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Biology of immature stages and host range characteristics of *Sudauleutes bosqi* (Coleoptera: Curculionidae), a candidate biological control agent of exotic *Ludwigia* spp. in the USA

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Abstract

South American invasive plants in the genus *Ludwigia* (Onagraceae) degrade many riparian and aquatic ecosystems worldwide. Biological control may aid in the management of these exotic weeds, but data on the host specificity of *Ludwigia* natural enemies is limited. The biology and host range of *Sudauleutes bosqi* Hustache (Coleoptera: Curculionidae), an herbivore of *Ludwigia* spp. in South America, was studied to determine its suitability as a biological control agent for 3 exotic *Ludwigia* spp. (targets) in the US. Weevils maintained at 25 °C (± 1 °C) and a 14:10 h (L:D) photoperiod developed through 7 life stages, with a generation time from egg to adult of 17.6 (± 1.2) d when reared on the target weed *Ludwigia hexapetala* (Hook. & Arn.) Zardini, Gu & P. H. Raven (Onagraceae). There was no difference in mean body length between females (2.6 ± 0.1 mm) and males (2.5 ± 0.1 mm). No-choice and multiple-choice host range tests were conducted using 3 exotic *Ludwigia* spp. and 8 native US plant species. *Sudauleutes bosqi* larvae completed development on the 3 target weeds and 4 native plant species, and oviposition occurred on all but 1 of the plant species that supported larval development. In multiple-choice tests, *S. bosqi* oviposited on 9 of 11 plant species tested. Results indicate that host selection and development of *S. bosqi* is not limited to target weeds but also includes valued non-target species. Therefore, *S. bosqi* is not sufficiently host-specific for further consideration as a biological control agent of exotic *Ludwigia* spp. in the US and additional testing is not warranted.

Key Words: aquatic weeds; invasive species; water primroses; development; host specificity; *Auleutes bosqi*

Resumen

Las plantas invasoras sudamericanas del género *Ludwigia* (Onagraceae) degradan muchos ecosistemas ribereños y acuáticos en todo el mundo. El control biológico puede ayudar en el manejo de estas malas hierbas exóticas, pero los datos sobre la especificidad de hospedero de los enemigos naturales de *Ludwigia* son limitados. Se estudió la biología y el rango de hospederos de *Sudauleutes bosqi* Hustache (Coleoptera: Curculionidae), un herbívoro de *Ludwigia* spp. en América del Sur para determinar su sostenebilidad como agente de control biológico de 3 especies exóticas de *Ludwigia* spp. (objetivos) en los EE.UU. Los gorgojos mantenidos a 25 °C (± 1 °C) con un fotoperíodo de 14:10 h (L:D) se desarrollaron a lo largo de 7 estadios de vida, con un tiempo de generación de huevo a adulto de 17,6 (± 1,2) dias cuando se criaron en la maleza objetivo *Ludwigia hexapetala* (Hook. & Arn.) Zardini, Gu & PH Raven (Onagraceae). No hubo diferencia en la longitud corporal media entre las hembras (2,6 ± 0,1 mm) y los machos (2,5 ± 0,1 mm). Se realizaron pruebas de variedad de hospederos de opción múltiple y de elección múltiple utilizando 3 especies exóticas de *Ludwigia* spp. y 8 especies de plantas nativas de EE. UU. Las larvas de *Sudauleutes bosqi* completaron el desarrollo en las 3 malezas objetivo y las 4 especies de plantas nativas, y la oviposición sucedió en todas menos 1 de las especies de plantas que apoyaron el desarrollo de las larvas. En las pruebas de opción múltiple, *S. bosqi* ovipositó en 9 de las 11 especies de plantas analizadas.

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Los resultados indican que la selección de hospederos y el desarrollo de *S. bosqi* no se limita a las malas hierbas objetivo, sino que también incluyen valiosas especies no objetivo. Por lo tanto, *S. bosqi* no es lo suficientemente específico para el hospedero como para ser considerado como un agente de control biológico de especies exóticas de *Ludwigia* en los EE. UU. y no se garantizan pruebas adicionales.

