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Trait-based plant mortality and preference for native versus non-native seedlings by invasive slug and snail herbivores in Hawaii

Aaron B. Shiels
USDA, APHIS, National Wildlife Research Center, Aaron.B.Shiels@aphis.usda.gov

Martin K. Ennis
University of Hawaii at Hilo

Laura Shiels
University of Hawaii at Hilo

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Trait-based plant mortality and preference for native versus non-native seedlings by invasive slug and snail herbivores in Hawaii

Aaron B. Shiels · Martin K. Ennis · Laura Shiels

Abstract Non-native herbivores may alter plant communities through their preferential consumption of seedlings of different species. We assessed seedling herbivory by two invasive gastropod species in Hawaii, the giant African snail (*Achatina fulica*) and the Cuban brown slug (*Veronicella cubensis*). We hypothesized that six native species would suffer greater gastropod herbivory than four non-native species, and that species with short stature, thin leaves, and lacking physical defenses would suffer the greatest mortality from gastropods. Herbivory was measured during 13-day preference trials using enclosures that each contained four different woody species (two native, two non-native) and were assigned to one of three treatments: giant African snail, Cuban brown slug, or control (no gastropod). Discriminant function analysis was used to predict gastropod-induced seedling mortality from a suite of seedling characteristics. Native species did not always experience greater herbivory than non-natives species, and seedling mortality was 0–100 %. Native *Pipturus albidus* and *Clermontia parviflora* suffered 100 % mortality from *V. cubensis* herbivory, and *P. albidus*, *Psychotria hawaiiensis*, and *Myrsine lessertiana* suffered ≥80 % mortality from *A. fulica*. Two non-natives (*Fraxinus uhdei*, *Clidemia hirta*), and two natives (*Metrosideros polymorpha*, *Diospyros sandwicensis*), suffered little damage and no mortality. Non-native *Ardisia elliptica* suffered 10–30 % gastropod mortality, and non-native *Psidium cattleianum* mortality was 0–50 %. Leaf thickness best predicted species mortality caused by slugs and snails; some thicker-leaved species suffered most. Invasive snails and slugs threaten some native and non-native seedlings by directly consuming them. Current and future plant community structure in Hawaii may in part reflect the feeding preferences of invasive gastropods.

Keywords *Achatina fulica* · Enemy release hypothesis · Forest community structure · Hawaiian Islands · *Veronicella cubensis* · Woody seedling herbivory

A. B. Shiels (✉)
USDA, APHIS, National Wildlife Research Center, Hawaii Field Station, P.O. Box 10880, Hilo, HI 96721, USA
e-mail: ashiels@hawaii.edu

M. K. Ennis
College of Agriculture, Forestry and Natural Resource Management, University of Hawaii at Hilo, 200 W. Kawili Street, Hilo, HI 96720, USA

L. Shiels
Department of Biology, University of Hawaii at Hilo, 200 W. Kawili Street, Hilo, HI 96720, USA

Introduction

Native floras on oceanic islands are highly susceptible to displacement and extinction following the

introduction of non-native species (Kueffer et al. 2010; Oduor et al. 2010). Successful invasion of non-native plant species in island ecosystems is commonly explained by the enemy release hypothesis, which states that non-native species experience higher survival, growth, abundance, or reproduction in their introduced range relative to their native range because they have escaped enemies (e.g. herbivores, pathogens) that occur in their native range (Lockwood et al. 2007). Such release from enemies is often linked to the invader's increased competitive dominance over native species in their introduced range (Keane and Crawley 2002; Colautti et al. 2004). Whether herbivores damage native plants to a greater extent than non-native plants has been a topic of much interest; the enemy release hypothesis is just one of many possible explanations for such differences in native and non-native plant success (see Colautti et al. 2004 for a review).

Slug and snail herbivores have long been recognized as threats to plant growth and survival in both agricultural and non-agricultural settings (Mead 1961; Stephenson 1968; Mason 1970). Through selective feeding, slugs and snails can alter the species composition and relative abundances of plant communities directly by reducing and possibly eliminating the most attractive or palatable plant species (Hanley et al. 1995; Hulme 1996; Bruelheide and Scheidel 1999), or indirectly by allowing less palatable and less abundant species to persist (Buschmann et al. 2005; Hanley and Sykes 2009). Herbivory of seedlings rather than adults is generally expected to result in greater changes to plant community composition (Fenner et al. 1999).

Non-native slugs and snails are common, yet understudied and largely underappreciated pests of native plants on oceanic islands (Cowie 2001; Joe and Daehler 2008; Kappes et al. 2009). In the Hawaiian Islands, at least 38 non-native terrestrial slug and snail species have established, many of them introduced accidentally through the horticulture trade (Cowie et al. 2008). Hawaii lacks native slugs and the diets of the >700 species of native snails that were once present in Hawaii are largely unknown. The only native snails in Hawaii for which diets have been reported are some species of *Achatinella*, which feed on algae and fungi on leaf surfaces (Hadfield and Mountain 1980; Severns 1981). Native plant communities in Hawaii may be at great risk to damage by invasive slugs and snails because native plants are

likely to be less well defended against such novel herbivores as a result of the potential absence of tightly co-evolved relationships between native gastropod herbivores and native Hawaiian plants (Funk and Throop 2010).

