

# Field Release of *Syphraea uberabensis* (Coleoptera: Chrysomelidae) for the Biological Control of Cane Tibouchina, *Tibouchina herbacea,* (Melastomataceae), in Hawaii

Final Environmental Assessment, April 2023

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### I. Purpose and Need for the Proposed Action

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Pests, Pathogens, and Biocontrol Permits (PPBP) is proposing to issue permits for release of the flea beetle *Syphraea uberabensis* (Coleoptera: Chrysomelidae). This beetle would be used by a permit applicant for classical biological control (biocontrol) of cane tibouchina, *Tibouchina herbacea* (Melastomataceae) in the State of Hawaii.

Classical biological control of weeds is a control method where natural enemies from a foreign country are used to reduce exotic weeds that have become established in the United States. Several different kinds of organisms have been used as biological control agents of weeds: insects, mites, nematodes, and plant pathogens. Efforts to study and release an organism for classical biological control of weeds consist of the following steps (TAG, 2021):

- 1. Foreign exploration in the weed's area of origin.
- 2. Host specificity studies.
- 3. Approval of the exotic agent by PPBP.
- 4. Release and establishment in areas of the United States invaded by the target weed.
- 5. Post-release monitoring.

APHIS has the authority to regulate biological control organisms under the Plant Protection Act of 2000 (Title IV of Pub. L. 106–224). Applicants who wish to study and release biological control organisms into the United States must receive PPQ Form 526 permits for such activities. The PPBP received a permit application requesting environmental release of the flea beetle *S. uberabensis* from Brazil, and the PPBP is proposing to issue permits for this action. Before permits are issued, the PPBP must analyze the potential impacts of the release of this agent into the State of Hawaii.

This environmental assessment (EA) has been prepared, consistent with USDA, APHIS' National Environmental Policy Act of 1969 (NEPA) implementing procedures (Title 7 of the Code of Federal Regulations (CFR), part 372). It examines the potential effects on the quality of the human environment that may be associated with the release of *S. uberabensis* to control infestations of cane tibouchina in the State of Hawaii. This EA considers the potential effects of the proposed action and its alternatives, including no action. Notice of this EA was made available in the Honolulu Star-Advertiser on January 23 and January 24, 2023 for a 30-day public comment period. APHIS received two comments on the EA by the close of that comment period. Both comments were in support of the release of *S. uberabensis* in Hawaii.

The following information in this EA regarding cane tibouchina, *S. uberabensis*, and the host specificity testing conducted is from information submitted by M.T. Johnson, U.S. Forest Service (USFS), Pacific Southwest Research Station (Johnson, undated), and a draft environmental assessment prepared by Garcia and Associates (2019) for Hawaii Division of Forestry and Wildlife.

The permit applicant's purpose for releasing *S. uberabensis* is to reduce the severity of damage of cane tibouchina in the family Melastomataceae in Hawaii. Cane tibouchina (*Tibouchina herbacea*) is a noxious weed native to Southern Brazil, Uruguay, and Paraguay. In Hawaii, it naturalized and is locally abundant in disturbed mesic to wet forest on the islands of Hawaii, Lanai, Maui, Molokai, and Oahu. It can invade native forest through abundant production of tiny, easily dispersed seeds. Once established it forms dense stands and displaces native vegetation.

Control of this plant with herbicides and mechanical methods is expensive and is not effective in the long term. In addition, cane tibouchina, as well as other invasive plants in the family Melastomataceae (melastomes), have invaded remote areas that are difficult to access using conventional control methods. Therefore, there is a need to identify and release an effective, host-specific biological control organism against cane tibouchina in Hawaii.

### II. Alternatives

This section will explain the two alternatives available to PPBP: no action (no issuance of permits) and issuance of permits for environmental release of *S. uberabensis* into Hawaii. Although APHIS' alternatives are limited to a decision of whether to issue permits for release of *S. uberabensis*, we describe other methods currently used to control cane tibouchina in Hawaii. Use of these control methods is not an APHIS decision, and their use is likely to continue whether or not PPBP issues permits for environmental release of *S. uberabensis*.

The PPBP considered a third alternative but will not analyze it further. Under this third alternative, PPBP would issue permits for the field release of *S. uberabensis*. The permits, however, would contain special provisions or requirements concerning release procedures or mitigating measures, such as limited releases of *S. uberabensis* in Hawaii. There are no issues raised indicating that special provisions or requirements are necessary.

### A. No Action

Under the no action alternative, the PPBP would not issue permits for the field release of *S*. *uberabensis* for the control of cane tibouchina in Hawaii — the release of this biological control

agent would not occur, and current methods to control cane tibouchina in Hawaii will continue at current levels. Use of these methods is likely to continue even if PPBP issues permits for release of *S. uberabensis*, depending on the efficacy of the organism to control cane tibouchina. Presently, control of cane tibouchina in Hawaii is limited to chemical control using herbicides, mechanical control, and cultural control methods.

### 1. Chemical Control

Various herbicides have been reported to control cane tibouchina. These include: 1) application of undiluted triclopyr ester to the stem base; 2) triclopyr amine in foliar sprays and in cut-stump treatments; 3) glyphosate foliar application. Based on work with other plants in the family Melastomataceae (melastomes), cane tibouchina is likely also sensitive to the herbicides 2,4-D, dicamba, and metsulfuron methyl (Motooka et al., 2003; Loh et al., 2014).

### 2. Mechanical Control

Cane tibouchina seedlings can be controlled by hand pulling (Buddenhagen, 2022).

### 3. Cultural Control

Sanitation practices are practiced by conservation workers in Hawaii and involve the use of sitespecific camping and hiking gear in the most pristine sites, and cleaning of boots to prevent moving cane tibouchina seeds into new locations (Buddenhagen, 2022).

### B. Issue Permits for Environmental Release of Syphraea uberabensis

Under this alternative, PPBP would issue permits for the field release of *S. uberabensis* for the control of cane tibouchina in Hawaii. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures. *Syphraea uberabensis* is specific to cane tibouchina and a subset of invasive plant species within the melastome family, which contains no native species in Hawaii.

### 1. Syphraea uberabensis Taxonomic Information

Order: Coleoptera Family: Chrysomelidae Genus: Syphraea Species: Syphraea uberabensis Bechyné (1955) Common name: none *Syphraea uberabensis* is the insect that is proposed for release for biocontrol of *T. herbacea* and related weeds in Hawaii. *Syphraea uberabensis* is a small beetle that has been evaluated in its native Brazil between 1993 and 2009 and in containment in Hawaii since 2005. Adults and larvae feed externally on foliage and soft stems of *T. herbacea.*, causing enough damage to kill small plants. *Syphraea uberabensis* is host specific to a subset of species within the melastome family, which contains no native members in Hawaii.

**Taxonomy**: *Syphraea uberabensis* Bechyné (1955) is a flea beetle, classified under the tribe Alticini and the leaf beetle family Chrysomelidae. Flea beetles are similar to other leaf beetles but are characterized by having enlarged hind legs, which afford them the ability to leap/spring when disturbed, hence the common name. Flea beetles are herbivores that feed on various parts of the plant; some flea beetle species are important agricultural pests. They do not bite humans or animals. The genus *Syphraea* Baly (1876) includes more than 100 species and is found throughout South and Central America (Scherer, 1983).

**Description**: Adults: Adult beetles are deep metallic blue,  $2.8 \pm 0.10$  millimeters (mm) long and  $1.5 \pm 0.03$  mm wide. Larvae (immature stage): The length of a mature larva is 4.4–6.30 mm. The body is cream/yellowish with a brown head (Casari and Teixeira, 2011).

**Biology:** A life history study conducted in the quarantine facility in Hawaii showed that *S*. *uberabensis* reared on cane tibouchina have an adult life span ranging from 2 days to 127 days and averaged 78.2 days. *Syphraea uberabensis* samples of the quarantine colony had a sex ratio close to 1:1. Males and females developed and emerged at similar rates (Souder, 2008).

Survival and development of *S. uberabensis* was studied in the laboratory at five constant temperatures ranging from 12 to 28 °C. No egg or larval development occurred below 16 °C. Complete development to adulthood was only seen at 20 and 24°C. Mean time for development from egg to adult was 50.5 days at 20 °C and 31.5 days at 24 °C, fitting the expected pattern for insects in general: faster development at increasing temperatures. Although development was slightly faster at 28 °C than at 24 °C, flea beetle survivorship was reduced, and no adults developed at 28 °C (Souder, 2008).

**Habitat:** *Syphraea uberabensis* is tolerant of cool and moderate temperatures and is not expected to be restricted in range by temperatures in Hawaii, except perhaps in exceptionally warm habitats. (Souder, 2008). However, the potential of *S. uberabensis* as a biological control could be limited by humidity at the microhabitat level. In Brazil, *S. uberabensis* is found with its melastome hosts in boggy soils, similar to the areas where *Tibouchina* and *Pterolepis* species thrive in Hawaii. On the other hand, *Melastoma* in Hawaii can grow in relatively drier areas, such as young lava flows.

*Syphraea uberabensis* could be less effective against *Melastoma* in the drier parts of its range, because externally feeding larvae appear to be susceptible to drying (Raboin et al., 2009).

### 3. Geographic Range of S. uberabensis

*Syphraea uberabensis* is native to southern Brazil. The distributional range of the species is not well studied.

### 4. Potential Range of S. uberabensis in Hawaii

*Syphraea uberabensis* is tolerant of cool and moderate temperatures and is not expected to be restricted in range by temperatures in Hawaii, except perhaps in exceptionally warm habitats (Souder, 2008). However, the potential of *S. uberabensis* as a biological control could be limited by humidity at the microhabitat level. In Brazil, *S. uberabensis* is found with its melastome hosts in boggy soils, similar to the areas where the melastomes *Tibouchina and Pterolepis* species thrive in Hawaii. On the other hand, *Melastoma* species in Hawaii can grow in relatively drier areas – such as young lava flows. *Syphraea uberabensis* could be less effective against *Melastoma* species in drier habitats because its eggs and larvae appear to be susceptible to drying when humidity is not high.

### 5. Impact of S. uberabensis on Cane Tibouchina

*Syphraea uberabensis* causes extensive damage to cane tibouchina. Both larvae and adults feed on the leaves as well as the soft exterior of young stems (figs. 1 and 2). Cane tibouchina was not able to regrow well after attack by *S. uberabensis*, drying after a period of 2 weeks of insect feeding, both in the field and in the laboratory. The leaves were skeletonized, leaving only the stem and vein structures. Plant growth was reduced, and flowering and seed production were prevented (Wikler and Souza, 2008).



Figure 1. Syphraea uberabensis larvae feeding on cane tibouchina.



Figure 2. Syphraea uberabensis adults feeding on cane tibouchina.

### **III. Affected Environment**

### A. Cane tibouchina

### 1. Description and Taxonomic Information

#### Taxonomy

Phylum: Spermatophyta Subphylum: Angiospermae Class: Dicotyledonae Order: Myrtales Family: Melastomataceae Tribe: Melastomeae Genus: *Tibouchina* Species: *Tibouchina herbacea* (DC.) Cogn.