Palabras Claves: malas hierbas acuáticas; especies invasivas; prímulas de agua; desarrollo; especificidad del hospedero; *Auleutes bosqi*

Ludwigia species (Onagraceae) were introduced from Central and South America to locations worldwide as ornamentals in the mid-nineteenth century (Wagner et al. 2007; Grewell et al. 2016a). A select group from the largely aquatic *Ludwigia* section *Jussiaea* (Hoch et al. 2015) have invaded both aquatic and riparian ecosystems (Thouvenot et al. 2013; Kim et al. 2019) and now are considered among the most aggressive weeds in the world (Cronk & Fuller 2001). This is particularly evident in the western and southeastern coastal regions of the US where 4 *Ludwigia* taxa have naturalized in aquatic systems (Grewell et al. 2016a): *Ludwigia hexapetala* (Hook. & Arn.) Zardini, Gu & P. H. Raven, *Ludwigia peploides* (Kunth) P. H. Raven subsp. *peploides*, *Ludwigia peploides* (Kunth) P. H. Raven subsp. *montevidensis* (Spreng.) P. H. Raven, and *Ludwigia grandiflora* (Michx.) Greuter & Burdet (all Onagraceae). These species form dense mats that impact ecological processes in aquatic ecosystems, including the displacement of desired wildlife and vegetation (Stiers et al. 2011; Thouvenot et al. 2013; Grewell et al. 2016b, 2019; Khanna et al. 2018). They also impede navigation and interfere with recreational activities, irrigation, drainage, and agricultural production (Thouvenot et al. 2013; Grewell et al. 2016a). The invasive potential of these taxa often is attributed to habitat eutrophication, adaptation through hybridization, phenotypic plasticity, vegetative and sexual modes of reproduction, and a general lack of specialized herbivores in the introduced range that regulate plant population growth (Grewell et al. 2016a, b; Reddy et al. 2021).

Management of exotic *Ludwigia* spp. in the US has relied on physical and chemical methods (Thouvenot et al. 2013); however these options often provide short term control and require repeated annual treatments (Sarat et al. 2015, 2018; Grewell et al. 2016a), which also are costly. For example, in the US, the Division of Boating and Waterways, Sacramento, California, USA, spends $7 million per yr to control invasive plants, including *Ludwigia* spp., in the Sacramento–San Joaquin River Delta in northern California (Brusati 2009). In addition, *L. hexapetala* and *L. peploides* produce viable seeds with a high capacity for germination under a wide range of temperatures (Gillard et al. 2017a, b) resulting in persistent seedbanks that require long-term management programs (Grewell et al. 2019). Additional tools are needed, specifically in environmentally sensitive systems where herbicide use is limited or not permitted (Grewell et al. 2016a). One sustainable and long-term alternative under consideration since the 1970s is the use of natural enemies to control exotic *Ludwigia* spp. in the US (i.e., biological control) (Cordo & DeLoach 1982a, b). The first foreign explorations for natural enemies of *Ludwigia* spp. were conducted by Cordo and DeLoach (1982a, b) in Argentina where they reported 5 beetle species. A more recent and comprehensive survey was conducted by Hernández and Cabrera Walsh (2014) in Argentina, which enumerated 19 insect species across 6 feeding guilds that feed on *L. hexapetala*. Among the described species, the defoliating weevil *Sudauleutes bosqi* Hustache (= *Auleutes bosqi*) (Coleoptera: Curculionidae) (Colonnelli 2004), was observed commonly in both surveys feeding on *Ludwigia* spp. (Cordo & DeLoach 1982a; Hernández & Cabrera Walsh 2014).

Little is known concerning the life history and host specificity of *S. bosqi*. Adults are small, reddish-brown, and feed on the surface of leaves, removing about 14.3 mm^2 of foliar surface area per d (Cordo & DeLoach 1982a). The larvae prefer to feed on young apical leaves, and pupation occurs within a spherical cocoon at the base of the plant (Hernández & Cabrera Walsh 2014). Moreover, based on field observations, Cordo & DeLoach (1982a) suggested *S. bosqi* was a possible candidate biological control agent of exotic *Ludwigia* spp. in the US and that its host range probably was limited to the genus *Ludwigia* in Argentina. However, aside from reporting the basic life history of *S. bosqi* adults, neither study investigated the biology and physiological host range of *S. bosqi*. Formal host specificity testing is needed to quantify the diet breadth of *S. bosqi* in relation to the diverse native *Ludwigia* species in the US (Reddy et al. 2021). Filling this knowledge gap, coupled with the renewed interest in *Ludwigia* biological control in the last decades (Reddy et al. 2021), led to surveys in Argentina and Uruguay in 2019, with a specific focus on collecting and colonizing *S. bosqi* for the present study.

