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Herbivores on native and exotic *Senecio* plants: is host switching related to plant novelty and insect diet breadth under field conditions?

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Running title: Herbivore host switching on exotic *Senecio*

Abstract

1. Native herbivores can establish novel interactions with alien plants after invasion. However, it is unclear whether these new associations are quantitatively significant compared to the assemblages with native flora under natural conditions.

2. Herbivores associated with two exotic plants, namely *Senecio inaequidens* and *S. pterophorus,* and two coexisting natives, namely *S. vulgaris* and *S. lividus*, were surveyed in a replicated long-term field study to ascertain whether the plant-herbivore assemblages in mixed communities are related to plant novelty and insect diet breadth.

3. Native herbivores used exotic *Senecio* as their host plants. Of the 19 species of Lepidoptera, Diptera, and Hemiptera found in this survey, 16 were associated with the exotic *Senecio* plants. Most of these species were polyphagous, yet we found a higher number of individuals with a narrow diet breadth, which is contrary to the assumption that host switching mainly occurs in generalist herbivores.

4. The *Senecio* specialist *Sphenella marginata* (Diptera: Tephritidae) was the most abundant and widely distributed insect species (*ca.* 80% of the identified specimens). *Sphenella* was associated with *S. lividus*, *S. vulgaris*, and *S. inaequidens* and was not found on *S. pterophorus*. The presence of native plant congeners in the invaded community did not ensure an instantaneous ecological fitting between insects and alien plants.

5. We conclude that novel associations between native herbivores and introduced *Senecio* plants are common under natural conditions. However, plant novelty is not the only predictor of herbivore abundance due to the complexity of natural conditions.

Keywords

Senecio, plant-herbivore interactions, exotic plants, host switching, diet breadth, insect performance, Enemy Release hypothesis, Biotic Resistance hypothesis

Introduction

The introduction of an exotic plant into a new habitat may strongly affect the functioning of the invaded ecosystem (Vitousek et al., 1996). Novel plant species can outcompete native vegetation to cause decreases in plant abundance and diversity and thus affect the local arthropod assemblage at higher trophic levels (Levine et al., 2004). Thus, mixed communities formed by introduced aliens and native vegetation have been associated with lower insect abundance, biomass, species composition, diversity, and richness compared with non-invaded communities (Herrera & Dudley, 2003; Gerber et al., 2008). Herbivores, particularly those adapted to consume one or a few plant species, might be particularly susceptible to the replacement of native vegetation by novel plant species (Tallamy, 2004).

Despite the negative effects that invasions may have on native insects, alien plants also offer potential food resources that can be exploited by local herbivores. Immediately after invasion, exotic plants are expected to be released from coevolved enemies from the area of origin (Keane & Crawley, 2002). However, new assemblages between native herbivores from the invaded area and exotic plants are often established (Jaenike, 1990; Frenzel & Brandl, 2003; Graves & Shapiro, 2003; Tallamy et al., 2010; García-Robledo & Horvitz, 2011) through a process known as Native Enemy Host Switching (Keane & Crawley, 2002). Host switching may be advantageous for the local herbivore populations because insects, in addition to feeding on their original hosts, incorporate the novel plant as a complementary food source (Agosta, 2006). In California, where 34% of the butterfly species have been reported to oviposit or feed on exotic plants, the use of an alternative alien host allows some insect species to expand their geographic range and extend their breeding season (Graves & Shapiro, 2003).

To establish persistent interactions with novel species, insects need to recognize and select alien plants that coexist in the same habitats, and immatures have to display resistance mechanisms against novel plant chemical defenses to successfully develop on the new host (Després et al., 2007).

Polyphagous herbivores that have adapted to feed on multiple species and families exhibit more plastic mechanisms of host recognition, selection, and resistance compared with herbivores with a narrow diet range (Jaenike, 1990). Accordingly, it is generally accepted that, during the initial stages of invasion, host switching will be more frequent for generalist compared with specialist herbivores (Keaney & Crawley, 2002; Parker & Hay, 2005; Doorduin & Vrieling, 2011). The presence of native hosts that are phylogenetically related to the invading exotic plants, which thus exhibit similar phytochemistry, may also facilitate host switching for specialist insects (Janzen, 1968; Strong et al., 1984).

At least two hypotheses have been proposed for the prediction of the intensity of host switching by native generalist and specialist insects under field conditions when both native and exotic host plants are available. The Enemy Release hypothesis (Keane & Crawley, 2002) suggests that local insects will have a higher preference and exhibit better performance when they are associated with native hosts. Thus, in a mixed community formed by native and alien plants, local insects would preferably attack the native over the alien plants. In contrast, the Biotic Resistance Hypothesis (Levine et al., 2004; Parker & Hay, 2005) predicts that the newly introduced plants exhibit a lack of defensive mechanisms against a phytophagous attack; thus, alien plants may be more susceptible to herbivory damage compared with native plants. There is a large body of evidence supporting both hypotheses from experiments performed under controlled conditions, such as laboratory bioassays and common garden designs (Agrawal & Kotanen, 2003; Parker & Gilbert, 2007; Chun et al., 2010; Tallamy et al., 2010). However, field data on herbivore preference and performance in invaded communities are scarce (Hill & Kotanen, 2010), and it is unclear whether these new plant-herbivore associations that are established under natural conditions are quantitatively significant compared to the assemblages with native flora.

In this study, we investigated the plant-herbivore associations between native and exotic plant congeners under field conditions. We used *Senecio* (Asteraceae) as a model system because it is one of the largest genera of flowering plants and is represented by more than 200 indigenous species (Royal Botanic Garden Edinburgh, 2012) and 10 alien species (DAISIE European Invasive Alien Species Gateway, 2008) in Europe. The genus *Senecio* is characterized by the presence of pyrrolizidine alkaloids that are highly toxic to vertebrate and invertebrate herbivores and affect host selection and performance (Hartmann 1999). *S. inaequidens* DC., which is native to South Africa, is a successful plant invader in Europe (EPPO Panel on Invasive Alien Species, 2012). Accidentally introduced in central Europe in 1896 through wool imports (Ernst, 1998), *S. inaequidens* has spread to most European countries and to other parts of the world, including South and North America, Australia, and Africa, where it has colonized vineyards, pastures, and natural areas. Another South African alien, *S. pterophorus* DC., was first reported in continental Europe in 1982 in a wool industrial area near Barcelona, NE Iberian Peninsula (Casasayas, 1989). *S. pterophorus* is highly invasive in SE Australia, where it is considered a noxious weed subject to control, but its distribution in Europe is locally restricted to two areas in the Mediterranean basin: Catalonia (NE Iberian Peninsula) (Chamorro et al., 2006) and Liguria (NW Italian Peninsula) (Barberis et al., 1998; Castells et al., 2013).