Seedlings of only seven native species have been assessed for their vulnerability to invasive slugs in Hawaii despite invasive slugs being mentioned as potential threats to at least 59 native Hawaiian plant species (Joe 2006; Joe and Daehler 2008). There have been no studies of herbivory by invasive snails on seedlings of native species in Hawaii. Identifying the degree to which invasive gastropods damage forest seedlings will increase our understanding of plant-animal interactions involving invasive species, and help guide native species conservation efforts in areas where herbivorous slugs and snails have invaded. The objectives of our study were to: (1) determine preferences for seedlings of native and non-native species by invasive gastropod herbivores in Hawaii, and (2) determine if there are particular seedling traits that can be used to predict mortality levels attributable to invasive gastropod herbivores. Consistent with the expected patterns of the enemy release hypothesis, we hypothesize that native species will suffer greater herbivore damage and mortality than non-native species. Although many plant traits may influence herbivory levels, we also predict that seedlings that are relatively short-statured with thin leaves (Rathcke 1985; Hahn et al. 2011), as well as those species lacking recognizable defense traits (e.g., trichomes and latex), will suffer the greatest mortality from invasive gastropods.

Materials and methods

Gastropod and plant species tested

The two tropical gastropod species included in this study were the giant African snail (*Achatina fulica*) and the Cuban brown slug (*Veronicella cubensis*). These are among the most damaging gastropods in Hawaii and other parts of the U.S. (Staples and Cowie 2001; Cowie et al. 2009), and *A. fulica*, which is one of the largest land snails in the world (Mead 1961), has been designated as one of the world's most damaging pests (Lowe et al. 2000). In Hawaii, *A. fulica* and *V. cubensis* are among the five most frequently observed

gastropod species in horticultural settings (Cowie et al. 2008) and they are established in wet forests (Meyer and Cowie 2010).

Seedlings of 10 woody species (six native, four non-native) were chosen for study because they are among the most abundant species that commonly co-occur in wet forests in Hawaii where the two species of gastropods can coexist (see Wagner et al. 1999; Meyer and Cowie 2010). Although it would have been preferable for our native versus non-native herbivory comparison to have plant species paired according to phylogenetic relatedness and provenance (i.e., each native paired with a non-native from the same genus or family), this was not possible given the woody species that occur in wet forests in Hawaii. The six native species used in our study included 'ōhā wai (*Clermontia parviflora*; Campanulaceae), lama (*Diospyros sandwicensis*; Ebenaceae), 'ōhi'ā (*Metrosideros polymorpha*; Myrtaceae), kōlea (*Myrsine lessertiana*; Myrsinaceae), māmaki (*Pipturus albidus*; Urticaceae), and kōpiko (*Psychotria hawaiiensis*; Rubiaceae); the four non-native species included *Ardisia elliptica* (Myrsinaceae), *Clidemia hirta* (Melastomataceae), *Fraxinus uhdei* (Oleaceae), and *Psidium cattleianum* (Myrtaceae) (Fig. 1). *Clermontia parviflora* and *C. hirta* are shrubs and the remaining species are trees. Leaf hairs occur on *C. hirta* (long hairs) and *P. albidus* (short hairs), and *C. parviflora* exudes white latex sap when damaged. Aside from *F. uhdei*, which is common within and adjacent to *F. uhdei* plantation forests in Hawaii, the non-native species included in our study are recognized as important threats to native forests and are among the top 25 most problematic invasive plant species in Hawaii (Shiels 2011).

Mesocosms

Mesocosms (enclosures containing soil and seedlings) were constructed to allow plant growth while maintaining a tight seal to keep the gastropods contained. Thirty mesocosms were constructed from flower pots (30 cm diameter × 13 cm height) and veils of window screening (1.2 mm mesh). Gravel was placed in the bottom of the pots for drainage, and seedlings (grown from seed or transplanted from the forest) were planted in the pots with commercial potting soil. Mesh window screen was formed on a wire frame (14 gauge) to create an open ended cylinder (40 cm high, 30 cm diameter) that was slipped over the top edge of the pot

and held tightly against the pot using rubber bands. After each mesocosm was randomly assigned a treatment (see below) and labeled, and snails and slugs were introduced to the experimental pots, the screen cylinder was sealed by folding the top and using paper clips to keep the folds in place. Mesocosms were placed under 60 % shadecloth in the greenhouse and watered with an automated spray system. The greenhouse temperature during the study ranged from 18 to 35 °C.

Captive feeding trials

To test gastropod herbivory using the 10 plant species, we conducted three trials that differed according to plant species composition. For each trial, seedlings of four species (two native, and two non-native; chosen according to availability of appropriate sizes) were placed in each mesocosm; *P. cattleianum* was the only species present in all three trials. The four species used in each trial and dates of testing were: Trial 1: *C. hirta*, *M. polymorpha*, *P. albidus*, *P. cattleianum* (13–26 July 2011), Trial 2: *F. uhdei, M. lessertiana*, *P. cattleianum*, *P. hawaiiensis* (16–29 March 2012); and Trial 3: *A. elliptica*, *C. parviflora*, *D. sandwicensis*, *P. cattleianum* (28 April–11 May 2012). Therefore, our study did not include a full factorial test of all plant species combinations. Preference trials were favored over single species trials to more closely reflect the conditions that the gastropod species experience in Hawaiian wet forests. *Psidium cattleianum* was used in each trial as a reference species for (non-statistical) comparisons of seedling damage among the three trials, and because it is particularly common and spreading in most wet forests in Hawaii (Wagner et al. 1999). All seedlings were <25 cm tall, contained at least one true leaf (*D. sandwicensis* and *P. hawaiiensis* each have prominent cotyledons), and had acclimated for 3 days in the mesocosms when trials began (Fig. 1).