Therefore, the primary objective of this research was to test the hypothesis that *S. bosqi* is host specific to plants within the *Ludwigia* section *Jussiaea*, which is required for a suitable biological control agent in the US given that there are no native representatives of the *Jussiaea* (Reddy et al. 2021). To accomplish this goal, no-choice and multiple-choice host range tests were conducted with an initial suite of 11 plant species that represented 3 exotic *Ludwigia* targets (*L. hexapetala*, *L. peploides* subsp. *peploides*, and *L. peploides* subsp. *montevidensis*) and 8 native US taxa. In addition, biological characteristics of *S. bosqi* were investigated to aid in interpreting herbivore performance across host plants, and thus supplement existing knowledge for this species.

Materials and Methods

ORIGIN AND REARING OF *SUDAULEUTES BOSQI*

Sudauleutes bosqi were collected from *L. hexapetala* plants on the edge of Laguna del Diario (34.8969904°S, 55.0033590°W), Uruguay during Mar 2019. The nascent *S. bosqi* colony was exported from Uruguay under scientific collection permit N° 9/2019 supplied by the Dirección Nacional de Medio Ambiente and imported under USDA APHIS-PPQ permit #P526P-19-03070 to a USDA-ARS containment facility in Albany, California, USA. Species identity was confirmed by the USDA-ARS Systematic Entomology Laboratory at the Smithsonian Institution, National Museum of Natural History, Washington, DC, USA, based on specimens in the US National Collection, and identified by Enzo Colonnelli. Vouchers were deposited in that institution. The colony was maintained on a laboratory benchtop under ambient temperature (20–25 °C), lighting, and humidity conditions. Adults were kept in a cylindrical 947 mL plastic containers (14.5 cm H × 11.5 cm D) with a piece of fine mesh cloth integrated into the lid to allow air circulation and prevent condensation. Approximately 15 adults per container fed and reproduced on a bouquet composed of 3 excised *L. hexapetala* stems (15 cm long) inserted into a plastic floral water tube. Stems were changed weekly. Between feedings, water was added to floral tubes as needed to maintain plant turgor. Periodically, older bouquets harboring eggs were retained and reared to augment colony numbers. The colony was reared exclusively on *L. hexapetala*, originally collected from the Sacramento–San Joaquin River Delta in northern California (38.002453°N, 121.568594°W). All subsequent biology and host range experiments were conducted in an environmental chamber set to constant 25 °C (± 1 °C), with a 14:10 h (L:D) photoperiod.

EGG DEVELOPMENT OF *SUDAULEUTES BOSQI*

Fresh bouquets of *L. hexapetala* stems were provided to all adult colony containers described above. Plant material was removed after 24 h and all eggs were collected. Individual eggs were cut from foliage, mixed with individuals from other colony containers, and randomly spread across 15 replicate Petri dishes (90 mm diam; Fisher Scientific, Waltham, Massachusetts, USA). Each Petri dish contained 10 eggs that were placed carefully on sterile filter paper (Whatman No. 2; Fisher Scientific, Waltham, Massachusetts, USA) moistened with water prior to sealing the dish with Parafilm® (Bemis Company, Inc., Neenah, Wisconsin, USA) to avoid desiccation. Replicated Petri dishes were arranged in a completely randomized design in a chamber and their position was rotated daily when egg hatching was monitored. Water was added as needed to keep the filter paper moist. Mean development time (d) and egg viability (larval hatching proportion: larvae hatched divided by eggs monitored) per Petri dish were calculated. Additionally, egg size was measured from 20 randomly selected eggs that originated from different parental females. Eggs were measured from pole to pole across the long side using a dissecting microscope (Olympus Corporation, Shinjuku, Tokyo, Japan) equipped with an ocular micrometer (Nikon, Minato, Tokyo, Japan).

LARVAL DEVELOPMENT OF *SUDAULEUTES BOSQI*

Twenty neonate larvae (≤ 24 h old) from the egg development study were collected at random and transferred individually using a fine brush onto the young leaves of a *L. hexapetala* stem (10 cm long) inserted into a floral water tube situated within an enclosed cylindrical 237 mL plastic container (4.0 cm H × 11.5 cm D). Fresh stems were provided weekly and water in the floral tube was replenished 3 times per wk. Larvae were monitored daily and developmental stage (visualized by the presence of exuviae) was recorded until adult metamorphosis. On the d each molt occurred, head capsule size was measured at the widest point (genae) using a dissecting microscope (Olympus Corporation, Shinjuku, Tokyo, Japan) equipped with an ocular micrometer (Nikon, Minato, Tokyo, Japan). Subsequently, the number of instars, head capsule size (mm) of each instar, and development time (d) of each stage were calculated. Total development time from egg to adult was calculated by adding mean egg, larval, and pupal development times. Finally, the length of 30 randomly selected adults from the colony (15 females and 15 males) was measured from the most forward part of the head (at the frons between the eyes) to the last abdominal segment. Because it was not possible to separate the sexes using morphological characters, females were identified by conducting 48 h oviposition tests; adults were placed singly in filter paper-lined Petri dishes containing a *L. hexapetala* leaf, with wet cotton wrapped around the petiole base. Water was added to the cotton after 24 h to prevent wilting. After 48 h, foliage was checked for the presence of eggs to differentiate females from males.

