.

under the tip of the female's elytra. He then slid his middle legs into a groove in the female's elytra just above the middle pair of legs. He patted her humeri with his front legs and then his antenna before lowering himself backward for copulation (Fig. 2f). Females often stridulate when handled and in a few instances the female was heard stridulating during copulation.

Oviposition was seen on three occasions between 1900 and 2300 h. The female excavated a small hole in the stem with her rostrum (Fig. 2g) and deposited a single egg into it. She then used her abdomen to cover the egg with frass and exudate. The time to dig the hole

and cover the egg averaged 28 ± 4 min ($n = 3$). The first female monitored from eclosion laid 59 eggs in 29 wk (2.0 ± 0.4 eggs per week) before dying. The second female had laid 39 eggs and was still alive after 18 wk (1.7 ± 0.5 eggs per week), when observations stopped.

Host Specificity Testing: Adult Feeding and Oviposition. Feeding was observed on all melastome species offered under no-choice conditions (Fig. 4), but significantly less feeding was observed on two nonmelastome (Myrtaceae) species ($F = 11.54$; df $= 1,19$; $P = 0.003$). No feeding occurred on *Eugenia truncata,* and only very light feeding on leaves of *Psidium guajava.*
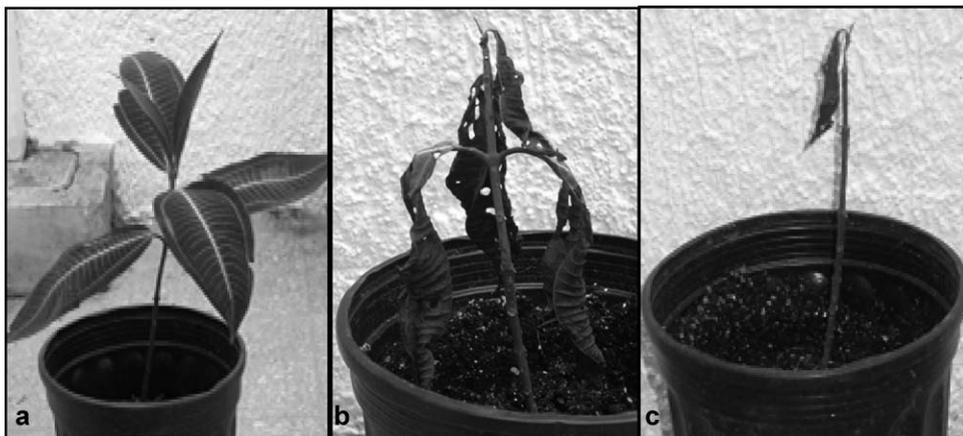

Fig. 3.   Damage to *M. calvescens* sapling after (a) 0, (b) 1, and (c) 2 wk of feeding by a pair of *C. melastomae* adults.
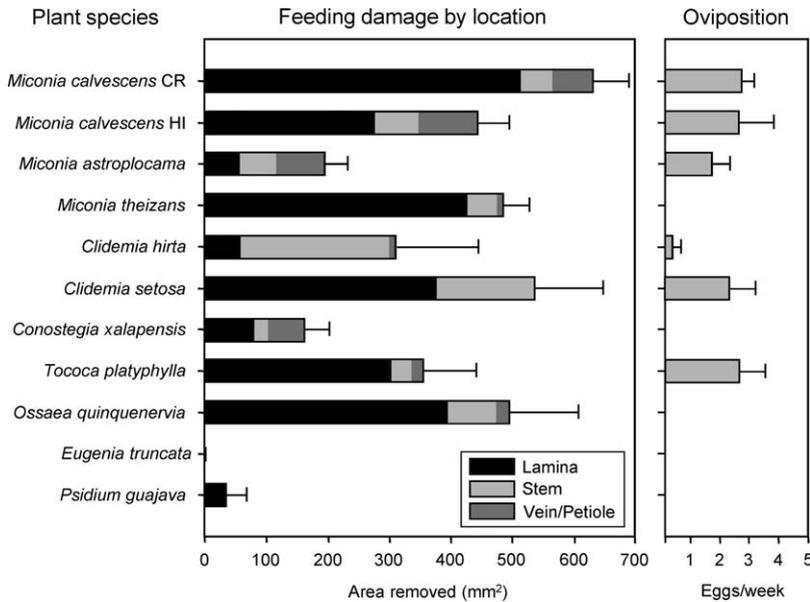
Fig. 4. Feeding (left) and oviposition (right) of *C. melastomae* on eight species of Melastomataceae and two Myrtaceae. Two populations of *M. calvescens* tested: CR, Costa Rica; HI, Hawaii.

Feeding damage was significantly greater on *M. calvescens* than on other *Miconia* species ($F = 5.40$; df = 1,19; $P = 0.03$), but feeding on *Miconia* species overall did not differ significantly from other melastomes ($F = 0.41$; df = 1,19; $P = 0.44$). Differences in damage on Costa Rican and Hawaiian biotypes of *M. calvescens* were not significant ($F = 0.19$; df = 1,19; $P = 0.67$).