Gastropods were collected from the vicinity of Hilo, island of Hawaii. They were not fed but were held in plastic ventilated containers (approximately 9,000 cm^3) containing about 5 ml of water; a water-soaked cloth was draped over the containers to maintain a humid environment. We held them for 14 days prior to the beginning of each trial without food to minimize the likelihood that they would reject all seedlings offered in the trials, as was the case in

Fig. 1 The 10 species of woody seedlings used in the feeding trials: **a** *Ardisia elliptica* (non-native), **b** *Clermontia parviflora* (native), **c** *Clidemia hirta* (non-native), **d** *Diospyros sandwicensis* (native), **e** *Fraxinus uhdei* (non-native), **f** *Metrosideros polymorpha* (native), **g** *Myrsine lessertiana* (native), **h** *Psidium cattleianum* (non-native), **i** *Pipturus albidus* (native), **j** *Psychotria hawaiiensis* (native). For scale, each seedling container is 5 cm on a side

previous trials in Hawaii in which food was not withheld (Joe 2006).

For each trial, the 30 mesocosms were divided into three groups of 10: one group received two *V. cubensis* (5.2 ± 0.2 cm (mean ± SE) long), one group received a single *A. fulica* (7.6 ± 0.5 cm shell length), and one group was the control with no gastropods. Placing two individuals in each *V.*

cubensis treatment mesocosm minimized the difference in herbivore biomass between *A. fulica* and *V. cubensis* treatments. Treatments were assigned randomly, as was the location of each mesocosm on the greenhouse bench. Once gastropods were placed in mesocosms, seedlings were monitored 24 h later, at day six, and at day 13. At the end of each trial, percentage leaf area loss (i.e., leaf damage) for each

seedling was determined by subtracting the number of leaves at day 13 from the number of leaves at day 0. Leaves that were not fully intact were counted as a fraction of a leaf (0.25, 0.50, or 0.75) based on the proportion of leaf tissue remaining and visually estimated to the nearest quarter. A fully intact leaf was scored 1.0. Seedling mortality at each monitoring period was also recorded; a seedling was considered dead when: (1) the stem had been chewed through at the lower 2.5 cm of the seedling and all leaves were missing from the remaining rooted stem section, or (2) when the entire seedling stem was missing. All slugs and snails were recovered from the mesocosms at the end of the trial.

Seedling morphological characteristics that predict mortality due to gastropods

Thirteen seedling characteristics were predicted to affect levels of herbivory. These included 12 physical measurements (Table 1) and the ratio of carbon to nitrogen concentrations. All seedlings used for morphological measurements were from the same batches as those used in each trial, and were the control seedlings or other seedlings that were not offered to gastropods. We used a leaf scanner (LICOR 3100) to estimates the average leaf area per individual and the average leaf area per species ($n = 8$ individuals; each individual had 2–8 leaves). For *D. sandwicensis* and *P. hawaiiensis*, the cotyledons were noted and included in leaf area measurements. Leaf thickness was measured using a digital micrometer at three random points, avoiding main veins, from the outer 5 mm of each leaf blade. The readings from the three points on each leaf were averaged, and at least three leaves per plant were measured (range 3–8 leaves; $n = 8$ individuals per species). All measurements of leaf area and thickness were made on fully hydrated leaves (as evidenced by moist soil and absence of flaccidity). Leaf and total plant (above ground) dry mass were determined after oven-drying samples at 60 °C for 5 days. Stem diameter was measured just above the root collar. Leaf and stem fleshiness were calculated as [(wet mass − dry mass)/wet mass) * 100]. Total percentage carbon and nitrogen concentrations were determined by combustion of dried and ground above-ground seedling tissue ($n = 5$ individuals per species) using a LECO CN2000 analyzer.

Statistical analysis

All statistical analyses were conducted in *R* (version 2.12.0; R Development Core Team 2010), and post hoc multiple comparisons were conducted if there were significant ($P < 0.05$) differences among plant species using package "pgirmess" in *R*. Percentage leaf area loss from seedlings and seedling survival at the end of each trial were compared among species for each treatment using Kruskal–Wallis tests and Fisher's exact tests, respectively. Discriminant function analysis (DFA) was used to determine how seedling characteristics were related to mortality levels (high, 60–100 %; medium, 20–59 %; low, 0–19 %). Three DFAs were performed, one for *V. cubensis*, one for *A. fulica*, and a combined one for *V. cubensis* and *A. fulica*. For the combined DFA, mortality levels reflected the gastropod species that caused the greatest average mortality for each plant species. Average values for each seedling characteristic for each species (Table 1) were used in the analysis. Because of significant pairwise correlations between some seedlings characteristics (i.e., correlation coefficients >0.65 when $P < 0.05$), only six of the 13 seedling characteristics were used in the DFAs. The six characteristics, with appropriate transformations to meet normality assumptions, were leaf area, leaf thickness, leaf fleshiness, stem fleshiness, seedling height (log-transformed), and carbon : nitrogen. The DFAs were conducted using package "MASS" in *R*.

