Syphraea uberabensis (Coleoptera: Chrysomelidae) potential agent for biological control of *Tibouchina herbacea* (Melastomataceae) in the archipelago of Hawaii, USA

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Summary

Biological invasions are one of the major threats to Hawaii's biodiversity. Herbacious glory tree, *Tibouchina herbacea* Cogn. (Melastomataceae), native to America, is regarded as one of the harmful plant species due to its fast growth, small wind dispersed seed and the absence of natural enemies. Since 1998, potential agents have been studied in Brazil for a classical biological control. This paper presents results from host range and impact tests conducted under field and laboratory conditions for *Syphraea uberabensis* Bechyné (Coleoptera: Chrysomelidae), which is indicated as having great potential as a control agent for *T. herbacea*. A detailed description of the adults of this insect has not been published, and we describe it in this paper.

From a group of 20 species of plants in ten families investigated, *S. uberabensis* fed only on the two species of *Tibouchina* demonstrating that *Tibouchina* supplies the physiological and biological needs of this insect. *S. uberabensis* completes its life cycle on the leaves of *T. herbacea* and does not attack other plant parts. During summer months, the life cycle is completed in approximately 35 days, lengthening to 80 days in the cooler months. The main impact to the plant was caused by the third-instar larvae and adults, and the damage can kill plants in less than 2 weeks. In laboratory conditions, 25% of leaf damage caused leaf death and leaf drop. A batch of *S. uberabensis* was sent to the Quarantine Service of USDA in Hawaii in 2005 where further host-specificity tests are being conducted.

Keywords: host range, impact tests, *Tibouchina*, Hawaii.

Introduction

Herbaceous glory tree, *Tibouchina herbacea* Cogn., native to southeast Brazil, Uruguay, Paraguay and Argentina, has become a particularly troublesome species in the Hawaiian Archipelago (Almasi, 2000). Its vigorous spread by tiny seeds and sprouts is beyond conventional control techniques, and it has been the target of extensive field research in Brazil since early exploratory work was conducted in 1994 by Burkhart (1994). Since 1998, a biological control research program targeting this aggressive and tenacious weed has been underway in southern Brazil, and potential biological agents are being evaluated. Among them, the flea beetle *Syphraea uberabensis* Bechyné (1955) is the highest ranked potential candidate according to the field and laboratory studies conducted in Irati, Brazil (Mueller and Wikler, 2001). Though some species of *Syphraea* are known to feed on the roots of the target weed, *S. uberabensis* has only been observed feeding on leaves.

The genus *Syphraea* is described by Baly (1876) as oval, compact, small black or blue-black flea beetles. *S. uberabensis* are 3–4 mm in length and have a dark blue color. The antennas have robust articles from the base to the apex compared with the anterior tibia; the elytra have simple and very fine punctuations (Bechyné, 1955). This paper provides further description of *S. uberabensis*, its biology and its impact on *T. herbacea* and results of host specificity tests.

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Methods and materials

Seedlings of *T. herbacea* from different provenances were reared for the experiments in greenhouses at the Irati campus. Cultures of *S. uberabensis* were obtained from *T. herbacea* and *Tibouchina cerastifolia* Cogn. plants that occurred naturally at the Irati Campus and Mananciais da Serra, Paraná State and from the locality of Nova Petrópolis, Rio Grande do Sul State.

Insect rearing, biological observations and experiments were conducted under laboratory conditions in a controlled temperature room ranging from 29 ± 2C° day to 22 ± 2C° night, under artificial light banks on a 12-h photo phase. Morphological descriptions were made of insects that were reared under these conditions.

Biological observations and host range experiments were conducted in Petri dish (10-cm diameter) and in plastic bottles of 200 ml, with wet filter paper at the bottom. Leaves of *Tibouchina* species were used to feed individual larva and adult *Syphraea*. The larvae and adults were examined every second day, and host plant leaves were substituted daily to reduce the incidence of diseases and to provide fresh food.

In the plastic bottles, seedlings of both species of *Tibouchina* plants (20 to 30 cm tall) were placed in about 10 cm of sterilized soil collected from the same sites as the plants to provide the whole plants to the insects. Frass was not removed, and no additional plant material was provided.

Based on the phylogenetic system of Cronquist (1981), 20 plant species that were closely related to the *T. herbacea* and occurred in similar habitats were selected for host-specificity tests (Hight *et al.*, 2003). Plants from the families Alzateaceae, Crypteroniaceae, Oliniaceae, Penaeaceae, Punicaceae, Rhynchocalycaceae, Sonneratiaceae and Trapaceae were not included in the tests due their absence in the study region.

For the no-choice tests, neonate's larvae reared at the laboratory and adults collected in the Irati Campus field were placed in Petri dish containing moist filter paper and leaves from the test plant. Four replicates of each test plant were used. After all larvae and adults had died in each dish, the leaves were observed for feeding indicated by scraping of leaf epidermal cells and for larval frass. Leaf area and consumed areas were measured by tracing the leaf outlines and the eaten areas on millimetre paper and counting the areas damaged or missing. Counts of the eggs and initial instars were conducted using a Wild stereoscopic microscope at 10× and 40×. Data were analyzed by conventional statistics using Microsoft® Excel.