Females laid multiple eggs on the Costa Rican and Hawaiian biotypes of *M. calvescens*, *M. astroplocama*, *C. setosa*, and *T. platyphylla* (Fig. 4). One egg was laid on *C. hirta* in three tests. There was no oviposition on other species. Although adults in this experiment (mostly collected from *M. calvescens* at the El Angel site) did not oviposit on *M. theizans*, adults at San Ramon appeared to do so continuously in nature. Larvae developed within all species on which oviposition occurred, but development to pupation was not confirmed.

Although *C. melastomae* adults fed on various parts of young *M. calvescens*, feeding was greatest on the leaf lamina (78% of damage in area) versus stems (11%) and veins/petioles (11%) (Kruskal-Wallis: $H = 72.39$; $P < 0.001$). Oviposition occurred in various locations on *M. calvescens*, but females preferred to deposit eggs in the stem internode (Kruskal-Wallis: $H = 26.87$; $P < 0.001$). Of 92 eggs on *M. calvescens*, 55% were in stem internodes, followed by leaf abscission scars (18%), leaf veins/petioles (15%), apical leaf buds (7%), and stem nodes (4%).

Discussion

The potential success of a classical weed biological control program depends on accurate knowledge of the biology and life history of the natural enemy being considered, as well as its interactions with the target host and with other organisms (e.g., Hokkanen 1985). That baseline knowledge was the main focus of this study.

Distribution, Biology, and Rearing. *C. melastomae* is distributed from Mexico to Ecuador. The environmental conditions that *C. melastomae* would be subjected to in Hawaii are similar to the conditions under which it lives in Costa Rica. This is important as unfavorable environmental conditions are a common cause for establishment failure (Hokkanen 1985, Wapshere et al. 1989). Further quarantine testing in Hawaii will be needed to determine if *C. melastomae* will survive, reproduce and thrive in Hawaiian forest ecosystems. Because *C. melastomae* is not abundant in Costa Rica and because it would be advantageous to release large numbers of individuals at each introduction site, further studies are required on its reproductive capacity, dispersal ability, and host finding ability.

C. melastomae appears to have an extended life cycle with overlapping generations. Our fecundity tests were inconclusive because of small sample size, but weevils survived and reproduced for several months in captivity. More recent data from *C. melastomae* reared in quarantine in Hawaii have demonstrated that average fecundity (150 eggs per female; M. T. Johnson, unpublished data) is only moderately lower than that of other weevil species considered as successful biocontrol agents, such as *Mogulones cruciger* (Ceutorhynchinae) on houndstongue, *Cynoglossum officinale* L. (192 eggs per female) (Schwarzlaender 1997) or *Microlarinus lareynii* (Jacquilin du Val) (Lixinae) (193 eggs per female) (Kirkland and Goeden 1978a) and *M. lypriformis* (Wollaston) (246 eggs per female) (Kirkland and Goeden 1978b) on punc-

turevine, *Tribulus terrestris* L. Overlapping generations would mean that *C. melastomae* attack the weed from the inside (larvae) and outside (adults) at the same time, creating greater potential for effective control.

C. melastomae can be reared successfully under greenhouse conditions. Compared with the long life cycle of this weevil, our attempts to develop a rearing program were underway for a relatively brief period. Despite this, the rearing protocol produced 229 eggs, 45 larvae, 18 pupae, and 8 adults during the duration of this study and demonstrated potential for building up colony numbers. Trees as small as 48.5 cm in height and 11.1 mm in diameter were used successfully to rear adults, eliminating disturbance and stress associated with transfer of larvae. Rearing large numbers of weevils within containment facilities or in field nurseries for large-scale releases appeared entirely feasible.

Feeding and Oviposition. Adults and larvae of *C. melastomae* feed on *M. calvescens*, and all life stages occur on the plant in Costa Rica. Feeding by adults and/or larvae can cause plant injury and death, and larval breathing holes and adult emergence holes in the stem are a potential entrance site for pathogens.

Adult specificity tests indicated that *C. melastomae* oviposit (and larvae develop) on other melastome species (Hawaiian biotype of *M. calvescens*, *M. astroplocama*, *C. hirta*, *C. setosa*, and *Tococa platyphylla*) and feed on other melastome species (all melastomes offered). Thus, this insect should be classified as a melastome-generalist.

A number of tribes and subfamilies of weevils are usually (though not always) host specific or oligophagous (O'Brien 1995) and this characteristic makes them potentially useful in biological control programs. The potential for oligophagous *C. melastomae* to oviposit and feed on other melastome species is not particularly worrisome in this case because there are no native Melastomataceae in Hawaii. A generalist melastome-feeder may even be encouraged in Hawaii, as many of the state's most problematic weeds are in this family (Wester and Wood 1977, Smith 2002).