Results

Captive feeding trials

For the species in trial 1, the only significant differences in *A. fulica* and *V. cubensis* treatments were that *P. albidus* (native) seedlings lost more leaves (100 % loss) than the other three species (*A. fulica*: $\chi^2 = 26.33$, $df = 3$, $P < 0.0001$; *V. cubensis*: $\chi^2 = 34.16$, $df = 3$, $P < 0.0001$; Fig. 2a), and no *P. albidus* seedlings survived beyond day 6 days for *A. fulica* treatments (Fig. 3a) and day 13 for *V. cubensis* treatments, whereas 100 % of the other species survived (Fig. 3b) (Fisher's exact two-sided test, $P < 0.0001$ for both *A. fulica* and *V. cubensis* treatments). The only other significant difference in trial 1 was that in the control treatments *M. polymorpha* seedlings (native) lost greater leaf area

Table 1 Seedling measurements (mean ± SE) for the 10 plant species used in captive-feeding trials with invasive snails and slugs (n = 5–30 seedlings per measurement; see text)

Species	Leaf					Stem		Total plant				
	Mass (mg)	Length (cm)	Thick-ness (mm)	Area (cm²)	Fleshi-ness (%)	Diameter (cm)	Fleshi-ness (%)	Mass (mg)	Height (cm)	Leaf area (cm²)	Carbon (%)	Nitrogen (%)
Ardisia elliptica[a]	2.1 ± 0.4	6.2 ± 0.4	0.22 ± 0.01	4.1 ± 0.5	80 ± 1	0.19 ± 0.02	80 ± 2	12.2 ± 2.6	5.1 ± 0.2	20.7 ± 2.8	42.6 ± 0.5	1.2 ± 0.1
Clermontia parviflora	1.8 ± 0.2	4.2 ± 0.8	0.24 ± 0.01	4.9 ± 0.6	85 ± 1	0.27 ± 0.03	87 ± 1	16.1 ± 3.3	5.2 ± 0.3	22.6 ± 2.8	42.5 ± 0.2	2.3 ± 0.2
Clidemia hirta[a]	4.0 ± 0.4	5.4 ± 0.4	0.20 ± 0.01	7.9 ± 0.9	58 ± 5	0.18 ± 0.01	61 ± 1	29.3 ± 7.1	4.8 ± 0.4	38.4 ± 6.2	44.9 ± 0.5	1.5 ± 0.2
Diospyros sandwicensis	3.4 ± 0.2	4.6 ± 0.3	0.28 ± 0.00	3.4 ± 0.2	62 ± 1	0.10 ± 0.01	50 ± 1	17.5 ± 1.5	6.1 ± 0.2	14.6 ± 0.9	45.5 ± 0.3	1.1 ± 0.2
Fraxinus uhdei[a]	1.0 ± 0.1	2.5 ± 0.2	0.18 ± 0.01	2.1 ± 0.2	67 ± 3	0.08 ± 0.01	58 ± 1	2.7 ± 0.2	3.0 ± 0.2	10.7 ± 1.6	44.9 ± 0.4	1.3 ± 0.2
Metrosideros polymorpha	0.6 ± 0.1	2.0 ± 0.1	0.24 ± 0.01	0.9 ± 0.2	71 ± 0	0.08 ± 0.01	61 ± 1	9.1 ± 2.6	4.9 ± 0.4	7.8 ± 1.0	46.4 ± 0.3	1.1 ± 0.1
Myrsine lessertiana	0.9 ± 0.1	1.5 ± 0.3	0.19 ± 0.01	1.6 ± 0.2	71 ± 2	0.09 ± 0.01	69 ± 2	1.5 ± 0.0	2.9 ± 0.2	6.6 ± 0.8	43.5 ± 0.2	1.4 ± 0.3
Psidium cattleianum[a]	1.1 ± 0.1	1.8 ± 0.3	0.21 ± 0.00	2.2 ± 0.3	75 ± 0	0.06 ± 0.00	51 ± 1	4.6 ± 1.0	5.1 ± 0.2	22.6 ± 3.2	46.2 ± 0.7	1.2 ± 0.1
Pipturus albidus	3.2 ± 0.6	5.3 ± 0.4	0.25 ± 0.01	11.5 ± 1.9	81 ± 1	0.22 ± 0.03	88 ± 1	24.1 ± 6.4	12.3 ± 0.7	59.0 ± 12.2	35.9 ± 0.5	2.4 ± 0.3
Psychotria hawaiiensis	1.9 ± 0.1	2.4 ± 0.1	0.33 ± 0.01	3.5 ± 0.3	80 ± 1	0.16 ± 0.01	80 ± 1	3.0 ± 0.2	2.9 ± 0.1	19.1 ± 3.2	43.5 ± 0.3	1.1 ± 0.1

[a] Non-native species

than the other three species ($\chi^2 = 9.47$, $df = 3$, $P = 0.0237$; Fig. 2a).

In trial 2, the only significant difference for percentage leaf area loss was that *A. fulica* (but not *V. cubensis*) caused less damage to *F. uhdei* (non-native) than to the other three species (*A. fulica*: $\chi^2 = 19.78$, $df = 3$, $P = 0.0002$; *V. cubensis*: $\chi^2 = 1.06$, $df = 3$, $P = 0.7879$; control: $\chi^2 = 2.05$, $df = 3$, $P = 0.5613$; Fig. 2b). A similar pattern was evident for seedling survival. *Achatina fulica* did not kill any *F. uhdei*, yet they killed all but one *M. lessertiana* (native), all but two *P. hawaiiensis* (native), and half *P. cattleianum* (non-native) within 13 days (Fig. 3c). Thus, survival among species differed significantly ($P < 0.0001$) in the *A. fulica* treatments. *Veronicella cubensis* only killed native species in trial 2 (three *M. lessertiana* and two *P. hawaiiensis*), but there was no significant difference in survival among species in the *V. cubensis* treatments ($P = 0.1383$; Fig. 3d). The only seedling mortality in control treatments was one *P. cattleianum*.