To characterize host switching among plant congeners under natural conditions, we conducted a replicated field survey of the phytophagous insect community associated with two exotic plants, namely *Senecio inaequidens* and *S. pterophorus*, and two coexisting native plants, namely *S. vulgaris* L. and *S. lividus* L. We determined the abundance and identity of any insects feeding on vegetative and reproductive plant tissues during the complete plant reproductive period to answer the following questions: 1) Does the herbivore abundance depend on the plant origin (native *vs.* exotic)?; 2) Is host switching related to insect diet breadth?; and 3) Does the consumption of exotic plants affect insect performance?

MATERIALS AND METHODS

Study sites

The present study was conducted at Montseny Natural Park (2°16'E 41°42' N), which is 60 km NE of Barcelona (NE Iberian Peninsula). The rainfall ranges from 860 to 1050 mm, and the mean temperature is 11.2ºC. Our main objective was to evaluate the assemblages occurring under field conditions between phytophagous insects and host plants with two contrasting coevolutionary histories (indigenous and exotic plants that are novel to the local fauna) that are also phylogenetically close. Based on the flora distributional data obtained from the Biodiversity Data Bank of Catalonia (Font, 2012) and the literature (Pino et al., 2000; Chamorro et al., 2006; Caño et al., 2007), we selected four species from the genus *Senecio* that are widely distributed within the Park: the exotic species *S. inaequidens* DC. and *S. pterophorus* DC., which have been present in the Park since the 1990s, and the native species *S. vulgaris* L. and *S. lividus* L. A preliminary field screening in various types of habitats, slopes, and altitudes was conducted to locate areas within the Park where the four species coexist. We selected six 600-m-diameter circular areas that are located at an altitude between 500 and 900 m on the south slope of the park and separated by at least 2 km (Fig. 1). By surveying a relatively small area, we aimed to ensure that the four species were exposed to the same arthropod communities and microclimate conditions. Thus, differences in the insect abundance across the plant species were expected to be mostly related to the insect preference and performance and not to differences in the flora and fauna spatial distributions. The common habitats within the sampling sites were open *Quercus ilex* forests, *Q. suber* plantations, forest margins, and abandoned crop fields. Other native *Senecio* species reported in the park (*S. adonidifolius* and *S. viscosus*) were absent in the selected locations. In April and May 2009, four to 32 individual plants from each *Senecio* species and location (a total of 479 individuals) were tagged using a plastic label attached to the plant stem. A GPS was used to ensure that the selected individuals were located within the targeted area. The plants were labeled during their initial reproductive stage, when the flower heads were still developing, and monitored during the entire reproductive period. The locality that showed the highest insect abundance in 2009 was Vallfornés, and we surveyed the herbivory in this location during one additional year (2010) using a similar protocol, through which a total of 116 additional individuals were tagged.

Insect survey

The insect survey was part of a larger study to determine the effects of herbivores and pathogens on plant fitness and alien plant invasive capacity. Every 10 to 15 days from April to December 2009 at all six localities (22 to 26 visits in each locality) and from May to November 2010 at Vallfornés (14 visits), the labeled plants were studied to determine if any insects were feeding on the vegetative and reproductive parts. On each sampling date, the plants were first inspected for aphids, and the presence or absence was recorded individually for each labeled plant. The shoots were then inspected, and the herbivores were counted, collected, and transported to the laboratory for identification. The predators collected during plant inspection, such as the Syrphidae (Diptera) *Eupeodes corollae* (Fabricius, 1794), *Scaeva dignota* (Rondani, 1857), and *Scaeva pyrastri* (Linnaeus, 1758), were not included in the study. To assess the presence of phytophagous insects growing within the flower heads, all non-dispersed fructified capitula (a total of 31,370 for 2009 and 2010) were dissected *in situ* or transported to the laboratory.

The flower heads were longitudinally dissected to determine the presence of insects growing inside. The heads that contained larvae were carefully closed and placed into a vial at room temperature to raise the adults. The Lepidoptera larvae collected on plant shoots were reared in plastic containers at room temperature by supplying leaf material from the host plant in which they were found. Food was added as necessary until the insects pupated. The pupae found inside the flower heads were maintained in glass vials at room temperature until the adults emerged. With the exception of *Orthosia cerasi* (Lepidoptera: Noctuidae), which was identified at the larval stage due to its unique morphology, the emerged adult moths were sent to specialist entomologists for identification. The mortality of early instar larvae reared in the laboratory was relatively high, and some individuals were thus not identified at the species level. In these cases, the insects were sorted by order and family. The empty pupal cases found inside the flower heads were also classified by order and family based on morphological similarities with pupal cases reared in the laboratory until the emergence of the adults. The aphids for taxonomic identification were collected only once in May 2010, and care was taken to include individuals of different colors, sizes, and morphologies. The labeled *S. pterophorus* plants at Vallfornés had no associated aphids in 2010, although significant amounts were recorded in 2009. Thus, in May 2011, we collected aphid subsamples from three *S. pterophorus* individuals at Can Tarrer (CT) for identification purposes. Some Lepidoptera larvae were parasitized, and the emerged parasitoid adults were sent for identification. Due to the lack of taxonomical information, the identity of most parasitoid species remained unresolved. The insect taxonomy was performed by internationally recognized specialists (see acknowledgements for details).

To estimate the effects of host plants on insect performance, the adult body mass of the two most abundant species was determined: the fruit fly *Sphenella marginata* and its parasitoid *Pteromalus* sp. Fruit fly specimens collected in the field as pupae that produced morphologically intact adults (N = 388 for *Sphenella marginata* and N = 100 for *Pteromalus* sp.) were oven-dried for 24 h at 65°C and weighted on a precision scale (MX5 Microbalance, Mettler Toledo).

Herbivore host breadth

Data on the associations between herbivore species and host plants were obtained from the relevant scientific literature. Different sources were used for each arthropod family: Noctuidae (Robinson et al., 2010), Geometridae (Mironov, 2003), Pyralidae (Roesler, 1973), Agromyzidae (Spencer, 1989; 1990), Tephritidae (White, 1988), and Aphididae (García Prieto & Nieto Nafría, 2005; Blackman & Eastop, 2006). The number of host genera and families for each phytophagous species were counted.