HOST RANGE EXPERIMENTS: TEST PLANTS

The test plant list was comprised of 11 taxa from the Onagraceae: 3 exotic *Ludwigia* targets (*L. hexapetala*, *L. peploides* subsp. *peploides*, and *L. peploides* subsp. *montevidensis*), 7 native taxa (*Ludwigia polycarpa* Short & Peter, *Ludwigia repens* J. R. Forst., *Ludwigia palustris* (L.) Elliott, *Epilobium ciliatum* Raf. subsp. *ciliatum*, *Epilobium canum* (Greene) P. H. Raven, *Clarkia amoena* (Lehm.) A. Nelson & J. F. Macbr., and *Oenothera elata* Kunth subsp. *hookeri* (Torr. & A. Gray) W. Dietr. & W. L. Wagner [all Onagraceae]) and *Ludwigia decurrens* Walter (Onagraceae), a congener that is sympatric with the target weeds and native to eastern-central US. *Ludwigia decurrens* is non-native to California where it established around 2011 as a noxious weed in rice fields (Kelch 2015). The native test species (non-targets) were selected based on their phylogenetic relationship to the 3 target species (Reddy et al. 2021). All test species were used in both no-choice and multiple-choice host range experiments. Plants were propagated over time in a greenhouse under controlled temperature (20–32 °C), a 14:10 h (L:D) photoperiod, and ambient humidity conditions. They were incorporated into host-range tests as available, and always included *L. hexapetala* as the control.

NO-CHOICE DEVELOPMENT AND OVIPOSITION TESTS

Four neonate larvae (≤ 24 h old) randomly were assigned a host plant species and transferred with a fine brush onto the young leaves of a 10 cm long stem (experimental unit) inserted into a floral water tube. Five replicate stems were placed individually in a cylindrical 473 mL plastic container (7.5 cm H ´ 11.5 cm D) (4 neonates × 5 replicate stems = 20 larvae per test plant species; *n* = 11 plant species). Larvae were transferred to fresh stems of their assigned test plant species twice per wk. Water in the floral tubes was replenished 3 times per wk during which time the larvae were observed under a dissecting microscope (Olympus Corporation, Shinjuku, Tokyo, Japan) to record survival and developmental stage. Larval survival rate (proportion) and mean development time from first instar to adult (*n* = 4 larvae per replicate) were calculated for each replicate stem.

The resulting adults from the no-choice development tests were collected and grouped by emergence date. Following the colony rearing methods described above, adults were kept in a rearing container for 1 wk to allow sexual maturation and mating, and they were fed the plant species from which they emerged. Females were identified by conducting 48 h oviposition tests described above, then each was paired with 1 male and placed in a 473 mL plastic container together with a bouquet of 2 to 3 stems (10 cm long) of the plant species on which the female was reared. This process was repeated until 5 replicate females per test plant were evaluated. Some test plant species had less replicates evaluated because the number of emerged females dictated the number of replicates. Adult males (1–4 wk old) from the colony were used if there were not enough males from the experiments described above. Eggs were collected from each bouquet after 8 to 10 d and counted under a dissecting microscope (Olympus Corporation, Shinjuku, Tokyo, Japan). Eggs from each female then were placed on moistened filter paper as described above and egg viability (hatching) was monitored daily until all eggs hatched or became shriveled (indicating mortality). Water was added to the filter paper as needed during monitoring. Subsequently, the number of eggs oviposited and egg viability (larval hatching proportion: larvae hatched divided by eggs oviposited) was calculated for each replicate female.

MULTIPLE-CHOICE OVIPOSITION TESTS

Experiments were conducted using colony adults (1–4 wk old). Gravid females were identified by conducting 48 h oviposition tests as described above. Five adult pairs were placed in a plastic container (36 cm L × 28 cm W × 24 cm H) together with 3 to 5 bouquets (1 bouquet per test plant). Five replicate bouquets per plant species were assessed (5 replicates × 11 plant species = 55 bouquets total). Each bouquet was composed of 2 stems (15 cm long) from a single test plant species inserted into a floral water tube as a potential source for feeding and oviposition. The side walls of the container were modified with a piece of fine mesh cloth to allow air circulation and prevent condensation within the container. Adults were collected and returned to the colony after an oviposition period of 4 d and eggs oviposited on each bouquet

were counted. The presence of feeding damage was noted, but not quantified. The experimental setup was repeated over time in 3 separate trials where a different set of plant species, including *L. hexapetala* as the control, was tested (i.e., 5, 5, and 3 plant species per trial).

