PLANT INVASION SUCCESS: INVESTIGATING THE ROLES OF HERBIVORY AND PLANT-SOIL FEEDBACKS

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DEDICATION

For my family, friends, and teachers. Thank you for sharing this journey with me.
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ABSTRACT

Understanding why some non-native plants become successful invaders while most fail to invade can help in assessing risks of invasion. Plants that have evolved defensive traits to resist and/or tolerate herbivory may be more successful invaders since herbivory can potentially prevent species from establishing or regulate existing populations. Plant-soil feedbacks may also enhance dominance of invasive species through allelopathy, changes in soil chemical properties, and interactions with soil biota that promote their own growth and suppress recruitment and growth of co-occurring species. I investigated herbivory as barrier to post-establishment invasion success by conducting three sets of experiments that compared invasive and non-invasive woody species in Hawaii. I measured: seedling acceptability to two generalist herbivores in laboratory no-choice feeding trials, foliar herbivory on seedlings in a field common garden, and seedling tolerance to simulated foliar herbivory in a greenhouse. I also investigated the role plant-soil feedbacks play in the dominance of an invasive tree in Hawaii, Ardisia elliptica, by conducting greenhouse soil feedback experiments to determine if A. elliptica positively impacts its own growth and suppresses growth of two co-occurring species. I found that:

1. There was no general difference in resistance to herbivores between invasive and non-invasive species in feeding trials or the common garden experiment.
2. There was no general difference in tolerance to simulated herbivory between invasive and non-invasive species.
3. There was no evidence of positive soil feedbacks promoting A. elliptica growth.
4. Soil taken from beneath *A. elliptica* did not suppress growth of two co-occurring species.

Thus, herbivory and plant-soil feedbacks do not appear to be playing a strong role in post-establishment invasion success of the species I investigated, suggesting that we still lack a comprehensive understanding of what factors drive invasion success or failure.
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CHAPTER 1
INTRODUCTION

Woody plant invasions

One of the central problems in invasion biology is to understand factors that allow some non-native species to become successful invaders (established and spread well beyond its introduction site: Richardson et al. 2000; Daehler 2001). Case studies of successful species have often focused on identifying plant life history traits that are common to successful invaders (Daehler & Strong 1993; Reichard & Hamilton 1997; Lloret et al. 2005; van Kleunen, Weber & Fischer 2010b). However, the invasion process can be viewed as a series of stages (i.e., transport, establishment, spread, and impact; Lockwood, Hoopes, and Marchetti 2007) with progressively fewer species moving to subsequent stages. A complementary way of addressing factors that promote invasion success is to investigate the barriers non-native plants are met with that may prevent them from moving from one stage to another. This approach is especially useful because the majority of species that are introduced are expected to fail to invade (Williamson & Fitter 1996). In plants, barriers to invasion can include biotic resistance through herbivory, pathogens, or competition (Creed & Sheldon 1995; Case & Crawley 2000; Emer & Fonseca 2010), a mismatch of environmental conditions (Closset-Kopp, Saguez & Decocq 2010), genetic constraints such as bottlenecks and founder effects (Ellstrand & Elam 1993; Crawford & Whitney 2010), a lack of mutualists such as pollinators or dispersers (Nadel, Frank & Knight Jr. 1992; Pringle et al. 2009), and a lack of sufficient propagule pressure (Von Holle & Simberloff 2005; Simberloff 2009).

Studies of woody plant invaders have gained importance in recent years because of an increasing recognition of their threats to biodiversity and conservation efforts (Keppel & Watling 2011; Richardson et al. 2013). Non-native woody species have been intentionally introduced for centuries for commercial, cultural, and aesthetic value making management of these species complex (Webster, Jenkins & Jose 2006; Wilgen & Richardson 2013). Rejmánek and Richardson (2013) report a total of 751 woody non-native species that are naturalized and spreading globally. This represents 434 tree and 317 shrub species from 90 families. The relative size and longevity of woody species
means they can affect broad ecological and community processes in their introduced ranges. For example, in a survey of 48 lowland forest sites on the island of Hawaii currently dominated by non-native tree species, Mascaro et al. (2008) reported that native plant regeneration was almost completely absent from the understory of the invasive trees. Invasive red mangrove (*Rhizophora mangle* L.) alters infaunal and epifaunal sediment community structure of near-shore sandy habitats in Hawaii by effectively lowering water velocity, increasing sedimentation rates, and making the soil environment anoxic (Siple & Donahue 2013). Introduced woody species have also been shown to drastically alter water cycles (Görgens & van Wilgen 2004) and nutrient availability (Vitousek & Walker 1989). Despite the prevalence of woody plant species invasions and documentation of their impacts, we still lack a complete understanding of why non-native woody plants succeed in new environments.

**Herbivory**

Herbivores can regulate establishment and spread of populations because they directly consume seedlings (Hulme 1994; Jackson & Bach 1999) and photosynthetic or storage tissue, decreasing growth rate and reproductive output (Huntly 1991; Levine, Adler & Yelenik 2004; Schmitz 2008). Herbivory can play a role in invasions because non-native plants that experience relatively high levels of herbivory may fail to invade. Conversely, plants that experience decreased levels of herbivory may be more likely to be successful invaders. Studies of a wide variety of plant types in different ecosystems to date have reached inconsistent conclusions about the influence of herbivory on invasion success or failure. Some studies have observed associations between high herbivory and invasion failure (Carpenter & Cappuccino 2005; Cappuccino & Carpenter 2005; Jogesh, Carpenter & Cappuccino 2008), high herbivory and invasion success (Parker & Gilbert 2007; Ashton & Lerdau 2008; Bohl Stricker & Stiling 2013), or a lack of pattern between herbivory and invasion success (Han *et al.* 2008; Matter *et al.* 2012; Dawson *et al.* 2014). Thus, even though herbivory is widely expected to have a role in plant invasions, we still lack a complete understanding of how and when herbivores regulate establishment and spread of non-native species. Understanding how non-
native plants defend against or manage herbivory may provide the key for determining whether herbivory broadly impacts invasion success. There are two types of plant defense traits: resistance and tolerance. First, resistance traits deter herbivory from occurring. Second, tolerance traits allow the plant to mitigate the consequences of herbivory they cannot avoid. Non-native plants that invest in defensive traits may be more likely to succeed in the environment.

Plant resistance traits can be either structural or chemical. Structural resistance traits are any anatomical traits that directly deter herbivory and increases relative fitness. Resistance traits can include the presence of spines and thorns (spinescence), hairs (trichomes), hardened leaves (sclerophyly), or impregnated mineral deposits (Hanley et al. 2007). While most structural traits are constitutive, some can be induced by herbivore damage to protect from further attack. For example, trichomes have been shown to increase in density following herbivore attack in some species (Traw & Dawson 2002; Dalin et al. 2008). Chemical resistance traits are adaptations to produce compounds within the plant that are not involved in photosynthesis or general metabolism. These can be either constitutive or induced and are very diverse across plant taxa (Wink 2003). For example, Biere et al. (2004) demonstrated that Plantago lanceolata L. produces high levels of iridoid glycoside (a monoterpane derivative), as deterrents against both insect herbivory and attack by fungal pathogens.

The second mechanism plants can use to manage herbivory is tolerance, defined as the maintenance of fitness in damaged plants compared to undamaged plants (Strauss & Agrawal 1999; Boege, Barton & Dirzo 2011). Most plants exhibit some capacity to recover from damage, but tolerance is highly variable by species, often depending on plant type (i.e. woody vs. herbaceous), ontogeny, or herbivore identity (Massad 2013). Considering that tolerance allows plants to mitigate the negative consequences of herbivory that they cannot avoid, tolerance might be particularly important for non-native plants in new habitats where they lack specific resistance against the novel herbivores. This could be especially important in tropical environments where herbivory rates are relatively high (Coley & Barone 1996). While studies are limited, there is some evidence that tolerance may play a role in invasions of some

**Plant-soil feedbacks**

Processes that directly or indirectly allow invaders to reduce recruitment of neighboring plants and increase their own relative abundance could be important in invasion success. In particular, invasive plants may succeed through feedbacks with the soil that increase their individual performance or decrease the performance of conspecifics, resulting in their dominance (plant-soil feedbacks: Bever, Westover & Antonovics 1997; van der Putten et al. 2013). The rhizochemical dominance hypothesis proposed by Daneshgar and Jose (2009), suggests that invasion success may be related to many soil-mediated processes including allelopathy in the introduced range (i.e., novel weapons: Callaway & Ridenour 2004) and alterations to soil chemical properties. Allelopathy occurs when plants produce chemicals that are either harmful or beneficial to neighboring plants or microorganisms and alter typical plant community dynamics (Albuquerque et al. 2011). The novel weapons hypothesis suggests that when plants are transported to a new environment, the existing plant community in the introduced range may not have evolved resistance to the allelopathic chemicals released by the invader, thus a non-native plant may reduce germination and growth of existing plant community members, leading to dominance of the non-native. Several temperate species have allelochemicals that may have played a role in their invasion success (*Centaurea diffusa* Lam., Hierro and Callaway 2003; *Centaurea maculosa* Lam., Thorpe et al. 2009; *Ranunculus ficaria* L., Cipollini and Schradin 2011). Much less is known about the prevalence of allelopathy in the tropics. Non-native plants may also negatively impact naïve plant communities in the invaded range by altering chemical properties of the soil environment directly or indirectly through their presence, which may promote invasion success (Ehrenfeld 2003; Weidenhamer & Callaway 2010). For example, invasive species can alter many soil chemical properties including moisture content (Cline, Uresk & Rickard 1977), pH (Boswell & Espie 1998), salinity (Vivrette & Muller 1977), carbon to nitrogen ratios (Stock, Wienand & Baker 1995), macronutrient
fluxes (Ashton et al. 2005) and levels of certain chemical elements that may affect plant growth such as potassium (Mitchell et al. 1997).

Positive and negative plant-soil feedbacks can also occur for colonizing plants as they interact with soil biota (Reinhart & Callaway 2006; van der Putten et al. 2013). The net direction and strength of the feedback depends on the balance of the negative effects of soil borne herbivores, enemies, and pathogens (Beckstead & Parker 2003) relative to the positive benefits of mycorrhizal fungi, nitrogen fixing bacteria and other organisms (Vitousek & Walker 1989; Duda et al. 2003; Pringle et al. 2009; Muthukumar & Udaiyan 2010). While negative soil feedback can be common in native plants, there is evidence that introduced plants experience positive soil feedbacks with soil biota in the introduced range (Klironomos 2002; Inderjit & van der Putten 2010). For example black cherry (*Prunus serotina* Ehrh.) was found to experience negative soil feedbacks due to pathogens in its native range in the United States, but experienced release from pathogens and positive soil feedbacks due to soil biota in its introduced range in Europe, accounting for increased germination and growth (Reinhart et al. 2003). However, relatively little is known about how prevalent beneficial plant-soil feedbacks mediated by soil biota are in woody tropical invaders.

**Research themes, questions, and approach**

Understanding the mechanisms by which non-native plants become successful invaders can help reduce their spread or prevent their entry into novel communities. My dissertation examines two mechanisms of invasion success in several woody plant species in Hawaii: herbivory (Chapters 2-4) and plant-soil feedbacks (Chapter 5). Herbivory may be an important barrier to invasion because it has the potential to prevent species from establishing and/or regulate spread of existing populations. Subsequently, plants that have evolved defensive traits to resist and/or tolerate herbivory should be more likely to succeed as invaders. Additionally, non-native plants may alter soil biotic or abiotic conditions thereby aiding their invasion through positive soil feedbacks that promote invader spread or negative impacts upon existing plant communities.
Research Theme 1: The role of herbivory in plant invasions

Research Questions:

1) Do invasive species have higher resistance to herbivores than non-invasive species?
2) Are highly invasive species able to tolerate herbivore damage more than non-invasive species?

I address these questions through laboratory, greenhouse, and field experiments in Hawaii. Hawaii is a unique place to study mechanisms of invasion partly because of the sheer number of invasive species. Hawaii is home to well over 2000 non-native plant species (Staples & Herbst 2005) that occur in both cultivated and natural or semi-natural settings. Conducting research in Hawaii provides the opportunity to compare many invasive and non-invasive species, which is one of the most explicit methods of determining the mechanisms of invasions (van Kleunen et al. 2010a). While there is generally little information on species that have failed to reach the establishment phase (Zenni & Nuñez 2013), Hawaii has an abundance of species that have established but do not spread (non-invasive), as well as species that reproduce and spread widely beyond their introduction sites (invasive) (Richardson et al. 2000). Furthermore, Hawaii has reasonably well-documented records of the extent of spread of non-native species, through local floras (Little, Skolmen & Sinclair 2003; Staples & Herbst 2005) and other sources (Staples, Herbst & Imada 2000; Imada 2012) which allow for informed decisions about which species are invasive and non-invasive. This is important because conservative estimates of time since introduction can also be determined, and species can be selected that are known to be non-invasive, despite having had ample opportunities to invade.

In Chapter 2, I compared plant resistance to herbivory in invasive and non-invasive species (addressing Question 1) in laboratory no-choice feeding trials of plant seedlings using two generalist invertebrate herbivores that are abundantly found on Oahu, the slug *Laevicaulis alte* (Ferussac, 1822), and the grasshopper, *Cnococephalus*
**saltator** (Saussure). I investigated resistance in invasive and non-invasive species grouped by family (slug trials: Table 2.1; grasshopper trials: Table 2.2). No-choice feeding trials were used to estimate maximum potential acceptability to herbivores among species without introducing additional confounding factors that can occur in choice experiments (van Driesche & Murray 2004). I hypothesized that invasive species would have lower consumption by herbivores (high resistance to herbivory), inferring that this would help explain invasive species success.

In Chapter 3, I continue to address whether plant resistance influences invasion success (Question 1) by measuring herbivory directly in the field. A common garden experiment was conducted on the island of Oahu, planting seeds of 5 invasive and 4 non-invasive species (Table 3.1). Individual plant mean percent leaf area loss due to herbivores was measured following germination at two, three, four, and five months since planting. I predicted that seedlings of non-invasive species would experience more herbivore damage than invasive species in the field, inferring that this contributes to their invasion failure. I was also interested in the role that herbivory plays in plant performance. To address this relationship, plant height was measured during the last three sampling periods and performance was calculated as relative height growth rate (RhGR) at two time intervals (3-4 and 4-5 months since planting). I predicted that as herbivore damage increases, subsequent performance (RhGR) would decrease.

In Chapter 4, I address whether tolerance to herbivory is an important aspect of invasion success (Question 2), by simulating herbivory damage mechanically (50% foliar loss) in a greenhouse and measuring tolerance response in 8 invasive and 8 non-invasive species (Table 4.1). Seedlings were grown for two weeks following damage, and biomass was analyzed to determine whether damaged plants regrew to levels comparable to undamaged controls. I predicted that invasive plant seedlings would have higher herbivory tolerance than non-invasive non-native plants, inferring that this would help explain invader success. In order to identify plant traits that are potentially associated with tolerance prior to damage, pre-damage biomass allocation traits were measured including root to shoot ratio, relative growth rate, cotyledon mass fraction, root mass fraction, and seed mass. I predicted that increased values of all these traits
would enhance seedling tolerance due to the importance of stored reserves in regrowth following damage.

Research Theme 2: The role of plant-soil feedback in plant invasion.

Some non-native species dominate plant communities in their introduced ranges, and drastically impact community dynamics. Soil mediated processes that promote growth of the invader and/or suppresses growth of neighbors (i.e., plant-soil feedbacks) could facilitate invader dominance. I chose to investigate whether plant-soil feedbacks could be influencing the invasion success of a shrub in Hawaii, Ardisia elliptica Thunb. (Myrsinaceae). Native to southeastern Asia, A. elliptica has become invasive in parts of North America and several Pacific islands, including Hawaii (Staples & Herbst 2005). This species has been observed as an ornamental plant in Hawaii at least since 1917 (Rock 1917) but now commonly forms dense stands in low to mid elevation wet forest habitats. However, we know relatively little about what factors allow this species to be a successful invader.

Research Questions:
1) Does A. elliptica promote its own growth through positive plant-soil feedbacks?
2) Does A. elliptica suppress the growth of co-occurring competitors through plant-soil feedbacks?