Data analyses

The abundance of herbivores (Lepidoptera and Diptera) and parasitoids was estimated as the total number of insects found on each individual plant corrected by the sampling effort, *i.e.*, the number of visits per plant. The abundance of Hemiptera Aphididae was estimated as the percentage of visits during which an individual plant was associated with aphids, which was recorded as presence or absence. The herbivore abundances were calculated throughout the entire plant reproductive season and the overlapping reproductive period for all surveyed *Senecio* species (May and June) during 2009 and 2010. The differences across plant species and locations (in 2009) were analyzed through ANOVA. The count data obtained in this study is not expected to follow a normal distribution even after transformation. However, ANOVAs can be performed if the variances are homogeneous without outliers and the sample size is large, even if the data are not normally distributed (Anabel Blasco, Statistical Services, Universitat Autònoma de Barcelona). Thus, to conduct the statistical analyses, the data were first transformed to stabilize the variances. The dependent variables expressed as mean values were square-root transformed, and the percentages were Anscombe-transformed [2 * square root (x + 3/8)]. Two-way ANOVA with the species as the fixed factor and the location as the random factor was performed to analyze the herbivore and parasitoid abundance in 2009. One-way ANOVA was performed to test the differences in the herbivore (Lepidoptera, Diptera, and Hemiptera) and parasitoid (Diptera Tachinidae and Hymenoptera) abundance across species in 2010. The differences in the body mass for the Diptera *Sphenella marginata* and its parasitoid *Pteromalus* sp. associated with *Senecio* species was tested independently through one-way ANOVA. Tukey's post-host tests were conducted on all of the analyses to test the differences among *Senecio* species with $P < 0.05$, including only the plant species which exhibited an insect abundance different from 0. The statistical analyses were performed using the SPSS 19 software (IBM).

Results

A total of 2,865 phytophagous specimens of Lepidoptera and Diptera were collected throughout the field surveys in 2009 and 2010 on the two native (*Senecio vulgaris* and *S. lividus*) and two exotic (*S. inaequidens* and *S. pterophorus*) host plants. Additionally, a total of 3,732 aphid colonies were recorded on 278 different individual plants.

The Lepidoptera abundance was generally higher on the exotic plant species compared with the native plant species in 2009 and 2010 if the entire sampling period was considered, with the exception of *S. pterophorus* and *S. lividus*, for which no statistically differences were found between species in 2009 (Fig. 2). However, when the comparison was restricted to May and June, which is the overlapping flowering period, no significant differences were found across the different plants (Fig. 3). The significant interaction between the species and locations in 2009 for the two time periods analyzed indicates that the type and intensity of the interactions between the plants and Lepidoptera differed among the localities (Table 1).

S. lividus exhibited the highest levels of Diptera compared with *S. vulgaris* and *S. inaequidens* during the entire reproductive plant season and during May and June in 2009 and 2010 (Fig. 2 and Fig. 3). *S. pterophorus* was not associated with Diptera in any location or sampling year.

The Hemiptera abundance varied across species in 2009 and 2010, although this trend was only marginally significant for the entire reproductive period in 2009 (Table 1). The levels of Hemiptera, however, were not consistent across the years. In 2009, the exotic *Senecio* species presented significantly higher levels of Hemiptera compared to the native *Senecio* species (*S. inaequidens* exhibited the highest levels) during the two sampling periods considered (Fig. 2 and Fig. 3). In 2010, the levels of Hemiptera were lowest in *S. pterophorus* compared with the other species during the two sampling periods, whereas the abundance in *S. inaequidens* was highest during May and June (Fig. 2 and Fig. 3).

The parasitoid abundance did not differ between the plant species in 2009. In 2010, this trend was significant (Table 1), but post-hoc comparisons did not reveal any differences between any pairs of species (data not shown).

One third of the collected Lepidoptera and Diptera larvae were reared to adulthood and identified at the species level. The rest of the specimens (17 Noctuidae, eight Geometridae, 117 Pyralidae Lepidoptera, and 1,809 Tephritidae Diptera) were classified at the family level. Of the 19 insect species identified, 14 were associated with the alien *S. inaequidens* and *S. pterophorus*. Of these, only five (two Diptera and three Hemiptera) were also found on native *Senecio* host plants, which indicates a process of host switching within plant genera. The remaining nine species, all of which were Lepidoptera, were absent on the native *Senecio* species, which suggests that their original host plants belonged to other species that were not included in this study.

Sixteen species (84%) were polyphagous (Table 2 and Fig. 4). However, the analysis of the number of individuals collected revealed that the specialist herbivores were predominant (Fig. 4). The *Senecio* specialist *Sphenella marginata* (Diptera: Tephritidae) was the most abundant and widely distributed species, representing 20% of the collected and 80% of the identified specimens. *Sphenella* was found in 46.4% of the *S. lividus* plants, 20.0% of the *S. inaequidens* plants, and 8.2% of the *S. vulgaris* plants and was not present in any *S. pterophorus* plants. Other abundant specialists were *Aphis haroi* (Hemiptera: Aphididae), which was found in all *Senecio* species, and *Phycitodes albatella* (Lepidoptera: Pyralidae), which was associated with the exotic species *S. inaequidens* and *S. pterophorus* (Table 2). Of the herbivores reared in the laboratory, 7.6% were parasitized to yield a total of 205 specimens from eight different species (Table 3). The most abundant parasitoid species was Hymenoptera *Pteromalus* sp., which is hosted by a Diptera Tephritidae species, most likely

Sphenella marginata, and associated with *S. vulgaris*, *S. lividus***,** and *S. inaequidens* (Table 3). The hosts of the other parasitoid species could not be determined.

The performance of *Sphenella* and the parasitoid *Pteromalus* varied across the host plants. The *Sphenella* specimens feeding on *S. lividus* presented a significantly higher body mass compared with the insects associated with *S. vulgaris* and *S. inaequidens* (one-way ANOVA, F = 26.11, $P < 0.001$; Fig. 5). Specimens of *Pteromalus* associated with *S. lividus* were also larger compared with specimens associated with *S. vulgaris* and *S. inaequidens* (one-way ANOVA, F = 6.58, $P < 0.005$; Fig. 5).