Synonyms: Arthrostemma herbacea DC.; Arthrostemma hirsutissimum DC.; Pterolepis herbacea (DC.) Triana)

*Tibouchina* Aubl. is a genus containing about 350 species ranging from Mexico, West Indies, to northern Argentina. The center of diversity is in southeastern Brazil. *Tibouchina* is classified in the tribe Melastomeae, which contains several related genera (e.g., *Arthrostemma, Dissotis, Melastoma*, and *Pterolepis*) that also have naturalized in Hawaii (Wagner et al., 1999).

**Description** Cane tibouchina is a semi-woody, upright shrub. Young stems are angled and hairy. Leaves are 3 inches long by 1.4 inches wide, hairy, and with five to seven prominent veins. Flowers are pink, with four petals and bright yellow anthers. The fruit is cuplike, small, 0.2 inches long by 0.2 inches wide. The seeds are very small and numerous (Motooka et al., 2003). Many of the hairs covering the leaves, stems, and fruits are gland-tipped, so that plants leave an oily, scented residue when touched. The growth form of cane tibouchina is very different between the populations in Brazil and Hawaii. In Brazil, it rarely grows above 1 meter (m) in height and dies back each year. In Hawaii, it can grow up to 3–4 m and the previous year's stems can survive the dormant period, forming rank sprawling stems from which new shoots arise the following year. It forms dense thickets that are difficult to traverse and smother adjacent vegetation, gradually increasing the size of the infestation (Almasi, 2000; Smith, 2002).

Cane tibouchina spreads by producing many seeds that are the size of fine sand, as well as vegetatively. Each multi-stemmed plant can produce hundreds of 5-mm wide seed capsules (fruiting hypanthia), with each capsule producing up to 700 seeds that fall or blow distances up to several meters (Almasi, 2000). The tiny seeds can be transported by birds, rats, pigs, water, and

human foot and vehicular traffic. Plants also can reproduce vegetatively by growing roots along leaf nodes or producing new shoots from rhizomes (Almasi, 2000). Rats and birds are claimed to be dispersers in Hawaii, despite the fact that the plant does not produce fleshy fruit (Almasi, 2000; Motooka et al., 2003). Feral swine likely spread the seeds and could possibly spread stem fragments, as areas disturbed by feral swine are often completely taken over by this plant (Buddenhagen, 2022).

### B. Areas Affected by Cane Tibouchina

### 1. Native and Worldwide Distribution

Cane tibouchina is native to South America, including Brazil, Argentina, Paraguay, and Uruguay.

### 2. Present Distribution in Hawaii

Cane tibouchina was introduced to Hawaii as an ornamental (Motooka et al., 2003) and was first collected in Hawaii Island in 1977. It subsequently colonized Maui by 1982. It is widely established on Hawaii and Maui and has been found on Lanai, Molokai, and Oahu (Wagner et al., 1999; Wysong et al., 2007; Imada, 2012).

### 3. Habitats Where Cane Tibouchina is Found in Hawaii

Cane tibouchina is found in swamps, meadows, and forests in its native range (Wagner et al., 1999). It naturalized in mesic and wet areas between 100 m and 1,600 m in Hawaii (SPREP, 2000). A habitat modeling study in Kohala Mountain indicates that cane tibouchina is most frequently found in partially shaded wet forests above 300 m and is positively associated with feral swine disturbance (Purell, 2006).

### C. Plants Related to Cane Tibouchina in Hawaii

### 1. Native and Non-Native Relatives

Information regarding plants taxonomically related to cane tibouchina is included because closely related plant species have the greatest potential for attack by *S. uberabensis* if it is released in Hawaii.

No plants in the family Melastomataceae are native to Hawaii, and nine of the 15 species naturalized in Hawaii have been declared state noxious weeds (Medeiros et al., 1997). Some of these species are discussed below.

#### Secondary Target Species: Related Weeds in Melastomataceae

During host specificity tests that are discussed later in this document, the researchers discovered that *S. uberabensis* fed and successfully developed and reproduced on several invasive melastomes that are suitable targets for the proposed release of *S. uberabensis* (Souder, 2008; Raboin et al., 2009). These include *Melastoma septemnervium, Melastoma sanguineum, Pterolepis glomerata*, and *Tibouchina longifolia*, all of which have invaded native wet forest habitats in Hawaii. *Melastoma septemnervium*, in particular, is widely distributed on Hawaii Island, where it has been recognized as a threat for many years (Jacobi and Warshauer, 1992). Each of these melastome species is likely to increase in population and expand in range in the absence of additional management attempts such as biocontrol by *S. uberabensis*.

#### Melastoma septemnervium Lour. - Asian melastome

**Taxonomy:** *Melastoma septemnervium* belongs to the tribe Melastomeae and the genus *Melastoma* L., which comprises 22 species centered in Southeast Asia and extending to India, South China, Japan, northern Australia, and Oceania. *Melastoma septemnervium* was previously known in Hawaii by the synonyms *Melastoma candidum* D. Don and *Melastoma malabathricum* auct. non L.: Sims.

**Description:** Shrubs or small trees 2–5 m tall; young branches are quadrangular, densely covered with appressed brown scales. Leaves are elliptic to ovate, 4–11 by 2–6 centimeters (cm), upper surface rough with bristly hairs, lower surface with fine hairs but also with scales on the principal veins of the leaves like those of the young branches. Inflorescences are two- to seven-flowered, petals are usually five, purple to pink, 2.5–3.2 cm long, 1.5–2.3 cm wide; fruit is a bell-shaped, 5-celled, fleshy capsule, 8–12 by 7–10 mm, densely covered with scales. (Wagner et al., 1999; Meyer, 2001).

**Distribution:** Native to northern Vietnam, southern China, and Taiwan (Meyer, 2001). In Hawaii, it is naturalized on Kauai (Wahiawa Bog), Oahu (Kalihi, Maunawili Valleys), and Hawaii Islands. One individual was found on the island of Maui in 2002 and removed (Penniman et al., 2011).

**Reproduction and Dispersal:** The fruit ruptures at maturity, exposing red-black pulp and yellow seeds (Meyer, 2001). Fruits are dispersed by birds (Smith, 1985).

**Impact:** *Melastoma septemnervium* was cultivated and is now naturalized in mesic to wet areas and bog margins from sea level to 700 m in Hawaii (Wagner et al., 1999). It forms dense stands up to 2 m tall shading out understory (Smith, 1985; Jacobi and Warshauer, 1992).

#### Melastoma sanguineum Sims - fox-tongued melastoma

**Taxonomy:** There are three recognized varieties of *M. sanguineum*: var. *sanguineum*, var. *laevifolium*, and var. *ranauense* (Meyer, 2001). *Melastoma sanguineum* var. *sanguineum* is known to hybridize with *M. candidum* in southeastern China (Liu et al., 2014).

**Description:** Shrubs or small trees 2–4 (up to 8) m tall; quadrangular young branches and petioles sparsely covered with spreading, smooth hairs 5–15 mm long; leaves lanceolate-elliptic, 10–20 cm long, 2–6 cm wide, surface rough or smooth; petiole 10–30 mm long, with red bristles, 5–9 mm long. Inflorescences two- to seven-flowered, petals usually 6, purplish pink, 2.5–4.7 cm long, 2.7–3.5 cm wide; Fruits bell-shaped, 6-celled, fleshy capsules, 8–19 by 8–18 mm, covered with spreading or incurved, basally flattened hairs. (Wagner et al. 1999; Meyer, 2001).

**Reproduction and Dispersal:** Like *M. septemnervium*, the fruit is a fleshy capsule which splits open exposing yellow pulp with orange seeds, which are bird-dispersed.

**Distribution:** In China, it occurs on open slopes, thickets, grasslands, woodland margins on low hills, trailside; below 400 m (Chen and Renner, 2007). In Hawaii, it was once cultivated and has naturalized since at least 1957, occurring on the Island of Hawaii in Keaukaha and along the highway between Volcano and Hilo. One individual was found on the island of Maui in 2004 and removed (Penniman et al., 2011).

**Impact:** Although *M. sanguineum* has not dispersed on the same scale as *M. septemnervium*, it is thought to have similar potential to form dense monotypic thickets and crowd out native vegetation (Penniman et al., 2011).

#### Pterolepis glomerata (Rottb.) Miq.- false meadowbeauty

**Taxonomy:** *Pterolepis* (DC.) Miq. consists of 15 taxa of herbs and small shrubs with center of diversity in Brazil (Renner, 1994; Almeda and Martins, 2015). Taxonomic treatment of the Hawaiian population of *P. glomerata* by Wagner et al. (1999) did not include sub-specific ranking, which the authors considered weakly defined. *Pterolepis* is closely related to the old world Melastomeae, which diverged around 11–12 million years ago (Renner and Meyer, 2001).

**Description:** Erect, basally woody herbs or subshrubs up to 0.5 m tall; young branches somewhat squared, with stiff hairs. Leaves ovate to elliptic, 1.4–4.5 cm long, 0.6–1.6 cm wide, both surfaces sparsely to moderately bristled, petioles 1–5 mm long. Flowers usually 3–5 in terminal tight

clusters; four petals white, pink or violet, 10–15 mm long, 10–14 mm wide; larger anthers pink, 3–4 mm long, smaller anthers yellow, 2.5–3.5 mm long. Fruiting hypanthium 4–6 mm long, 2–5 mm wide, covered with simple and branched hairs. Seeds ca. 0.5 mm long (Wagner et al., 1999).

**Distribution:** *Pterolepis glomerata* occurs from the Dominican Republic (Hispaniola) and Puerto Rico over the Lesser Antilles and Trinidad to Venezuela, the Guianas, and south to Santa Catarina in Brazil; reaching adjacent Paraguay and Bolivia (Renner, 1994; Wagner et al., 1999). In Hawaii, it naturalizes on Kauai, Oahu, Molokai, Lanai, and Hawaii Islands (Imada, 2012). It was first collected on Oahu in 1949 (Wagner et al., 1999).

**Reproduction and Dispersal:** *Pterolepis glomerata* reproduces by seeds and vegetative fragmentation. About 500 seeds can be found in a capsule. The seeds are dispersed by birds and water (Ramirez and Brito, 1998; Wagner et al., 1999).

**Habitat/Ecology:** In Hawaii, the species is not cultivated, but weedy and locally naturalized in mesic to wet disturbed sites and trail margins (Wagner et al., 1999). It is considered among the invasive plants that threaten many endangered plants on Oahu (FWS, 2012).

#### Tibouchina longifolia (Vahl) Baill. Ex Cogn.

**Taxonomy:** *Tibouchina longifolia* (Vahl) Baill. ex Cogn. (Synonyms: *Rhexia longifolia* Vahl.) belongs to the pantropical melastome family (Melastomataceae). *Tibouchina* Aubl. is a genus containing about 350 species ranging from Mexico, West Indies, to northern Argentina (Wagner et al., 1999). The center of diversity is in southeastern Brazil.

**Description:** *Tibouchina longifolia* is a weedy shrub 0.5–2 m tall. Leaves are narrowly elliptic to lanceolate with dense smooth hairs, 3.5–11.5 cm long and 1–3 cm wide. Flowers are white and approximately 0.5 inches in diameter with five petals 5–7 mm long and 2.5–4 mm wide. Anthers are 1.5–2 mm long, fruiting hypanthium is 4–4.5 mm long and 3–4 mm wide. Seeds are very small, typically 0.25–0.5 mm long (Wagner et al., 1999).