Results

Description of adults of *S. uberabensis*

Body: Elongated, slightly broader posteriorly; robust legs; thorax, abdomen, legs and antennae relatively covered with fine short hairs; coloration deep metallic blue, 2.8 ± 0.10 mm long and 1.5 ± 0.03 mm wide.
Head: Top of the head with strong depressed area before frontal calli; punctuation behind frontal calli, longitudinal carina narrow, elevated, eyes small and oblong, entire; antennae more or less thickened.
Thorax: Pronotal punctures small, larger antebasal sulcus but not deeply impressed; lateral margins of pronotum narrow; prosternal narrow with dense and long setation, extending a little beyond posterior margin of procoxae; metasternum densely covered with fine soft hairs.
Elytra: Large punctures densely distributed and deeply impressed toward the base getting a little apex; elytra not smooth; few short setae on posterior margin of elytra.

Biology of *S. uberabensis*

Most information presented in this paper is from the laboratory experiments. When possible, we have provided ranges for summer and winter field conditions. Mating occurred predominantly on the under surface of the leaf toward the apex of the leaf, although, in both laboratory and field situations, we occasionally observed mating on the upper surface. Mating occurred during the night, early hours of daylight and evening, but on rainy days and periods of high humidity, it occurred throughout the day. Mating was effected by the female partially opening their elytra not only to facilitate the appropriate juxtaposition of the male but also allowing for a quick disassociation in case of predation. We observed that mating frequency was reduced when food was scarce, especially in autumn and winter when the plants were in decline or reduced to their overwintering, short shoots.

The eggs were white and elliptical, measuring 0.58 ± 0.01mm by 0.25 ± 0.01mm. Oviposition was observed only in the laboratory because it occurred principally during the cooler hours of darkness and to a lesser extent the early morning or late afternoon. The female pushed the leaf hairs apart with the hind legs and deposited eggs among the hairs on both surfaces of the leaf and on the stem. On *T. herbacea*, the eggs were interspersed between the abundant leaf hairs, but on *T. cerastifolia*, the eggs were cemented on the leaf cuticle. Eggs were normally laid singly, although rarely two or three eggs were found together. It was not known if these eggs were laid at the same time or that females later visited the same site (Wikler and Souza, 2005). Egg laying began 8 ±3 days after copulation in the lab and approximately 12 days after copulation in the field in autumn. The maximum number of eggs oviposited during a single session of 3 h in the lab was 42. The average number of eggs laid in the Petri dishes was 44 ±0.5 per week, although in the first eight weeks, it is higher than 65 eggs per couple and during this period most individuals died (n = 57 mating pairs; Fig. 1). Eggs took between 12 and 21 days to hatch, and the emerging larvae were active and mobile.

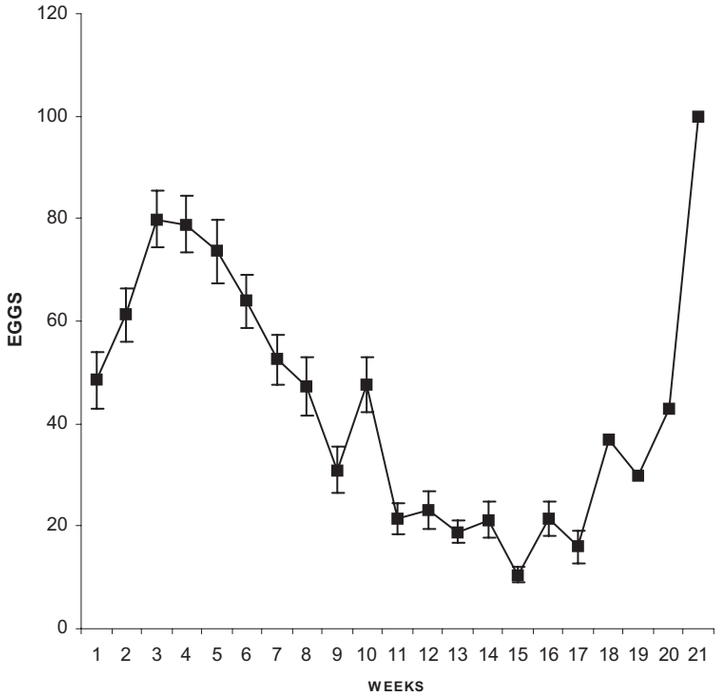

Figure 1. Weekly average number of eggs oviposited by mated females of *Syphraea uberabensis* (Coleopera: Chrysomelidae) under laboratory conditions in a controlled temperature room.