Discussion

Two hypotheses have been proposed to explain the changes in the plant-herbivore assemblages in invaded communities. The Enemy Release hypothesis (Keane & Crawley, 2002) suggests that local herbivores are better adapted to their native host plants than to the alien plants and that these herbivores thus have a higher preference toward the native plant species. Conversely, the Biotic Resistance hypothesis (Levine et al., 2004; Parker & Hay, 2005) states that exotic plants are more susceptible to local herbivores compared with native hosts because the exhibit a lack of resistance mechanisms to reduce herbivore damage. In this study, Lepidoptera species were more abundant on the exotic species *S. inaequidens* and *S. pterophorus* than in the native species *S. lividus* and *S. vulgaris*, which is in accordance with the Biotic Resistance hypothesis. To determine whether this pattern was caused by a higher insect preference for exotic species and not by differences in plant phenology, we compared the herbivore abundance during the overlapping reproductive period for all *Senecio* species (May-June). When the plants co-occurred, the exotic and native plants were equally affected by Lepidoptera. These results suggest that the extended plant phenology of the exotic plants favored new associations with local insects in absence of native *Senecio* plants and that host switching also occurred when both native and exotic plants were available. None of the above mentioned hypotheses explicitly predicts similar herbivory for native and exotic plants, but the absence of enemy release in exotic *Senecio* species is more suggestive of a biotic resistance mechanism. Similarly, the higher or equal levels of Hemiptera in *S. inaequidens* (and in *S. pterophorus* in 2010) compared with the native *Senecio* species also supports the Biotic Resistance hypothesis. In contrast, the abundance of Diptera was more consistent with the Enemy Release hypothesis because no insect specimens were found on any individual *S. pterophorus* plant during any sampling period and the amount of Diptera in *S. inaequidens* was lower compared with *S. lividus*. However, *S. inaequidens* was not released from Diptera herbivory compared with *S. vulgaris*.

The more pronounced host switching in *S. inaequidens* compared with *S. pterophorus* for Hemiptera and Diptera could be explained by differences in the plant invasion history. A longer time since introduction may facilitate host shifting because insect populations are more likely to interact with novel plants and they have a longer time to acclimate or adapt to its unique chemistry (Hawkes, 2007; Mitchell et al., 2010). Because *S. pterophorus* was introduced in Europe more recently than *S. inaequidens*, local herbivores may be more reluctant to use *S. pterophorus* as a host. In Australia, where *S. pterophorus* was introduced 50 years before than in Europe, the levels of herbivory due to host switching are significantly increased (Castells et al., 2013), which suggests that a longer coexistence time between local insects and novel plants enhances host switching.

Most of the identified species in this survey were polyphagous. However, contrary to the assumption that host switching in invaded communities is mainly performed by generalist herbivores (Keane & Crawley, 2002; Parker & Hay, 2005; Doorduin & Vrlieling, 2011), we found that herbivores with a narrow diet breadth were more abundant on exotic plants than polyphagous herbivores. *Sphenella marginata* (Diptera: Tephritidae), which is the most abundant species found in this study, is a fruit feeder specialized on the genus *Senecio* (e.g., *S. aquaticus, S. erucifolius, S. jacobaea, S. paludosus, S. squalidus, S. sylvaticus, S. viscosus,* and *S. vulgaris*) (White, 1988) and was found to be associated with the native species *S. vulgaris* and *S. lividus* and the exotic species *S. inaequidens* in this study. Other common insect species found in exotic *Senecio* species were also specialists. *Aphis haroi* (Hemiptera: Aphididae), which was previously reported on *S. pyrenaicus* (García Prieto & Nieto Nafría, 2005), and *Phycitodes albatella* (Lepidoptera: Pyralidae), which is a microlepidoptera that has been reported on three genera of Asteraceae (*Crepis, Solidago,* and *Senecio*) (Roesler, 1973), were found to be associated with *S. inaequidens* and *S. pterophorus*.

Host switching by specialist insects may be facilitated by the presence of native species that are phylogenetically close to the introduced plants, e.g., belonging to the same genera, in the invaded community (Mitchell et al., 2006; Verhoeven et al., 2009). Herbivores that have adapted to feed on native plants may readily establish novel interactions with exotic congeners through an immediate ecological fitting because these exotic plants share similar physical and chemical traits with the herbivores' original hosts that facilitate recognition, selection, and resistance (Janzen, 1968; Strong et al., 1984; Pearse & Hipp, 2009; Verhoeven et al., 2009). Under these conditions, we expected that insects specialized on native *Senecio* would easily shift to exotic *Senecio* species. However, the presence of native congeneric plant species in the invaded community was not sufficient to readily determine host switching: *Sphenella marginata* shifted from the native *Senecio* species to *S. inaequidens* but not to *S. pterophorus.* The lack of association between *S. pterophorus* and *Sphenella marginata* could not be explained by an asynchrony of plant and insect life cycles. Tephritidae develop and pupate within *Senecio* flower heads, and their occurrence is thus limited by the plant's reproductive stage. However*,* *S. pterophorus* was blooming at the time that the flies were seeking flower heads for oviposition. In fact, *Sphenella marginata* specimens were observed approaching *S. pterophorus* in the field (EC personal observation), which suggests that insects recognized the plant as a potential host. The pre-adaptations of *Sphenella marginata* for the consumption of native *Senecio* were not sufficient to ensure the establishment of successful associations with *S. pterophorus*. Different species of *Senecio* share the same type of chemical defenses, namely pyrrolizidine alkaloids (Hartmann, 1999), but insects might adapt to feed on individual chemical compounds rather than groups of chemicals (Macel et al., 2005). The *S. pterophorus* chemical composition may constitute a novel weapon (*sensu* Callaway & Ridenour, 2004) that regulates the new assemblages with local herbivores.

We also explored whether changes in the host use translated to differences in performance in the two most abundant insects that were surveyed: *Sphenella marginata* and the parasitoid *Pteromalus* sp. Both species presented the highest body mass when associated with *S. lividus* compared with *S. vulgaris* and *S. inaequidens*. These results are highly consistent with host selection in the field. Thus, insects feeding on *S. lividus*, which is the most preferred host plant, exhibited a better performance than insects feeding on the less attacked *S. vulgaris* and *S. inaequidens*. Positive correlations between preference and performance are expected to occur when females ovipositing on the selected host plants maximize their fitness (Jaenike, 1990). *Sphenella marginata* performed better when feeding on *S. lividus*, but the use of *S. inaequidens* was not disadvantageous in the absence of *S. lividus* plants. In fact, *Sphenella marginata* populations most likely benefit from the consumption of *S. inaequidens* because the presence of reproductive individuals of *S. inaequidens* in Montseny Natural Park during the fall offers an alternate food source when no other *Senecio* species are available.