**Distribution:** *Tibouchina longifolia* is native to the Neotropics and widespread from Mexico and the West Indies to Bolivia and Brazil (Wagner et al., 1999). It was first collected in Hawaii in 1983 in the Puna District and is now established in the wild (Wagner et al., 1999).

**Reproduction and Dispersal:** In Hawaii, *T. longifolia* is now naturalized in native 'ōhi'a forests on Hawaii Island. It has been propagated by cuttings and cultivated by humans in the past, However, it is now recognized as a noxious weed. Mechanisms for natural dispersal are not

documented but are likely the same as for related species (USGS, 2003).

### **IV. Environmental Consequences**

### A. No Action

Under the no action alternative, *S. uberabensis* will not be released for biocontrol of cane tibouchina and other invasive melastomes in Hawaii. Control of the target weeds will be limited to mechanical, chemical, and cultural control methods.

### 1. Impact of Cane Tibouchina and Other Invasive Melastomes on the Environment

The main environmental consequence of the No Action alternative is continued degradation of native forests, which harbor large numbers of native plants and animals. Cane tibouchina and other invasive melastomes have altered the species composition in open and slightly open wet and mesic sites in Hawaii by becoming new dominant species there, especially in the remaining relatively intact high-altitude forests of the islands of Hawaii and Maui (Buddenhagen, 2022). It is likely to affect successional patterns of the dominant forest trees in mesic and wet habitats of Hawaii (Buddenhagen, 2022). Cane tibouchina can also clog waterways (Almasi, 2000).

### 2. Impact from the Use of Other Control Methods

The continued use of chemical, mechanical, and cultural controls at current levels in Hawaii would result if the "no action" alternative is chosen and may continue even if permits are issued for environmental release of *S. uberabensis* in Hawaii.

### a. Chemical Control

For infestations that are easily accessible and limited in size, chemical control can be a preferred method because it has the advantage of short response time and minimal initial resource investment required. However, for infestations in large areas or remote locations, chemical controls are infeasible or economically prohibitive, and likely will lead to continued population increase and range expansion of the target weeds.

### b. Mechanical Control

Similar to chemical control, hand-pulling can be effective for rapid removal of infestations that are small in size and easily accessible. However, for large areas and remote locations, this method is not effective because the sites are too difficult to access.

### c. Cultural Control

Sanitation practices to reduce movement of seeds and plant parts are useful to prevent new infestations but will not eliminate already-established infestations.

These impacts from the use of other control methods may have environmental consequences even with the implementation of the biological control alternative, depending on the efficacy of *S*. *uberabensis* to reduce cane tibouchina and other invasive melastome infestations in Hawaii.

### B. Issue Permits for Environmental Release of Syphraea uberabensis

### 1. Impact of S. uberabensis on Non-target Plants

Host specificity of *S. uberabensis* to cane tibouchina and invasive melastomes in Hawaii has been demonstrated through scientific literature and host range testing. If the candidate biological control agent only attacks one or a few plant species closely related to the target weed, it is considered to be very host specific. Host specificity is an essential trait for a biological control organism proposed for environmental release.

### a. Host Specificity Testing

Quarantine host range testing was conducted to determine the specificity of *S. uberabensis* to cane tibouchina and to determine if nontarget plants in Hawaii could be at risk of attack by *S. uberabensis*. Host specificity of *S. uberabensis* to cane tibouchina and certain invasive melastomes has been demonstrated through host specificity testing. If the candidate biological control agent only attacks one or a few plant species closely related to the target weed, it is considered to be very host-specific. Host specificity is an essential trait for a biological control organism proposed for environmental release.

#### Site of Quarantine Studies in Hawaii

*Syphraea uberabensis* eggs and larvae on cane tibouchina cuttings were shipped in July 2005 from Universidade Estadual Centro-Oeste in Irati, Parana State, Brazil to the Hawaii Volcanoes National Park Quarantine Facility (HVNPQF).

### Host Specificity Testing Methodology

The initial colony of S. uberabensis consisted of approximately 50 adult flea beetles. Cane

tibouchina cuttings collected around Glenwood and Volcano, Hawaii (700–1200 m) were used to maintain colony insects. In HVNPQF, the environmental conditions ranged from 18–24° C, 20–95 percent relative humidity (RH), with a natural photoperiod (approximately 12–12 hours light:dark). Flea beetles were reared on fresh leaf cuttings of *T. herbacea* over moistened paper towel in 150mm x 25mm circular petri dishes. The moistened towel maintained a level of humidity inside the petri dish that kept plant material from wilting. Each dish was filled with 30–40 newly emerged adults (roughly 1:1 sex ratio). Deteriorating and heavily damaged leaves were removed and replaced with new cuttings every other day, and each petri dish was changed completely approximately twice per week. When adults began to lay eggs, the egg bearing leaves were removed and recombined in equal proportions from different source dishes to maintain a diverse genetic pool. Larvae were reared on fresh leaf cuttings in large petri dishes, similar to adults. Large third instars were transferred to petri dishes with moistened vermiculite to simulate soil for pupation. Beetles completed a full generation cycle in approximately two months.

Potential host preferences were evaluated on a total of 58 plant species in 30 families. Test plants were selected based on the centrifugal phylogenetic method proposed by Wapshere (1974). The test list included six plant species requested by the U.S. Fish and Wildlife Service because of their ecological importance, as well as a variety of species with economic significance in Hawaii (table 1). Potted plants were grown with a standard medium of half potting soil and half cinder under automated irrigation and either direct sunlight (1,200 m elevation at HVNPQF) or 73 percent shade cloth (300 m elevation at Waiakea Experiment Station, University of Hawaii College of Tropical Agriculture and Human Resources, Hilo). Cuttings were made from wild plants growing in the vicinities of Volcano and Hilo, Hawaii. Two common forms of Hawaii's dominant forest tree, *Metrosideros polymorpha*, were tested: with glabrous (smooth) and pubescent (hairy) leaves. All plants and cuttings were maintained without pesticides and were inspected and cleaned to remove pests and previous damage before testing.

Order	Family	Tribe	Species	Common name
Myrtales	Melastomataceae	Melastomeae	Tibouchina herbacea	cane tibouchina
Myrtales	Melastomataceae	Melastomeae	Tibouchina longifolia	none
Myrtales	Melastomataceae	Melastomeae	Tibouchina urvilleana	glorybush
Myrtales	Melastomataceae	Melastomeae	Pterolepis glomerata	none
Myrtales	Melastomataceae	Melastomeae	Heterocentron subtriplinervium	pearl flower
Myrtales	Melastomataceae	Melastomeae	Melastoma septemnervium	none
Myrtales	Melastomataceae	Melastomeae	Melastoma sanguineum	none
Myrtales	Melastomataceae	Melastomeae	Dissotis rotundifolia	none
Myrtales	Melastomataceae	Microlicieae	Arthrostemma ciliatum	none
Myrtales	Melastomataceae	Dissochaeteae	Medinilla cumingii	none
Myrtales	Melastomataceae	Miconieae	Clidemia hirta	Koster's curse
Myrtales	Melastomataceae	Miconieae	Miconia calvescens	miconia

**Table 1.** Plant species used for *Syphraea uberabensis* host specificity testing, listed in order of phylogenetic relation to the target weed, *Tibouchina herbacea*.

Order	Family	Tribe	Species	Common name
Myrtales	Melastomataceae	Miconieae	Tetrazygia bicolor	none
Myrtales	Myrtaceae	Not listed	Metrosideros polymorpha *	ohia lehua
Myrtales	Myrtaceae	Not listed	Syzygium cumini	Java plum
Myrtales	Myrtaceae	Not listed	Syzygium malaccense	mountain apple
Myrtales	Myrtaceae	Not listed	Syzygium jambos	rose apple
Myrtales	Myrtaceae	Not listed	Psidium cattleianum	strawberry guava
Myrtales	Myrtaceae	Not listed	Eugenia uniflora	Surinam cherry
Myrtales	Lythraceae	Not listed	Lythrum maritimum	none
Myrtales	Lythraceae	Not listed	Cuphea carthagenensis	none
Myrtales	Lythraceae	Not listed	Cuphea ignea	cigar flower
Myrtales	Lythraceae	Not listed	Cuphea hyssopifolia	false heather
Myrtales	Onagraceae	Not listed	Fuchsia magellanica	fuchsia
Myrtales	Onagraceae	Not listed	Epilobium ciliatum	none
Myrtales	Onagraceae	Not listed	Oenothera laciniata	evening primrose
Myrtales	Combretaceae	Not listed	Terminalia catappa	tropical almond, false
5			11	kamani
Sapindales	Anacardiaceae	Not listed	Mangifera indica	mango
Sapindales	Rutaceae	Not listed	Citrus limon	lemon
Sapindales	Sapindaceae	Not listed	Dodonaea viscosa*	ʻa'ali'i
Malvales	Malvaceae	Not listed	Hibiscus arnottianus	hibiscus
Malvales	Thymelaeaceae	Not listed	Wikstroemia sandwicensis	akia
Brassicales	Caricaceae	Not listed	Carica papaya	papaya
Fabales	Fabaceae	Not listed	Acacia koa *	koa
Fabales	Fabaceae	Not listed	Erythrina sandwicensis	wiliwili
Fabales	Fabaceae	Not listed	Pisum sativum	pea
Fabales	Fabaceae	Not listed	Sophora chrysophylla *	mamane
Rosales	Rosaceae	Not listed	Rubus ellipticus	Himalayan raspberry
Rosales	Rosaceae	Not listed	Rubus hawaiensis	akala
Rosales	Rosaceae	Not listed	Fragaria vesca	strawberry
Rosales	Urticaceae	Not listed	Pipturus albidus	mamaki
Malpighiales	Passifloraceae	Not listed	Passiflora spp.	passion flower
Ericales	Ericaceae	Not listed	Vaccinium calvcinum	ohelo
Ericales	Theaceae	Not listed	Camellia sinensis	tea
Gentianales	Apocynaceae	Not listed	Alyxia stellata	maile
Gentianales	Rubiaceae	Not listed	Coffea arabica	coffee
Solanales	Solanaceae	Not listed	Physalis peruviana	poha
Lamiales	Myoporaceae	Not listed	Myoporum sandwicense *	naio
Asterales	Goodeniaceae	Not listed	Scaevola chamissoniana	naupaka
Proteales	Proteaceae	Not listed	Macadamia integrifolia	macadamia
Alismatales	Araceae	Not listed	Anthurium sp.	anthurium
Asparagales	Asparagaceae	Not listed	Cordyline fruticosa	ki
Asparagales	Orchidaceae	Not listed	Arundina graminifolia	bamboo orchid
Asparagales	Orchidaceae	Not listed	<i>Cymbidium</i> sp.	cymbidium
Zingiberales	Zingiberaceae	Not listed	Hedychium gardnerianum	kahili ginger
Laurales	Lauraceae	Not listed	Persea americana	avocado
Gleicheniales	Gleicheniaceae	Not listed	Dicranopteris linearis	uluhe
Polypodiales	Dicksoniaceae	Not listed	Cibotium glaucum *	hapuu pulu

\*Ecologically significant native species tested on request of U.S. Fish and Wildlife Service.

Researchers conducted no-choice and multi-choice tests using adult and larval S. uberabensis to

determine its host range. See appendix A for a complete description of host specificity testing and results.