In Chapter 4, I conducted a soil feedback study with Ardisia elliptica. To address whether A. elliptica experiences positive soil feedbacks that may explain its dominance (Question 1), a shade house experiment was conducted investigating A. elliptica growth in rhizosphere soil from heavily invaded A. elliptica compared to un-invaded sites. To address Question 2, concerning whether A. elliptica suppresses growth and establishment of other plant species through plant-soil feedbacks, I compared growth of seedlings two co-occurring species, the native species Bidens sandvicensis, and the non-native Spathodea campanulata, in soil differing in history of A. elliptica invasion. I predicted that rhizosphere soil from A. elliptica plants would a) promote A. elliptica
growth, and b) decrease growth of co-occurring species. I was also interested in the role soil biota might play in the plant-soil feedbacks, so I used a soil sterilization treatment as part of the above shade house experiments to look at growth with and without soil biota. I predicted that soil communities will a) enhance growth of *A. elliptica*, and b) decrease growth of co-occurring species.
CHAPTER 2
SEEDLING ACCEPTABILITY TO TWO GENERALIST HERBIVORES IN HAWAII DOES NOT EXPLAIN PLANT INVASION SUCCESS OR FAILURE

Matthew H. Lurie and Curtis C. Daehler

Abstract

Biotic resistance by generalist herbivores has been widely recognized as a potential barrier for introduced plant species. However we still lack a complete understanding of how and when generalist herbivores regulate spread and impact of non-native plant species pre- and post- naturalization. Hawaii has an abundance of introduced generalist invertebrate and vertebrate herbivores. In this study we used confamilial groups of non-native invasive and non-invasive woody plant species in Hawaii to determine whether these two groups differed in their seedling acceptability to two non-native generalist herbivores, the slug Laevicaulis alte and the grasshopper Conocephalus saltator. We used no-choice feeding trials to test the hypothesis that invasive species have lower acceptability to generalist herbivores, helping to explain the success of invasive plants compared with non-invasive species. We found species-specific differences in non-native seedling acceptability, and thus resistance, to both slug and grasshopper herbivores in feeding trials. However, contrary to our hypothesis, invasive species did not have significantly lower seedling acceptability by either slugs or grasshoppers. Several highly invasive species had relatively high levels of herbivore acceptability, suggesting that generalist invertebrate herbivory may not be a strong barrier to invasion in Hawaii at the seedling stage.
Introduction

Invasions of non-native species are widely recognized as one of the major threats to biodiversity and ecosystem stability at local, regional, and global scales (Vitousek et al. 1997; Lockwood et al. 2007; Ehrenfeld 2010). Plant invasions in particular can impact community structure (Pyšek & Pyšek 1995; Dunbar & Facelli 1999), fire regimes (Hughes, Vitousek & Tunison 1991; Brooks et al. 2004), hydrology (DiTomaso 1998; Dyer & Rice 1999), and nutrient cycling (Vitousek & Walker 1989; Evans et al. 2001). Studies of woody plant invaders (i.e. trees and shrubs) have gained importance in recent years because of an increasing recognition of their threats to biodiversity and conservation (Keppel & Watling 2011; Richardson et al. 2013). Rejmánek and Richardson (2013) report a total of 751 woody non-native species that are naturalized and spreading globally (434 tree and 317 shrub species from 90 families). Non-native woody species have been introduced intentionally for centuries for commercial, cultural, and aesthetic reasons making management of these species complex (Webster et al. 2006; Wilgen & Richardson 2013). The relative size and longevity of woody species means they can affect broad ecological and community processes in their introduced ranges. One challenging aspect of woody species invasions is that they may have long time lags between initial introduction and subsequent spread, with impacts going unnoticed until populations are very large (Kowarik 1995; Frappier et al. 2003).

Successful plant invaders are species that have established and spread well beyond their introduction sites (Richardson et al. 2000; Daehler 2001). One of the central problems in invasion biology is to understand factors that make a particular non-native species a successful invader. Case studies of successful invasive species have often focused on identifying plant life history traits that are common to them (Daehler & Strong 1993; Reichard & Hamilton 1997; van Kleunen et al. 2010b). For example, in a comparison of over 350 non-native species across five Mediterranean Islands, Lloret et al. (2005) found that large leaf size, summer flowering, long flowering period, and dispersal by wind or vertebrates was positively associated with species abundance. Studies of this type have led to useful recommendations for managers, especially in the
form of risk assessments (Daehler et al. 2004; van Klinken, Panetta & Coutts 2013). The majority of species that are introduced will fail to invade (Williamson & Fitter 1996) or may be invasive in one location but not others (Colautti & MacIsaac 2004). The invasion process can be viewed as a series of stages (i.e. transport, establishment, spread, and impact; Lockwood, Hoopes, and Marchetti 2007). Thus a complementary way of addressing invasion success is to investigate the barriers non-native plants meet and that may prevent them from progressing from one stage to the next.

In plants, barriers to invasion can include a lack of sufficient propagule pressure (Von Holle & Simberloff 2005; Simberloff 2009), mismatch of environmental conditions (Closset-Kopp et al. 2010), genetic constraints such as bottlenecks and founder effects (Ellstrand & Elam 1993; Crawford & Whitney 2010), lack of mutualists such as pollinators or dispersers (Nadel et al. 1992; Pringle et al. 2009), and biotic resistance (Creed & Sheldon 1995; Case & Crawley 2000; Emer & Fonseca 2010). Biotic resistance has received a lot of attention in the invasion literature since Elton (1958) suggested that a diverse community in the invaded range could better resist establishment of non-native species through herbivory, competition, or disease. This can occur in two ways. First, biotic resistance stops invaders prior to establishment. For plant species, this means that biotic resistance would decrease either overall growth or survival rates to such low levels that they cannot maintain a population (lambda < 1) and the species fails to naturalize. This type of biotic resistance leading to failed invasions before naturalization (consistent reproduction and sustained populations; Richardson et al. 2000) is often difficult to detect (Zenni & Nuñez 2013). Second, the pre-existing community may simply limit the growth and spread of an introduced plant (lambda ~ 1) such that it is not recognized as invasive. Herbivores in particular may regulate populations because they directly consume photosynthetic or storage tissue, decreasing growth rate and reproductive output (Huntly 1991; Levine et al. 2004; Schmitz 2008), and often kill seedlings (Hulme 1994; Jackson & Bach 1999).

Most studies of herbivory focus on mature plants, however the seedling stage is often the most vulnerable to herbivore attack (Fenner, Hanley & Lawrence 1999). This is partially because herbivores can consume a large portion of plant tissue compared to herbivore’s size. In the field, individual seedling herbivory varies widely, from 0 - 100 %.
of tissue removed (Clark & Clark 1985; Coley & Barone 1996). Reports of non-native herbivore damage rates at early growth stages across a large number of individuals are rare in tropical forests. At least one study, observed mean foliar tissue loss to herbivory ranging from 1-15% in ten invasive and 18 non-invasive species from several families in a rainforest in Tanzania (Dawson, Burslem & Hulme 2009b), however there is little information on whether these foliar damage rates impact plant performance. Other studies have shown that cotyledon removal can have long lasting effects on plant performance and fitness (Dalling & Harms 1999; Kitajima 2003). For example, complete removal of cotyledons at the seedling stage has been shown to decrease subsequent plant performance in terms of growth and reproductive output at the mature stage (Hanley & Fegan 2007). Thus, herbivory at the cotyledon stage may play an important role in limiting invasions.

Plant species that have effective defensive traits should be better able to overcome biotic resistance due to generalist herbivores in the introduced range. Consequently, herbivory has been widely acknowledged as playing a role in plant invasions and some studies have found support for the relationship that invasive species experience less herbivory (Cappuccino & Carpenter 2005; Jogesh et al. 2008; Bohl Stricker & Stiling 2013). For example, in a common garden experiment measuring leaf damage on seven species of invasive and non-invasive plants (Centaurea spp.) in North America, Jogesh et al. (2008) found that the invasive plants had significantly less leaf damage than the non-invasive ones, suggesting that the former have more effective defense traits. For example, one of the invasive species, Centaurea maculosa Lam., has strong anti-herbivore chemical defenses. These results were consistent with a study by Carpenter and Cappuccino (2005), who surveyed 39 introduced Canadian plant species varying in invasiveness, showing that highly invasive plants had lower levels of leaf damage. However, the association between invasion success and low herbivore damage has not been supported in many other studies (Carpenter & Cappuccino 2005; Liu, Stiling & Pemberton 2007; Parker & Gilbert 2007; Ashton & Lerdau 2008; Dawson et al. 2009b; Matter et al. 2012). These mixed results suggest that despite the fact that herbivory is being broadly recognized as a factor that is relevant to invasion success, we still lack a complete understanding of this process. A lack of pattern between
herbivory and invasion success may be due to many studies broadly grouping non-native species in a single ‘invasive’ category. The single invasive category approach has primarily been used to investigate traits of non-native species compared to native species (Joe & Daehler 2008; Chun, van Kleunen & Dawson 2010) and look at differences in plant traits of an invasive species between the introduced and native range (Schaffner et al. 2011) but lacks the ability to explain failure of introduced species which are often difficult to detect (Zenni & Nuñez 2013). Fortunately there is a growing body of research recognizing the importance of multi-species direct comparisons between invasive and non-invasive species (van Kleunen et al. 2010b; Dehnen-Schmutz 2011).

The goal of our study was to determine whether lower acceptability to generalist herbivores could help explain invader success in non-native woody plant species in Hawaii. Hawaii is home to over 2000 non-native plant species (Staples & Herbst 2005) which occur in both cultivated and natural or semi-natural settings. Because direct comparisons between invasive and non-invasive plant species in their introduced range is one of the most explicit methods of identifying the mechanisms of invasions (van Kleunen et al. 2010a), we compared plant resistance in invasive and non-invasive species grouped by family (Table 2.1 and 2.2). We chose two herbivores from different phyla that are common in Hawaii, the slug Laevicaulis alte (Ferussac, 1822) and the grasshopper Conocephalus saltator (Saussure) because we were broadly interested in resistance to common generalists. We conducted laboratory no-choice feeding trials to estimate maximum potential herbivore resistance among plant species without introducing additional confounding factors that can occur in choice experiments (van Driesche & Murray 2004). We hypothesized that non-invasive plants would suffer higher rates of consumption or damage compared to invasive plants suggesting that resistance to herbivory helps explain invader success.
Methods

Plant selection

Seedling herbivory is particularly important in Hawaiian mesic and wet forests where non-native generalist herbivores are abundant (Stone 1985; Joe & Daehler 2008), so invasive and non-invasive plant species adapted to tropical mesic or wet conditions were selected for study (Table 2.1 and Table 2.2). To ensure invasion opportunity, widely planted species with a minimum residence time (Richardson & Pyšek 2006) of at least 50 years, as determined by the earliest record in the Bishop Museum Herbarium (http://nsdb.bishopmuseum.org/) were used in our study.

We designated species as invasive or non-invasive (Richardson et al. 2000) using local floras (Little et al. 2003; Staples & Herbst 2005), reports of extent of spread (Staples et al. 2000; Imada 2012) and consultations with invasive plant experts. All of the invasive species are widely naturalized on the island of Oahu and generally across multiple islands. The non-invasive species do not appear to be spreading or naturalizing and in some cases exist only in cultivation. Fruits and seeds of all species were collected from Oahu, primarily from roadside plants and easily accessible trails and landscaping surrounding the Manoa Valley, with the exception of Solanum melongena seeds, which were purchased from a local nursery. Due to constraints of fruiting season, seed longevity, and germination for each species, some plant species used for slug feeding trials (Table 2.1) differed from those used for grasshopper feeding trials (Table 2.2). We chose confamilial groups of invasive and non-invasive species to control for family-level phylogenetic differences in defense mechanisms.

Herbivore selection

We tested acceptability of invasive and non-invasive plants to two non-native generalist herbivores found in Hawaii using no-choice feeding trials. The slug, Laevicaulis alte, has been present in Hawaii since approximately 1900 (Cowie 1998) and has been recorded on Oahu, Kauai, Maui, and Hawaii Island (Cowie 1997). We also used a grasshopper, Conocephalus saltator, native to central and South America (Hebard 1927) that has been a widespread agricultural pest since the 1920s and is
known to be a generalist omnivore (Swezey 1928; Illingsworth 1931) found in open, disturbed habitats. Both herbivores were abundant in low to mid elevation forest sites and cultivated habitats where the plant species used in feeding trials can be found (pers. obs.). Slugs and grasshoppers were collected from the University of Hawaii at Manoa campus.

Feeding trials

All plants were germinated either in Petri dishes with a moist paper towel in a growth chamber (Percival Environmental Controller model I-30VL) on a 12 h day / 12 h night cycle at 25 °C / 21 °C or in seed trays containing soil mixture in a glass house at the University of Hawaii at Manoa campus. Species of Fabaceae required pretreatment prior to germination. *Acacia confusa*, *Leucaena leucocephala*, and *Enterolobium cyclocarpum* seeds were placed in a glass container, covered with 100 °C water, and allowed to soak for 2 mins. The seeds were then placed in room temperature water for 24 h prior to planting. *Tamarindus indica* seeds were mechanically scarified with a razor blade. Feeding trials were conducted with seedlings in the cotyledon stage of growth. In some species, the first pair of foliar leaves had also begun to develop at the beginning of the trial and continued to grow during the trial. In most cases cotyledon size was larger than that of foliar leaves during feeding trials.

We tested seedling acceptability to the slug *Laevicaulis alte* with five invasive plants and five non-invasive plants (Table 2.1). Following germination and growth to the cotyledon stage, seedlings were transplanted into slug feeding chambers. Three to eight replicate feeding chambers were used for each plant species (N = 3-8). The feeding chamber consisted of clear plastic trays. Each tray had bottom dimensions of 21 cm x 21 cm and top dimensions of 24 cm x 24 cm with a depth of 5 cm. The bottom tray was filled approximately 3 cm deep with a 4:1:1 ratio mixture of “Niu Hawaiian Style All Purpose Potting Soil,” 0.5 inch granite gravel, and perlite. For each plant species, sixteen seedlings were transplanted into the tray in rows of four, spaced 3 cm apart. Slugs were collected in the field and starved for 3-5 days in the lab prior to feeding trials to help ensure consistent feeding motivation among individuals and among trials. We selected similar sized slugs for all experiments (mean slug mass 13.98 g, standard
deviation 2.84 g). Four slugs were randomly chosen, weighed and placed in each tray. Multiple slugs were used in each feeding chamber to reduce the influence of the feeding behavior of a single individual on the results. After slugs had been placed in the tray, a second tray was inverted for use as a lid and sealed with tape to prevent the slugs from escaping. A 5 cm x 5 cm square hole was cut out of the lid and covered with fiberglass mesh to allow airflow. Feeding chambers were placed in a growth chamber (Percival Environmental Controller model I-30VL) with a 12 h day / 12 h night cycle at 25 °C / 21 °C respectively and 80 % relative humidity. The number of seedlings consumed each day was measured after six days. In general, when slugs consumed a seedling they removed the entire cotyledons, leaving a severed stem. In a small number of these cases a portion of the cotyledon remained (< 10 % of seedling tissue) and these were considered ‘consumed’ because the result of this damage was seedling mortality (pers. obs.).

We tested seedling acceptability to the grasshopper *Conocephalus saltator* in six invasive and six non-invasive plants (Table 2.2). Following germination and growth to the cotyledon stage, seedlings were transplanted into grasshopper feeding chambers. Feeding chambers were made from 600 mL clear plastic cups covered with a fiberglass mesh. Seedlings were weighed and their roots were wrapped in moist cotton to prevent desiccation. A single seedling was placed into a 30 mL plastic cup and positioned in the bottom of the feeding container. Grasshoppers were collected in the field and starved for 24 hours. A single grasshopper was weighed and then placed in each cup. Only one grasshopper was used because in pilot studies antagonistic behaviors were observed when multiple grasshoppers were placed together. The average mass of grasshoppers used was 0.113 g (standard deviation 0.084 g). Between five and ten replicate chambers were used for each plant species. Feeding chambers containing the herbivore and seedling were placed in a growth chamber (Percival Environmental Controller model I-30VL) with a 12 h day / 12 h night cycle at 25 °C / 21 °C respectively and 80 % relative humidity for 24 h. Pilot studies indicated that plant biomass change caused by desiccation was highly variable so initial and final biomass changes were not indicative of actual herbivore consumption. Instead, herbivory was assessed at the end of the 24 h period by visually estimating herbivore damage (e.g., foliar or stem tissue.
removed) on individual seedlings using a dissecting microscope. Visible chewing damage was estimated for whole seedlings and scored in 10 % intervals (i.e., 0 % = 0, 1-10 % = 1, 11-20 % = 2, 21-30 % = 3, 31-40 % = 4, 41-50 % = 5, 51-60 % = 6, 61-70 % = 7, 71-80 % = 8, 81-90 % = 9, 91-100 % = 10). Any seedling parts that were detached from the plant as a result of chewing were included as herbivore damage. This included leaves that were detached because the stem was severed.

Statistics

To determine if seedling acceptability to generalist slug and grasshopper herbivores differed between invasive and non-invasive species, we used a linear mixed effects model. This approach allowed us to control for phylogenetic variation and measure the influence of herbivore size on seedling acceptability. Significance testing for mixed models followed a “step down” approach (West, Welch & Galecki 2007). Normal quantile-quantile plots of residuals and scatter plots of predicted values versus residuals were used to test model assumptions. Likelihood ratio tests using a chi squared distribution with one degree of freedom were used to assess significance of effects based on maximum likelihood for fixed effects and restricted maximum likelihood for random effects. For all mixed models, the covariance structure type was variance components. Other covariance structures were investigated but did not significantly change the model so are not included in the results. Outliers were identified, but removing them did not alter conclusions so they were left in. Post hoc Tukey’s adjusted least square mean analysis examined pair-wise differences between significant fixed effects with more than two levels. All statistical analyses, including mixed model analyses were performed using SAS for Windows version 9.4 (2013).