Final Remarks

Host preference is a main factor for the determination of new assemblages between insects and exotic plants. However, under natural conditions, trophic interactions are additionally constrained by other factors that are not directly related to preference, such as the spatial and temporal distributions of insects and plants, the presence of native plant congeners, the plant invasion history, the abundance of alien species relative to native hosts, the composition of the local insect community, and the phenological synchrony between plants and herbivores (Janzen, 1968; Graves & Shapiro, 2003). Laboratory bioassays can provide valuable information on the ability of insect species to recognize, select, and develop in new hosts, but these studies are insufficient to elucidate which interactions will actually occur in the field. Unfortunately, few of the studies that have evaluated host switching accounted for the complexity and idiosyncrasy of natural conditions. This study was designed to provide information on the new plant-herbivore interactions that are established in the field when native plan congeners are present. We performed a long-term replicated plant survey in relatively small areas to ensure that all of the plant species were exposed to the same arthropod communities and microclimate conditions. However, we acknowledge that other factors, such as plant abundance in the surveyed areas and differences in the biological features between native and exotic plants, including plant phenology and chemical defenses, may also determine the host switching process. Our results show that plant novelty was not the only predictor of herbivore abundance. Host switching was generally increased for Lepidoptera and Hemiptera, as expected by the Biotic Resistance hypothesis, but decreased of Diptera, in accordance with the Enemy release hypothesis. Of the exotic species surveyed, *S. inaequidens*, which had coexisted longer with the local fauna in the study site, generally experienced higher herbivory compared with the exotic *S. pterophorus*. Contrary to previous results, host switching was quantitatively more common for specialist insects, but the presence of phylogenetically related native species in the invaded community did not ensure an instantaneous association between specialist insects and exotic plants. In conclusion, the insect order, plant phenology, herbivore diet breadth, and time since plant introduction may affect the host switching among *Senecio* congeners. Despite the limitations of field surveys, this work highlights the importance of the study of plant-herbivore associations under the complexity of natural conditions to understand not only the potential interactions that could be established based on insect preference and performance but also the realized interactions under the constraints of field conditions.

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Table 1. Effect of plant species and sampling location on the herbivore and parasitoid abundance during the entire reproductive period and during the overlapping reproductive period. The statistical results were obtained through ANOVA analyses between all of the plant species (May-June). The significant differences (*P* < 0.05) are indicated in bold.

				Entire reproductive period		**May-June**	
				F	*P-value*	F	*P-value*
2009	Herbivores						
		Lepidoptera	Species	1.666	0.214	0.915	0.457
			Location	0.928	0.488	0.670	0.652
			Species x Location	3.453	**<0.001**	1.826	**0.037**
		Diptera	Species	37.051	**<0.001**	27.349	**<0.001**
			Location	5.861	**0.002**	6.244	**0.002**
			Species x Location	1.250	0.231	1.056	0.397
		Hemiptera	Species	3.098	0.058	3.527	**0.045**
			Location	1.193	0.358	1.440	0.273
			Species x Location	7.451	**<0.001**	10.729	**<0.001**
	Parasitoids		Species	1.498	0.249	0.527	0.670
			Location	3.668	**0.018**	3.032	**0.039**
			Species x Location	1.236	0.241	1.351	0.181
2010	Herbivores						
		Lepidoptera	Species	10.368	**<0.001**	1.863	0.140
		Diptera	Species	22.692	**<0.001**	26.542	**<0.001**
		Hemiptera	Species	16.462	**<0.001**	24.794	**<0.001**
	Parasitoids		Species	7.138	**<0.001**	5.282	**0.002**

Table 2. Phytophagous insects feeding on native and exotic *Senecio* host plants at Montseny Natural Park. The sampling was conducted during the reproductive stage for each *Senecio* species in 2009 and 2010. The presence of Lepidoptera and Diptera at a particular month of the year is indicated by roman characters. The aphids were sampled for identification once in May 2010 at Vallfornés; the species presence is indicated by an asterisk.

					Natives		Exotics		
Order, Family	**Species**	**N[1]**	**Plant part[2]**	**Type of feeding[3]**	***Senecio vulgaris***	***Senecio lividus***	***Senecio inaequidens***	***Senecio pterophorus***	**Diet Breadth (Num. gen/fam[4])**
Plant reproductive range (sampling period)					XI-VI	IV-VII	V-XII	V-VII	
Lepidoptera									
Noctuidae	*Autographa gamma* (Linnaeus 1758)	1	V/R	Chewing	-	-	IX	-	75/24
Noctuidae	*Helicoverpa armigera* (Hubner 1808)	4	V/R	Chewing	-	-	XI-XII	-	102/40
Noctuidae	*Heliothis peltigera* (Denis & Schiffermuller 1775)	1	V/R	Chewing	-	VI	-	-	27/12
Noctuidae	*Orthosia cerasi* (Fabricius 1775)	2	V	Chewing	-	-	VI	-	4/4
Noctuidae	*Thysanoplusia orichalcea* (Fabricius 1775)	1	V	Chewing	-	-	X	-	57/24
Geometridae	*Eupithecia absinthiata* (Clerk 1759)	1	R	Chewing	-	-	XI	-	26/7
Geometridae	*Eupithecia unedonata* Mabille 1968	1	R	Chewing	-	-	XI	-	3/3
Geometridae	*Eupithecia virgaureata* Doubleday 1861	3	R	Chewing	-	-	XI	-	21/6
Geometridae	*Gymnoscelis rufifasciata* (Haworth 1809)	1	R	Chewing	-	-	IX	-	79/41
Pyralidae	*Phycitodes albatella* (Ragonot 1887)	22	R	Chewing	-	-	VII, XI	VII	3/1
Diptera									
Agromyzidae	*Chromatomyia horticola* (Goureau 1851)	7	V	Leaf mining	-	IV-V	V	-	268/36
Tephritidae	*Sphenella marginata* (Fallen 1814)	709	R	Chewing	V-VI	V-VII	VI-XI	-	1/1
Hemiptera									
Aphididae	*Aphis fabae* Scopoli 1763	3	V	Sucking	*	*	-	-	574/109
Aphididae	*Aphis haroi* Nieto Nafría 1974	63	V	Sucking	*	*	*	*[5]	1/1
Aphididae	*Aulacorthum solani* Kaltenbach 1843	3	V	Sucking	*	*	-	-	344/76
Aphididae	*Brachycaudus cardui* Linnaeus 1758	1	V	Sucking	-	*	-	-	115/21
Aphididae	*Brachycaudus helichrysi* (Kaltenbach 1843)	8	V	Sucking	*	-	-	*[5]	285/49
Aphididae	*Macrosiphum euphorbiae* (Thomas 1878)	3	V	Sucking	-	*	*	-	302/82
Aphididae	*Myzus persicae* Sulzer 1776	5	V	Sucking	*	*	-	-	650/125

[1] Number of Lepidoptera and Diptera specimens identified. In the case of aphids, the number of samples collected, each of which contains several individuals, is shown.