DATA ANALYSES

Data were tested for normality using Shapiro-Wilk tests. Larval hatch and survival data (proportion) were arcsine square-root transformed; eggs per female, eggs per plant, and adult body length data were square-root transformed; and mean larval development data were $\log_{10}$ transformed to normalize results prior to analyses. One-way ANOVAs were then used to compare body length between female and male adults and to compare larval survival, mean larval development time, eggs per female, and egg viability (larval hatching proportion per female) among plant species in no-choice tests. A linear mixed model was used to test for the effect of test species on oviposition (eggs per plant) in multiple-choice tests. Post-hoc pairwise comparisons between test species were made with Tukey's HSD ($\alpha = 0.05$). Plant species on which larvae failed to survive or females did not oviposit were omitted from the analyses. All analyses were conducted using JMP® PRO, version 15 (SAS 2019).

Results

LIFE HISTORY OF *SUDAULEUTES BOSQI*

Sudauleutes bosqi completes 7 stages during development: egg, 3 larval instars, prepupa, pupa, and adult. Generation time from egg to adult was 17.6 ± 1.2 d (range 17.3–21.3 d; *n* = 12; hereafter means are reported with ± 1 SD) when feeding on *L. hexapetala* and reared at 25 °C.

Females oviposited eggs singly below the epidermis of the leaf lamina into holes chewed by the female, usually on the margins but occasionally in the interior of the leaf. Eggs were light yellow and slightly oval in shape with symmetrical round poles. Mean length was 0.5 ± 0.04 mm (range 0.4–0.6 mm; *n* = 20). Mean development time from oviposition to hatch was 3.3 ± 0.4 d (range 2.7–3.8 d; *n* = 15), and an average proportion of 0.9 ± 0.1 (range 0.6–1; *n* = 15) of those eggs were viable.

Neonate larvae were transparent yellow with a black head. Average development time of the first, second, and third larval instars were 2.6 ± 1.1 d (range 2–6.5 d; *n* = 19), 2.3 ± 1 d (range 1–5.5 d; *n* = 19), and 2.6 ± 1.1 d (range 2–5.5 d; *n* = 11), with an average head capsule size of 0.2 ± 0.01 mm (*n* = 20), 0.4 ± 0.03 mm (*n* = 19), and 0.5 ± 0.03 mm (*n* = 19), respectively. Larvae ceased feeding at the end of the third larval stage, the body became opaque yellow and moved to the base of stems (i.e., bouquet). The larvae then formed a spherical pupal case that was attached to a moist surface, typically where the stems meet the lid of the plastic water tube but occasionally on a substrate at the bottom of the rearing container. Because not all larvae built a pupal case, it was possible to measure the duration of the prepupal stage for some individuals. Mean prepupal and pupal periods were 1.5 ± 0 d (*n* = 8) and 6.6 ± 1.3 d (range 5–10 d; *n* = 12). Total larval development time (neonate to adult) was 14.3 ± 1.2 d (range 14–18 d; *n* = 12).

Newly emerged adults were tan and turned reddish brown after ≤ 24 h. Adults were observed feeding on apical and older leaves of *L. hexapetala*. There was no difference ($F = 1.94$; df = 1,28; $P = 0.174$) between average body length of females (2.6 ± 0.1 mm; range 2.4–2.9 mm; *n* = 15) and males (2.5 ± 0.1 mm; range 2.4–2.6 mm; *n* = 15).

NO-CHOICE HOST RANGE TESTS

Sudauleutes bosqi larvae did not survive on 4 native plant species (*E. canum*, *E. ciliatum* subsp. *ciliatum*, *O. elata* subsp. *hookeri*, and *L. decurrens*), but successfully completed development on the remaining 7 plant species tested: 3 exotic *Ludwigia* targets and 4 native species (Table 1). However, larval survival proportion did not differ among plant species that supported complete development ($F = 1.75$; df = 6,43; $P = 0.132$). In contrast, mean larval development time differed across plant species ($F = 12.01$; df = 6,20; $P < 0.0001$), which ranged from 15.75 (*L. peploides* subsp. *montevidensis*) to 24.00 d (*L. repens*). Development was faster on *L. peploides* subsp. *montevidensis* than on *L. hexapetala*, *L. repens*, and *L. palustris* (Tukey's HSD test, $P \leq 0.05$). Development was slower on the native *L. repens* than on the 3 *Ludwigia* target weeds (*L. hexapetala*, *L. peploides* subsp. *peploides*, *L. peploides* subsp. *montevidensis*) and on *C. amoena* (Tukey's HSD test, $P \leq 0.05$). Within the 3 *Ludwigia* target weeds, larval development time differed between *L. hexapetala* and *L. peploides* subsp. *montevidensis* ($P = 0.046$), but not between *L. hexapetala* and *L. peploides* subsp. *peploides* ($P = 0.216$) or between *L. peploides* subsp. *peploides* and *L. peploides* subsp. *montevidensis* ($P = 0.956$).