For slug feeding data (i.e., the number of seedlings consumed after six days in the feeding chamber), the mixed model tested the main effect of invasion category (invasive or non-invasive) as a fixed factor. The combined mass of the four slugs used in each feeding chamber was included as a fixed factor covariate. The interaction of slug mass and invasion category was included as a fixed factor. For grasshopper feeding data (i.e., the seedling herbivore damage score after 24 hours), the mixed model tested the main effect of invasion category (invasive or non-invasive) as a fixed
factor. Grasshopper mass and initial seedling mass were included as fixed covariates in the model. For both herbivore types, species nested within family was included as a random factor. Additionally, a 'group effect' (“SAS system for Windows” 2013) of family was included in the model that allowed for heterogeneity in the covariance structure of the random variable for each level of species nested within family. A summary of model construction can be viewed in the appendices (slug trials: Appendices 2.1 and 2.2; grasshopper trials: Appendices 2.3 & 2.4).

Results

Slugs feeding trials

Slugs consumed on average 4.59 ± 0.70 (mean ± standard error) (29 %) of the seedlings across all species by the sixth day. Species means are shown in Figure 2.1. They consumed on average 15.3 ± 0.7 seedlings of *Alstonia macrophylla* and 14.0 ± 0.8 of *Solanum melongena*. On average, fewer than half the seedlings of the remaining species were consumed (Fig. 2.1). They did not consume any of the *Tabebuia aurea* (non-invasive; Bignoniaceae) or *Tamarindus indica* (non-invasive; Fabaceae).

Other than species differences, none of the effects tested in the mixed model contributed significantly to the variance in the number of seedlings consumed (Table 2.3). There was no significant difference in the number of invasive and non-invasive seedlings consumed (5.4 ± 2.6, mean ± standard error, 4.4 ± 2.7, respectively; $\chi^2 (1) = 0; P = 1.0$; Fig. 2.2). Slug mass (i.e., the total mass of four slugs used in each feeding chamber) did not contribute significantly to the variance in the final model ($\chi^2 (1) = 0.1, P = 0.752$). There was also no significant interaction between slug mass and invasion category ($\chi^2 (1) = 0.8, P = 0.371$). The random factor of species nested within family did contribute significantly to the variance in the final model ($\chi^2 (1) = 59.5, P <0.001$).

Grasshopper feeding trials

Seedlings in grasshopper feeding trials had an overall damage score of 0.75 ± 0.27 (mean ± standard error) out of a possible score of 10, representing less than 10 % average damage. Species means are shown in Figure 2.3. Only in three trials was a
damage score of 10 (100% damage) recorded, twice in *Carissa macrocarpa* and once in *Nerium oleander*. The highest average damage rate also occurred in *Carissa macrocarpa* (non-invasive; Apocynaceae; 2.89 ± 1.39) and *Nerium oleander* (non-invasive; Apocynaceae; 2.22 ± 1.22), indicative of aboveground tissue damage of 20-30%. In contrast, *Enterolobium cyclocarpum* (non-invasive; Fabaceae) and *Alstonia macrophylla* (invasive; Apocynaceae) received no damage.

Other than species differences, none of the effects tested contributed significantly to the variance in the damage score on seedlings (Table 2.4). There was no significant difference in damage scores between invasive and non-invasives (0.49 ± 0.29, mean ± standard error, 0.58 ± 0.32, respectively; \( \chi^2 (1) < 0.1, P > 0.999; \) Fig. 2.4) Initial seedling mass did not contribute significantly to the variance in the final model (\( \chi^2 (1) = 0.1, P =0.752 \)) nor did grasshopper mass (\( \chi^2 (1) = 0.2, P =0.654 \)). Species nested within family was a significant contributor of variance in the overall model (\( \chi^2 (1) = 5.5, P = 0.019 \)).

**Discussion**

Herbivory has been widely acknowledged as playing a role in plant invasion success (Blossey & Notzold 1995; Levine & D’Antonio 1999; Keane & Crawley 2002). However, we found herbivore acceptability was not significantly greater for seedlings of invasive woody species than for seedlings of non-invasive woody species. Investigations of the relationship between herbivory and invasion have generally focused on leaf damage estimates in the field for juvenile and adult plants from a wide variety of growth forms. Of these studies, the prediction that invaders will have high resistance is supported in some cases but not all. Several studies have shown that invaders have higher resistance through significantly lower herbivore damage estimates in direct comparisons of invasive and non-invasive species (Cappuccino & Carpenter 2005; Jogesh et al. 2008; Bohl Stricker & Stiling 2013) or demonstrated a significant negative relationship between herbivore damage and plant invasion status (Carpenter & Cappuccino 2005). For example, Cappuccino and Carpenter (2005) choose nine invasive and nine non-invasive species in north-eastern North America and measured
total leaf area loss due to chewing, gall making, and mining insects in field surveys. The authors found that median leaf damage in the invasive species was lower than 1% compared to non-invasive species, which had a median leaf damage of ~5%. These rates are within the damage score range we observed for seedlings in grasshopper feeding trials (0-10%). Two additional studies demonstrated weak support for the trend of lower damage in invasive species overall, but did not show significant differences (Han et al. 2008; Dawson et al. 2014). Conversely, a study of foliar damage rates on temperate vines (Ashton & Lerdau 2008) and pathogen susceptibility and herbivore attack on clover species (Parker & Gilbert 2007) found that invasives actually showed lower resistance rates than non-invasives. Additional studies found no relationship between resistance and invasiveness (Liu et al. 2007; Matter et al. 2012). For example, Matter et al. (2012) found that for trees and shrubs surveyed in ornamental settings of Cincinnati, Ohio, where pesticides were not being used, there was no difference in leaf damage between 12 non-invasive and 11 invasive species. Liu et al. (2007) also did not observe significant differences in leaf damage between the Florida invasive Eugenia uniflora and three of its non-invasive congeners, though use of a single invasive species prohibits broad conclusions. Given these mixed results, we still lack a complete understanding of how and when generalist herbivores regulate the spread and impact of plant species pre- and post-naturalization. However, the hypothesis of stronger herbivore resistance among successful invaders, relative to non-invaders, is not supported in the seedlings of woody species we investigated.

Estimating herbivore resistance by measuring seedling acceptability to common generalist herbivores in a controlled environment is one of the most direct ways to measure potential for herbivore influence. In addition to our study, we are aware of only one other study that compared acceptability to generalist herbivores in a controlled setting. Jogesh et al. (2008) studied feeding acceptability of non-native plant species to two native generalist grasshoppers. In their first set of feeding trials they presented the American grasshopper, Schistocerca americana (Drury), with cut leaves measuring 6.25 cm² from nine non-native species in the Asteraceae and Brassicaceae families differing in invasion status as measured by prominence on local and federal invasion lists. Unlike our study, the authors observed a relationship between increasing invasiveness and
decreasing amount of leaf material consumed by *S. americana*. However the findings from their second set of feeding trials, where the authors compared acceptability of 7 non-native species in the knapweed genus *Centaurea* (Asteraceae), including 3 invasive and 4 non-invasive species to the red-legged grasshopper *Melanoplus femurrubrum* (DeGeer), showed mixed results. Even though two invasive species had the least amount of leaf area eaten by *M. femurrubrum*, the amounts were not significantly different from the majority of non-invasive species. Thus their observations of strong species specific differences in feeding acceptability with *M. femurrubrum* more closely matched our own observations with a generalist slug and grasshopper herbivore in Hawaii. Since manual leaf cutting may affect constitutive or induced defensive traits (Schmelz, Alborn & Tumlinson 2001), our study may more accurately represent plant defensive conditions herbivores are met with when they encounter undamaged seedling in the field.

We found some invasive species with high acceptability to herbivores, contrary to our expectation. This has been previously observed in common garden experiments (Parker & Gilbert 2007; Ashton & Lerdau 2008). For example, Ashton and Lerdau (2008) noted that four juvenile invasive temperate vine species showed more leaf damage by insects and mammal herbivores than four non-invasive species over the course of two growing seasons. Parker and Gilbert (2007) also noticed that in seven introduced clover species in California, the two most invasive species, *Medicago arabica* L. (Huds.) and *Medicago polymorpha* L., had higher levels of herbivore and pathogen attack than non-invasive species. In our study, the invasive plant *Alstonia macrophylla* (Apocynaceae) had nearly 100% of its seedlings consumed in all slug feeding trials. In grasshopper feeding trials, 100% of seedlings of the invasive species *Melaleuca quinquenervia* (Myrtaceae) showed chewing by grasshoppers. Despite their acceptability to herbivores, there are several reasons why these plant species may still be invasive in the landscape. First, herbivores are probably met with a variety of food items in the field which they may encounter more frequently or have preference for compared with a given introduced species. Thus, a species that is frequently eaten in our acceptability trials may never or only rarely be consumed in the field. Second, if some individuals pass the vulnerable seedling stage by recruiting to safe sites, a
subsequent high growth rate or high reproductive capacity could make up for high seedling mortality in terms of population growth. This is certainly possible with *A. macrophylla* and *M. quinquenervia*, which are prolific in terms of wind-dispersed seeds and have high germination rates in the lab (pers. obs.). Plants may also escape herbivory through phenological traits that match dispersal and growth with predictable fluctuations in herbivore population densities. However, generalist herbivores are ubiquitous in the environment year round in Hawaii so it is unlikely that phenology solely explains invasion success. High propagule pressure has also been suggested as a mechanism to overcome invasion barriers, especially associated with establishment (Eschtruth & Battles 2009; Simberloff 2009). For example, highly acceptable species may be able to overcome biotic resistance through repeated introductions that make seedlings more likely to find safe sites. This seems possible for *M. quinquenervia*, which is commonly used as a landscape tree in urban environments around Oahu. Third, plants may be able tolerate herbivory by reducing the impact herbivory has on the fitness of the plant once damage has occurred (Strauss & Agrawal 1999; Boege *et al.* 2011). In other words, invasive plants may still be able to succeed in the environment even if herbivore damage is high. Though tolerance was not directly investigated, our slug acceptability trials do not seem to support this mechanism because seedlings with high herbivore damage generally did not survive (pers. obs.). We did not observe seedling mortality following grasshopper trials, but grasshopper damage rates were relatively low.

We also identified non-invasive species with low levels of acceptability to herbivores, indicating high resistance. In slug feeding trials fewer than 12 % of the seedlings of the non-invasive species *Nerium oleander*, *Tabebuia aurea*, and *Tamarindus indica* were consumed. In grasshopper feeding trials, the non-invasive *Enterolobium cyclocarpum* was not damaged. Because high resistance in these species does not confer invasion success, other barriers are probably preventing significant population spread. Seedlings may have structural or chemical traits that confer resistance to generalist herbivores but lack vigor in terms of plant performance to compete with neighboring plants. Also, genetic constraints, biotic resistance due to
competitors and/or pathogens, or lack of mutualists could cause failure to invade in these species.

Conclusion

An abundance of generalist herbivores in Hawaii suggests that biotic resistance to invasion is at least a possibility, and we would expect generalist enemies to suppress some introduced species. However, lab feeding trials suggest no general difference in acceptability to generalist herbivores between plants that have become successful invaders and those that failed to invade, with high variability among species. It seems likely that invaders use a variety of strategies to overcome biotic resistance from generalist herbivores (including escape, tolerance, or high propagule pressure) while non-invasive species may be resistant to herbivores but encounter other barriers (such as competition or lack of mutualists) that prevent their invasion, though these are areas for further investigation.
<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Invasion Category</th>
<th>Growth Form</th>
</tr>
</thead>
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<tr>
<td>Fabaceae</td>
<td><em>Acacia confusa</em> Merr.</td>
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<td>Tree</td>
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<td><em>Tamarindus indica</em> L.</td>
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<td>Tree</td>
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<td>Invasive</td>
<td>Tree</td>
</tr>
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<td><em>Tabebuia aurea</em> (Manso) Benth. &amp; Hook. f. ex S. Moore</td>
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<td>Tree</td>
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<tr>
<td>Apocynaceae</td>
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<td>Myrtaceae</td>
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<td></td>
<td><em>Callistemon viminalis</em> (Sol. ex Gaertn.) G. Don</td>
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<td><em>Solanum melongena</em> L.</td>
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Table 2.2. Invasive and non-invasive plant species used in grasshopper seedling acceptability trials.

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<th>Family</th>
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<th>Invasive Category</th>
<th>Growth Form</th>
</tr>
</thead>
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<td>Apocynaceae</td>
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<td>Tree</td>
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<td><em>Thevetia peruviana</em> (Pers.) K. Schum.</td>
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<td><em>Nerium oleander</em> L.</td>
<td>Non-Invasive</td>
<td>Large shrub</td>
</tr>
<tr>
<td>Fabaceae</td>
<td><em>Acacia confusa</em> Merr.</td>
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<td>Tree</td>
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<td><em>Tamarindus indica</em> L.</td>
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<td>Invasive</td>
<td>Tree</td>
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<tr>
<td></td>
<td><em>Tabebuia aurea</em> (Manso) Benth. &amp; Hook. f. ex S. Moore</td>
<td>Non-Invasive</td>
<td>Tree</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td><em>Melaleuca quinquenervia</em> (Cav.) S.F. Blake</td>
<td>Invasive</td>
<td>Tree</td>
</tr>
<tr>
<td></td>
<td><em>Callistemon viminalis</em> (Sol. ex Gaertn.) G. Don</td>
<td>Non-Invasive</td>
<td>Tree</td>
</tr>
</tbody>
</table>
Table 2.3. Results of mixed effects model analysis for slug seedling acceptability trials. The dependent variable is the number of seedlings consumed by day six of acceptability trials. Bolded variables are significant at $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Effect Type</th>
<th>Variable</th>
<th>Test Statistic $\chi^2$ (1)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed</td>
<td>Invasion Category</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Slug Mass</td>
<td>0.1</td>
<td>0.752</td>
</tr>
<tr>
<td></td>
<td>Invasion Category x Slug Mass</td>
<td>0.8</td>
<td>0.371</td>
</tr>
<tr>
<td>Random</td>
<td>Species (Family)</td>
<td>59.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 2.4. Results of mixed effects model analysis for grasshopper seedling acceptability trials. The dependent variable is the seedling damage score (0-10). Bolded variables are significant at $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Effect Type</th>
<th>Variable</th>
<th>Test Statistic $\chi^2(1)$</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed</td>
<td>Seedling Mass</td>
<td>0.1</td>
<td>0.752</td>
</tr>
<tr>
<td></td>
<td>Grasshopper Mass</td>
<td>0.2</td>
<td>0.654</td>
</tr>
<tr>
<td></td>
<td>Invasion Category</td>
<td>&lt;0.1</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>Random</td>
<td>Species (Family)</td>
<td><strong>5.5</strong></td>
<td><strong>0.019</strong></td>
</tr>
</tbody>
</table>
Figure 2.1. Mean number of seedlings of invasive (dark bars) and non-invasive species (light bars) consumed during slug feeding trials. Error bars represent ±1 standard error.