#### b. Summary of Host Specificity Results

Testing revealed *S. uberabensis* to be narrowly host-specific within the family Melastomataceae and it was able to complete development on only five plant species in Hawaii, all invasive plants within the Melastomataceae. Larvae and naïve adults showed a somewhat broader range of feeding compared to mature adults in tests lasting a few days; however, low levels of feeding outside the normal host range is a common result of no-choice tests, in which insects are unable to seek out preferred hosts (Heard, 2002). Longer test periods demonstrated that only a few melastome species support survival to maturity and oviposition. Choice tests demonstrated the same few melastome species to be highly preferred over other related plants.

Egg laying was negligible on all plants tested except *Tibouchina herbacea*, *Tibouchina longifolia*, Pterolepis glomerata, Melastoma septemnervium and Melastoma sanguineum. Furthermore, these species were the only plants that supported the complete life cycle of S. uberabensis. Eggs laid in very low numbers on other species may have been a result of egg dumping, which occurs with some insects when a female's egg load exceeds a maximum threshold (Papaj, 2000; Wang and Horng, 2004). Feeding and minor egg laying suggested that a few melostomes (*Tibouchina* urvilleana, T. bicolor, Heterocentron subtriplinervium and Dissotis rotundifolia) might be marginal hosts; however, longer development tests showed that these plants are unlikely to sustain populations of S. uberabensis. If introduced to Hawaii, it is possible that S. uberabensis could be found in association with these plants where they grow in proximity to hosts that support complete development. Additional association could be observed on the non-melastome Terminalia catappa (Combretaceae), which experienced minor feeding damage in host specificity tests. However, no sustained development occurred during long-term larval and adult tests on this plant, and in Hawaii T. catappa typically occurs at coastal sites where the preferred melastome hosts are not common. Feeding observed in no-choice testing on plants like *T. catappa* is less likely to occur when *S.* uberabensis can move to a preferred host (Heard, 2002). Choice tests confirmed this, showing negligible feeding and egg laying by S. uberabensis on T. catappa, regardless of presence or absence of highly preferred hosts. See Appendix A for a complete description of host specificity testing and results.

If *S. uberabensis* successfully controls the target melastome species, the sites previously occupied by these plants can become available to other plants. In the less degraded wet forest, native plants may benefit from the natural resources previously occupied by the target species. In more degraded plant communities, the target species are more likely to be replaced by other non-native species

present nearby. Controlling existing populations of cane tibouchina will help to prevent its spread to new locations and islands.

### 2. Impact of S. uberabensis on Cane Tibouchina and Other Invasive Melastomes

The direct effect of *S. uberabensis* on the target weeds is the reduction of abundance through larval and adult feeding on the plants (herbivory). *Syphraea uberabensis* feeding has the potential to significantly reduce the abundance and distributional range of cane tibouchina and other invasive melastomes in Hawaii wherever the insect and the plants interact. The level of control, however, will likely depend on the physical and biological environments at release sites and is expected to vary by location. If *S. uberabensis* successfully establishes at release sites, it is expected to disperse and expand its range throughout each island over time. Unaided dispersal between islands is unlikely but human-mediated dispersal of *S. uberabensis*, especially as eggs or larvae, is possible.

The preferred melastome hosts of S. uberabensis are all considered serious weeds in Hawaii (HDOA, 1992; Jacobi and Warshauer, 1992; Almasi, 2000; Motooka et al., 2003). Of these plants, T. longifolia has the most limited distribution and appears least likely to have significant ecological interaction with S. uberabensis. If cane tibouchina and M. septemnervium can maintain substantial populations of S. uberabensis, these might help suppress T. longifolia and prevent it from spreading. Cane tibouchina and *M. septemnervium* overlap geographically across large areas, which could increase establishment and impacts of S. uberabensis. Melastoma sanguineum is ecologically similar to *M. septemnervium* but less widely distributed. Impacts of biocontrol by *S.* uberabensis would likely be swifter and more severe on T. herbacea than M. septemnervium and *M. sanguineum*, which grow to large woody shrubs. Increased herbivory of *M. septemnervium*, which has been targeted but not adequately impacted by past introductions of other biocontrols (Conant et al., 2013), would have potential benefit to extensive forest watersheds in Hawaii (Jacobi and Warshauer, 1992). The final host, P. glomerata, is a less prominent invader but broadly distributed in wet forests and pastures, including mountain areas on the island of Oahu where it has limited overlap with the other melastome hosts. Although P. glomerata appears to be equally suitable as a host for S. uberabensis, longer development times on this plant might delay the impacts of biocontrol (Souder, 2008).

### 3. Impact on Human and Animal Health

*Syphraea uberabensis* is a small beetle. This insect poses no risk to humans, livestock, or wildlife. It will not sting or bite, and feeds only on cane tibouchina and other invasive, non-native melastome plants.

#### 4. Impact on Native Fauna

Native fauna is expected to benefit from the release of *S. uberabensis* if control of the target species is successful because cane tibouchina and invasive melastomes pose a threat to the remaining native ecosystems on which native animals depend. There is no evidence that native fauna use the target species to an appreciable degree. A small number of native fauna might be indirectly affected by release of *S. uberabensis* if the target weeds are utilized for food or shelter. However, the effect is expected to be insignificant, as the native fauna that adapted to use the introduced species would be generalists, capable of using alternative plant species. Successful control or elimination of the target weeds will not threaten the existence of these generalist species.

The family of insects to which *S. uberabensis* belongs, Chrysomelidae, is not native to Hawaii and is represented by relatively few introduced species. Although there are a few pest chrysomelids in Hawaii, they have not been actively targeted for biocontrol. Therefore, there is not a known threat of specialized natural enemies affecting *S. uberabensis*. Its populations can be expected to be subject to predation by some generalist predators and diseases that affect beetles broadly. These natural enemies may increase in abundance where populations of *S. uberabensis* grow large, but such interactions are expected to be localized and temporary given the fluctuating nature of the beetle populations on their host plants.

#### 5. Socio-economic Impacts

The release of the any biocontrol agent poses a risk to socioeconomic environment when the biocontrol agent causes negative effects on non-target species that are socio-economically important. This may be caused by direct predation, competition, or secondarily when the results of the action cause socio-economic impact. However, release of *S. uberabensis* into Hawaii's environment is not expected to have negative socio-economic impacts. The successful control of invasive melastomes will benefit the environment and can release the resources used in chemical and mechanical control efforts for other purposes. The proposed action will not significantly change the land use of areas affected by cane tibouchina and other invasive melastomes. The results of successful control of the invasive weeds would improve the integrity of the native forest, which is crucial to the conservation of biodiversity as well as watershed value. Recreational use of the affected area is expected to benefit from the proposed action. The target species can degrade the recreational value of natural lands by colonizing areas including trails and forests. Therefore, the proposed action is expected to benefit recreation. This insect and its relatives are not known to be a nuisance such as by attraction to lights or mass migration or aggregation. *Syphraea uberabensis* is unlikely to become nuisance to residents and visitors.

### 6. Uncertainties Regarding the Environmental Release of S. uberabensis

Once a biological control agent such as *S. uberabensis* is released into the environment and becomes established, there is a slight possibility that it could move from the target plant (cane tibouchina and other invasive melastomes) to attack nontarget plants. Host shifts by introduced weed biological control agents to unrelated plants are rare (Pemberton, 2000). Native species that are closely related to the target species are the most likely to be attacked (Louda et al., 2003). If other plant species were to be attacked by *S. uberabensis*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *S. uberabensis* generally spread without intervention by man. In principle, therefore, release of this biological control agent at even one site must be considered equivalent to release over the entire area in which potential hosts occur, and in which the climate is suitable for reproduction and survival. However, significant non-target impacts on plant populations from previous releases of weed biological control agents are unusual (Suckling and Sforza, 2014).

In addition, this agent may not be successful in reducing cane tibouchina and other invasive melastome populations in Hawaii. Worldwide, biological weed control programs have had an overall success rate of 33 percent; success rates have been considerably higher for programs in individual countries (Culliney, 2005). Actual impacts on cane tibouchina and other melastomes by *S. uberabensis* will not be known until after release occurs and post-release monitoring has been conducted (see appendix B for release protocol and post-release monitoring plan). It is expected that *S. uberabensis* will reduce populations of invasive melastomes in Hawaii.

### 7. Cumulative Impacts

"Cumulative impacts are defined as the impacts on the environment which results from the incremental impact of the action when added to other past, present and reasonably foreseeable future actions regardless of what agencies or person undertakes such other actions" (40 CFR 1508.7).

Control of cane tibouchina and other invasive melastomes is carried out by various federal, state, local, and private organizations in Hawaii. For instance, the Oahu Invasive Species Committee requests that people report finds of cane tibouchina to them and they will remove it properly (<u>Cane Tibouchina (Cane Ti) — Oahu Invasive Species Committee (OISC) (oahuisc.org)</u>. Park rangers at Hawaii Volcanoes National Park work to control a variety of invasive plants including cane tibouchina and *Tibouchina urvilleana* (<u>Threats to the Parks: Hawaii Volcanoes National Park Struggles with Invasive Plants</u> | <u>National Parks Traveler</u>).

Release of S. uberabensis is not expected to have any negative cumulative impacts in Hawaii

because of its host specificity to invasive melastomes. Release of *S. uberabensis* will not preclude other agencies or organizations from working to control these plants and if effective, would assist them with their efforts. Effective biological control from introduced *S. uberabensis* may not only provide safe, effective, and long-term control of invasive melastomes such as cane tibouchina, but it may also result in reduced use of herbicides against these plants.

No other agents have been released in Hawaii for biological control of cane tibouchina or other melastomes; therefore, no competitive interactions between agents are expected. No other agents have been released in Hawaii for biological control miconia; therefore, no competitive interactions between agents are expected. The USFS is proposing to release a butterfly for biocontrol of miconia (*Miconia calvescens*) in the family Melastomataceae in Hawaii. However, that butterfly does not feed on cane tibouchina, nor does *S. uberabensis* feed on miconia. Thus, it is not expected that the two insects will interact or with one another.

### 8. Endangered Species Act

Section 7 of the Endangered Species Act (ESA) and ESA's implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of federally listed threatened and endangered species or result in the destruction or adverse modification of critical habitat.

**U.S. Fish and Wildlife Service Assessment:** There are currently 474 federally listed species under the jurisdiction of the U.S. Fish and Wildlife Service in the State of Hawaii (FWS, ECOS, 2022), the majority of which are flowering plants.

**Mammal assessment:** The Hawaiian hoary bat is the only listed mammal in Hawaii. This bat would not use *T. herbacea* or other potential melastome hosts as a roost because they do not have the height characteristics of roost trees. They are also not reported to be used as roost trees. Therefore, release of *S. uberabensis* will have no effect on the Hawaiian hoary bat.