In trial 3, *A. fulica* fed less on *D. sandwicensis* (native) than on *A. elliptica* (non-native) ($\chi^2 = 11.45$, $df = 3$, $P = 0.0095$; Fig. 2c), but seedling survival following *A. fulica* exposure did not differ significantly among species ($P = 0.0602$) despite three of the 10 replicates of both *C. parviflora* (native) and *A. elliptica* (non-native) being killed by *A. fulica* (Fig. 3e). The only other significant differences in trial 3 were in *V. cubensis* treatments in which *C. parviflora* seedlings lost more leaves (100 % loss; $\chi^2 = 27.57$, $df = 3$, $P < 0.0001$; Fig. 2c) and survival was lower (0 % survival after 6 days; $P < 0.0001$; Fig. 3f) than the other three species.

Seedling morphological characteristics that predict mortality due to gastropods

Based on the six seedling characteristics (each characteristic represented by averaging individuals for each species) for the 10 plant species, discriminant functions clearly separated the species' vulnerability to the three mortality levels (high, medium, low) resulting from herbivory by *A. fulica* (Fig. 4a), *V. cubensis* (Fig. 4b), and *A. fulica + V. cubensis* (Fig. 4c). For *V. cubensis*, discriminant function 1 clearly separated seedling mortality levels without
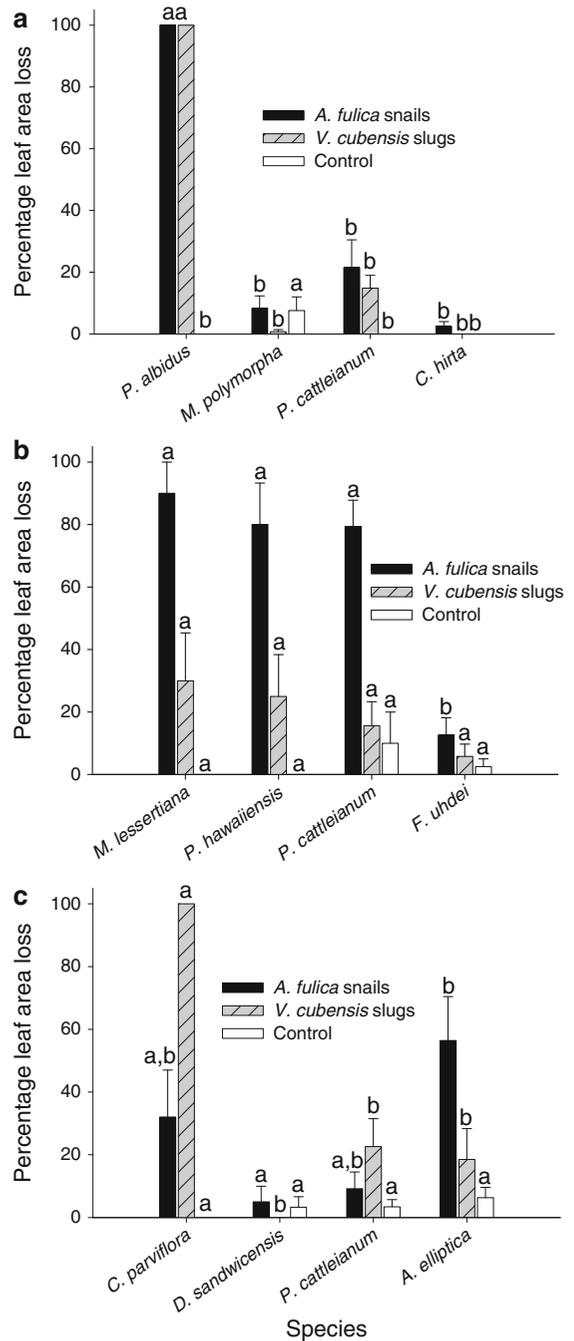

Fig. 2 Mean (SE) percentage leaf area loss at 13 days for the four species of woody seedlings that were exposed to one of three treatments: *Achatina fulica* snails, *Veronicella cubensis* slugs, or control (no gastropods) in trial 1 (**a**), trial 2 (**b**), and trial 3 (**c**). Within a given treatment, values indicated by the same lowercase letter were not significantly different (i.e., $P > 0.05$). $n = 10$ for each plant species in each treatment