[2] V = Vegetative (leaves or stems); R = reproductive (flowers or fruits).

[3] Type of feeding for Lepidoptera and Diptera at the immature stage.

[4] Number of genera and families used as host plants based on the literature (see Materials and Methods).

[5] Sampling performed on May 2011.

Table 3. Parasitoids associated with herbivores feeding on native and exotic *Senecio* host plants at six locations in Montseny Natural Park. The collection month for each species is indicated by roman characters.

				Natives		**Exotics**	
Order, Family	**Species**	**N**	**Feeding strategy**	***Senecio vulgaris***	***Senecio lividus***	***Senecio inaequidens***	***Senecio pterophorus***
Diptera							
Tachinidae	*Voria ruralis*	1	Endoparasitoid	-	-	IX	-
Hymenoptera							
Braconidae	*Aleiodes* sp.	1	Endoparasitoid	-	-	VI	-
Braconidae	*Bracon* sp.	1	Ectoparasitoid	-	VI	-	-
Eulophidae	*Chrysocharis pubicornis* (Zetterstedt 1838)	2	Endoparasitoid	-	V	V	-
Ichneumonidae	*Hyposoter didymator* (Thunberg 1824)	4	Endoparasitoid	-	VI	-	VI
Ichneumonidae	*Tromatobia* sp.	1	Endoparasitoid	-	VI	-	-
Ichneumonidae	*Syrphophilus bizonarius* (Gravenhorst 1829)	1	Endoparasitoid	-	-	VI	-
Pteromalidae	*Pteromalus* sp.	194	Endoparasitoid	V-VI	V-VII	VI-XI	-

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Figure captions

Fig. 1 Study sites at Montseny Natural Park, Catalonia, NE Spain. Each dot represents a 600-m-diameter location where native (*S. vulgaris*, *S. lividus*) and alien *Senecio* species (*S. inaequidens* and *S. pterophorus)* co-occur. Between four and 32 individual plants per species and location were surveyed for the presence of herbivores every 10 to 15 days during the plant reproductive period. The location codes are the following: Vallfornés –VF–, Can Bosc –CB–, Santa Susanna –SS–, Can Tarrer –CT–, Can Pere Poc –CP–, and Fogueres de Montsoriu –FM–. The park limits are indicated in bold.

Fig. 2 Abundance of Lepidoptera (a, b), Diptera (c, d), and Hemiptera (e, f) on two native (*S. vulgaris* –SV– and *S. lividus* –SL–) and two exotic plants (*S. inaequidens* –SI– and *S. pterophorus* –SP–) during the entire reproductive plant period in two consecutive years. The abundance was estimated as the number of specimens per plant (for Lepidoptera and Diptera) or the percentage of plants associated with an insect colony (for Hemiptera) divided by the number of visits (mean and SE). The different letters indicate significant differences among the plant species ($P < 0.05$ by Tukey's post-hoc test).

Fig. 3 Abundance of Lepidoptera (a, b), Diptera (c, d), and Hemiptera (e, f) on two native (*S. vulgaris* –SV– and *S. lividus* –SL–) and two exotic plants (*S. inaequidens* –SI– and *S. pterophorus* –SP–) during the overlapping reproductive period (May and June) in two consecutive years. The abundance was estimated as the number of specimens per plant (for Lepidoptera and Diptera) or the percentage of plants associated with an insect colony (for Hemiptera) divided by the number of visits (mean and SE). The different letters indicate significant differences among the plant species ($P < 0.05$ by Tukey's post-hoc test).

Fig. 4 Richness (a) and abundance (b) of herbivore insects associated with native (*S. vulgaris* –SV– and *S. lividus* –SL–) and exotic plants (*S. inaequidens* –SI– and *S. pterophorus* –SP–) classified by their diet breadth: a single plant genera, a single plant family, and more than one plant family. Values are standardized by the sampling effort (i.e. number of visits per plant).

Fig. 5 Adult body mass of the herbivore *Sphenella marginata* (a) and the parasitoid *Pteromalus* sp. (b) associated with the native *S. vulgaris* (SV) and *S. lividus* (SL) and the exotic *S. inaequidens* (SI). The different letters indicate significant differences ($P < 0.05$) between species. N = 123, 246, and 19, respectively.