Oviposition was monitored on the 7 test plant species that supported complete larval development (Table 1). However, *L. polycarpa* and *C. amoena* were excluded from the analyses because there was

Table 1. Larval survival and development (first instar to adult), oviposition, and egg viability of *Sudauleutes bosqi* on exotic *Ludwigia* and native test plant species in no-choice host range tests. Mean ± 1 SE (*n*).

Test plant	Larval survival (proportion)	Larval development (d)	Number of eggs per female	Larval hatching per female (proportion)[3]
Ludwigia hexapetala[1]	0.43 ± 0.08 (15) a	18.87 ± 0.61 (5) bc	36.38 ± 7.67 (8) a	0.82 ± 0.04 (8) a
L. peploides subsp. *peploides*[1]	0.50 ± 0.08 (5) a	16.70 ± 0.96 (5) cd	29.50 ± 7.50 (2) a	0.84 ± 0.12 (2) a
L. peploides subsp. *montevidensis*[1]	0.50 ± 0.16 (5) a	15.75 ± 0.25 (4) d	24.25 ± 6.05 (4) a	0.93 ± 0.05 (4) a
L. polycarpa[2]	0.10 ± 0.06 (5) a	19.00 ± 0.00 (2) abcd	1.00 (1)[4]	1.00 (1)[4]
L. repens[2]	0.20 ± 0.15 (5) a	24.00 ± 1.00 (2) a	9.50 ± 2.50 (2) a	0.700 ± 0.003 (2) a
L. palustris[2]	0.35 ± 0.07 (10) a	21.55 ± 0.50 (5) ab	0.00 (2)[4]	n/a[4,5]
Clarkia amoena[2]	0.35 ± 0.1 (5) a	16.50 ± 0.73 (4) cd	4.00 (1)[4]	0.14 (1)[4]
Epilobium canum[2]	0 (5)[4]			
E. ciliatum subsp. *ciliatum*[2]	0 (5)[4]			
Oenothera elata subsp. *hookeri*[2]	0 (5)[4]			
L. decurrens[2]	0 (5)[4]			

Means within a column followed by different lowercase letters are significantly different ($P \leq 0.05$; ANOVA and Tukey's HSD test).
[1]Target weed; [2]native species; [3]egg viability (larval hatching proportion = larvae hatched divided by eggs oviposited) was calculated for each replicate female; [4]excluded from analysis; [5]no larval hatching data available as no oviposition occurred.

only 1 replicate (of 5) each where 1 and 4 eggs were oviposited (larval hatching proportion of 1.00 and 0.14), respectively. *Ludwigia palustris* also was excluded because no oviposition occurred on this test plant. For the remaining 4 test plant species, no difference in total eggs per female (F = 1.22; df = 3,12; P = 0.346) or larval hatching proportion (F = 2.73; df = 3,12; P = 0.090) was observed among plant species.

MULTIPLE-CHOICE HOST RANGE TESTS

Of the 11 plant species tested, *S. bosqi* did not oviposit on *L. decurrens* and *E. canum* (Table 2). The number of eggs oviposited per plant differed among test plant species (F = 22.95; df = 8,42.36; P < 0.0001). The highest number of eggs were oviposited on *L. hexapetala*, which differed from *L. palustris*, *C. amoena*, *E. ciliatum* subsp. *ciliatum*, and *O. elata* subsp. *hookeri* (Tukey's HSD test, $P \leq 0.05$). *Clarkia amoena*, *E. ciliatum* subsp. *ciliatum*, and *O. elata* subsp. *hookeri* received the lowest number of eggs and they differed from *L. peploides* subsp. *peploides*, *L. peploides* subsp. *montevidensis*, *L. polycarpa*, and *L. repens* (Tukey's HSD test, $P \leq 0.05$). Oviposition did not differ among the 3 *Ludwigia* target weeds (Tukey's HSD test, $P > 0.05$). Adult feeding damage was observed on all test plant species, except *E. canum* and *L. decurrens*, and minimally on *C. amoena*, *E. ciliatum* subsp. *ciliatum*, and *O. elata* subsp. *hookeri*.