Fig. 3 Percentage of each plant species that survived exposure to *Achatina fulica* snails, or *Veronicella cubensis* slugs, in trial 1 (**a, b**), trial 2 (**c, d**), and trial 3 (**e, f**). *n* = 10 for each species. Note that some symbols overlap
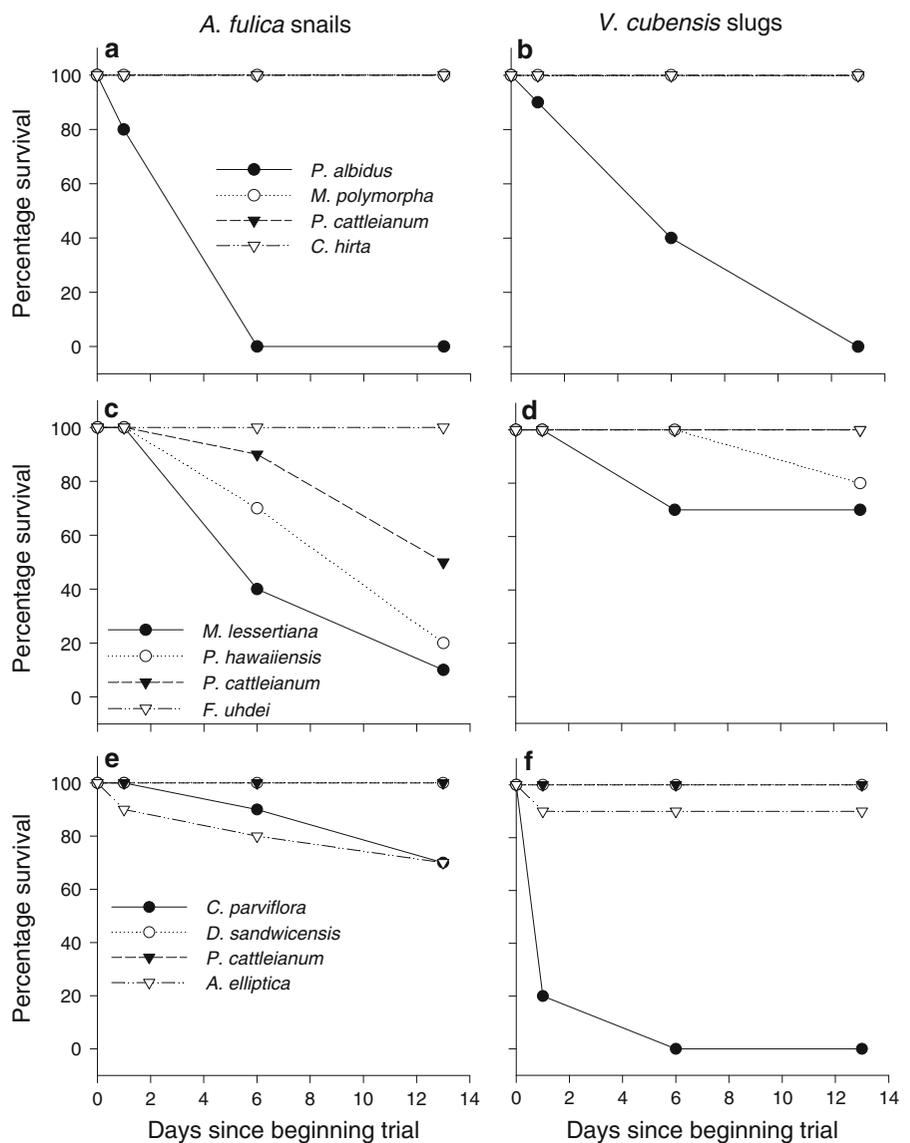
error because there was no overlap in mortality levels along discriminant function 1 axis (Fig. 4b), whereas discriminant functions 1 and 2 were necessary to separate seedling mortality levels due to *A. fulica* herbivory (Fig. 4a). This slight difference in the importance of discriminant functions between *V. cubensis* and *A. fulica* is also indicated by the amount of variation explained by discriminant function 1, which was 87, 99, and 67 %, for *A. fulica*, *V. cubensis*, and *A. fulica* + *V. cubensis*, respectively. The remaining variation in each dataset was explained by discriminant function 2. Coefficients of linear

discriminant functions, which are measures of the relative contributions of each seedling characteristic to each discriminant function, are shown in Table 2. Leaf thickness was the seedling characteristic that contributed most to each discriminant function, and therefore contributed most toward group (high, medium, low mortality) separations. Moreover, leaf thickness helped separate mortality levels resulting from *V. cubensis* herbivory slightly better than for mortality levels caused by *A. fulica* herbivory, as indicated by discriminate function 1 separations (Fig. 4) and coefficients of linear discriminant functions (Table 2).
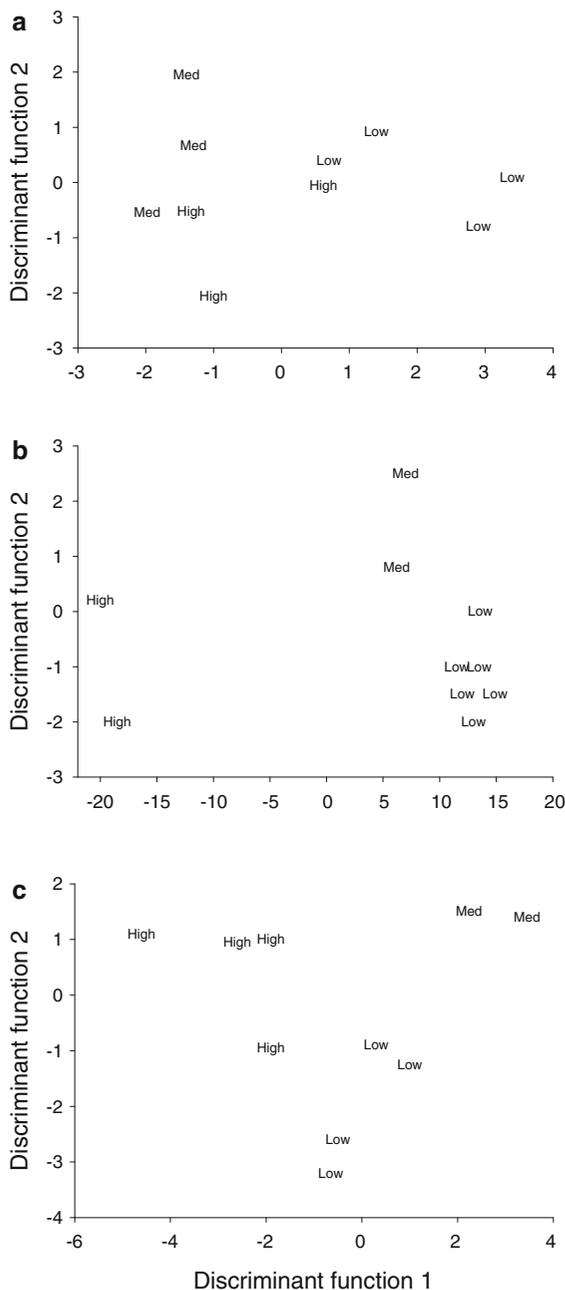
Fig. 4 Plots of discriminant function scores, based on six seedling characteristics, for each of the 10 plant species grouped according to mortality level (high, medium, and low) from *Achatina fulica* snails (**a**), *Veronicella cubensis* slugs (**b**), and snails + slugs (**c**) herbivory in the discriminant function analyses