**Bird assessment:** Ten birds are federally listed in Hawaii. Only the Hawaii akepa is a forestinhabiting bird species, although the Hawaiian goose may inhabit scrub forests as well as a variety of other habitats. The Hawaii akepa uses old growth forest trees and is not reported to use *T*. *herbacea* or other potential melastome hosts for nesting or foraging, and it is an insectivore (FWS, 2006a). No federally listed birds in Hawaii are dependent on or are reported to specifically forage on *T. herbacea* or other invasive melastomes. Although invasive melastomes are bird-dispersed species, there is no evidence that listed birds use these species as a food source. Therefore, APHIS has determined that release of *S. uberabensis* will have no effect on the Hawaii akepa, Hawaiian goose, or other listed birds in Hawaii. **Reptile assessment:** Four sea turtles are federally listed in Hawaii (green (Central North Pacific DPS), hawksbill, leatherback, and olive ridley). Melastomes are not considered primary constituent elements of the critical habitat of these species; thus, release of *S. uberabensis* will have no effect on their critical habitat. There is no information indicating specific interactions between listed sea turtles and invasive melastomes, and sea turtles would not use them in any way; thus, removal of the plant from the environment would have no effect on them. Therefore, APHIS has determined that release of *S. uberabensis* will have no effect on listed sea turtles in Hawaii.

**Snail assessment:** Four snails are federally listed in Hawaii (*Partulina semicarinata*, Lanai tree snail; *Partulina variabilis*, Lanai tree snail; *Newcombia cumingi*, Newcomb's tree snail; and *Erinna newcombi*, Newcomb's snail). Newcomb's snail (*Erinna newcombi*) is an aquatic snail.

Lanai tree snail (*Partulina semicarinata*) is found on the following native host plants: *Metrosideros polymorpha, Broussaisia arguta* (kanawao), *Psychotria* spp. (kopiko), *Coprosma* spp. (pilo), *Melicope* spp. (alani), and dead *Cibotium glaucum* (tree fern, hapuu). Occasionally the snail is found on nonnative plants such as *Psidium guajava* (guava), *Cordyline australis* (New Zealand tea tree), and *Phormium tenax* (New Zealand flax). (Hadfield, 1994).

Lanai tree snail (*Partulina variabilis*) is found on the following native host plants: *Metrosideros polymorpha, Broussaisia arguta, Psychotria* spp., *Coprosma* spp., *Melicope* spp., and dead *Cibotium glaucum*. Occasionally *Partulina variabilis* is found on nonnative plants such as *Psidium guajava* and *Cordyline australis* (Hadfield, 1994). Historically, *Partulina variabilis* was found in wet and mesic *Metrosideros polymorpha* forests on Lanai.

Newcomb's tree snail (*Newcombia cumingi*) has been documented living on small, older *Metrosideros polymorpha* (ohia) primarily in areas with dense cover by *Dicranopterus linearis* (uluhe fern) (Thacker and Hadfield, 1998), though other hosts that support suitable microbes might also be used by the tree snail.

Melastomes are not reported as host plants for these tree snails. Thus, release of *S. uberabensis* will have no effect on these snails. Release of *S. uberabensis* will also have no effect on the designated critical habitat of listed snails in Hawaii. Melastomes are not a physical or biological feature essential to the conservation of any listed snail.

**Insect assessment:** *Hylaeus* species are adapted to forage on pollen and nectar resources from a diversity of native plants, and rarely use non-native floral forage (Daly and Magnacca, 2003). Native yellow-faced bees have not been observed to forage on invasive melastomes, and any use

of the targeted plants would be peripheral to their primary foraging on native species. The seven *Hylaeus* species which are currently listed are known from dry to mesic forest habitats. Their range does not overlap significantly with the range of cane tibouchina or other targeted melastomes, which are invasive predominantly in wet to mesic forests.

Blackburn's sphinx moths are found in dry to mesic forest habitats. Larvae can develop on a range of native and non-native plants in the Solanaceae. In addition to using known larval hosts like the native and endangered aiea (*Nothocestrum* spp.) and tree tobacco (*Nicotiana glauca*), it also has the ability to develop fully on the native glossy nightshade (*Solanum americanum*) and *Solanum sandwicense* in a laboratory setting. *Syphraea uberabensis* would not affect the solanaceous host plants of this moth.

The naiads (immature stage) of damselflies are aquatic and both adults and naiads are predaceous. Eggs are laid on aquatic vegetation. Thus, release of *S. uberabensis* would have no effect on the blackline Hawaiian damselfly, crimson Hawaiian damselfly, flying earwig Hawaiian damselfly, oceanic Hawaiian damselfly, orangeblack Hawaiian damselfly, or Pacific Hawaiian damselfly or designated critical habitat.

Several of the listed *Drosophila* spp. picture wing flies occur in wet to mesic forests, where melastomes invade (table 2). However, melastomes are not hosts for these flies. In addition, invasion of wet forests by *T. herbacea* and other melastomes may adversely affect these fly species and their critical habitat if they outcompete their plant hosts such as *Cyanea* spp. and *Clermontia* spp. Release of *S. uberabensis* would have no effect on listed *Drosophila* spp. or their critical habitat in Hawaii. There is a potential that release could be beneficial to these flies if it can reduce the competition between invasive melastomes and their primary host plants, but this is dependent on the efficacy of *S. uberabensis* in reducing invasive melastomes.

Species	Island	Habitat type	Primary host plant(s)
Dropsophila aglaia	Oahu	mesic forest	Urera glabra
Dropsophila hemipeza	Oahu	mesic forest	Cyanea spp., Lobelia spp., Urera kaalae
Dropsophila montgomeryi	Oahu	mesic forest	Urera kaalae
Dropsophila obatai	Oahu	dry to mesic	Pleomele aurea, Pleomele forbesii
		forest	
Dropsophila substenoptera	Oahu	wet forest	Cheirodendron spp., Tetraplasandra spp.
Dropsophila tarphytrichia	Oahu	mesic forest	Charpentiera spp.
Dropsophila heteroneura	Hawaii	mesic to wet	Cheirodendron spp., Clermontia spp.,
		forest	Delissea spp.

**Table 2.** Distribution of 12 Hawaiian picture-wing flies by island, general habitat type, and primary host plant (FWS, 2006b).

Species	Island	Habitat type	Primary host plant(s)
Dropsophila mulli	Hawaii	wet forest	Pritchardia beccariana
Dropsophila ochrobasis	Hawaii	mesic to wet	Clermontia spp., Marattia spp., Myrsine
		forest	spp.
Dropsophila differens	Molokai	wet forest	Clermontia spp.
Dropsophila musaphilia	Kauai	mesic forest	Acacia koa
Dropsophila neoclavisetae	Maui	wet forest	<i>Cyanea</i> spp.

**Crustacean assessment:** Two Anchialine pool shrimp (*Procaris hawaiana* and *Vetericaris chaceorum*) are listed in Hawaii. Biological control of melastomes is not expected to result in increased runoff or sedimentation of waterbodies. In addition, biological control may reduce the use of herbicides for invasive plant control that could runoff or drift into aquatic habitat and could directly adversely affect listed crustaceans (acute or chronic exposure). There is no information indicating specific interactions between listed crustaceans and melastomes. Although invasive plants are listed as a threat to these species, melastomes are not mentioned (FWS, 2020a;b).

Melastomes are not considered a primary constituent element of the critical habitat of any listed crustacean and are not reported as occurring in the habitat of any listed crustacean. APHIS has determined that release of *S. uberabensis* will have no effect on listed crustaceans or their designated critical habitats.

#### Plant assessment:

Potential for attack of federally-listed plants by *S. uberabensis*: There are no federally listed plants belonging to the same family as the target weeds (Melastomataceae).

Souder (2008): Host specificity tests were carried out in the quarantine facility in Hawaii. Nochoice tests (also known as starvation tests) were conducted on 35 plant species found in Hawaii, including 12 native species that are considered significant components of native plant communities. Feeding by beetles was mainly, but not completely, restricted to the family Melastomataceae. Larvae and young adult beetles fed at very low levels on a few introduced nonmelastomes, mainly *Terminalia catappa* (Combretaceae) and *Cuphea* species (Lythraceae). Persistence of beetle populations on these plants did not appear to be possible, because they did not support larval development to adulthood, and they were not accepted by mature beetles for oviposition. There are no federally-listed species in Hawaii in the family Lythraceae or the family Combretaceae.

Larvae and naïve adults showed a somewhat broader range of feeding compared to mature adults in tests lasting a few days, however low levels of feeding outside the normal host range is a

common result of no-choice tests, in which insects are unable to seek out preferred hosts (Heard, 2002). Longer test periods demonstrated that only a few melastome species support survival to maturity and oviposition. Choice tests demonstrated the same few melastome species to be highly preferred over other related plants.

Egg laying was negligible on all plants tested except *Tibouchina herbacea*, *Tibouchina longifolia*, *Pterolepis glomerata*, and *Melastoma septemnervium*. Furthermore, these species were the only plants that supported the complete life cycle of *S. uberabensis*. Eggs laid in very low numbers on other species may have been a result of egg dumping, which occurs with some insects when a female's egg load exceeds a maximum threshold (Papaj, 2000; Wang and Horng, 2004). Feeding and minor egg laying suggested that a few Melastomataceae (*T. urvilleana*, *T. bicolor*, *H. subtriplinervium* and *D. rotundifolia*) might be marginal hosts, however longer development tests showed that these plants are unlikely to sustain populations of *S. uberabensis*.

Potential impact by *S. uberabensis* removal of invasive melastomes on federally-listed plants: Cane tibouchina invades wet and mesic forests that are disturbed (especially by pigs and landslides), though it can grow in shaded areas. It forms dense stands in pastures and disturbed forests, out-competing native species. It is listed among the invasive plants that are considered the most serious habitat modifying species (Medeiros and Loope, 2013). See table 3 for federallylisted plant species in Hawaii that have been adversely impacted by invasion of *T. herbacea*.

Plant Species	Listing	Effect	References
	Status		
Clermontia lindseyana	Endangered	Competition- monopolizing	FWS, 2010
(Campanulaceae)		resources; Competition -shading	
Clermontia peleana (Campanulaceae)	Endangered	Competition- monopolizing	FWS, 2008
		resources; Competition- shading	
Cyanea duvalliorum (Campanulaceae)	Endangered	Competition- monopolizing	Oppenheimer,
		resources; Competition- shading	2008
Cyanea gibsonii (Campanulaceae)	Endangered	Competition- monopolizing FWS, 1995	
		resources; Competition- shading	
Cyanea maritae (Campanulaceae)	Endangered	Competition- monopolizing Oppenheimer,	
		resources; Competition- shading	2008.
Cyanea mceldowneyi	Endangered	Competition- monopolizing FWS, 1997	
(Campanulaceae)		resources; Competition- shading	
Cyrtandra munroi (Gesneriaceae)	Endangered	Competition- monopolizing FWS, 1995	
		resources; Competition- shading	

**Table 3.** Federally-listed plant species in Hawaii adversely affected by *Tibouchina herbacea*(From: Buddenhagen, 2022).

Plant Species	Listing	Effect	References
	Status		
Gahnia lanaiensis (Cyperaceae)	Endangered	Competition- monopolizing	FWS, 1995
		resources; Competition- shading	
Phyllostegia glabra var. lanaiensis	Endangered	Competition- monopolizing	FWS, 1995
(ulihi phyllostegia) (Lamiaceae)		resources; Competition- shading	
Pteris lydgatei (Lidgate's brake)	Endangered	d Competition- monopolizing FWS, 2009	
(Pteridaceae)		resources; Competition- shading	
Santalum lanaiense (Santalaceae)	Endangered	Competition- monopolizing	FWS, 1995
		resources; Competition- shading	
Viola lanaiensis (Hawaii violet)	Endangered	Competition- monopolizing	FWS, 1995;
(Violaceae)		resources; Competition- shading	Havran et al.,
			2012

During host specificity tests, it was found that *S. uberabensis* fed and successfully developed and reproduced on several invasive melastomes that are suitable targets for the proposed release of *S. uberabensis* (Souder, 2008; Raboin et al., 2009). These include *Tibouchina longifolia*, *Pterolepis glomerata*, *Melastoma sanguineum*, and *Melastoma septemnervium*, all of which have invaded native wet forest habitats in Hawaii. *Melastoma septemnervium*, in particular, is widely distributed on Hawaii Island, where it has been recognized as a threat for many years (Jacobi and Warshauer, 1992). Each of these melastome species is likely to increase in population and expand in range in the absence of additional management attempts such as biocontrol by *S. uberabensis*.