Figure 1

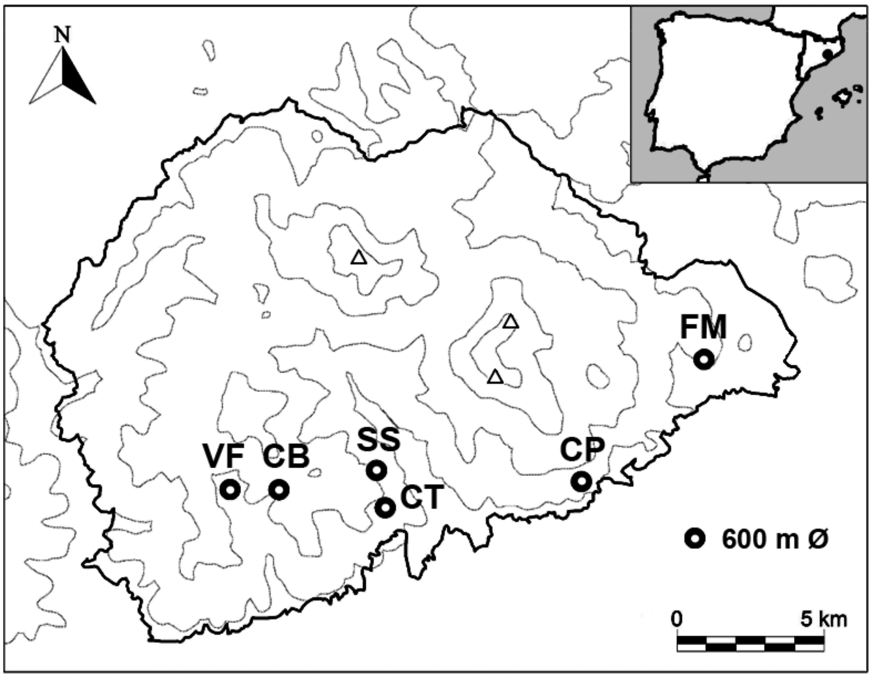

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Figure 2

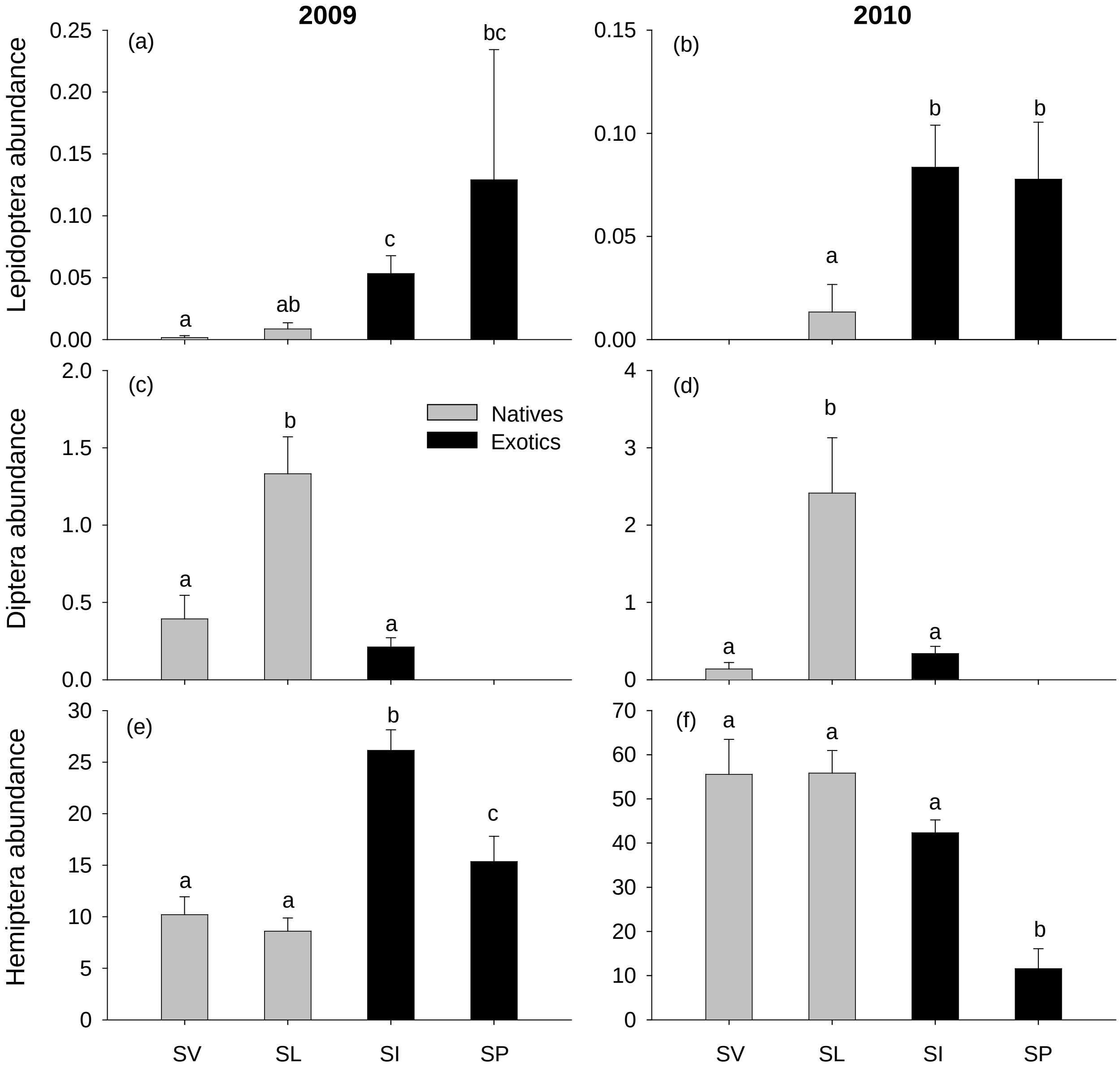
2009
2010
Lepidoptera abundance
Diptera abundance
Hemiptera abundance
(a)
0.25
0.20
0.15
0.10
0.05
0.00
a
ab
c
bc
(b)
0.15
0.10
0.05
0.00
a
b
b
(c)
2.0
1.5
1.0
0.5
0.0
a
b
a
Natives
Exotics
(d)
4
3
2
1
0
a
b
a
(e)
30
25
20
15
10
5
0
a
a
b
c
(f)
70
60
50
40
30
20
10
0
a
a
a
b
SV
SL
SI
SP
SV
SL
SI
SP