Seedling height had the second greatest contribution to each discriminant function, as indicated by the second highest coefficient value in each of the three discriminant function analyses (Table 2).

Discussion

Generalizations regarding damage and mortality from invasive herbivores that are solely based on plant origin (native vs. non-native) are not supported in our study and some species do not fit the expected pattern of the enemy release hypothesis. For example, native *D. sandwicensis* and *M. polymorpha* seedlings suffered very little damage and no mortality, whereas non-native *P. cattleianum* and *A. elliptica* seedlings suffered up to 30–50 % mortality from *A. fulica* and *V. cubensis* herbivory. There are many factors that can affect herbivory (e.g., plant characteristics, phylogenetic relatedness, herbivore behavior, plant species composition) and contribute to the lack of differences in levels of herbivory between native and non-native species observed in our study and in previous studies (Buschmann et al. 2006; Funk and Throop 2010; Oduor et al. 2010). For the seedling characteristics that we included in our analysis, leaf thickness and seedling height showed the greatest promise for predicting species mortality caused by *V. cubensis* and *A. fulica*. From our choice trials, the native plants that are most at risk (i.e., suffered ≥80 % mortality) to *A. fulica* are *P. albidus*, *P. hawaiiensis*, and *M. lessertiana*, and the species most at risk to *V. cubensis* are *P. albidus* and *C. parviflora*. We suggest that the feeding preferences revealed in our trials be used to predict patterns of seedling herbivory in the field, and that they be applied to conservation and restoration projects in ecosystems where these two gastropod species have invaded.

Few studies have been able to assess the impacts of particular herbivore species without containing them. *Achatina fulica* and *V. cubensis* generally preferred the same plant species in our mesocosm feeding trials. However, *C. parviflora* tended to suffer greater leaf area loss and mortality from *V. cubensis* than *A. fulica*, and *M. lessertiana*, *P. hawaiiensis*, and to a lesser extent *P. cattleianum* (for trial 2 only) and *A. elliptica*, tended to suffer greater leaf area loss and mortality by *A. fulica* than *V. cubensis*. In a previous study (Joe 2006), there were no significant differences in food preference among five slug species (*Laevicaulis alte*, *Limacus flavus*, *Limax maximus*, *Meghimatium striatum*, *V. cubensis*), which was attributed to the absence of tightly co-evolved relationships between slugs and native Hawaiian plants, and/or small sample sizes and high variation resulting from the majority of the slugs

Table 2 Coefficients of linear discriminant functions (DF) from discriminant function analyses in which six seedling characteristics for each of the 10 species of woody seedlings were used to assess high, medium, and low mortality due to invasive snail (*Achatina fulica*) and slug (*Veronicella cubensis*) herbivores

Characteristic	A. fulica		V. cubensis		A. fulica ± V. cubensis	
	DF 1	DF 2	DF 1	DF 2	DF 1	DF 2
Leaf area	−0.37	−0.12	−1.84	−0.04	−0.60	−0.50
Leaf thickness	10.78	−17.7	132.33	−16.52	49.02	14.83
Leaf fleshiness	−0.33	0.07	0.14	0.01	−0.21	−0.33
Stem fleshiness	0.08	−0.04	−0.10	−0.02	0.06	0.07
Seedling height	3.82	4.06	10.61	8.02	−3.83	4.01
Carbon: nitrogen	−0.03	0.05	−1.79	0.04	−0.40	−0.09

failing to feed within the 48 h observation period. Similar to our findings, Joe and Daehler (2008) determined that *C. hirta* and *P. cattleianum* seedlings did not experience significant mortality when exposed to slugs. However, in trial 2 with *A. fulica*, *P. cattleianum* seedlings suffered 50 % mortality despite *A. fulica*'s tendency to prefer the two native species, *P. hawaiiensis* (80 % mortality) and *M. lessertiana* (90 % mortality). Higher *P. cattleianum* mortality in trial 2 relative to the other trials involving *A. fulica* highlights how the composition of the plant community (or species present in choice trials) may influence herbivory and mortality of each species. *Clidemia hirta* and *P. cattleianum* are recognized as two of the world's most problematic non-native species (Lowe et al. 2000). The limited herbivory on these two plant species in our study, and/or the greater palatability of additional co-existing plant species, may contribute to the invasion success of *C. hirta* and *P. cattleianum*, and may serve as an example of how changes to plant community structure in Hawaiian forests could result from feeding preferences of invasive gastropods.