APHIS has determined that the release of *S. uberabensis* will have no effect listed plants or their critical habitats in Hawaii due to non-target attack. It is possible that there could be a beneficial effect to certain listed plants, but this depends on the efficacy of *S. uberabensis* in reducing invasive melastomes in Hawaii.

National Oceanic and Atmospheric Administration (NOAA) Fisheries Species Assessment:

The proposed release of *S. uberabensis* would have no effect on federally-listed species or critical habitat under the jurisdiction of the National Marine Fisheries Service (table 4) (NOAA Fisheries, 2022). There would be no interaction between *S. uberabensis* and these species.

Common Name	Scientific Name	ESA Listing Status
Blue Whale	Balaenoptera musculus	Endangered
False Killer Whale -	Pseudorca crassidens	Endangered with critical
Hawaiian Insular		habitat
Fin Whale	Balaenoptera physalus	Endangered
North Pacific Right Whale	Eubalaena japonica	Endangered

**Table 4.** Species in Hawaii under NOAA Fisheries jurisdiction that are protected under the Endangered Species Act (NOAA Fisheries, 2022).

Common Name	Scientific Name	ESA Listing Status
Sei Whale	Balaenoptera borealis	Endangered
Sperm Whale	Physeter macrocephalus	Endangered
Hawaiian Monk Seal	Neomonachus schauinslandi	Endangered with critical
		habitat
Central North Pacific Green	Chelonia mydas	Threatened
Turtle		
Hawksbill Turtle	Eretmochelys imbricata	Endangered
Leatherback Turtle	Dermochelys coriacea	Endangered
North Pacific Loggerhead	Caretta caretta	Endangered
Turtle		
Olive Ridley Turtle	Lepidochelys olivacea	Threatened
Giant Manta Ray	Manta birostris	Threatened
Oceanic Whitetip Shark	Carcharhinus longimanus	Threatened

## V. Other Issues

### A. Equity and Underserved Communities

In Executive Order (EO) 13985, Advancing Racial Equity and Support for Underserved Communities Through the Federal Government, each agency must assess whether, and to what extent, its programs and policies perpetuate systemic barriers to opportunities and benefits for people of color and other underserved groups. In EO 12898, Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations, Federal agencies must identify and address disproportionately high and adverse human health or environmental impacts of proposed activities.

Consistent with these EOs, APHIS considered the potential for disproportionately high and adverse human health or environmental effects on any minority populations and low-income populations. APHIS did not identify any disproportionately high or adverse environmental or human health effects from the field release of *S. uberabensis*. The preferred action will not have disproportionately high or adverse effects to any minority or low-income populations.

Federal agencies also comply with EO 13045, Protection of Children from Environmental Health Risks and Safety Risks. This EO requires each Federal agency, consistent with its mission, to identify and assess environmental health and safety risks that may disproportionately affect children and to ensure its policies, programs, activities, and standards address the potential for disproportionate risks to children. Consistent with EO 13045, APHIS considered the potential for disproportionately high and adverse environmental health and safety risks to children. No aspects of the proposed field release of *S. uberabensis* could be identified that would have disproportionate effects on children.

### **B. Cultural Assessment**

ASM Affiliates Hawaii, a Heritage and Cultural Resource Management firm, prepared a Cultural Impact Assessment (CIA) for the Hawaii Department of Land and Natural Resources (DLNR), Division of Forestry and Wildlife (DOFAW), and Hawaii Department of Agriculture (HDOA) for the proposed release of *S. uberabensis* statewide in Hawaii (Brandt, 2019). This assessment is part of the administrative record for this EA and is available upon request. The primary focus of the report was to understand the cultural and historical context of cane tibouchina and other weedy melastomes with respect to Hawaii's host culture. It includes a cultural-historical context of the settlement of the Hawaiian Islands by early Polynesian settlers and the transformation of their beliefs and practices associated with the land following western contact, an overview of the history of biocontrol in Hawaii, and a discussion of potential impacts as well as appropriate actions and strategies to mitigate such impacts.

To identify individuals knowledgeable about traditional cultural practices and/or uses associated with the affected environment, a public notice was submitted to the Office of Hawaiian Affairs (OHA) for publication in the May 2019 issue of their monthly newspaper, Ka Wai Ola. While no responses were received from the public notice, 45 individuals were contacted via email and/or phone regarding the preparation of the CIA report. Twenty people responded to the request with either brief comments, referrals, or acceptance of the interview request. ASM Affiliates conducted a total of eight interviews. The interviewees were asked a series of questions regarding their background, and their experience and knowledge of the target species. Additional questions focused on any known cultural uses, traditions, or beliefs associated with any of the target species. The interviewees were then asked about their thoughts on the cultural appropriateness of using biocontrol agents and whether they were aware of any potential cultural impacts that could result from the use of biocontrol and whether they had any recommendations to mitigate any identified cultural impacts or any other thoughts about the proposed action.

A review of the cultural-historical background in addition to the consultation efforts yielded no reported cultural use for cane tibouchina nor is there any historical evidence to suggest that this plant is crucial to any particular ethnic groups' cultural history, identity, practices, or beliefs. Although cane tibouchina is not culturally significant, the wet forest habitat in which it thrives is culturally significant because it contains many culturally important indigenous and endemic taxa, which are still utilized in certain Hawaiian cultural practices. Some of these wet forest resources

are also associated with certain Hawaiian cultural beliefs. Based on the information derived from the cultural-historical background and from the insight shared by the consulted parties, it is the assessment of this study that the release of the proposed biocontrol agent, *S. uberabensis*, will not result in impacts to any valued cultural, historical, or natural resources. Conversely, if no action is taken to further reduce remaining populations of cane tibouchina and other highly invasive melastomes from claiming more of Hawaii's wet forest habitat, impacts to this valued resource would be anticipated. While no specific cultural impacts were identified through the CIA, the consulted parties shared valuable insight, concerns, and recommendations that could reduce the potential for any future impacts and improve public transparency regarding the effectiveness of biocontrol as a conservation management strategy. Several key themes emerged from the consultation efforts: 1) maintain stringent pre- and post-release testing and monitoring; 2) improved community transparency and input; 3) active and ongoing public outreach and education; 4) improve efforts to limit the introduction of potentially harmful invasive species.

### C. Climate Change

Climate change will affect Hawaii in many ways as a result of rising air temperatures, changing rainfall patterns, rising sea levels, and increased risk of extreme drought and flooding (Keener et al., 2018).

### 1) Impact of Climate Change on Proposed Action

Climate change is affecting Hawaii, resulting in sea level rise, coastal and inland flooding, and coastal erosion (State of Hawaii, 2022). These will lead to land becoming unusable, and structures, roads, cultural sites, and other assets at risk (State of Hawaii, 2022). Changing climate, including increased flooding events, could possibly affect the ability of *S. uberabensis* to establish and control cane tibouchina and other invasive melastomes.

### 2) Impact of Proposed Action on Climate Change

Sources of greenhouse gas emissions as a result of permitting the environmental release of *S*. *uberabensis* would include (1) vehicle use by the permittee and cooperators during biocontrol agent delivery and monitoring in the field, and greenhouse gas releases associated with heating and cooling the facilities used for the rearing of *S. uberabensis*. It is not possible to predict the number of site visits or distance traveled to those sites. Initially, these visits would be expected to be more frequent as *S. uberabensis* is distributed and monitoring activities are conducted by the permittee and cooperators. Over time, as the agent establishes and spreads on its own, site visits would be expected to decrease. Rearing of *S. uberabensis* occurs in the Hawaii Volcanoes National Park

Quarantine Facility (HVNPQF). Rearing of *S. uberabensis* would contribute only a small portion of greenhouse gas produced by the facility. In addition, if *S. uberabensis* is successful in reducing the invasion of cane tibouchina and other melastomes into new locations, the greenhouse gas emissions from vehicles used to apply insecticides or cultural methods to control them would be reduced.

### VI. Agencies, Organizations, and Individuals Consulted

This EA was prepared and reviewed by personnel from APHIS, USFS, and Garcia and Associates. The addresses of participating APHIS units and any applicable cooperators are provided below.

Garcia and Associates 146 Hekili St., Suite 101 Kailua, Hawaii 96734

U.S. Department of Agriculture Animal and Plant Health Inspection Service Policy and Program Development Environmental and Risk Analysis Services 4700 River Road, Unit 149 Riverdale, MD 20737

U.S. Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine Pests, Pathogens, and Biocontrol Permits 4700 River Road, Unit 133 Riverdale, MD 20737–1236

U.S. Department of Agriculture Forest Service Pacific Southwest Research Station Institute of Pacific Island Forestry 60 Nowelo St. Hilo, Hawaii 96720

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# **Appendix A. Host Specificity Testing Methods and Results**

# **Design of Host Specificity Tests**

Test plant lists are developed by researchers for determining the host specificity of biological control agents of weeds. Test plant lists are usually developed on the basis of phylogenetic relationships between the target weed and other plant species (Wapshere, 1974). It is generally assumed that plant species more closely related to the target weed species are at greater risk of attack than more distantly related species.

The host specificity test strategy as described by Wapshere (1974) is "a centrifugal phylogenetic testing method which involves exposing to the organism a sequence of plants from those most closely related to the weed species, progressing to successively more and more distantly related plants until the host range has been adequately circumscribed." Researchers do not pursue release of biological control agents that do not demonstrate high host specificity to the target weed.

Insect rearing: Syphraea uberabensis eggs and larvae on T. herbacea cuttings were shipped in July 2005 from Universidade Estadual Centro-Oeste in Irati, Parana State, Brazil to the Hawaii Volcanoes National Park Quarantine Facility (HVNPQF). The shipment resulted in an initial colony of approximately 50 adult flea beetles. Abnormal growth of potted Tibouchina plants in HVNPQF limited rearing the flea beetle on live plants. Therefore, T. herbacea cuttings collected around Glenwood and Volcano, Hawaii (700-1,200 m) were used to maintain colony insects. In HVNPQF, the environmental conditions ranged from 18–24° C, 20–95 percent relative humidity (RH), with a natural photoperiod (approximately 12–12 hour light:dark). Flea beetles were reared on fresh leaf cuttings of T. herbacea over moistened paper towel in 150 mm x 25 mm circular petri dishes. The moistened towel maintained a level of humidity inside the petri dish that kept plant material turgid. Each dish was filled with 30–40 newly emerged adults (roughly 1:1 sex ratio). Deteriorating and heavily damaged leaves were removed and replaced with new cuttings every other day, and each petri dish was changed completely approximately twice per week. When adults began to lay eggs, the egg bearing leaves were removed and recombined in equal proportions from different source dishes to maintain a diverse genetic pool. Larvae were reared on fresh leaf cuttings in large petri dishes, similar to adults. Large third instars were transferred to petri dishes with moistened vermiculite to simulate soil for pupation. Beetles completed a full generation cycle in approximately two months.