Species families are abbreviated in brackets after species names (Apoc = Apocynaceae, Myrt = Myrtaceae, Fab = Fabaceae, Big = Bignoniaceae, Sol = Solanaceae). No seedlings of *Tabebuia aurea* (non-invasive; Bignoniaceae) and *Tamarindus indica* (non-invasive; Fabaceae) were consumed.
Figure 2.2. Diagram of mean seedlings consumed out of sixteen possible by day six of slug seedling acceptability trials for invasive and non-invasives species. Error bars represent ±1 standard error.
Figure 2.3. Diagram of mean grasshopper damage score (0-10) for species in 24 hour seedling acceptability trials. Error bars represent ±1 standard error. Species families are abbreviated in brackets after species names (Apoc = Apocynaceae, Myrt = Myrtaceae, Fab = Fabaceae, Big = Bignoniaceae). Dark bars are invasive species. Light bars are non-invasive species. *Alstonia macrophylla* (invasive; Apocynaceae) and *Enterolobium cyclocarpum* (non-invasive; Fabaceae) had zero damage in all trials. *Tamarindus indica* (non-invasive; Fabaceae) had a standard error of zero.
Figure 2.4. Diagram of mean grasshopper damage score for invasive and non-invasive species. Error bars represent ± 1 standard error.
Acknowledgements

This research was funded in part by a University of Hawaii at Manoa Botany Department Research Grant. We thank Danielle Frohlich and the Oahu Early Detection Program for assistance with species selection. Bonnie Liu and several undergraduate volunteers provided valuable support in the laboratory.
CHAPTER 3
HERBIVORE DAMAGE IN SEEDLINGS OF INVASIVE AND NON-INVASIVE WOODY PLANT SPECIES: A COMMON GARDEN STUDY IN HAWAII

Matthew H. Lurie, Jennifer L. Bufford, and Curtis C. Daehler

Abstract

Herbivory has been widely recognized as a potential invasion barrier for introduced plant species. However we still lack a complete understanding of how and when herbivores regulate spread and impact of introduced plant species pre- and post-naturalization. The goals of our investigation were to 1) examine whether species that failed to invade experienced higher levels of herbivory compared to invasive species, 2) assess whether plant performance was influenced by natural levels of herbivore damage, and 3) determine if herbivory levels in seedlings changed over time. We chose to investigate early growth because the seedling stage is the most vulnerable to attack and critical for establishment and spread of many introduced species. We conducted a common garden experiment at two locations on Oahu, in the Hawaiian Islands, representing a range of tropical mesic environments. We planted seeds of five invasive and four non-invasive woody plant species in ten plots at each site. Individual plant mean percent leaf area missing due to herbivores was measured at two, three, four, and five months since planting. Relative height growth rate (RhGR) was calculated for two time intervals to assess whether performance was impacted by herbivory. Individual plant herbivory ranged from zero to 100%. However mean foliar herbivory was low for all species (< 2 %). There was no significant difference in foliar herbivory between invasive and non-invasive species and damage rate did not change significantly over time. Herbivore damage also did not predict plant performance in the following month at either time interval investigated. Whether invasive or not, seedling establishment and growth of the introduced woody plant species we investigated generally do not appear to be regulated by herbivory.
Introduction

Invasions of non-native species are widely recognized as one of the major threats to biodiversity and ecosystem stability at local, regional, and global scales (Vitousek et al. 1997; Lockwood et al. 2007; Ehrenfeld 2010). Invasive plants in particular can impact community structure (Pyšek & Pyšek 1995; Dunbar & Facelli 1999), fire regimes (Hughes et al. 1991; Brooks et al. 2004), hydrology (DiTomaso 1998; Dyer & Rice 1999), and nutrient cycling (Vitousek & Walker 1989; Evans et al. 2001). Only a small fraction of plant species that are introduced become established, and an even smaller number spread beyond introduction sites and dominate communities (Williamson & Fitter 1996; Richardson et al. 2000; Daehler 2001). One of the central problems in invasion biology is to understand factors that allow some non-native species to become successful invaders while others fail. Because the invasion process can be viewed as a series of stages (i.e. transport, establishment, spread, and impact; Lockwood, Hoopes, and Marchetti 2007), a valuable way of understanding the invasion process is to experimentally investigate the barriers non-native plants are met with that may prevent them from moving from one stage to another. In plants, barriers to invasion can be caused by a lack of sufficient propagule pressure (Von Holle & Simberloff 2005; Simberloff 2009), a mismatch of environmental conditions (Closset-Kopp et al. 2010), genetic constraints such as bottlenecks and founder effects (Ellstrand & Elam 1993; Crawford & Whitney 2010), a lack of mutualists such as pollinators or dispersers (Nadel et al. 1992; Pringle et al. 2009), and biotic resistance by herbivores, pathogens, or competitors (Creed & Sheldon 1995; Case & Crawley 2000; Emer & Fonseca 2010).

Herbivory in particular has the potential to be a substantial invasion barrier because plants may be immediately exposed to herbivores in the introduced community, where they consume photosynthetic, structural, or storage tissue, potentially decreasing plant growth rates and reproductive output (Huntly 1991; Levine et al. 2004; Schmitz 2008) and even causing mortality (Hulme 1994; Jackson & Bach 1999; Green & Juniper 2004). In terms of population dynamics, herbivores may stop invaders prior to establishment by decreasing either overall growth or survival rates to
such low levels that they cannot maintain a population and the species fails to naturalize. Herbivores may also limit the growth and spread. Thus, introduced plants that experience relatively high levels of herbivory may fail to invade, while those that experience lower levels of herbivory may be more likely to be successful invaders. Studies to date, however, have reached inconsistent conclusions about the influence of herbivory on invasion success or failure. Some studies have observed associations between high herbivory and failure (Carpenter & Cappuccino 2005; Cappuccino & Carpenter 2005; Jogesh et al. 2008), high herbivory and success (Parker & Gilbert 2007; Ashton & Lerdau 2008; Bohl Stricker & Stiling 2013), or a lack of any association between herbivory and invasion success (Han et al. 2008; Matter et al. 2012; Dawson et al. 2014), though these studies investigated a wide variety of plant types in different ecosystems. Relatively few studies have investigated invasion success in tropical forests, even though herbivory has been suggested as an important regulator of tropical forest plants, partially due to the abundance and diversity of phytophagous insects compared to other ecosystems (Dirzo & Boege 2008). The few studies that have investigated tropical forest species in field surveys have generally shown little evidence that herbivores determine invasion success or failure (Liu et al. 2007; Dawson, Burslem & Hulme 2009a).

Given the paucity of studies in tropical habitats and general lack of consensus on the importance of herbivory in invasion success or failure, our objective was to test for an association between herbivore damage and invasion failure using woody plants in Hawaii. We focused on non-native woody plant species because they drastically alter landscapes and change biodiversity (Webster et al. 2006). Since herbivory rates can be heavily influenced by location within the canopy or understory (Reynolds & Crossley 1997), season (Aide 1992; Barone 2000), and climate (Kim 2014), we conducted a common garden experiment to minimize the influence of extrinsic factors. We investigated herbivory in nine non-native woody plant species that differ in invasion status (invasive vs. non-invasive). We measured monthly foliar damage on seedlings from two to five months after planting. We focused on young plants because many herbivores target the seedling stage (Crawley 1983; Mills 1983; Franks, Kral & Pratt 2006; Joe & Daehler 2008) and because seedling recruitment is a critical aspect of
population establishment and spread (Fenner 1987). We predicted that seedlings of non-invasive species would experience more herbivore damage than invasive species in the field, contributing to their invasion failure. We also measured relative growth rates at two time periods in order to test if there is a link between herbivore damage and performance. We predicted that herbivore damage would be associated with decreased growth. Because physical and chemical defenses have been shown to increase over time in young woody plants (Barton & Koricheva 2010), we also predicted that herbivory rates would decrease over the course of the experiment, as plants developed or allocated more resources to defense.

Methods

Plant Species

Seedling herbivory is particularly important in wet forests (Green & Juniper 2004; Joe & Daehler 2008), and so a species pool was developed that included invasive (N = 5) and non-invasive (N = 4) plants that are adapted to wet or mesic forests in Hawaii and that produced viable seeds (Table 3.1). All species had a minimum residence time of at least 50 years, as determined by the earliest record in the Bishop Museum Herbarium (http://nsdb.bishopmuseum.org/). We designated species as invasive or non-invasive (Richardson et al. 2000) using local floras (Little et al. 2003; Staples & Herbst 2005) and reports of extent of spread (Staples et al. 2000). Where information on species was not available we consulted invasive plant experts to establish whether a species was invasive or not. All of the invasive species are widely naturalized on Oahu and generally across multiple Hawaiian Islands. The non-invasive species have been planted around the Hawaiian Islands, but do not appear to be spreading or naturalizing to date and are rarely, if ever, found outside of cultivation in the Hawaiian Islands. Fruits and seeds for all species were collected from the island of Oahu, primarily from botanical gardens, public landscaping, and private gardens and from multiple plants and locations. Seeds of most species were collected just before the start of the experiment,
although seeds of some species were up to six months old prior to planting. Seeds from multiple plants and locations were mixed prior to planting.

Site selection

A common garden experiment was conducted at two replicate sites on Oahu to compare the impacts of herbivory on invasive and non-invasive species, one at the Harold T. Lyon Arboretum and one at the Waimanalo Research Station, both affiliated with the University of Hawaii at Manoa. These sites were chosen to represent a range of mesic environmental conditions. Lyon Arboretum is in a lowland wet forest (~150 meters above sea level) on the leeward side of Oahu, receiving approximately 3840 mm of rainfall annually spread evenly throughout the year (Giambelluca et al. 2013). The arboretum is a large, semi-managed environment planted with native and non-native species. The area assigned to this experiment was initially dominated by non-native forbs and grasses. Prior to the experiment, the area had been routinely mowed. The experimental area was cleared by spraying with glyphosate in April 2010 and was covered with weed cloth until planting in September. Waimanalo Research Station is a lowland agricultural research facility (~20 meters above sea level) on the windward side of Oahu. Waimanalo receives approximately 1080 mm of rainfall throughout the year (Giambelluca et al. 2013) and has more pronounced wet/dry season with most rainfall occurring between November and March. The experimental area designated was adjacent to and partially under the canopy of an abandoned macadamia (Macadamia integrifolia Maiden & Betche) grove, with an understory dominated by guinea grass (Urochloa maxima (Jacq.) R. Webster). The plot was sprayed with glyphosate in March 2010 and remained essentially bare through the dry season up to the time of planting in October. For the analysis and discussion we refer to the arboretum as the “wet site” and Waimanalo as the “dry site.”

Common Garden

Common gardens were set up at the wet and dry sites. Each common garden consisted of ten 1.5 m x 2 m plots, arranged to capture a variety of light environments (i.e., a range from open to closed canopy). The surrounding vascular plant community in
the plots at both sites was composed entirely of non-native species. In each plot, nine plastic rings (10 cm diameter, 4 cm high) were half buried in the soil and secured by garden staples where necessary. Rings were spaced approximately 25 cm apart with a 25 cm buffer around the edges of the plot. Rings were initially cleared of any residual vegetation within, and seeds of a randomly assigned species were scattered in the ring and lightly covered with soil from within the ring. For most species, ten seeds were planted in a ring, however only five seeds of *Kigelia africana* were used because of limited availability. For *Thevetia peruviana*, five fruits were planted in the ring because the woody endocarp represents the natural dispersal unit. Fruits of *Thevetia peruviana* have between one and four seeds each. Seeds were planted in late September 2010 at the wet site and mid-October 2010 at the dry site. Germination was monitored twice weekly. Since the space available within a ring was not sufficient to support multiple plants over an extended growing period, after more than half the seedlings in a ring had two fully expanded true leaves, seedlings were randomly thinned to one seedling per ring, resulting in one seedling of each species per plot. In most cases, thinning was done between one and three months after planting. In a few cases, when the randomly chosen seedling appeared unusually stressed (presumably due to abiotic stress), we randomly chose a different individual in the ring. If any plots had rings where seeds did not germinate or no seedlings survived, we transplanted thinned seedlings.

Data collection

Visible above-ground leaf damage was measured on individuals two, three, four, and five months after planting. Although the agents of foliar damage were rarely seen, observed patterns of damage could be reasonably attributed to herbivory, as opposed to wind, rain or other abiotic sources of damage. We did not distinguish herbivore damage by particular herbivore species because we were primarily interested in net damage to plants by the herbivore community (Hill and Kotanen 2009). At each month, we counted the number of foliar leaves and estimated damage on each leaf to the nearest percent. Damage estimates were regularly calibrated between two primary field observers when data were collected. For month five at the dry site, four plots out of ten were accidently mowed and were not included in the final analysis. At month three,
four, and five, we also measured the height of each individual as the distance (to the nearest 0.5 cm) from the ground to the apical meristems.

Mean leaf damage

We calculated mean percent leaf damage for each plant at each sampling date as the total damage observed on all leaves combined divided by the number of leaves. In plots where seedlings had not been thinned, damage was averaged across all individuals in the ring.

Plant performance

We calculated plant performance at months four and five as the relative change in plant height from the previous month. Relative height growth rate (RhGR) was calculated for each individual as follows:

\[ RhGR_m = \left( \frac{\ln(H_m) - \ln(H_{m-1})}{T_d} \right) \]

where \( RhGR_m \) is the relative height growth rate for month \( m \), \( H_m \) is the plant height for month \( m \), \( H_{m-1} \) is the plant height for month \( m-1 \), and \( T_d \) is the time in days between the two sampling periods for \( H_m \) and \( H_{m-1} \).

Analysis

In order to determine if foliar damage in the field differed between invasive and non-invasive species, foliar damage proportions were compared using a linear mixed effects model. We also estimated the effect of months since planting within the same model using a repeated measures analysis to determine if damage was changing with time. Separate analyses using linear mixed effects models were conducted to determine if damage in the field at months three and four predicted plant performance the following month, in terms of relative height growth rate.
Statistics

All statistical analyses were performed using SAS software, Version 9.4 for Windows. (2013). Linear mixed effects models were performed using Proc Mixed in SAS. Significance testing used F tests based on Restricted Maximum Likelihood (ReML) estimation with Satterthwaite degrees of freedom calculations. Normality of calculated variables and homogeneity of variances were assessed using normal quantile-quantile plots of residuals and scatter plots of predicted values versus residuals. A log(X+1) transformation was used for mean foliar damage to better meet normality assumptions in the model. The covariance structure type for random effects was variance components. Other covariance structures were investigated but did not significantly improve the model, assessed by looking for smaller AICc values. Outliers were identified, but removing them did not alter conclusions so they were left in. Post hoc Tukey’s analysis examined pair-wise differences between significant fixed effects with more than two levels. Least square mean foliar damage rates were back transformed and confidence limits were calculated for results.

To address the influence of invasion category on predicting mean foliar damage and investigate how damage changed over time, we conducted a mixed effects model analysis. The factors invasion category (invasive vs. non-invasive), site (wet vs. dry), time since planting (2-5 months) were treated as fixed factors. Months since planting was treated as a within subjects effect and individual plants located within plots were treated as the subject. This effect was modeled using an autoregressive covariance structure that allows for correlation between time points to be larger when they are closer together. We investigated all possible interactions between fixed factors. Both species identity and plot number within each common garden were treated as random effects. A ‘group effect’ of invasion category was included in the model that allowed for heterogeneity in the covariance structure of the random variable species for due to invasion category.

To address whether mean foliar damage could predict plant performance we used two separate mixed effects models. The first model used the relative height growth rate (RhGR) between month three and four since planting as a dependent variable and the mean foliar damage at month three as a fixed continuous predictor variable. The
second model used the RhGR between month four and five since planting as a dependent variable and the mean foliar damage at month four as a fixed continuous predictor variable. Each model also included site and invasion category and their interaction as fixed effects. Both species identity and plot number within each common garden were treated as random effects. A ‘group effect’ of invasion category was included in the model that allowed for heterogeneity in the covariance structure of the random variable species for due to invasion category.

Results

Herbivore damage

Individual plants experienced foliar damage ranging from 0-100%. Herbivores removed on average 1.0% of leaf tissue for invasive species (95% confidence interval (CI) = 0.3, 1.9) and 0.8% of leaf tissue for non-invasive species (95% CI = 0.3, 1.5). Invasion category did not account for a significant amount of variance in mean foliar damage ($F_{1,7.03} = 0.12, P = 0.744$; Fig. 3.1). Mean foliar damage at the “wet site,” 1.0% (95% CI = 0.5, 1.7), was not significantly different than mean foliar damage at the “dry site,” 0.8% (95% CI = 0.4, 1.4)($F_{1,17.6} = 0.51, P = 0.484$; Fig. 3.1). There was a trend of increasing herbivore foliar damage over time, with mean foliar damage at two months after planting being 0.7% (95% CI = 0.3, 1.2) and increasing to 1.1% (95% CI = 0.6, 1.8) after five months after planting, however this effect was not significant ($F_{3,481} = 1.07, P = 0.361$) nor was there any notable pattern of invasive species differing in foliar damage compared to non-invasive species over time ($F_{3,477} = 0.47, P = 0.700$; Fig. 3.2). None of the remaining interactions between fixed factors explained a significant amount of variance in herbivore damage (Table 2). The amount of foliar tissue removed for each species varied (Fig. 3.3) with Justicia betonica (invasive: Acanthaceae) having the highest mean foliar herbivory, 1.9% (95% CI = 1.3, 2.5), and Tabebuia heterophylla (invasive: Bignoniaceae) having the lowest, 0.3% (95% CI = 0.0, 0.6).
Plant performance

Herbivore damage did not influence plant performance the following month in terms of relative height growth rate (RhGR) for month three (F\(_{1,113} = 0.12, P = 0.731\)) or month four since planting (F\(_{1,89} = 1.04, P = 0.310\)). There was no significant difference in performance between invasive and non-invasive species at either time period (Table 3.3). Plants at the dry site experienced four-fold greater RhGR relative to the wet site between month three and four since planting (t = 3.75, d.f. = 93, P <0.001; Fig. 3.4a). However, the effect of site was not significant between month four and five (Table 3.3; Fig. 3.4b). Additionally the interaction between invasion and site was not significant at either time period (Table 3.3). Species varied in their performance at both time periods (Fig. 3.5). The highest mean growth rates occurred during the three to four months planting period, 0.017 cm cm\(^{-1}\) d\(^{-1}\) (standard error (SE) ± 0.003) for Justicia betonica (invasive: Acanthaceae) and 0.015 cm cm\(^{-1}\) d\(^{-1}\) (SE ± 0.002) for Asystasia gangetica (invasive: Acanthaceae) (Fig. 3.5a). The lowest performance value was a negative growth rate -0.001 cm cm\(^{-1}\) d\(^{-1}\) (SE ± 0.005) for Tabebuia aurea (non-invasive: Bignoniaceae) between four and five months since planting.