Discussion

Life history characteristics and host range of *S. bosqi* were examined as part of a risk assessment to determine its potential as a candidate biological control agent in the US. Particular interest in this species was placed as previous reports prioritized this species for consideration (Cordo & DeLoach 1982a). Weevils have been particularly successful in controlling invasive plants throughout the world, including *Eichhornia crassipes* (Mart.) Solms (water hyacinth; Commelinales: Pontederiaceae), *Salvinia molesta* D.S. Mitch. (giant salvinia; Salviniales: Salviniaceae), *Pistia stratiotes* L. (water lettuce; Alismatales: Araceae), and *Carduus* thistles (*Carduus acanthoides* L. and *Carduus nutans* L.; Asterales: Asteraceae) (O'Brien 1995; Julien & Griffiths 1999; Kok 2001; Herrick & Kok 2010). Beyond biological control, data reported here also have relevance to general life history characteristics of *S. bosqi*, because no information on its biology has been reported until now, except for adult body length (about 2.5 mm) (Cordo & DeLoach 1982a). Results from this study show that there is no difference in length between *S. bosqi* females (2.6 mm) and males (2.5 mm). *Sudauleutes bosqi* belongs to the minute seed weevil subfamily Ceutorhynchinae (Colonnelli 2004) and its life history is similar to that of 2 confamilials and biological control agents, *Euhrychiopsis lecontei* Dietz (milfoil weevil) and *Rhinoncomimus latipes* Korotyaev (mile-a-minute weevil) (both Coleoptera: Curculionidae). The size of *S. bosqi* adults and egg length (about 0.5 mm) were similar to *E. lecontei*, which are 2 to 3 mm and 0.5 mm, respectively (MAISRC 2021). Generation time of *S. bosqi* (about 17 d) is 3 and 5 to 6 d faster than *E. lecontei* (Mazzei et al. 1999) and *R. latipes*, respectively, at 25 °C (Hough-Goldstein et al. 2016). The fast generation time of *S. bosqi* greatly facilitated its rearing and experimentation during host specificity testing. The early larval instars are not difficult to rear. Higher mortality was observed, however, between the late third instar and pupal stage, possibly due to pupal requirements for a moist environment that was difficult to maintain in the laboratory setting (Cordo & DeLoach 1982a).

We found no evidence to support the hypothesis that *S. bosqi* is host specific to species within the *Ludwigia* section *Jussiaea*, herein represented by the target weeds *L. hexapetala*, *L. peploides* subsp. *peploides*, and *L. peploides* subsp. *montevidensis*. Under no-choice conditions, *S. bosqi* larvae fed and completed development on 7 of the 11 plant species tested: all 3 target weeds and 4 native species (*L. polycarpa*, *L. repens*, *L. palustris*, and *C. amoena*). There was no difference in *S. bosqi* survival among host species, but development time varied across species with higher levels of variability among the native species (Table 1). Interestingly, no development occurred on the closely related *L. decurrens* as compared to more distantly related hosts. We hypothesize that unlike other *Ludwigia* species, *L. decurrens* is not part of the host range of *S. bosqi* because the growth habitat requirements of this plant preclude any association between *S. bosqi* and *L. decurrens*. In its native range, *L. decurrens* is found in habitats similar to that of other *Ludwigia* species from the *Macrocarpon* section and *S. bosqi* has not been found on these plant species (ADS personal observation). In contrast, *Ludwigia* species associated with *S. bosqui* (*L. hexapetala* and *L. peploides* spp.) have growth habits similar to that of *L. repens* and *L. palustris*, which also are part of the host range of *S. bosqi*.

While larval survival and development provides important insights to host specificity, comparing adult fitness between individuals reared on different species can reveal sublethal effects of suboptimal hosts. Oviposition patterns separated test plant species that supported complete development into 2 groups: the 3 target weeds versus the 4 native species (Table 1). The number of eggs oviposited per female ranged from 24 to 36 on the target weeds but was consistently lower (0–9.5 eggs per female) on 4 native plant species (*L. polycarpa*, *L. repens*, *C. amoena*, and *L. palustris*). The combined effect of limited oviposition (*L. palustris*) and replication (*L. polycarpa* and *C. amoena*) in no-choice tests precluded comparing oviposition between a greater sample of

Table 2. Eggs oviposited by *Sudauleutes bosqi* females on exotic *Ludwigia* and native test plant species in multiple-choice host range tests. Mean number of eggs ± 1 SE (*n*).