**Test Plants:** Potential host preferences were evaluated on a total of 58 plant species in 30 families. Test plants were selected based on the centrifugal phylogenetic method proposed by Wapshere (1974). The test list also included six plant species requested by the U.S. Fish and

Wildlife Service because of their ecological importance, as well as a variety of species with economic significance in Hawaii (Table 1). Potted plants were grown with a standard medium of half potting soil and half cinder under automated irrigation and either direct sunlight (1,200 m elevation at HVNPQF) or 73% shade cloth (300 m elevation at Waiakea Experiment Station, University of Hawaii College of Tropical Agriculture and Human Resources, Hilo). Cuttings were made from wild plants growing in the vicinities of Volcano and Hilo, Hawaii. Two common forms of Hawaii's dominant forest tree, *Metrosideros polymorpha*, were tested: with glabrous and pubescent leaves. All plants and cuttings were maintained without pesticides and were inspected and cleaned to remove pests and previous damage before testing.

# **Results of Host Specificity Tests**

### Summary of Results

Adult beetles, 3-4 mm in length, feed and lay eggs on leaves and soft stems of their host plants. Larvae feed externally on leaves as well. Specificity tests indicated the host range of *Syphraea uberabensis* is restricted to a few melastome species, all non-native and considered invasive in Hawaii. The results of no-choice starvation tests and multi-choice testing consistently identified the potential Hawaiian hosts as: *Tibouchina herbacea, Tibouchina longifolia, Pterolepis glomerata, Melastoma septemnervium,* and *Melastoma sanguineum*.

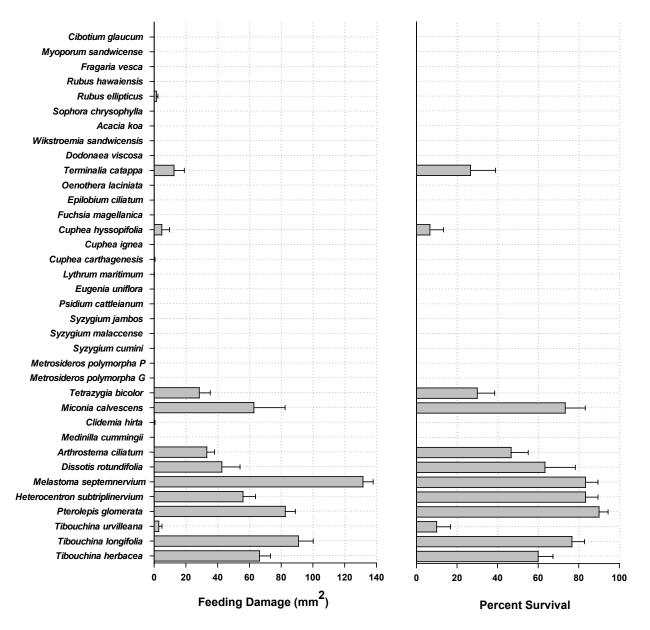
In no-choice tests, substantial feeding by *Syphraea* beetles was observed on no more than seven melastome species, and egg laying was further restricted to the five mentioned species, all in the tribe Melastomeae, which includes American and Asian species (figure 2). In no-choice tests with larvae (figure 1), these same melastomes supported high rates of survival, while a few other melastomes supported lower rates of survival, and other plants did not support survival beyond the second instar (table 2).

Multi-choice testing with adult beetles (figure 5) revealed strong preferences for feeding and oviposition in the same species identified as probable hosts during no-choice trials (table 3, figures 3 and 4). Feeding within tribe Melastomeae occurred at significantly higher levels than in other tribes (p < 0.01). When the preferred hosts were excluded in reduced multi-choice tests, adult insect feeding decreased dramatically. In the absence of the preferred hosts, oviposition increased slightly on other species within family Melastomataceae, with the greatest increase in oviposition occurring on *Tibouchina urvilleana*. Although closely related to preferred host plants, this weedy shrub was rarely accepted by *S. uberabensis* for feeding or egg laying. It appears to be an unlikely host because its leaves are well protected by dense hairs.

The Hawaiian ranges of *T. herbacea, T. longifolia, P. glomerata, M. septemnervium*, and *M. sanguineum* overlap considerably. Although *S. uberabensis* showed a clear preference for *T. longifolia* in laboratory tests, it is unlikely that this preference will have a significant impact in the Hawaiian environment because *T. longifolia* is so scarce compared to *T. herbacea* and other potential hosts. A more likely scenario is that *S. uberabensis* will negatively impact widespread *T. herbacea*, while perhaps helping prevent *T. longifolia* from spreading.

*Syphraea uberabensis* is tolerant of cool and moderate temperatures and is not expected to be restricted in range by temperatures in Hawaii, except perhaps in exceptionally warm habitats (Souder, 2008). However, the potential of *S. uberabensis* as a biological control could be limited by humidity at the microhabitat level. In Brazil, *S. uberabensis* is found with its melastome hosts in boggy soils, similar to the areas where *Tibouchina* and *Pterolepis* thrive in Hawaii, so these hosts should be highly susceptible. On the other hand, *Melastoma* in Hawaii can grow in relatively drier areas – such as young lava flows. *Syphraea uberabensis* could be less effective against *Melastoma* in drier habitats because its eggs and larvae appear to be susceptible to drying when humidity is not high.

#### **Results: Larval Feeding and Survival**



**Figure A-1.** Feeding and survival of newly hatched *S. uberabensis* larvae after 7 days on potted plants under no-choice conditions (means  $\pm$  standard errors; 6 replicates, 5 larvae per replicate). Genetic relationship to *Tibouchina herbacea* increases from top **to** bottom. Two leaf forms of *Metrosideros polymorpha* were tested: glabrous (G), and pubescent (P).

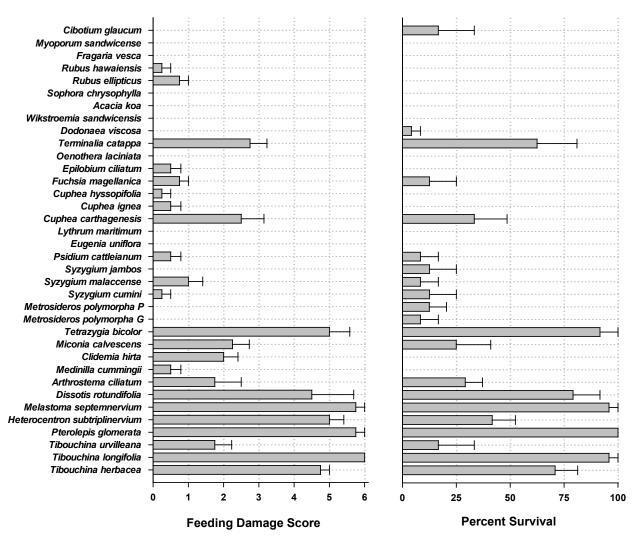
#### **Results: Larval Survival and Development**

Test Plant*	2 <sup>nd</sup> Instar	3 <sup>rd</sup> Instar	Pupa	Adult	
Tibouchina herbacea	$80.0 \pm 4.1$	$70.0 \pm 4.1$	$67.5 \pm 4.8$	$62.5 \pm 4.8$	
Tibouchina longifolia	$82.5 \pm 2.5$	$77.5 \pm 4.8$	$75.0 \pm 2.9$	$62.5 \pm 2.5$	
Tibouchina urvilleana	0	Not applicable (NA)	NA	NA	
Pterolepis glomerata	$90.0\pm4.1$	$85.0 \pm 2.9$	$80.0\pm4.1$	$67.5 \pm 2.5$	
Heterocentron subtriplinervium	$50.0 \pm 4.1$	$30.0 \pm 7.1$	$25.0 \pm 6.5$	$15.0 \pm 6.5$	
Melastoma septemnervium	$82.5 \pm 8.5$	$75.0 \pm 5.0$	$67.5 \pm 2.5$	$62.5 \pm 2.5$	
Dissotis rotundifolia	$42.5 \pm 11.1$	$27.5 \pm 4.8$	$17.5 \pm 7.5$	$12.5 \pm 4.8$	
Arthrostema ciliatum	0	NA	NA	NA	
Medinilla cummingii	0	NA	NA	NA	
Clidemia hirta	0	NA	NA	NA	
Miconia calvescens	$37.5 \pm 8.5$	$22.5 \pm 9.5$	0	NA	
Tetrazygia bicolor	$32.5 \pm 8.5$	$17.5 \pm 2.5$	$10.0 \pm 4.1$	0	
Cuphea carthagenensis (Lythraceae)	0	NA	NA	NA	
Cuphea hyssopifolia (Lythraceae)	5.0 ± 2.9	0	NA	NA	
<i>Terminalia catappa</i> (Combretaceae)	15.0 ± 6.5	0	NA	NA	

\*Plants without family indicated in table are in Melastomataceae.

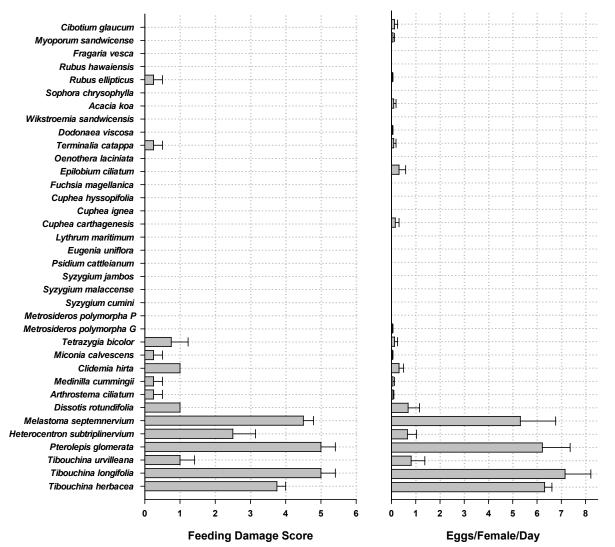
**Table A-1.** Survival of *S. uberabensis* from newly hatched first instars to successive developmental stages on fresh plant cuttings under no-choice conditions (mean percentage  $\pm$  standard error; 4 replicates 10 larvae per replicate). Plant species were selected base on occurrence of at least minor levels of larval feeding in 7-day tests.

#### **Results: Young Adult Feeding and Survival**



**Figure A-2.** Feeding and survival of naïve adult *S. uberabensis* after 7 days on potted plants under no-choice conditions (means  $\pm$  standard errors; 4 replicates). Naïve adults (3 male and 3 female per replicate) were newly emerged from pupation in vermiculite (<12 hours old) and had not been exposed to any plant material prior to testing. Feeding score: 0 = no damage, 1 = fewer than 10 pinholes, 2 = less than 1 cm<sup>2</sup> damaged, 3 = 1-2 cm<sup>2</sup>, 4 = 2-3 cm<sup>2</sup>, 5 = 3-4 cm<sup>2</sup>, 6 = greater than 4 cm<sup>2</sup> damaged.