Although no eggs were laid on the two species of Myrtaceae offered, in one instance a small amount of feeding occurred on *Psidium guajava* (guava) under no-choice conditions. Further testing of guava, which is grown commercially in Hawaii, and other nontarget Myrtaceae species are needed. Starvation no-choice tests, in which adults are left on single plant species such as *P. guajava* for a longer period of time, will be useful for further elucidating host range. Single-choice and multiple-choice experiments should also be performed to determine host plant preferences.

A Complex of Natural Enemies for *M. calvescens*. Since 2002 scientists at UCR and the Federal University of Viçosa (Brazil) have been evaluating several potential biological control agents for *M. calvescens*. The goal is to develop a suite of host-specific enemies that significantly impact *M. calvescens* in a variety of ways. Although cumulative impacts of multiple agents appears to be unimportant in most successful weed biocontrol programs (Denoth et al. 2002), the aggressive spread and growth of woody *M. calvescens* may constitute the unusual case where multiple agents are needed.

To date only one biocontrol agent has been introduced to Hawaii for *M. calvescens*. The host-specific fungus *Colletotrichum gloeosporioides* f. sp. *miconiae* (Phyllachorales: Phyllachoraceae) was isolated from *M. calvescens* in Brazil, where it caused leaf necrosis leading to premature leaf drop and retarded growth and development, and released in Hawaii in 1997 (Killgore et al. 1998, 1999). It has begun to spread and is having a visible impact on at least one of the major infestations there (Smith 2002).

Other possible control agents include the gregarious caterpillar, *Euselasia chrysippe* (Bates) (Lepidoptera: Riodinidae), whose feeding causes considerable leaf damage (Allen 2007, Nishida 2010); the fungal pathogen, *Coccodiella miconiae* (Duby) Hino and Katuamoto (Phyllachorales: Phyllachoraceae), that causes black pimple, a disease leading to leaf deformation and premature drop (Killgore 2002); *Ditylenchus* sp. (Tylenchida: Anguinidae), a nematode that causes galling of leaves and shoots of *M. calvescens* and other melastomes (Barreto et al. 2005); the fruit-feeding weevil, *Anthonomus monostigma* Champion (Coleoptera: Curculionidae), that causes premature fruit drop and appears to reduce seed germination (Chacón 2007); a leaf rolling pyralid moth, *Salbia lotanalis* (Lepidoptera: Pyralidae), that can cause 50% defoliation (Picanço et al. 2005); a leaf rasping sawfly, *Atomacera petroa* (Hymenoptera: Argidae); and a fruit feeding moth, *Mompha* sp. (Lepidoptera: Coleophoridae) (Badenes-Perez et al. 2008).

Role of *C. melastomae* in *M. calvescens* Control. *C. melastomae* is complementary to the assemblage of natural enemies of *M. calvescens* discussed above. Unlike the other agents being considered, *C. melastomae* is a larval stem borer. Previous successful biocontrol projects have shown that herbivores that destroy the vascular or mechanical support tissues and those that prevent seed production (for annual weeds) have the greatest potential as weed biocontrol agents, while sap-feeders and defoliators show less potential and leaf miners/gall-formers possess the lowest potential (Harris 1973, Goeden 1983). Those that attack the weed in a combination of ways *may* be among the most sought-after (Hokkanen 1985). In addition to its stem-boring larvae, *C. melastomae* adults also feed on stem tissue, petioles, and leaf veins and lamina, contributing to its desirability as a candidate for control programs.

A sapling feeder would be a valuable addition to the current management strategy for *M. calvescens* in Hawaii. When *M. calvescens* trees are physically removed or sprayed aerially the resulting openings in the canopy lead to a flush in seed bank germination (Loope 1996). Incorporating the release of a sapling-damaging biocontrol agent at this time might substantially reduce the number of saplings and, in turn, decrease the number of trees that survive to reproduce. *C. melastomae* showed a preference for young trees at Costa Rican field sites, and its detrimental effects on these

smaller plants demonstrated its potential usefulness in this situation.

If released into Hawaii, *C. melastomae*, like any biocontrol agent, would interact with other organisms in the novel ecosystem. Problems often arise in weed biocontrol programs when parasites, predators and pathogens attack the introduced agent to such an extent that it fails to establish or becomes incapable of building effective populations (Hokkanen 1985). Thus far, the only known natural enemy of *C. melastomae* is a species of the braconid genus *Capitonius. Capitonius* species attack Coleoptera larvae (Buprestidae, Cerambycidae, Curculionoidea) embedded in plant tissue; however, unlike many other parasitoids of weevils, members of this genus are solitary endoparasitic koinobionts, which keep their host alive for some time before emergence (Shaw 2006). The genus has not been recorded from Hawaii and thus would not present a problem in Hawaii if care is taken to prevent its importation along with *C. melastomae.*

C. melastomae appears worth pursuing as a classical biological control agent for *M. calvescens* in Hawaii for several reasons: its environmental requirements appear to be met in *M. calvescens*-infested areas of Hawaii; it can be reared under greenhouse conditions; its host range may be restricted to species of Melastomataceae, all of which are alien and often invasive in Hawaii; and it causes damage leading to mortality of *M. calvescens* saplings.

Acknowledgments

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