Discussion

Contrary to our prediction, non-invasive species did not experience more herbivore damage (i.e., high resistance from the herbivore community) than invasive species. Thus we were unable to explain invasion failure based solely on herbivory. The few studies that have compared herbivory between young invasive and non-invasive plants have found mixed results. Dawson et al. (2014) looked at seedlings of 11 invasive and 7 non-invasive herbaceous species in a common garden in Switzerland and found mean foliar damage ranged from 7-13 % for invasive and non-invasive species but there was no statistical difference between the two groups at the end of four months. In contrast, a common garden study of four invasive and three non-invasive species of Centaurea in Ottawa, Canada, found that three month old seedlings of invasive species suffered significantly less herbivory than those of non-invasive species, and this was suggested to be a key factor in explaining their invasion (Jogesh et al.)
A common garden experiment using four confamilial pairs of invasive and naturalized non-invasive temperate vine species in the northeastern United States found that by the end of the second growing season invasive species had statistically more herbivore damage than non-invasive species (Ashton & Lerdau 2008). These mixed results suggest the pattern of herbivore influence on invasion success may be largely species specific or perhaps dependent on plant growth form (woody vs. herbaceous vs. vines). Also the majority of studies have focused on temperate ecosystems, thus differences in herbivore influence between temperate and tropical ecosystems should be explored. In the only study we found that compared foliar damage of young woody plants in a tropical setting (Dawson et al. 2009a), highly invasive and non-invasive plants did not experience significantly different levels of herbivory. In that study, field surveys of foliar herbivory were conducted on seedlings and juveniles of 10 invasive and 18 non-invasive species from several plant families in a rainforest in Tanzania. Thus, for the two studies of tropical forests to date, Hawaii and Tanzania, herbivory at early growth stages is not an important predictor of invasion success or failure.

We observed instances of high herbivory on an individual basis, but the majority of plants in our experiment experienced only low levels of herbivory, having mean tissue damage loss of < 2 % (Fig. 3.3). The rates of seedling foliar loss to herbivory can be highly variable by species (< 1 % - 25 %; de la Cruz & Dirzo 1987; Dawson et al. 2009) The magnitude of foliar damage we observed is similar to the lowest mean damage rates in at least two other studies of introduced plants in tropical settings. In a common garden in forest plots in Hawaii comparing native and non-native species, Joe and Daehler (2008) showed that after 6 months, two woody invasives, *Clidemia hirta* (L.) D. Don (Melastomaceae) and *Psidium cattleianum* Sabine (Myrtaceae), averaged approximately 7 % and 1 % respectively of leaf area missing due to herbivores. Notably, at least 6 herbivorous insect species have been introduced to Hawaii to control *C. hirta* (Dewalt, Denslow & Ickes 2004). In surveys of foliar damage in seedlings and juveniles in a tropical forest in Tanzania, Dawson et al. (2009a) observed that herbivory ranged from approximately 1 % in the case of *Toona ciliata* Roem. (invasive: Myrtaceae) to approximately 12 % for a species of *Eugenia sp.* (non-invasive: Myrtaceae).
It is also possible that our study underestimated the damage that is occurring in the field. A limitation of using visual estimations of herbivore damage is that they generally underestimate damage because measurements may not include leaves or even whole plants that are completely eaten (Lowman 1984). For example during our experiment, we observed a small number of seedlings that were present during one sampling period and absent the next. Because we were unable to identify the specific cause of death (e.g. abiotic stress, trampling, herbivory, or disease) we did not record these plants as being consumed by herbivores. However, if we assumed that all of these missing seedlings were consumed by herbivores, damage rates are still only 1.4 % for invasives and 2.1 % for non-invasives. Thus, even our estimates of maximum possible damage are still low compared to many other seedling herbivory studies in tropical forests (e.g., ~ 10 %; de la Cruz & Dirzo 1987), which could indicate a difference in types and abundances of herbivores between Hawaii and other tropical forests. Most native Hawaiian herbivores are extinct or severely reduced in abundance (James 1995). Hawaii now has an abundance of introduced herbivores, including mammals, insects, and mollusks serving that ecological role. However we lack an understanding of how a broad range of introduced herbivores interacts with a broad range of introduced plant species. It’s possible that introduced herbivores preferentially target native plants in Hawaii which generally lack strong herbivore defenses (Scowcroft & Giffin 1983; Howarth 1985; Bowen & Van Vuren 1997; Joe & Daehler 2008) and this might account for low rates of seedling herbivory in introduced species.

If the low rates of damage we observed were regulating establishment and spread of populations, we would expect to see a correlation between increasing herbivory and decreasing plant performance. However, herbivore damage did not predict subsequent plant height growth at the two time periods we measured. The assumption that herbivory impacts performance is prevalent in the literature (Clark & Clark 1985; Louda, Keeler & Holt 1990) but there is relatively little evidence showing direct links in woody species. For example, juveniles of 41 woody species in a Panamanian tropical forest showed no link between short term herbivory and relative growth rate (Coley 1983). Another study of three canopy rain forest trees (Fabaceae) in Africa, showed that seedling herbivory was not associated with a relative height growth
rate over the course of the first dry and wet seasons (Norghauer & Newbery 2014). Plants may be able to tolerate (maintain relative fitness or performance) after relatively low, non-fatal herbivore damage. Or it may be that there is a tradeoff between performance and herbivore avoidance traits (e.g. physical or chemical defenses) in some species. Additionally, despite height being an important performance trait in light limited forest environments, it may be that herbivory is linked with decreases in other performance traits in Hawaii. Another possible explanation is that foliar herbivory impacts performance at a longer time scale than that of our experiment. For example, Eichhorn et al. (2010) also observed small damage rates (< 1 %) on mature leaves in five lowland tropical forest species in Malaysia. They found that damage did not correspond with relative growth rate in terms of stem diameter at the time of herbivory, but that the leaves with the highest levels of herbivory were the least likely to survive one year later. There is some evidence that a similar process may be occurring in Hawaii. Of the nine species we investigated, three invasive species (Asystasia gangetica, Justicia betonica, Dolichandra unguis-cati) and two non-invasive species (Tecoma stans and Kigelia africana) had decreased survival after one year if they experienced higher rates of herbivore and pathogen attack (J. Bufford unpublished data).

Our finding that non-invasive species fail to invade despite relatively low herbivory damage suggests that there are other barriers that may be preventing them from establishing and becoming successful invaders. For example Kigelia africana (Bignoniaceae) has large woody fruits that are consumed by large African mammals (e.g. elephants and giraffes) in the native range and then seeds are passed through the herbivore’s digestive system (Theuerkauf et al. 2000). In Hawaii, large herbivores are not present to aid in dispersal, which may hinder spread beyond cultivated settings and into surrounding habitats. Furthermore, genetic constraints and other forms of biotic resistance due to competitors and/or pathogens could cause failure to invade in these species.

The highest damage rate we observed was in an invasive species, Justicia betonica (Acanthaceae). This was contrary to our expectation that highly invasive species would have the lowest levels of herbivory, but similar results have observed
previously in common garden experiments (Parker & Gilbert 2007; Ashton & Lerdau 2008). For example, Parker and Gilbert (2007) noticed that in seven introduced clover species in California, the two most invasive species, *Medicago arabica* (L.) Huds. and *Medicago polymorpha* L., had higher levels of herbivore and pathogen attack than the other non-invasive species. There are several reasons why these species may still be invasive despite higher damage from herbivores than other introduced species. Invasive plants with high rates of herbivory may be able to tolerate herbivory by reducing the impact herbivory has on the fitness of the plant once damage has occurred (Strauss & Agrawal 1999; Boege et al. 2011). Thus, invasive plants may still be able to succeed in the environment even if herbivore damage is high. High propagule pressure has also been suggested as a mechanism to overcome invasion barriers, especially associated with establishment (Eschtruth & Battles 2009; Simberloff 2009). For example, highly acceptable species may be able to overcome biotic resistance through repeated introductions that make seedlings more likely to find safe sites. This seems possible for *J. betonica* which is commonly used as a landscape plant around Oahu. Highly invasive species may also have high reproductive output (e.g. seeds per fruit) which could also be relevant for increasing the probability of finding safe sites.

We did not see a significant increase or decrease in foliar damage between two and five months after planting. We predicted that herbivory would decrease with time based on evidence that woody species increase both physical and chemical defenses during the seedling and juvenile phases (Barton & Koricheva 2010). Eichhorn et al. (2010) similarly did not notice a change in herbivory rates on seedlings of five tropical tree species in the Dipterocarpaceae over the course of a year. One explanation for a lack of temporal pattern is that the defensive capacity of woody species does not change considerably over time in young plants or that we would not have been able to detect a significant decrease in plant herbivory given that damage rates were so low (< 2 %) at the start of the experiment. Given that both increases (Ashton & Lerdau 2008) and decreases (Fritz *et al.* 2001) in herbivory rates have also been observed over growing seasons, it is difficult to make broad generalizations about patterns of herbivory over time.
One limitation to our study is that the non-invasive species we used were from one family (Bignoniaceae), while invasives were from several families (Acanthaceae, Apocynaceae, and Bignoniaceae). Closely related species often share similar chemical and structural defense traits (Wink 2003). Since plant palatability has been shown to correlate with specific defense traits (Poorter et al. 2004), herbivory rates may be tied with phylogeny, though they have rarely been investigated in the context of invasions. Thus it is possible that family level differences in our study could be influencing the apparent lack of an invasion category effect. However, when we compared species from only the Bignoniaceae family (one invasive species vs. five non-invasive species), mean damage rates were 0.5 % for the invasive species and 0.8 % for non-invasive species but this difference was not significant. This suggests that family level effects are not biasing our results.

**Conclusion**

Despite herbivory being well-recognized as a factor potentially affecting plant invasions, foliar herbivore damage in young, woody introduced species was not related to invasion failure or success in Hawaii. Average herbivore damage was relatively low (< 2 %) for all species but within the range of some other studies of herbivory in introduced plants. Non-invasive species may fail to invade in Hawaii despite low levels of herbivory because of a lack of mutualists, genetic constraints, or biotic pressure in the form of competition or pathogen stress. The low levels of herbivory we observed may indicate that population dynamics of many introduced species in Hawaii are generally not strongly regulated by herbivory. Along these lines, herbivore damage was not related to plant performance in terms of relative height growth rate, adding to a growing consensus that a relationship between herbivory and performance is not always evident. Furthermore we predicted that herbivory would decrease with time because of increasing investment in chemical or physical defense traits as seedlings developed into juveniles. Contrary to our expectation, herbivory did not change significantly over time suggesting that low herbivory rates at the start of the experiment
may prevent detection of decreases in herbivory rates and consequently inferences about changes in defensive traits over time are limited.
Table 3.1. Invasive and non-invasive plant species used in common garden experiments at the Harold T. Lyon Arboretum (“wet site”) and at the Waimanalo Research Station (“dry site”).

<table>
<thead>
<tr>
<th>Invasion Category</th>
<th>Family</th>
<th>Species Name</th>
<th>Habit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive</td>
<td>Bignoniaceae</td>
<td><em>Tabebuia heterophylla</em> (DC.) Britton</td>
<td>Tree</td>
</tr>
<tr>
<td></td>
<td>Acanthaceae</td>
<td><em>Asystasia gangetica</em> (L.) T. Anderson</td>
<td>Shrub</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Justicia betonica</em> L.</td>
<td>Shrub</td>
</tr>
<tr>
<td></td>
<td>Apocynaceae</td>
<td><em>Thevetia peruviana</em> (Pers.) K. Shum.</td>
<td>Tree</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Dolichandra unguis-cati</em> (L.) L.G. Lohmann</td>
<td>Vine</td>
</tr>
<tr>
<td>Non-Invasive</td>
<td>Bignoniaceae</td>
<td><em>Kigelia africana</em> (Lam.) Benth.</td>
<td>Tree</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Tabebuia aurea</em> (Silva Manso) Benth. &amp; Hook. f. ex S. Moore</td>
<td>Tree</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Tecoma capensis</em> (Thunb.) Lindl.</td>
<td>Shrub</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Tecoma stans</em> (L.) Juss. ex Kunth</td>
<td>Tree</td>
</tr>
</tbody>
</table>
Table 3.2. ANOVA table for fixed effects generated from a linear mixed effects model for mean herbivore damage of five invasive and four non-invasive species in a common garden experiment in Hawaii.

<table>
<thead>
<tr>
<th>Fixed Factor</th>
<th>F Value</th>
<th>df1 / df2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>0.51</td>
<td>1 / 17.6</td>
<td>0.484</td>
</tr>
<tr>
<td>Time</td>
<td>1.07</td>
<td>3 / 481</td>
<td>0.361</td>
</tr>
<tr>
<td>Invasion Category</td>
<td>0.12</td>
<td>1 / 7.03</td>
<td>0.744</td>
</tr>
<tr>
<td>Site x Time</td>
<td>1.98</td>
<td>3 / 481</td>
<td>0.116</td>
</tr>
<tr>
<td>Site x Invasion Category</td>
<td>2.29</td>
<td>1 / 482</td>
<td>0.131</td>
</tr>
<tr>
<td>Time x Invasion Category</td>
<td>0.47</td>
<td>3 / 477</td>
<td>0.700</td>
</tr>
<tr>
<td>Site x Time x Invasion Category</td>
<td>0.22</td>
<td>3 / 477</td>
<td>0.880</td>
</tr>
</tbody>
</table>
Table 3.3. Results of mixed effects models showing the relationship between relative height growth rate (RhGR) and the fixed factors. RhGR between months three and four since planting used herbivore damage at month three as a continuous predictor variable. RhGR between months four and five since planting used herbivore damage at month four as a continuous predictor variable. Significant effects are bolded.

<table>
<thead>
<tr>
<th>Fixed Factors</th>
<th>RhGR (3 - 4 months)</th>
<th>RhGR (4 - 5 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F Value</td>
<td>df1/df2</td>
</tr>
<tr>
<td>Herbivore Damage</td>
<td>0.12</td>
<td>1 / 113</td>
</tr>
<tr>
<td>Site</td>
<td>14.09</td>
<td>1 / 16.6</td>
</tr>
<tr>
<td>Invasion Category</td>
<td>0.04</td>
<td>1 / 7.46</td>
</tr>
<tr>
<td>Site x Invasion Category</td>
<td>2.21</td>
<td>1 / 104</td>
</tr>
</tbody>
</table>
Figure 3.1. Mean percent foliar damage for individuals of invasive and non-invasive species at the dry site and the wet site. Error bars represent 95% confidence limits.
Figure 3.2. Mean percent foliar damage for both invasive and non-invasive species over four months. Error bars represent 95% confidence limits.
Figure 3.3. Mean percent herbivore damage over the course four sampling periods (2-5 months since planting) at the wet and dry site. Error bars represent 95% confidence limits. A list of species is shown in Table 3.1.
Figure 3.4. Relative height growth rate (RhGR) for invasive and non-invasive species at wet and dry sites between a) three and four months since planting, and b) four and five months since planting. Error bars represent ±1 standard error.
Figure 3.5. Species level mean relative height growth rate (RhGR) for invasive and non-invasive species at wet and dry sites between a) three and four months since planting, and b) four and five months since planting. Error bars represent ±1 standard error. Full species names are listed in Table 3.1.
Acknowledgements

We would like to thank Lyon Arboretum and Waimanalo Research Station for access to field sites and for logistical support. We thank several undergraduate and graduate volunteers for helping setup and planting seeds for the common garden experiment.
CHAPTER 4
PRE-DAMAGE BIOMASS ALLOCATION AND NOT PLANT INVASIVENESS PREDICT TOLERANCE TO DAMAGE IN SEEDLINGS OF WOODY SPECIES IN HAWAII

Matthew H. Lurie, Kasey E. Barton, and Curtis C. Daehler

Abstract

Understanding the mechanisms by which non-native plants become successful invaders can help us to reduce or prevent their entry into novel communities. Plants that have evolved defensive traits to resist and/or tolerate herbivory may be more successful as invaders since herbivory can potentially regulate existing populations or prevent species from establishing. Tolerance might also be important because it allows plants to mitigate the negative consequences of herbivory that they cannot avoid. The goals of this study were to test whether tolerance is an important driver of plant invasion success, and to identify plant traits that are associated with tolerance. A greenhouse experiment was conducted to measure seedling tolerance to simulated herbivory through mechanical damage (50 % leaf removal) of 16 non-native woody plant species differing in invasion status (invasive vs. non-invasive) in Hawaii. Pre-damage biomass allocation traits were measured including root to shoot ratio, relative growth rate, cotyledon mass fraction, root mass fraction, and seed mass. Seedlings were grown for two weeks following damage, and biomass was analyzed to determine whether damaged plants regrew to levels similar to undamaged controls.

Tolerance did not differ between invasive and non-invasive species, but there was significant variation among species. While a few species fully tolerated damage with respect to survival and regrowth, most damaged seedlings remained significantly smaller than control seedlings two weeks after damage. Even though species in our study generally under-compensated for tissue loss, in terms of total biomass, tolerance
was positively related to pre-damage investment in cotyledons and high root to shoot ratio adding to our understanding of defense mechanisms, especially in young plants. The lack of higher tolerance in highly invasive species suggests that tolerance may not be an important explaining invasion success in Hawaii.
Introduction

Invasions by non-native species are widely recognized as one of the major threats to biodiversity and ecosystem stability at local, regional, and global scales (Vitousek et al. 1997; Lockwood et al. 2007; Ehrenfeld 2010). Plant invasions in particular can impact community structure (Pyšek & Pyšek 1995; Dunbar & Facelli 1999), fire regimes (Hughes et al. 1991; Brooks et al. 2004), hydrology (DiTomaso 1998; Dyer & Rice 1999), and nutrient cycling (Vitousek & Walker 1989; Evans et al. 2001). Even though non-native plants are transported around the globe at an astonishing rate, only a small percentage of these introduced plants establish sustainable populations and become invasive (Richardson et al. 2000). Understanding the mechanisms by which non-native plants become successful invaders can help us to reduce or prevent their entry into novel communities. This is especially true for woody species that are major components of terrestrial ecosystems. Studies of woody plant invaders have gained importance in recent years because of an increasing recognition of their threats to biodiversity and conservation (Keppel & Watling 2011; Richardson et al. 2013). Rejmánek and Richardson (2013) report a total of 751 woody non-native species (434 tree and 317 shrub species from 90 families) that are naturalized (consistent reproduction and sustained populations; Richardson et al. 2000) and spreading globally. Non-native woody species have been intentionally introduced for centuries for commercial, cultural, and aesthetic value, making management of these species complex (Webster et al. 2006; Wilgen & Richardson 2013).