#### **Results: Mature Adult Feeding and Survival**



**Figure A-3.** Feeding and oviposition by mature adult *S. uberabensis* after 4 days on potted plants under no-choice conditions (means  $\pm$  standard errors; 4 replicates). Before testing, adults were reared in petri dishes on *T. herbacea* cuttings for 30 days, removed from food for 24 hours, and then transferred as mating pairs into enclosures on potted plants (2 males and 2 females per replicate). Mature beetles fed more selectively than naïve adults which had no prior feeding experience on *T. herbacea* (figure A-2). However, testing naïve adults for longer periods showed that only a few melastome species support survival to maturity and oviposition (table A-2).

#### **Results: Fecundity and Development**

Test Plant	Number	Life Span	Pre-	Eggs per	F1	F2
	females	(days)	oviposition	female	Survival	Survival
			length		egg to	egg to 3rd
			(days)		adult	instar
					(n=eggs	(n=eggs
					collected)	collected)
Tibouchina	12	$50.3\pm3.4$	$23.4\pm0.5$	$173 \pm 38$	38% (94)	67% (100)
herbacea						
Tibouchina	10	$70.1\pm8.0$	$40.3\pm3.3$	$175\pm36$	43% (90)	72% (100)
longifolia						
Tibouchina	7	$51.4\pm4.9$	$31.6 \pm 1.5$	$36 \pm 13$	0% (50*)	Not
urvilleana						applicable
Pterolepis	14	$98.8\pm7.4$	$58.6\pm6.5$	$220\pm31$	51% (93)	79% (100)
glomerata						
Heterocentron	10	$25.8\pm3.8$	$23.0\pm0$	6 ± 6	0% (11*)	Not
subtriplinervium						applicable
Melastoma	11	$63.6\pm7.5$	$29.6\pm2.5$	$207\pm37$	42% (91)	71% (100)
septemnervium						
Tetrazygia	11	$52.9\pm5.6$	$45.3\pm6.9$	$17 \pm 12$	0% (40*)	Not
bicolor						applicable

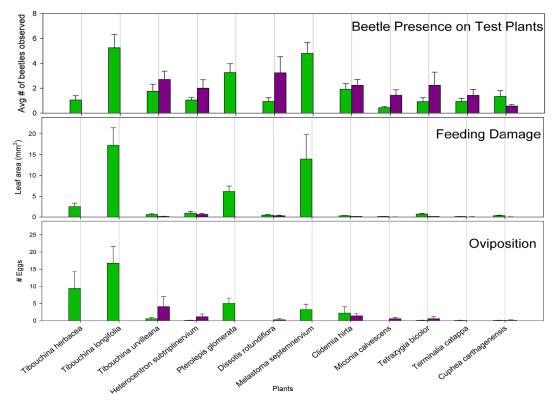
\*Collected every egg laid by females on this test plant.

**Table A-2.** Female lifespan, pre-oviposition period, and total fecundity (means  $\pm$  standard errors), and survival of offspring for *S. uberabensis* male-female pairs fed fresh plant cuttings under no-choice conditions. All plants belong to the family Melastomataceae.

This test was initiated with naïve adults caged on foliage of a potted plants of 13 species (30 beetles per plant). After 14 days surviving individuals were separated into single male-female pairs and placed in petri dishes with plant cuttings. Only four plants of 13 species tested sustained naïve adults to maturation, oviposition, and development of F1 generation adult beetles: *T. herbacea, T. longifolia, P. glomerata,* and *M. septemnervium.* F1 progeny were reared on each of these four plant species, producing viable F2 eggs and larvae that developed to third instars before testing was terminated. Plant species *T. urvilleana, H. subtriplinervium,* and *T. bicolor* supported survival of beetles to maturation and egg-laying, but larvae did not survive. No beetles survived beyond 14 days on plant species *Arthrostemma ciliatum, Clidemia hirta, Miconia calvescens, Cuphea carthagenesis,* and *Terminalia catappa*; some beetles survived on *Dissotis rotundifolia* but did not produce any eggs. (For these reasons, results for these six plant species are not shown above).

There were significant differences between *T. herbacea*, *T. longifolia*, *P. glomerata*, and *M. septemnervium* in female lifespan (H = 17.90, df = 3, P  $\ge$  0.001) and pre-oviposition time (H =

17.74, df = 3, P  $\ge$  0.001), but no significant differences in the total number of eggs laid (H = 1.46, df = 3, P = 0.691) or in daily oviposition rates (H = 1.73, df = 3, P = 0.631).



**Results: Adult Choice Tests** 

**Figure A-4.** Location, feeding, and oviposition of *S. uberabensis* in multi-choice testing over 3 days in an arena (40x40x40 cm) with cut stems of several plant species (means  $\pm$  standard errors). Plant species are listed from left to right in order of decreasing genetic relationship to *Tibouchina herbacea*. Green bars represent Full tests (12 replicates, 12 test plants), and purple bars represent Reduced tests, for which the highly preferred host plants were removed (7 replicates, 8 test plants). Feeding and egg laying decreased greatly overall when preferred host plants were removed, and egg laying increased only slightly on the non-preferred melastomes, mainly *Tibouchina urvilleana*. *Terminalia catappa* is in the family Combretaceae and *C. carthagenesis* is in the family Lythraceae.

## **Researcher's Conclusions From Host-Specificity Testing**

Testing revealed *S. uberabensis* to be narrowly host-specific within the family Melastomataceae and able to complete development on only five plant species in Hawaii. Larvae and naïve adults showed a somewhat broader range of feeding compared to mature adults in tests lasting a few days; however, low levels of feeding outside the normal host range is a common result of no-choice tests, in which insects are unable to seek out preferred hosts (Heard, 2002). Longer test periods demonstrated that only a few melastome species support survival to maturity and oviposition. Choice tests demonstrated the same few melastome species to be highly preferred over other related plants.

Egg laying was negligible on all plants tested except *Tibouching herbacea*, *Tibouching longifolia*, Pterolepis glomerata, Melastoma septemnervium, and Melastoma sanguineum. Furthermore, these species were the only plants that supported the complete life cycle of S. uberabensis. Eggs laid in very low numbers on other species may have been a result of egg dumping, which occurs with some insects when a female's egg load exceeds a maximum threshold (Papaj, 2000; Wang and Horng, 2004). Feeding and minor egg laying suggested that a few Melastomataceae (T. urvilleana, T. bicolor, H. subtriplinervium, and D. rotundifolia) might be marginal hosts. However, longer development tests showed that these plants are unlikely to sustain populations of S. uberabensis. If introduced to Hawaii, it is possible that S. uberabensis could be found in association with these plants where they grow in proximity to hosts that support complete development. Additional association could be observed on the non-melastome Terminalia catappa, which experienced minor feeding damage in host specificity tests. However, no sustained development occurred during long-term larval and adult tests on this plant, and in Hawaii, T. catappa typically occurs at coastal sites where the preferred melastome hosts are not common. Feeding observed in no-choice testing on plants like T. catappa is less likely to occur when flea beetles can move to a preferred host (Heard, 2002). Choice tests confirmed this, showing negligible feeding and egg laying by S. *uberabensis* on *T. catappa*, regardless of presence or absence of highly preferred hosts.

It is interesting to note that two suitable hosts of *S. uberabensis*, *Melastoma septemnervium* and *Melastoma sanguineum*, originate from Asia, and that ancestors of this plant genus likely diverged from neotropical ancestral hosts of *S. uberabensis* an estimated 11 to 12 million years ago (Renner and Meyer, 2001). Molecular analyses place the three genera, *Melastoma*, *Pterolepis*, and *Tibouchina*, all in the same clade (Clausing and Renner, 2001). Thus, host range results are consistent with a long coevolutionary relationship between *S. uberabensis* and members of these taxa.

The preferred melastome hosts of S. uberabensis are all considered serious weeds in Hawaii

(HDOA, 1992; Jacobi and Warshauer, 1992; Almasi, 2000; Motooka et al., 2003). Of these plants, T. longifolia has the most limited distribution and appears least likely to have significant ecological interaction with the potential biocontrol agent. If *T. herbacea* and *M. septemnervium* can maintain substantial populations of S. uberabensis, these might help suppress T. longifolia and prevent it from spreading. The species T. herbacea and M. septemnervium overlap geographically across large areas, which could facilitate establishment and impacts of S. uberabensis generally. M. sanguineum is ecologically similar to M. sanguineum but less widely distributed. Impacts of biocontrol by S. uberabensis would likely be swifter and more severe on T. herbacea than M. septemnervium and M. sanguineum, which grow to large woody shrubs. Increased herbivory of M. septemnervium, which has been targeted but not adequately impacted by past introductions of other biocontrols (Conant et al., 2013), would have potential benefit to extensive forest watersheds in Hawaii (Jacobi and Warshauer, 1992). The final host, P. glomerata, is a less prominent invader but broadly distributed in wet forests and pastures, including mountain areas on the island of Oahu where it has limited overlap with the other melastome hosts. Although P. glomerata appears to be equally suitable as a host for S. uberabensis, longer development times on this plant might delay the impacts of biocontrol (Souder, 2008).

*Syphraea uberabensis* is tolerant of cool and moderate temperatures, and it is not expected to be restricted in range by temperatures in Hawaii, except perhaps in exceptionally warm habitats (Souder, 2008). However, its potential as a biological control could be limited by humidity at the microhabitat level. In Brazil, *S. uberabensis* is found with its melastome hosts in boggy soils, similar to the areas where *T. herbacea* and *P. glomerata* thrive in Hawaii, so these hosts should be highly susceptible. On the other hand, *Melastoma* spp. can grow in drier areas – such as young lava flows. *Syphraea uberabensis* could be less effective against *Melastoma* in dry habitats because its eggs and larvae appear to be susceptible to drying when humidity is not high.

Note: References included in this section are listed in "VII. References" section of the EA.

# Appendix B. Release and Post-release monitoring plan for S. uberabensis

*Syphraea uberabensis* will be removed by the permittee from a source colony maintained at the Hawaii Volcanoes National Park Quarantine Facility. This colony will originate from insects collected from southern Brazil and screened to eliminate associated natural enemies via rearing through their life cycle in petri dish containers before import and again in quarantine. Roughly 30 insects at a time will be removed from quarantine as adult beetles newly emerged from pupation, independent of host plant material and other potential contaminants. Adults will be used to establish colonies reared in petri dishes at U.S. Department of Agriculture and Hawaii Department of Agriculture insectaries in Volcano, Hilo, and Honolulu. Offspring from rearing colonies will be used for environmental releases at selected locations statewide.

Initial sites for environmental release of *S. uberabensis* will be selected on east Hawaii island, west Maui, and the Koolau Mountains of Oahu. Sites will be monitored by the permittee to gain information on rates of establishment and dispersal on the target plants *Tibouchina herbacea* (Hawaii and Maui) and *Pterolepis glomerata* (Oahu). Sites on Hawaii island will be monitored by the permittee as well for spread of the insect from *T. herbacea* to nearby alternate hosts *P. glomerata* and *Melastoma septemnervium*. At all sites, monthly surveys for adult and larval insects will provide temporal data on population dynamics of *S. uberabensis*. Bimonthly or quarterly visual and photographic monitoring of fixed quadrats of potential host plants will provide temporal data on impacts to target plants, including foliage cover, stem numbers and size, and reproductive rates. It is anticipated that monitoring will continue for two to three years to quantify sustained impacts and spread of initial populations.