Test plant	Number of eggs per test plant	Range in number of eggs per test plant
Ludwigia hexapetala[1]	32.53 ± 4.27 (15) a	11–70
L. peploides subsp. *peploides*[1]	22.60 ± 3.75 (n) ab	12–33
L. peploides subsp. *montevidensis*[1]	29.20 ± 5.82 (5) ab	13–45
L. polycarpa[2]	20.80 ± 3.28 (5) abc	10–29
L. repens[2]	12.20 ± 1.85 (5) ab	7–18
L. palustris[2]	3.60 ± 1.50 (5) bcd	0–9
Clarkia amoena[2]	0.40 ± 0.24 (5) cd	0–1
Epilobium ciliatum subsp. *ciliatum*[2]	1.20 ± 0.49 (5) cd	0–2
Oenothera elata subsp. *hookeri*[2]	1.80 ± 1.11 (5) d	0–5
E. canum[2]	0.00 (5)[3]	
L. decurrens[2]	0.00 (5)[3]	

Means within a column followed by different lowercase letters are significantly different (P £ 0.05; linear mixed model and Tukey's HSD test).
[1]Target weed; [2]native species; [3]excluded from analysis.

native plant species and the target weeds. Nevertheless, the number of eggs oviposited and subsequent viability of these eggs did not differ between the 3 target weeds and the native *L. repens* (Table 1), suggesting no apparent decrease in fitness over a generation of feeding exclusively on the test plant species. It is surmised from development and oviposition data that several native plants included in this study are likely to support sustained *S. bosqi* populations for more than the 1 generation.

Whereas *S. bosqi* larvae may lack host specificity, females can restrict host use through selective oviposition. Therefore, multiple-choice tests were conducted to provide insights to the herbivore's ovipositional host plant selection preferences. Herein, however, *S. bosqi* females did not demonstrate a strong ovipositional preference for species in the *Jussiaea* section of *Ludwigia* over native conspecifics (Table 2). Oviposition occurred on all but 2 (*L. decurrens* and *E. canum*) of the 11 plant species tested. Females oviposited the most eggs on the 3 target weeds and 2 native plant species (*L. polycarpa* and *L. repens*), but oviposition did not differ between these 5 species. In contrast, females oviposited significantly fewer eggs on a separate group of 4 native plant species (*L. palustris*, *C. amoena*, *E. ciliatum* subsp. *ciliatum*, and *O. elata* subsp. *hookeri*), but oviposition did not differ between these species as well. *Sudauleutes bosqi* also oviposited on plant species that do not support development. The weevil oviposited on *E. ciliatum* subsp. *ciliatum* and *O. elata* subsp. *hookeri*, yet larval development tests showed that larvae cannot complete development on these species. These data suggest that *S. bosqi* females can oviposit broadly among hosts that range from optimal to unacceptable suitability for larval survival.

Collectively, these data indicate that *S. bosqi* is not a specialist of the *Jussiaea* section but rather an oligophagous herbivore of *Ludwigia* spp., and possibly related species (e.g., *C. amoena*). *Sudauleutes bosqi* did not distinguish *Ludwigia* spp. from *C. amoena* during development, but preferred *Ludwigia* spp. to *C. amoena* in multiple-choice oviposition tests. The findings are consistent with field observations of *S. bosqi* by Cordo and DeLoach (1982a) and Hernández and Cabrera Walsh (2014), who recorded adults feeding on *Ludwigia peploides* (Kunth) P. H. Raven, *L. hexapetala*, *L. grandiflora*, *Ludwigia elegans* (Cambess.) H. Hara, and *Ludwigia leptocarpa* (Nutt.) H. Hara. These data also indicate that the physiological host range of *S. bosqi* does not mirror the phylogenetic relationship of the *Ludwigia* species and their more distant relatives (i.e., development/oviposition on *L. polycarpa*, *L. repens*, *L. palustris*, and *C. amoena* but not on *L. decurrens*) (Reddy et al. 2021).

Although our results demonstrate that *S. bosqi* is not a suitable biological control agent for invasive *Ludwigia* spp. in the US, these should not be extended to presume *S. bosqi* is equally unsuitable for biological control in other parts of the world where exotic *Ludwigia* spp. also are problematic. The data reported herein are the first to quantify larval developmental parameters of *S. bosqi*, which are critical for estimating the host range of this herbivore. *Sudauleutes bosqi* may still be considered for introduction elsewhere and these data can guide future host range testing as well as facilitate the rearing and handling of these weevils in general.

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