Figure 3

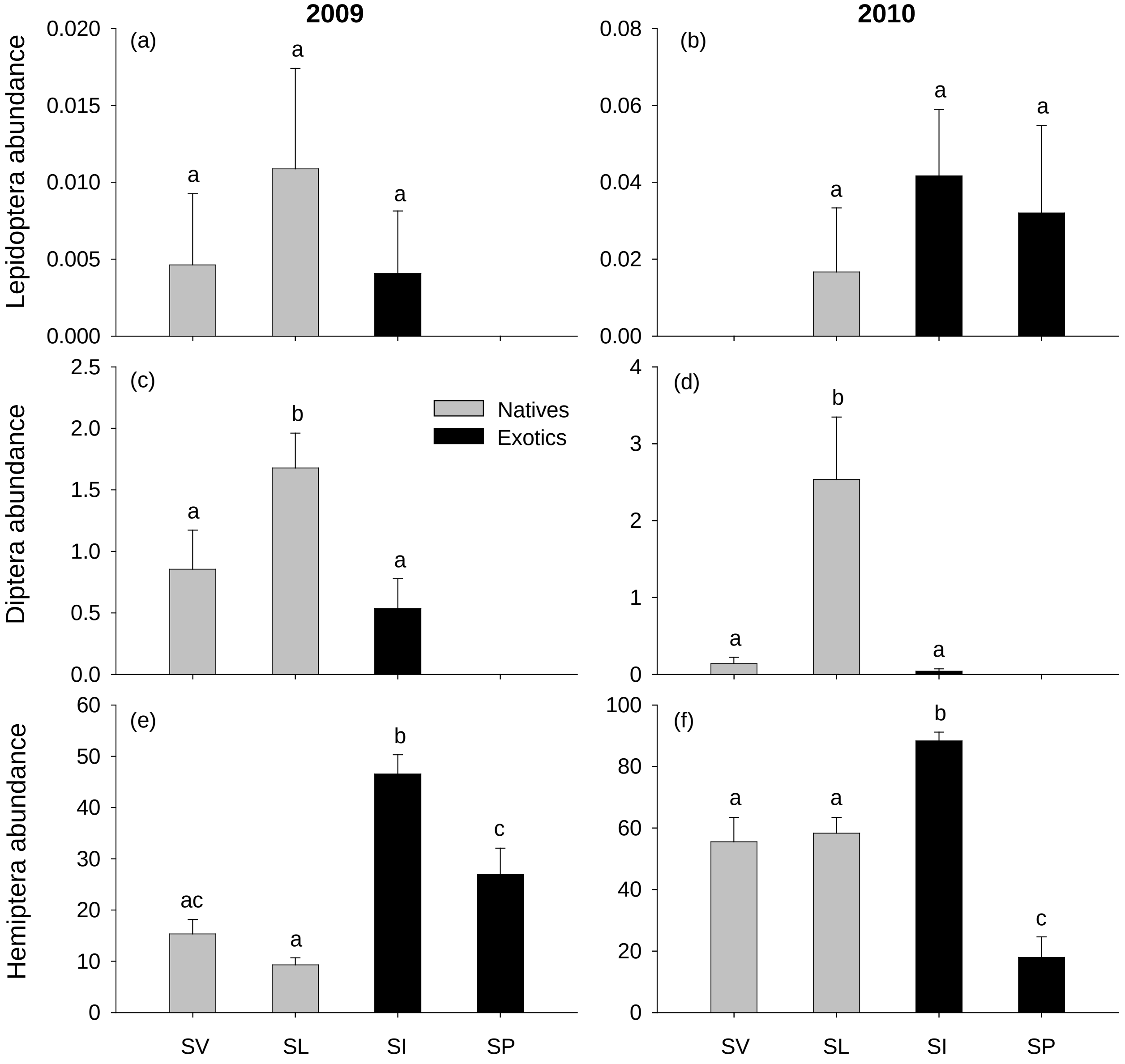

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Figure 4

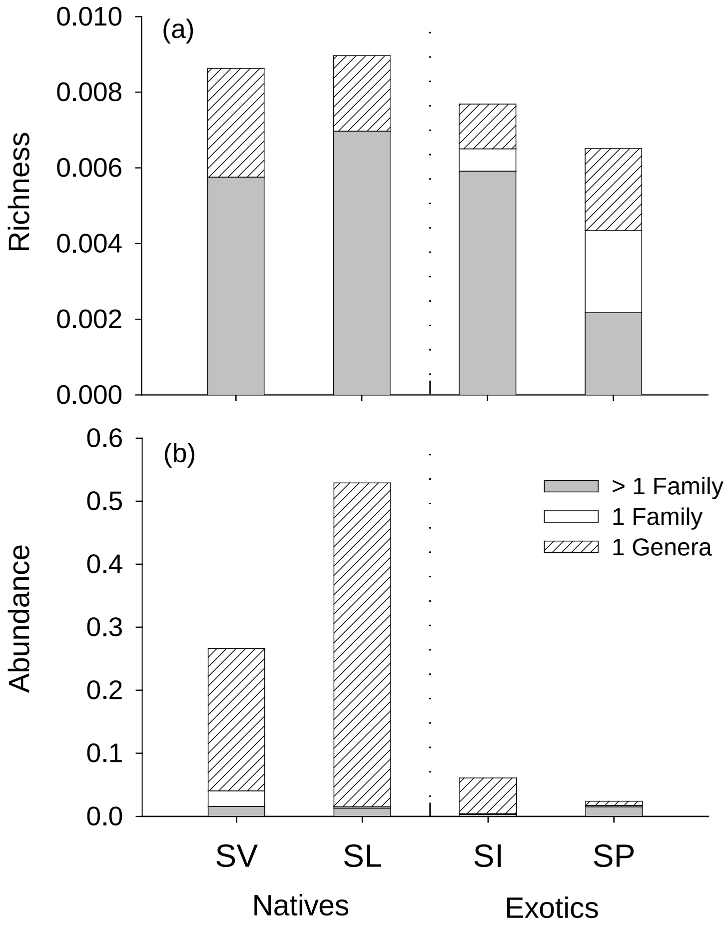

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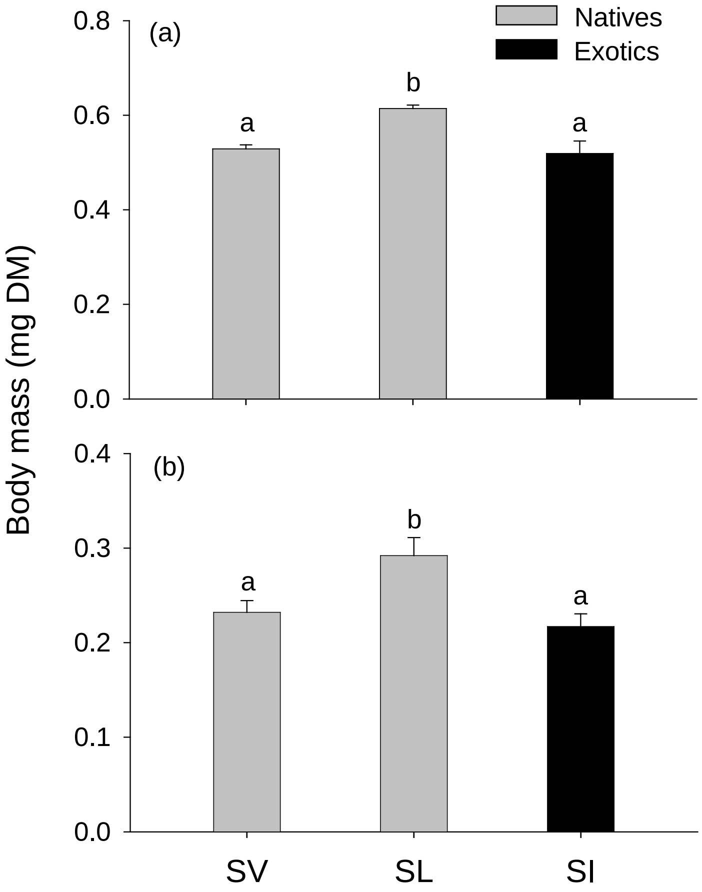
Figure 5



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