In plants, reasons for failure to invade include a lack of sufficient propagule pressure (Von Holle & Simberloff 2005; Simberloff 2009), a mismatch of environmental conditions (Closset-Kopp et al. 2010), genetic constraints such as bottleneck and founder effects (Ellstrand & Elam 1993; Crawford & Whitney 2010), a lack of mutualists such as pollinators, dispersers or species that aid in growth (Nadel et al. 1992; Pringle et al. 2009), and biotic resistance (Creed & Sheldon 1995; Case & Crawley 2000; Emer & Fonseca 2010). Herbivory has received a lot of attention in the invasion literature in terms of biotic resistance since Elton (1958) suggested that a diverse community could better resist establishment of non-native species through herbivory, competition, or
disease. Herbivory can affect introduced populations in two ways. First, herbivory may prevent potentially invasive species from establishing by reducing plant growth and survival to such low levels that populations never establish and the species fails to naturalize. This type of biotic resistance, leading to failed invasions before naturalization, is often difficult to detect (Zenni & Nuñez 2013). Second, herbivory may act to regulate established populations by reducing non-native plant fitness through the consumption of photosynthetic, structural or storage tissue, thereby limiting population growth and spread (Huntly 1991; Levine et al. 2004; Schmitz 2008; Stephens & Westoby 2014).

Plants have evolved a myriad of ways to defend themselves against enemies (Koricheva, Nykänen & Gianoli 2004). Investigations into plant defenses and their role in the invasion process have typically focused on plant resistance traits that directly deter herbivores, such as physical structures (e.g. thorns, spines, trichomes) or secondary metabolites. An additional component of plant defense that is poorly understood in terms of its relation to invasions is tolerance, defined as the maintenance of fitness in damaged plants compared to undamaged plants (Strauss & Agrawal 1999; Boege et al. 2011). Most plants exhibit some capacity to recover from damage, but tolerance is highly variable among species, often depending on plant type (i.e. woody vs. herbaceous), ontogeny, or herbivore identity (Massad 2013). Considering that tolerance allows plants to mitigate the negative consequences of herbivory that they cannot avoid, tolerance might be particularly important for non-native plants in new habitats where they lack specific resistance to novel herbivores. This could be especially important in tropical environments where herbivory rates are relatively high (Coley & Barone 1996). While studies are limited, there is some evidence that tolerance may play a role in invasions. For example, a study by Ashton and Lerdau (2007) found that the relative growth rate in 12 related temperate vine species following simulated herbivory through leaf clipping was higher in invasive species compared with non-invasive non-natives and native species. After observing high levels of herbivore damage in the field across all species, including highly invasive species, the authors suggest that high herbivore tolerance could be a mechanism of success if plants are unable to avoid herbivory.
Plants tolerate damage in a variety of ways (Tiffin 2000). Induced mechanisms of tolerance function after damage has occurred, often through processes that contribute to regrowth. Examples include activating dormant meristems (Tuomi, Nilsson & Åström 1994), reallocating resources from roots to shoots (Trumble, Kolodny-Hirsch & Ting 1993; Rivera-Solis et al. 2012; Hochwender et al. 2012; Barton 2013), increasing photosynthetic rates (Vanderklein & Reich 1999; Koricheva et al. 2004) and increasing growth rates (Nykänen & Koricheva 2004). On the other hand, constitutive mechanisms of tolerance involve traits that exist prior to damage and aid the plant’s ability to manage damage after it has occurred. Assuming growth rate prior to damage is related to growth rate after tissue loss (Weis, Simms & Hochberg 2000), plants with high pre-damage growth rates may have the machinery in place for high tolerance. Additionally, constitutive mechanisms of tolerance may involve biomass reallocation from stored reserves that accumulate before damage (e.g., high root to shoot ratios before damage may reflect stored reserves in roots that may then be used for survival and regrowth; Hochwender et al. 2012).

For seedlings, storage tissues in seeds may contribute to herbivory tolerance, leading to a significant positive relationship between seed size and tolerance (Armstrong & Westoby 1993; Harms & Dalling 1997; Bonfil 1998). There are two ways that large seededness could increase tolerance in seedlings. First, larger seeded species generally have larger seedlings, which may allow for better access to limiting resources in the environment (e.g. light, water, and nutrients), both pre- and post-damage (plant size effect; Leishman et al. 2000). Second, during early development larger seeded species may have a relatively large amount of storage reserves, manifested in endosperm, the hypocotyl, or cotyledons (reserve effect; Westoby et al. 1996). Although seedlings are rarely thought to have accumulated significant stored reserves, if these reserves are more slowly used by the plant during normal growth compared to small seeded species, a relatively larger portion of reserve tissue could be available for tolerance when damage occurs.

Even though we have learned a great deal about tolerance from previous studies, we still lack unifying concepts that could aid in addressing invasion success. Our overall objective was to test the association of herbivory tolerance with plant
invasion success using woody plants in Hawaii as a model system. Hawaii has experienced unprecedented rates of plant invasions, with more than 2100 non-native plant species occurring in both cultivated and natural or semi-natural settings (Staples & Herbst 2005). Many more species have probably been introduced, but have failed to establish sustainable populations or spread into native communities. Thus, it remains unclear whether certain traits contribute to the successful establishment of many non-native species while limiting the success of others. Herbivory may play a key role in this process. Most native Hawaiian herbivores are extinct or severely reduced in abundance (James 1995). However, Hawaii now has an abundance of introduced herbivores, including mammals, insects, and mollusks, which are consuming plants and pose serious threats to the native flora (Scowcroft & Giffin 1983; Howarth 1985; Bowen & Van Vuren 1997; Joe & Daehler 2008). Because many of these introduced herbivores particularly target the seedling stage (Joe & Daehler 2008), and because seedling recruitment is a critical aspect of population establishment and spread (Fenner 1987), we focus on herbivory tolerance in seedlings. Although seedling tolerance is generally predicted to be weak given the limited stored reserves and photosynthetic capacity of seedlings (Strauss & Agrawal 1999), there is evidence that plasticity in biomass allocation and high rates of photosynthesis can enhance seedling tolerance of herbivory, and that fast growing, weedy species can often tolerate high levels of damage, quickly catching up in size with undamaged control plants (Barton 2008, 2013; Barton & Koricheva 2010).

The goals of our investigation were:

1) To test whether tolerance to herbivory is an important driver of plant invasion success. We predict that invasive plant seedlings will have higher herbivory tolerance than non-invasive non-native plants.

2) To identify plant traits associated with seedling tolerance to herbivory across a range of different species. We predict that increased relative growth rate, root to shoot ratio, cotyledon mass fraction, and larger seed
size will enhance seedling tolerance because of the importance of stored reserves in regrowth following damage.

To address these goals, we performed a greenhouse experiment simulating herbivore damage through manual leaf tissue removal and application of jasmonic acid (JA), a jasmonate derivative. Jasmonates are signaling hormones induced by herbivore attacks and involved in the signal transduction pathways that lead to increases in plant resistance and tolerance following damage (Howe & Jander 2008; Heil & Ton 2008; Heil et al. 2012). Combining mechanical damage with the application of jasmonates, including the derivative jasmonic acid, has been shown to be an effective way to elicit plant responses that are similar to natural herbivory (Thaler et al. 1996; Constabel et al. 2000; Heil et al. 2001; Zas, Moreira & Sampedro 2011), which is especially useful when herbivores are unavailable or difficult to work with under controlled conditions (Babst et al. 2005; Hummel et al. 2007). We focused on non-native woody plant species because they drastically alter landscapes and change biodiversity (Webster et al. 2006) but have received less attention in the tolerance literature. We used sixteen non-native woody plant species that differ in invasion status (invasive vs. non-invasive). Because measuring fitness directly in long lived woody plant species is challenging, we used plant growth and survival, essential components of seedling performance, as metrics of fitness (Barton 2013).

Methods

Species selection and location

Seedling herbivory is particularly important in Hawaiian mesic and wet forests where non-native herbivorous mollusks are abundant (Stone 1985; Joe & Daehler 2008), and so invasive and non-invasive plant species adapted to tropical mesic or wet conditions were selected for study (Table 4.1). Only species with a minimum residence time (Richardson & Pyšek 2006) of at least 50 years, as determined by the earliest record in the Bishop Museum Herbarium (http://nsdb.bishopmuseum.org/) were included in our study. We designated species as invasive or non-invasive (Richardson
et al. 2000) using local floras (Little et al. 2003; Staples & Herbst 2005), reports of extent of spread (Staples et al. 2000; Imada 2012), and consultations with invasive plant species experts. All of the invasive species are widely naturalized on Oahu and generally across multiple islands. Non-invasive species have been planted around the islands for at least 50 years, but do not appear to be spreading or naturalizing to date. In some cases the non-invasive species exist only in cultivation. Fruits and seeds of all species were collected from the island of Oahu, primarily from roadside plants and easily accessible trails and landscaping surrounding Manoa Valley. Due to constraints of fruiting seasons, seeds of Grevillea robusta and Cestrum nocturnum were purchased from nurseries.

The experiment was conducted in a glass house on the University of Hawaii Manoa campus from June to December 2013. When possible, species in the same family were planted at the same time. Because germination times varied among species, experimental treatments were conducted at slightly different times within the experimental period. However, because Hawaii’s climate does not show dramatic seasonal climatic changes and the work was done in a glass house, temporal variation in species treatments is not likely to influence experimental outcomes. Glass house mean day and night temperature were 26.8 °C (std. dev. = 4.2) and 22.4 °C (std. dev. = 1.7) respectively throughout the experiment.

Mean seed mass was measured for each species prior to the experiment (N = 20 seeds). Because of the small size of Melaleuca quinquenervia and Callistemon viminalis seeds, an average mass of fifty seeds was used (N = 5 replicates). Between 200-1000 seeds for each species were planted at the start of the experiment in seed trays filled with a 2:1:1 media mixture of Pro-Mix BX (75-85% Canadian sphagnum peat moss, perlite, dolomitic and calcitic limestone, macro- and micronutrients, Glomus intraradices mycorrhizae inoculum), 3/4” granite chips, and perlite respectively. Plants were shaded with 30% aluminum shade cloth and watered four times daily using a misting system such that the soil mixture did not dry. Visible germination was recorded when cotyledons appeared. Seedlings were allowed to grow until they developed two foliar leaves, at which time, forty five seedlings were transplanted into 2.83 L ‘tall one’ treepots (Stuewe & Sons, Tangent, Oregon, USA) filled with the same media mixture as
above. Prior to transplanting, 2.5 mL of slow release fertilizer pellets (Osmocote) was added to the surface of each filled pot and mixed with the top 4 cm of the soil. Plants that died within 2-3 days of transplanting were replaced. Additionally, all pots from different species and treatments were randomly interspersed every 3-4 days on three neighboring glass house benches over the duration of the experiment.

* Treatments*

Cotyledon stage seedlings were randomly assigned to one of four treatment groups. The total sample size included 45 plants per species (N = 11 to 12 per treatment group) x 16 species, for a total of 720 plants. Two groups had half of each leaf removed by cutting them across the midvein with scissors to simulate herbivory. Because mechanical damage alone may not induce the full response to herbivory by animals, half of the damaged plants also received a one-time pulse of 0.5 micromol solution of jasmonic acid. The jasmonic acid solution was sprayed on seedlings until the surface of each seedling was saturated and beginning to drip. Jasmonic acid is the plant signaling hormone elicited in response to animal herbivory, and previous studies have demonstrated that foliar application of jasmonic acid enhances the induced response to simple mechanical damage, more closely mimicking actual herbivory (van Kleunen & Schmid 2003; Hoan, Ormond & Barton 2014). The defoliation only treatments were sprayed with water to mimic spraying with jasmonic acid. The remaining plants were controls that received no damage treatment. Half of these were harvested at the time of the damage treatments and were used to obtain pre-damage biomass data. The remaining control plants were also sprayed with water to mimic spraying in the jasmonic acid treatment and were harvested with the two damaged groups two weeks following treatment. Upon harvest, aboveground biomass was collected and separated into cotyledon, leaf, and stem portions. Belowground root biomass was harvested by removing roots of any debris and washing with water. Plant parts were dried in a drying oven at 60 °C for a minimum of one week or until constant mass, which was measured with a precision of 0.00001 g (Mettler Toledo New Classic MF).
Tolerance indices

Since we were interested in evaluating whole plant responses to herbivory, each species’ ability to compensate for lost tissue at the end of two weeks was determined by calculating a tolerance index \((T_i)\) following Strauss and Agrawal (1999). Tolerance indices were calculated for each species using the following equation,

\[
T_i = \frac{M_d}{M_c}
\]

where \(M_d\) and \(M_c\) are the mean total biomass of plants in the damaged treatments and control treatment respectively. Separate tolerance indices were calculated for the defoliation and defoliation plus jasmonic acid treatments for each species.

Pre-damage biomass traits

Several pre-damage biomass traits were measured for each species from the seedlings harvested at the time the damage treatments were applied. Relative growth rate (RGR) was calculated for each species using the equation,

\[
RGR = \frac{(M_t - M_s)}{T_p}
\]

where \(M_t\) is the mean total biomass of pre-damage seedlings, \(M_s\) is the mean seed mass, and \(T_p\) is the number of days between planting and the pre-damage biomass harvest. Two species, *Tamarindus indica* and *Macadamia integrifolia*, had negative relative growth rates at the start of the experiment because a large portion of their seed mass included a thick hard seed coat that was shed after plants germinated. Since the seed coat reflects conditions in nature, these species were still included in the analysis. Cotyledon mass fraction was calculated for each species by dividing the cotyledon mass of a seedling by the total biomass of that seedling and averaging among seedlings of that species. Root to shoot ratio was calculated for each species by averaging the ratio between root biomass and shoot biomass (i.e. cotyledons, leaves, stems) for each seedling among plants of that species.
Analysis

To determine if plant invasiveness could explain damage response in terms of above- and belowground growth for each individual, invasive and non-invasive species biomass data were compared using a linear mixed effects model. The ability to predict species level tolerance (e.g. tolerance indices) based on plant species invasiveness was also investigated using a linear mixed effects model. Since pre-damage plant traits can also be important precursors to tolerance response, seed mass, pre-damage root to shoot ratio, pre-damage relative growth rate, and pre-damage cotyledon mass fraction were evaluated as predictor variables by including them as fixed covariates in the mixed model. Plant family was used to control for phylogenetic differences between species in both growth and tolerance data. We also investigated pair-wise associations between pre-damage traits using correlation analyses.

Statistics

Significance testing for mixed models followed a “step down” approach (West et al 2007) and appropriate diagnostic considerations were made including normal quantile-quantile plots of residuals and scatter plots of predicted values versus residuals. Likelihood ratio tests using a chi squared distribution with one degree of freedom were used to assess significance of effects based on maximum likelihood for fixed effects and restricted maximum likelihood values for random effects. AICc values were also investigated but the results were similar to those of the likelihood tests and are not reported. For all mixed models, the covariance structure type was variance components. Other covariance structures were investigated but did not significantly change the model so are not included in the results. Outliers were identified, but removing them did not alter conclusions so they were left in. Post hoc Tukey’s adjusted least square mean analysis examined pair-wise differences between significant fixed effects with more than two levels. All statistical analyses that included mixed model analyses were performed using SAS software, Version 9.4 for Windows. (2013).

For plant growth data, the dependent variables, aboveground and belowground biomass were log transformed to meet normality and homoscedasticity assumptions. The main effects of damage type (defoliated, defoliated plus jasmonic acid, or control)
and invasion category (invasive or non-invasive) were treated as fixed factors. Species nested within plant family was included as a random factor. An interaction effect of treatment and invasion category was included to determine if compensation patterns between treatments changed depending on whether a plant is a successful invader or not. A ‘group effect’ (“SAS system for Windows” 2013) of family was included in the model that allowed for heterogeneity in the covariance structure of the random variable for each family level of species nested within family. For analyses of tolerance indices, invasion category (invasive or non-invasive) was included in the mixed model as a fixed factor. Pre-damage traits (e.g. seed mass, pre-damage root to shoot ratio, pre-damage relative growth rate, and pre-damage cotyledon mass fraction) were treated as fixed factor covariates in the model. The effect of plant family was included as a random factor and although not significant, it was retained to control for the effects of phylogeny. Separate mixed model analyses were conducted for tolerance indices using defoliated and defoliated-JA treatments. Model construction is summarized in the appendices (above- and belowground growth: Appendices 4.1 and 4.2; tolerance indices: Appendices 4.3 & 4.4).