Herbivory in our study (41 and 28 % average leaf area loss for *A. fulica* and *V. cubensis*, respectively) was within the range of 25 other tropical studies conducted in wet forest gaps (48 %) and understories (11 %) where a wider range of herbivore guilds were present (Coley and Barone 1996). Species-specific outcomes to gastropod herbivory are common; leaf area loss from slug herbivory can range among plant species from 2 to 100 %, and plant mortality can range from 0 to 19 % (Buschmann et al. 2006). Invasive slugs decreased seedling survival of two endangered native plants by 51 % in a Hawaiian forest (Joe and Daehler 2008). Four of the 10 species that were

included in our study did not suffer any mortality, yet *V. cubensis* caused 100 % mortality in two species and *A. fulica* caused ≥80 % mortality in three species. These high mortality levels for some species in our study relative to most plant species in other gastropod studies (e.g., Buschmann et al. 2006; Joe and Daehler 2008; Hahn et al. 2011) may have been influenced by our use of mesocosms, by the greater duration for which food was withheld prior to beginning each trial, and by the relatively large body sizes of *A. fulica* and *V. cubensis*.

Leaf thickness was the seedling trait that had the most influence on the level of mortality, and some of the species with relatively thick leaves tended to suffer the greatest mortality (Tables 1, 2). In previous studies elsewhere the thinnest leaves were the most preferred by gastropod herbivores (Dirzo 1980; Hahn et al. 2011); however, Funk and Throop (2010) found that native adult plants in Hawaii had significantly thicker leaves and suffered greater average leaf damage (ca. 4 %) during the dry season than the thinner non-natives species (ca. 2 % average leaf damage). Similarly, leaf thickness of native seedlings in our study (0.26 ± 0.02 mm) tended to be greater than that of non-native seedlings (0.20 ± 0.01 mm; Table 1). The second most influential trait affecting levels of mortality in the DFA was seedling height, yet there was no clear pattern among species regarding the most vulnerable height. For example, *P. albidus* had the tallest seedlings and suffered the greatest mortality, whereas *D. sandwicensis* was the next tallest species and suffered no mortality. The relatively low sample size (*n* = 10 species) precludes generalizations across species, but we suggest that leaf thickness and seedling height be the focus of future studies to develop trait-

based generalizations for predicting levels of invasive gastropod damage to seedlings.

Phylogenetic patterns may influence seedling herbivory in Hawaii, and future studies would benefit if phylogenetic relationships among test species could be better controlled for native versus non-native comparisons (e.g., Funk and Throop 2010). Although we expected trichomes and conspicuous sap to deter herbivores, native species with these characteristics were the most damaged. Species in Urticaceae were preferred and suffered the greatest mortality in our study (*P. albidus*) and that of Joe (2006) in which fresh (adult) leaves of endangered *Urera kaalae* were offered to slugs. Both *P. albidus* and *U. kaalae* have short and dense trichomes and relatively large leaves (Table 1; Wagner et al. 1999); *U. kaalae* also has resin glands on the leaves that contain copious amounts of sap that becomes greenish black when exposed to air (Wagner et al. 1999). Similarly, members of Hawaiian Campanulaceae contain white latex sap that is exuded when damaged. *Clermontia parviflora* in our study and *Cyanea superba* in that of Joe and Daehler (2008), both Campanulaceae, suffered among the greatest mortality from slugs (51–100 %). *Clermontia parviflora* also suffered 30 % mortality from *A. fulica* in our study, and it was one of the two species most preferred by *A. fulica* in Trial 3. Adult leaves of two additional Campanulaceae (*Cyanea grimesiana* and *Brighamia rockii*) were fed upon by five invasive slug species in captive feeding trials (Joe 2006), and fruit and seed of *Delissea rhytidosperma* (Campanulaceae) have been damaged by slugs in restoration sites on Kauai (Erwin and Young 2010). In Urticaceae and Campanulaceae 35 % (4 of 15) and 50 % (58 of 110), respectively, of their extant Hawaiian species are listed as rare or endangered (Wagner et al. 1999). By contrast, native *M. polymorpha* and non-native *P. cattleianum* (both Myrtaceae) are two of the most common tree species in forests in Hawaii and were among the least preferred species by gastropods (this study; Joe and Daehler 2008), insects, and pathogens (Funk and Throop 2010). Given the range of species tested for their vulnerability to invasive gastropods in Hawaii (Joe 2006; Joe and Daehler 2008; this study), we recommend that species in Urticaceae and Campanulaceae receive priority conservation attention to help protect them from herbivory by invasive gastropods.

Conclusion

An understanding of the range of threats to native plant species on oceanic islands is critical because of the shift in plant community dominance from native to non-native species in many natural areas (Meyer and Florence 1996; Mascaro et al. 2008; Cordell et al. 2009). *Achatina fulica* and *V. cubensis* have become widely established on both islands and continents, yet they are just two of 38 non-native gastropod species that have established in Hawaii, which highlights the need to examine more interactions of gastropod species with native and non-native plants in Hawaii. Although some native plant species are at high risk of consumption by *A. fulica* and *V. cubensis*, generalizations attempting to base vulnerability on plant origin (native vs. non-native) are not supported in our study. Plant species traits such as leaf thickness and seedling height are associated with species mortality, yet future studies with a larger species pool are recommended before applying such seedling characteristics to generalize across many plant species. By reducing particular species through preferential feeding on their seedlings, invasive gastropods may have caused and may continue to cause important, though largely unrecognized, changes in Hawaiian plant communities.

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