First-instar larvae were 1.95 ± 0.02 mm long, whitish and becoming yellowish on the second day. They have three to four segments, and the head is not well defined. This stage was brief lasting an average of 1.87 days. Feeding damage by these young larvae was almost imperceptible. The second-instar larva was light yellowish color, with five to seven segments and a well defined head, although it was often difficult to distinguish and was best observed from the underside. There was a small posterior protuberance. Initially, the larvae were 3 mm long, growing to 5 mm before ecdysis. This stage lasted from 6 to 12 days. Feeding damage was insignificant and did not exceed 1 cm^2 per week. Third-instar larvae were dark yellow, with seven segments, a well-defined head and a large posterior protuberance. It was 6 mm long. Feeding damage was more than 1.27 cm^2 per week, producing the most significant damage to the plant, greater even than the adult. This stage lasted 12 to 25 days, the first half feeding and the second half entering the soil in prepare for pupation. The pupae were initially the same color as the third-instar larva, darkening only a few days before eclosion.

Adults emerged as soon as 10 days after pupation, although in the field in winter it took at least 30 days. In the laboratory, of 48 insects, only 50% survived 7 weeks, 25% for 11 weeks and all had died by the end of the 21st week

Mortality during development of this insect was high; 65.7 ± 17.2% of the eggs survived to first instar, 55.8% ± 21.4 % to the second instar, 51.2% ± 20.0% to third instar and 7.6 ± 7.7% of eggs survived to adulthood. In only one collect in Rio Grande do Sul, two generalist Hemipterans (not yet identified) were found attacking the adult insects in the field. Fungi also attacked the larvae and pupae during periods of high humidity in the laboratory.

Feeding occurred without preference on both younger and older leaves. Visual observations of leaf consumption by adults and especially third-instar larvae was extensive and resulted in reduced plant viability, lack of flower maturity and reduced seed production (C. Wikler and P. Souza, unpublished data).

No-choice tests

In no-choice tests, *S. uberabensis* laid eggs on and larvae and adults fed on plants in the genus *Tibouchina* but not on any other test plant (Table 1). Additional feeding trials conducted in the laboratory showed that larvae completely defoliated *T. herbacea* in no-choice tests and were able to complete development to adults on this species. In choice tests, larval preferences for *T. herbacea* and *T. cerastifolia* were equal.

Effects on *T. herbacea* and *T. cerastifolia*

In the experiments of biological impact, adults and larvae of *S. uberabensis* demonstrated great potential

Table 1. The list of test plants used in the host specificity tests for *Syphraea S. uberabensis* and the results, where X indicates positive results.

Family	Species	Adults	Larvae	Eggs
Anacardiaceae	*Rhus sandwichensis* Gray			
	Schinus terebinthifolius Raddi			
	Lithraea brasiliensis Marchand			
Euphorbiaceae	*Manihot esculenta* Crantz			
	Phyllanthus tenellus Roxb.			
Lythraceae	*Lafoensia pacari* St. Hil.			
Melastomataceae	*T. herbacea* (DC.) Cogn.	X	X	X
	T. cerastifolia Cogn.	X	X	X
Monimiaceae	*Peumus boldus* Molina			
Myrtaceae	*Psidium cattleianum* Sabine (red form)			
	Psidium cattleianum Sabine (yellow form)			
	Psidium guava L.			
	Campomanesia xanthocarpa O. Berg.			
	Eugenia uniflora L.			
	Eucalyptus grandis W. Hill ex. Maiden			
Onagraceae	*Ludwigia* sp			
Poaceae	*Bambusa vulgaris*Schrad			
Rosaceae	*Pyrus malus* L.			
	Pyrus communis L.			
Thymelaeaceae	*Daphnopsis racemosa* Griseb			

to be used in the control of *T. herbacea* due the extensive damage caused to the plant. The leaves were skeletonized removing completely the plant matter, leaving only the stem and vein structure. As a consequence, plant growth was reduced, and flowering and consequently seed production were prevented.

The consumed leaf area was higher by the larvae (third instar) that in the adult stage. The consumption difference was not significant because, on average, the larvae consumed less than half of a square centimetre more than the adults. The weekly consumption area of the adults was on average 1.15 cm^2, approximately 12.8% of the total leaf area, and the consumption of the larvae was on average of 1.28 cm^2, approximately 14.2% of the total leaf area.

In the plastic bottles experiment, it was observed that consumption of about 25% of the leaf was enough to dry it out and cause leaf drop. The plant showed high vulnerability to the *S. uberabensis* attack, which caused the defoliation of all of the plants and they all died, on average, after 4 weeks.

In the field, as in the laboratory, the leaves of both *Tibouchina* species demonstrated low or no regenerating capacity after the attack of the *S. uberabensis*, drying soon after a period of 2 weeks of the insect damage.

Discussion

Laboratory and field investigations confirmed that *S. uberabensis* is strictly specific to the genus *Tibouchina* and therefore a safe biological control agent for *T. herbacea* in Hawaii. According to Harris (1971), the loss of mature leaves is normally most damaging to the plant, as these leaves represent the direct photosynthetic capacity of the plant. The attack by *S. uberabensis* is therefore meaningful, as no preferences based on the age of the leaves were found. As result of these studies and the potential of this insect as a biological control agent, 2000 insects were sent in 2005 to the Quarantine Service of USDA in Hawaii where further host-specificity tests are being conducted.

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