The association between covariates (relative growth rate, cotyledon mass fraction, seed mass, and root to shoot ratio) was investigated using a correlation analysis. *Macadamia integrifolia* had relatively large mean seed mass (8.6 g) compared with all other species which had mean seed masses < 1.0 g, so a non-parametric approach (Spearman’s Correlation) was used to test whether there was a monotonic relationship between variables and to reduce the influence of *M. integrifolia*.

**Results**

**Plant survival and growth**

All damaged seedlings of all species survived. Among control groups, a single *Melaleuca quinquenervia* seedling and two *Callistemon viminalis* seedlings did not survive. After harvest, there were significant differences among treatments (Table 4.2, Fig. 4.1; aboveground $\chi^2 (1) = 24.5$, $P < 0.001$; belowground $\chi^2 (1) = 15.1$, $P < 0.001$). Aboveground biomass of control plants was significantly higher than that of defoliated
treatment plants (t = -4.54, P < 0.001) and defoliated-JA treatment plants (t = -7.25, P < 0.001). Plants that received defoliation alone were significantly larger than plants receiving defoliation plus jasmonic acid, suggesting that jasmonic acid reduced tolerance (t = -2.69, P = 0.020). Similar patterns were detected for root biomass between damage and controls, with significantly greater biomass for control plants than plants from either defoliation only (t = -3.00, P = 0.010) or defoliation plus jasmonic acid (t = -5.07, P < 0.001). However, in this case there was no significant difference between the two defoliation treatments (-2.07, P = 0.105).

Invasion category and tolerance
Contrary to our prediction, invasive species did not tolerate seedling damage better than non-invasive non-natives (as evidenced by a non-significant invasion status x treatment interaction; Table 4.2). However, there was a significant effect of invasion category on mean plant biomass across treatments, with non-invasive plants having significantly greater aboveground and belowground biomass (Table 4.2). Furthermore, species varied significantly in their mean size (significant species effect) and in their tolerance of damage (significant species x treatment interaction; Table 4.2). The majority of species in the experiment failed to tolerate the damage completely as evidenced by tolerance indices less than 1.0 (Fig 4.2). However, Alstonia macrophylla, Thevetia peruviana, Solanum melongena, and Macadamia integrifolia nearly tolerated the defoliated only treatments, having tolerance indices of 0.972, 0.975, 0.936, and 0.926 respectively. In both the defoliated and defoliated-JA treatments, Callistemon viminalis plants over-compensated, having a tolerance index of 1.246 for defoliated treatments and 1.252 for the defoliated-JA. Tolerance indices did not significantly differ between invasion categories for either defoliated or defoliated-JA treatments (Table 4.3; Fig 4.3).

Pre-damage traits and tolerance
Several of the pre-damage traits we used as continuous predictors in our mixed model analyses were moderately correlated with each other. Root to shoot ratio was significantly negatively associated with cotyledon mass fraction (rho = -0.532, P =
0.034) and seed mass (rho = -0.615, P = 0.011). Relative growth rate was negatively associated with cotyledon mass fraction (rho = -0.571, P = 0.030). However, there was no association between relative growth rate and root to shoot ratio (rho = 0.232, P = 0.387) or seed mass (rho = -0.406, P = 0.119). There was also no association with cotyledon mass fraction and seed mass (rho = 0.424, P = 0.102).

The covariates cotyledon mass fraction and root to shoot ratio both had significant positive effects on tolerance in both defoliated and defoliated-JA treatments, revealing these traits as important mechanisms enhancing tolerance (Table 4.3). In contrast, relative growth rate and seed mass did not explain a significant amount of variance and were not included in the final model for either defoliated or defoliated-JA treatments (Table 4.3). The random effect of plant family was also not significant but was included in the final model to control for phylogeny.

Discussion

Invasions and Tolerance

Despite tolerance being a well-recognized component of plant defense in addition to resistance, we were unable to demonstrate a clear pattern of higher tolerance in highly invasive species. Seedlings of highly invasive species generally under-compensated for simulated herbivory, having tolerance indices (i.e., total biomass of defoliated treatments divided by that of controls calculated for each species) similar to non-invaders and less than 1.0 after two weeks. Investigations into the capacity of invasive plants to tolerate damage have been mixed; several studies of single invaders have shown low tolerance in some species (Bossdorf et al. 2004; Huang et al. 2010; Hanley 2012) and full tolerance in others (Rogers & Siemann 2004; Stastny, Schaffner & Elle 2005; Callaway, Kim & Mahall 2006; Gard et al. 2013). Hanley (2012) observed biomass under-compensation in seedlings of Plantago lanceolata L. from invasive populations in North America after mechanically defoliating 95 % of cotyledons and measuring total biomass after 21 days. In contrast, Callaway et al. (2006) investigated tolerance capacity in juveniles of the invasive plant, Centaurea solstitialis L., in southern California, following severe damage treatments consisting of removing all leaves from
basal rosettes and stems, and found that there was no difference in biomass of clipped plants versus controls after eight weeks, indicating high herbivore tolerance. These studies suggest that tolerance is generally species specific in invaders. However, broad characterizations are tenuous because differences observed between single invader studies may be attributable to differences in experimental design, such as the kind of herbivory applied (simulated vs. bioassays), the type of tissue removed (e.g. leaves, cotyledons, roots, stems), or the relative proportion of tissue removed. Studies of multiple invaders are less common but are useful because they directly address patterns in species variation and investigate plant traits that may confer invasion success. Pirk and Farji-Brener (2012) performed a greenhouse experiment investigating tolerance in juvenile plants of three invasive forb species from the Patagonian desert region in Argentina. Performance variables varied among species but all introduced species fully compensated for lost tissue in terms of final biomass twelve weeks following mechanical removal of 33% of leaf tissue area. Ashton and Lerdau (2008) examined tolerance in seedlings of four invasive and four non-native non-invasive temperate vine species in North America, following removal of 50% of the leaves and stems of six week old seedlings and cuttings to simulate mammalian herbivory in the greenhouse. To our knowledge, this is only other study that has examined multiple non-invasive species and tolerance. After seven weeks, Ashton and Lerdau measured tolerance in terms of the difference in relative growth rate between damaged and undamaged plants, finding that invasive species on average had comparable relative growth rates as controls, while relative growth rate was smaller in non-invasive species, suggesting that high herbivore tolerance could be a mechanism of success in vines. In contrast to both these studies, we did not observe full tolerance to herbivory in most woody introduced species. We also found that invasives had the same tolerance as non-invasives. Though more studies of multiple invaders are needed, it may be that high tolerance is linked to invaders in some ecosystems and not others (i.e. temperate and desert habitats vs. tropical forest) or that it is tied with plant growth habit (e.g. vines vs. woody species).

Given that the majority of highly invasive species in our study under-compensated for damage and that low tolerance has been found in other invasive
species (e.g., Bossdorf et al. 2004; Huang et al. 2010; Hanley 2012), understanding
why these species may still be invasive is important. First, plants may possess chemical
or structural defense traits (i.e., resistance or avoidance traits) that reduce or prevent
herbivory. For example, *Cestrum nocturnum* (invasive: Solanaceae), produces steroidal
saponins and tannins in leaf tissue (Mimaki *et al.* 2001; Patil *et al.* 2011) and *Grevillea
robusta* (invasive: Proteaceae) produces hydrogen cyanide in flowers and fruits (Ullah
*et al.* 2014). Saponins, tannins, and cyanide are known anti-herbivore secondary
metabolites (Wink 2003) that may make these species less palatable. Thus, having low
tolerance may not affect invasion success if these plants are being consumed with less
intensity in the field. Second, plants may physically ‘escape’ herbivory by recruiting to
safe sites. Subsequent high growth rate or high reproductive capacity of seedlings in
safe sites could make up for fitness losses due to low tolerance in seedlings outside of
safe sites. This is certainly possible with *G. robusta* (invasive: Proteaceae), *Spathodea
campanulata* (invasive: Bignoniaceae), and *Melaleuca quinquenervia* (invasive:
Myrtaceae). These invasive species are prolific in terms of wind-dispersed seeds and
have high germination rates in the lab (pers. obs.), which may increase chances of
finding, and recruiting to, safe sites. Similarly, high propagule pressure has been
suggested as a mechanism to overcome invasion barriers, especially in association with
establishment (Eschtruth & Battles 2009; Simberloff 2009). For example, less tolerant
species may be able to overcome biotic resistance through repeated introductions that
make seedling more likely to find safe sites. This seems possible for *M. quinquenervia*
(invasive: Myrtaceae), which is commonly used as a landscape tree in urban Oahu.
Lastly, plants may also escape herbivory through phenological traits that match
dispersal and growth with predictable fluctuations in herbivore population densities.
However, it is unlikely that phenology solely explains invasion success in Hawaii
because generalist herbivores are ubiquitous year round (pers. obs.).

The majority of the studies that have experimentally tested tolerance response in
non-native species measured plant performance after a much longer time than in our
study (1-6 months). It is possible that invasive species in our study did not have enough
time to fully tolerate damage. For example, Hanley (2012) observed invasive *Plantago
lanceota* not tolerating damage in terms of biomass after 21 days, but that reproductive
traits, including time to flower and the number of inflorescences, were similar across treatments after 150 days. Though Hanley did not measure biomass at the later date, this may indicate that the plants’ tolerance capacity increased with ontogeny. A delayed tolerance response has been noted in other studies (e.g. Haukioja and Koricheva 2000; Sacchi and Connor 2013) and the consequence may be that short-term studies in general underestimate tolerance responses. However, in our study, at least one non-invasive species, *Callistemon viminalis*, responded by over-tolerating. Thus, two weeks may be sufficient to observe a true, rapid tolerance response in some species. Future studies measuring tolerance over extended time periods are needed to support this argument.

*Tolerance related to pre-damage allocation of resources*

We found that investment of resources in storage organs, specifically roots and cotyledons, prior to damage increased tolerance to defoliation across species. This result adds to a growing consensus that pre-damage growth patterns determine tolerance to damage, perhaps even more than induced responses (van der Meijden, Wijn & Verkaar 1988; Harms & Dalling 1997; Hochwender, Marquis & Stowe 2000; Myers & Kitajima 2007; Stevens, Kruger & Lindroth 2008; Rivera-Solís et al. 2012; Barton 2013). However, very little of this research has explicitly focused on seedlings (but see Barton 2008, 2013). In general, seedlings are predicted to have low tolerance to damage because even relatively small herbivores can remove a large portion of biomass relative to the size of the plants they attack (Hanley et al. 2007). Yet there is evidence that some species, particularly weedy herbs with fast growth rates, can tolerate herbivory to the same degree as older ontogenetic stages (Barton & Koricheva 2010; Barton 2013). During germination and early development, plants rely heavily on storage reserves such as the seed and cotyledon, so it is logical that these types of organs provide resources so that plants can grow following herbivory (Erb et al. 2009). For example, Barton (2013) found that in the herbaceous perennial *Plantago major* L., tolerance to 50 % defoliation was related to high root to shoot ratios prior to damage, concluding that root biomass was an important driver of tolerance. Similarly, in a common garden experiment of seven understory species, Meyers and Kitajima (2007)
found that survival after a year following 100% defoliation of 3-18 week old seedlings was related to the initial amount of non-structural carbohydrates (e.g., starch and simple sugars) within the stems and roots. As in these studies, we found a positive association of tolerance with root to shoot ratio across many different species. Furthermore, we found that species with relatively large cotyledons for their size had higher tolerance to damage, providing additional support for the conclusion that pre-damage investment in storage organs (roots and cotyledons) are key mechanisms of seedling tolerance. Given that cotyledon mass fraction and root to shoot ratio were moderately correlated in our analysis, conclusions about the predictive power of each of these traits is limited due to collinearity. It may be that high cotyledon mass is important for tolerance, but requires a simultaneous investment in roots or vice versa, or that each of the traits is important singly. Studies with a larger number of replicates (at the level of species) could aid in clarifying these relationships.

Seed size, which is generally positively associated with cotyledon size (Kidson & Westoby 2000), was not correlated with plant tolerance or cotyledon mass fraction in our study, contrasting with previous studies in which increased seed size was associated with increased plant performance, including survival (Armstrong & Westoby 1993) and regrowth of above ground leaves (Harms & Dalling 1997; Green & Juniper 2004) following simulated herbivory. Our results thus suggest that the 'plant size effect' (Leishman et al. 2000) and 'reserve effect' (Westoby et al. 1996) are not universal mechanisms of tolerance in all species. Also, pre-damage relative growth rate (RGR) was not indicative of plant tolerance, contrary to our predictions and previous work (e.g., Myers and Kitajima 2007), perhaps because seedling defoliation initiates growth rate limitation. Constitutive pre-damage RGR should only be important in tolerance if plants can maintain high levels of growth following damage (Weis et al. 2000) and to assess this, post-damage measurements of RGR are needed. As RGR was negatively correlated with cotyledon mass fraction, which was a significant contributor to tolerance in our analysis, pre-damage RGR may still play an important role in tolerance.

**Jasmonic acid**

Combining mechanical damage with the application of jasmonates, including the
derivative jasmonic acid, has been shown to be an effective way to elicit plant responses that are similar to natural herbivory (Thaler et al. 1996; Constabel et al. 2000; Heil et al. 2001; Zas et al. 2011). Among the species we investigated, application of jasmonic acid reduced the growth of aboveground biomass compared to mechanical wounding alone. One possible explanation is that jasmonic acid induced changes in resistance traits (such as secondary compounds and physical defense traits) at the expense of tolerance. Additional studies incorporating bioassays with appropriate herbivores and measurements of resistance traits would reveal whether simultaneous induction of tolerance and mechanisms aimed at reducing herbivore damage is occurring in these species.

Conclusion

A suite of plant traits and environmental circumstances is likely to explain why some plants are invasive and others are not. Trying to identify patterns is a necessary goal for invasion ecology and is especially important in oceanic islands where plant invasions are a major threat to ecosystems. The findings presented in this paper suggest that a plant’s defensive investment in tolerance to herbivory is not an important driver of invasion success in seedlings of woody plants in Hawaii. Despite all plants surviving simulated herbivore damage, both invasive and non-invasive species in our study responded similarly and generally did not fully regrow total biomass to similar levels as controls. Given that we only allowed plants to grow for two weeks following damage, it may be that invasive species needed longer to fully compensate. However, we observed over-compensation in one non-invasive species, suggesting that plants can compensate for biomass loss, even over short time periods. Additionally, tolerance was related to increased investment in potential storage reserves, specifically cotyledons and roots, adding to our understanding of defense mechanisms, especially in young plants. The lack of higher tolerance in highly invasive species, relative to non-invasive species, may suggest that invaders use other traits to overcome herbivory invasion barriers, such as resistance traits (chemical or structural defenses) or escape from herbivores through occupation of safe sites.
<table>
<thead>
<tr>
<th>Family</th>
<th>Invasive Category</th>
<th>Species</th>
<th>Native Range</th>
<th>Recorded in Hawaii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabaceae</td>
<td>Invasive</td>
<td><em>Acacia confusa</em> Merr.</td>
<td>Taiwan and Philippines</td>
<td>1923</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Leucaena leucocephala</em> (Lam.) de Wit</td>
<td>Tropical America</td>
<td>1837</td>
</tr>
<tr>
<td></td>
<td>Non-Invasive</td>
<td><em>Tamarindus indica</em> L.</td>
<td>Tropical Africa and Asia</td>
<td>1919</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Cajanus cajan</em> (L.) Millsp.</td>
<td>Asia</td>
<td>1917</td>
</tr>
<tr>
<td>Solanaceae</td>
<td>Invasive</td>
<td><em>Cestrum nocturnum</em> L.</td>
<td>Central America</td>
<td>1928</td>
</tr>
<tr>
<td></td>
<td>Non-Invasive</td>
<td><em>Solanum melongena</em> L.</td>
<td>India</td>
<td>1824</td>
</tr>
<tr>
<td>Bignoniaceae</td>
<td>Invasive</td>
<td><em>Spathodea campanulata</em> P. Beauv.</td>
<td>West Africa</td>
<td>1915</td>
</tr>
<tr>
<td></td>
<td>Non-Invasive</td>
<td><em>Tabebuia aurea</em> (Manso) Benth. &amp; Hook. f. ex S. Moore</td>
<td>Brazil</td>
<td>1945</td>
</tr>
<tr>
<td>Apocynaceae</td>
<td>Invasive</td>
<td><em>Alstonia macrophylla</em> Wall. ex G. Don</td>
<td>Southeast Asia</td>
<td>1949</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Thevetia peruviana</em> (Pers.) K. Schum.</td>
<td>Central America</td>
<td>1920</td>
</tr>
<tr>
<td></td>
<td>Non-Invasive</td>
<td><em>Nerium oleander</em> L.</td>
<td>Mediterranean, Western Asia</td>
<td>1928</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Carissa macrocarpa</em> (Eckl.) A. DC.</td>
<td>South Africa</td>
<td>1925</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td>Invasive</td>
<td><em>Melaleuca quinquenervia</em> (Cav.) S.F. Blake</td>
<td>Western Australia</td>
<td>1924</td>
</tr>
<tr>
<td></td>
<td>Non-Invasive</td>
<td><em>Callistemon viminalis</em> (Sol. ex Gaertn.) G. Don</td>
<td>Eastern Australia</td>
<td>1933</td>
</tr>
<tr>
<td>Proteaceae</td>
<td>Invasive</td>
<td><em>Grevillea robusta</em> A. Cunn. ex R. Br.</td>
<td>Northern Australia</td>
<td>1880</td>
</tr>
<tr>
<td></td>
<td>Non-Invasive</td>
<td><em>Macadamia integrifolia</em> Maiden &amp; Betché</td>
<td>Eastern Australia</td>
<td>1909</td>
</tr>
</tbody>
</table>
Table 4.2. Results of linear mixed model with dependent variables of log transformed above- and belowground mass. Bolded variables are significant at $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Log Aboveground</th>
<th>Log Belowground</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test Statistic</td>
<td>P Value</td>
</tr>
<tr>
<td></td>
<td>$\chi^2 (1)$</td>
<td></td>
</tr>
<tr>
<td>Fixed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasion Category</td>
<td>11.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Treatment</td>
<td>24.5</td>
<td>&lt;0.001</td>
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<tr>
<td>Invasion Category x Treatment</td>
<td>2.3</td>
<td>0.129</td>
</tr>
<tr>
<td>Random</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species (Family)</td>
<td>107.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species x Treatment</td>
<td>5.5</td>
<td>0.019</td>
</tr>
</tbody>
</table>
Table 4.3. Results of linear mixed model with dependent variables of tolerance indices for defoliated and defoliated plus jasmonic acid (JA) treatments. Bolded variables are significant at $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Effect</th>
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<th></th>
<th></th>
<th>Defoliated-JA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test Statistic $\chi^2$ (1)</td>
<td>P Value</td>
<td>Estimate (S.E.)</td>
<td>Test Statistic $\chi^2$ (1)</td>
<td>P Value</td>
<td>Estimate (S.E.)</td>
</tr>
<tr>
<td>Seed Mass</td>
<td>0.1</td>
<td>0.751</td>
<td>--</td>
<td>2</td>
<td>0.157</td>
<td>--</td>
</tr>
<tr>
<td>Cotyledon Mass Fraction</td>
<td>5</td>
<td>0.025</td>
<td>0.668 (0.216)</td>
<td>10.5</td>
<td>0.001</td>
<td>0.865 (0.231)</td>
</tr>
<tr>
<td>Relative Growth Rate</td>
<td>0.2</td>
<td>0.655</td>
<td>--</td>
<td>3.6</td>
<td>0.058</td>
<td>--</td>
</tr>
<tr>
<td>Root to Shoot Ratio</td>
<td>4.5</td>
<td>0.025</td>
<td>0.628 (0.287)</td>
<td>4.7</td>
<td>0.030</td>
<td>0.783 (0.304)</td>
</tr>
<tr>
<td>Invasion Category</td>
<td>0.0</td>
<td>1.0</td>
<td>--</td>
<td>0.1</td>
<td>0.752</td>
<td>--</td>
</tr>
<tr>
<td>Family</td>
<td>2</td>
<td>0.157</td>
<td>--</td>
<td>1.8</td>
<td>0.179</td>
<td>--</td>
</tr>
</tbody>
</table>
**Figure 4.1.** Mean dried invasive and non-invasive plant aboveground and belowground biomass in grams at the end of two weeks for each of the treatments, control, 50% defoliation, and 50% defoliation plus jasmonic acid (JA). Error bars are ±1 standard error.
**Figure 4.2.** Tolerance indices of invasive and non-invasive species (total biomass of damaged plants divided by total biomass of control) for defoliated and defoliated plus jasmonic acid (JA) treatments. Values below one represent under-compensation and above one represent over-compensation.
**Figure 4.3.** Mean tolerance index for defoliated and defoliated plus jasmonic acid (JA) treatments for invasive and non-invasive species. Error bars represent ±1 standard error.
Acknowledgements

We thank Danielle Frohlich and the Oahu Early Detection project for their valuable insight into non-native species selection. Andy Taylor provided statistical insight. We thank Kari Bogner and Risha Mishima for their invaluable help in the greenhouse and in the laboratory. This work was made possible by a research grant from the University of Hawaii Manoa Graduate Student Organization. This research would also not have been possible without support from Dr. Travis Idol who allowed us to use greenhouse space managed by the Department Natural Resources and Environmental Management at the University of Hawaii at Manoa.
CHAPTER 5
TESTING THE ROLE OF SOIL FEEDBACKS IN THE INVASION SUCCESS OF
ARDISIA ELLIPTICA THUNB. IN HAWAI

Matthew H. Lurie, Jennifer L. Bufford, Meagan Rathjen, Curtis C. Daehler, and Inderjit

Abstract

Plant-soil feedbacks have been suggested to cause non-native species dominance, contributing to invasion success. Our goal was to identify impacts of an invasive tropical forest tree, Ardisia elliptica, in Hawaii, and examine whether plant-soil feedbacks influence dominance by A. elliptica. First, we surveyed stand structure characteristics of A. elliptica (stem density, height, DBH and canopy openness) in invaded areas. To assess if A. elliptica is associated with suppression of plant species diversity, we conducted surveys of plant species richness in habitats where A. elliptica had invaded and neighboring habitats where it was generally not present. Next, we tested whether A. elliptica dominance could be explained by plant-soil feedbacks. We conducted a shade house experiment using rhizosphere soil from heavily invaded A. elliptica habitats and nearby un-invaded habitats, comparing seedling growth of A. elliptica and two co-occurring species, the native Bidens sandvicensis, and the non-native Spathodea campanulata. We predicted that A. elliptica rhizosphere soil would have positive effects on growth of A. elliptica but negative effects on co-occurring species. To assess the possible influence of soil biota in plant-soil feedbacks, we also used soil sterilization treatments.

Ardisia elliptica invaded sites were characterized by a mostly closed A. elliptica canopy and had mean stem densities ranging from 6 - 11 saplings and adults m⁻² and 14 - 296 seedlings m⁻². Areas invaded by A. elliptica had significantly fewer plant species than those without it. We found no evidence of positive soil feedbacks promoting A. elliptica growth in its own rhizosphere soil or through soil biota. However, these results were inconclusive because seedlings, which were collected directly from the field, may have been exposed to soil mutualists from the A. elliptica rhizosphere.
before the start of the experiment. We also found no evidence that soil taken from beneath *A. elliptica* suppresses growth of two co-occurring species. This suggests that *A. elliptica* does not have strong allelopathic effects on its neighbors.
**Introduction**

Invasions by non-native plants can have widespread impacts at local and regional scales. Impacts in introduced ecosystems can include alteration of community structure (Pyšek & Pyšek 1995; Dunbar & Facelli 1999), fire regimes (Hughes *et al.* 1991; Brooks *et al.* 2004), hydrology (DiTomaso 1998; Dyer & Rice 1999), and nutrient cycling (Vitousek & Walker 1989; Evans *et al.* 2001). Several mechanisms have been suggested that may broadly explain invasion success, including availability of empty niche space (Elton 1958), fluctuating resources (Davis, Grime & Thompson 2000), evolution of increased competitive ability (Blossey & Notzold 1995), possession of superior competitive traits (Bakker & Wilson 2001), enemy release (Keane & Crawley 2002), and facilitation by existing non-native species (Simberloff & Von Holle 1999). Processes that directly or indirectly allow invaders to reduce recruitment of neighboring plants and increase their own relative abundance could be important in invasion success. In particular, invasive plants may succeed through feedbacks with the soil that increase their individual performance or the performance of conspecifics, resulting in their dominance (Bever *et al.* 1997). Positive soil feedbacks may occur through the introduction of new chemicals into the environment by the invasive species that negatively affect existing species relatively more than the introduced species ('novel weapons'; Callaway and Ridenour 2004). The presence of introduced plants may also change the mutualistic or pathogenic components of soil communities in ways that benefit the introduced plant, compared to native plants (Klironomos 2002; Reinhart & Callaway 2006).

Allelopathy occurs when plants produce chemicals that are either harmful or beneficial to neighboring plants or microorganisms and alter typical plant community dynamics (Albuquerque *et al.* 2011). Many types of plant secondary metabolites can act as allelochemicals, such as phenolics (Inderjit 1996; Kim & Lee 2011), terpenoids (Vokou *et al.* 2003), and alkaloids (Wink & Twardowski 1992). Allelochemicals have been shown to directly impact plant biology including changes in membrane permeability, protein synthesis, mineral uptake, hormone balance, and water relations (Rizvi *et al.* 1992) with implications for population dynamics in terms of growth,
reproduction, and survival. Allelopathic chemicals are released into the environment in a number of ways, including exudation, vaporization, leaching, or decomposition (Scognamiglio et al. 2013). Allelopathy has been suggested to play a role in the invasion success of certain species. The novel weapons hypothesis (Callaway & Ridenour 2004) suggests that when plants are transported to a new environment, the existing plant community in the introduced range may not have evolved resistance to the allelopathic chemicals released by the invader; thus, a non-native plant may reduce germination and growth of existing plant community members, leading to dominance of the non-native. For example, the polyphenol (±)-catechin, which is exuded from roots of Centaurea maculosa Lam., a forb that has invaded North American grasslands forming dense monotypic stands, inhibits the germination, growth, and survival of several species in its introduced range (Inderjit et al. 2008; Thorpe et al. 2009). Consistent with the novel weapons hypothesis, this negative effect does not seem to be as prominent in the native range.

Several temperate species have allelochemicals that may have played a role in their invasion success (Centaurea diffusa Lam., Hierro and Callaway 2003; Centaurea maculosa, Thorpe et al. 2009; Ranunculus ficaria L., Cipollini and Schradin 2011). Much less is known about the prevalence of allelopathy in the tropics. Allelopathy has been shown in Lantana camara L., a tropical American plant that has invaded tropical and subtropical forests and riparian zones in over 60 countries, suppressing surrounding vegetation with phenols (Sharma, Raghubanshi & Singh 2005). Allelochemicals leached into the soil from seeds of the tropical shrub, Sesbania virgata (Cav.) Pers., negatively affect growth of other plants with low resistance in areas invaded by S. virgata (Simões et al. 2008; El Id et al. 2015). Another tropical American shrub, Chromolaena odorata (L.) R. M. King and H. Robinson, exudes a unique chalcone compound, odoratin, through its roots into the rhizosphere. Pot experiments conducted by Zheng et al. (2015) reveal that rhizosphere soil collected from below C. odorata plants suppressed seedling emergence and growth in four herb and shrub species native to the invaded range in China, where odoratin is a novel chemical, but did not have severe negative effects on four different species from the native range in Mexico that may have evolved resistance to it.
Non-native plants may also negatively impact naïve plant communities in the invaded range by altering chemical properties of the soil environment directly or indirectly (Ehrenfeld 2003; Weidenhamer & Callaway 2010). Invasive species can alter soil moisture content (Cline et al. 1977), pH (Boswell & Espie 1998), salinity (Vivrette & Muller 1977), carbon to nitrogen ratios (Stock et al. 1995), macronutrient fluxes (Ashton et al. 2005), and levels of certain chemical elements that may affect plant growth, such as potassium (Mitchell et al. 1997). Because invasive plants may influence soil properties in many ways that potentially influence invasion success, a useful approach that integrates several hypothesis about soil interactions is the 'rhizochemical dominance' hypothesis proposed by Daneshgar and Jose (2009). This hypothesis suggests that invasion success is related to factors such as allelopathy in the introduced range (i.e. novel weapons) in addition to invader-mediated alterations to soil chemical properties. This hypothesis seems to explain invasion success of an Asian perennial grass, _Imperata cylindrica_ (L.) P. Beauv., that invades the southeastern United States and many other subtropical and tropical locations around the world. By producing allelopathic chemicals, _I. cylindrica_ has been shown to suppress growth of co-occurring species (MacDonald 2004). In addition, _I. cylindrica_ alters soil chemical properties by lowering soil pH and decreasing available nitrogen (Collins & Jose 2009). Because _I. cylindrica_ is highly tolerant of a wide range of chemical conditions in the soil and several co-occurring species are less tolerant, Collins and Jose (2009) suggest that rhizochemical interactions explain invader dominance. Thus, the rhizochemical dominance hypothesis might be a comprehensive explanation of why certain invasive species have positive soil feedbacks, such that their own colonization promotes continued invasion.

Positive and negative feedbacks can occur for colonizing plants as they interact with soil biota (Reinhart & Callaway 2006; van der Putten et al. 2013). The net direction and strength of the feedback depends on the balance of the negative effects of soil borne herbivores, enemies, and pathogens (Beckstead & Parker 2003) compared to the positive benefits of mycorrhizal fungi, nitrogen fixing bacteria and other organisms (Vitousek & Walker 1989; Duda et al. 2003; Pringle et al. 2009; Muthukumar & Udaiyam 2010). While negative feedback can be common in native plants, there is evidence that
introduced plants experience positive soil feedback in the introduced range (Klironomos 2002; Inderjit & van der Putten 2010). Niu et al. (2007) studied an invasive shrub *Ageratina adenophora* Sprengel, native to central America and invasive in many parts of the world including China. The authors found that in invaded sites *A. adenophora* altered soil chemistry through increased levels of nitrate, ammonium, potassium, and available phosphorous compared to un-invaded sites. Additionally, *A. adenophora* increased soil vesicular-arbuscular mycorrhizal fungi in invaded sites. A greenhouse experiment conducted by the authors suggested that soil biota from the un-invaded range had negative impacts on growth of three co-occurring native plant species, but not on *A. adenophora* and that soil biota positively affected dominance of *A. adenophora* in soil from the invaded range. The influence of soil biota was particularly highlighted when a decrease in dominance occurred when *A. adenophora* was grown in its own sterilized rhizosphere soil, indicating positive feedbacks with soil biota. In another study, black cherry *Prunus serotina* Ehrh. was found to experience negative soil feedbacks due to pathogens in its native range in the United States, but experienced release from pathogens and positive soil feedbacks due to soil biota in its introduced range in Europe, accounting for increased germination and growth (Reinhart et al. 2003). Little is known about how common plant soil feedbacks mediated by soil biota are in tropical environments. There is evidence of strong negative soil feedback affecting abundance and diversity generally in tropical forest species (Mangan et al. 2010), which suggests that tropical invaders have the potential to benefit from neutral or positive soil feedbacks in invaded ranges.

Only a small fraction of plant species that are introduced become established, and an even smaller number spread beyond introduction sites and dominate communities (Williamson & Fitter 1996; Richardson et al. 2000; Daehler 2001). Thus, one of the central problems in invasion biology is to understand factors that make a particular non-native species successful (van Kleunen et al. 2010a). *Ardisia elliptica* Thunb. (Myrsinaceae) is a tropical understory shrub or small tree native to southeastern Asia which has invaded many tropical forest habitats around the world including parts of North America and Hawaii (Staples & Herbst 2005) and has been rated as one of the 100 ‘worst’ global invasive species by Lowe et al. (2000). In Hawaii, *A. elliptica* has
been cultivated as an ornamental plant in since at least 1917 (Rock 1917), and is now naturalized on several islands including Oahu, Kauai, Maui, and Hawaii (Wagner, Sohmer & Herbst 1999). In many areas where A. elliptica is present, it is the dominant plant, commonly forming near-monotypic or high density stands (pers. obs.). However we lack descriptions of stand structure and a complete understanding of how A. elliptica impacts habitats where it is present. We also generally do not understand what factors make A. elliptica a successful invader in Hawaii. Our overall aim was to address this gap and assess the importance of plant-soil feedbacks in explaining its dominance in certain habitats. To address this, we conducted field surveys and performed plant soil-feedback studies with A. elliptica and two co-occurring species, the native Bidens sandvicensis Less. (Asteraceae) and non-native Spathodea campanulata P. Beauv. (Bignoniaceae).

The objectives of this investigation were:

1) To describe A. elliptica stand structure in invaded habitats
2) To determine if A. elliptica impacts plant species richness in areas where it has invaded. We predicted that invaded habitats would be associated with decreased plant species richness.
3) To determine if A. elliptica causes changes in the soil environment that promote A. elliptica growth and suppresses growth of co-occurring species. We predicted that rhizosphere soil from A. elliptica plants will a) promote A. elliptica growth, and b) decrease growth of co-occurring species.
4) To determine if A. elliptica benefits from plant-soil feedbacks with soil biota in areas where it has invaded. We predicted that A. elliptica will benefit more from soil biota compared to co-occurring species.
Methods

Field study sites

We conducted field surveys on the Hawaiian island of Oahu at the Harold T. Lyon Arboretum (21°19'58.60"N, 157°48'8.19"W) and Hoomaluhia Botanical Garden (21°23'17.85"N, 157°48'19.87"W). Lyon Arboretum is a lowland wet forest (~ 150 meters above sea level) on the leeward side of Oahu, receiving approximately 3840 mm of rainfall annually, spread evenly throughout the year (Giambelluca et al. 2013). Hoomaluhia Botanical Garden is a lowland wet forest (~ 380 meters above sea level) on the windward site of Oahu, receiving approximately 2000 mm of rainfall annually, with rain being heavier in November through March (Giambelluca et al. 2013). Initial field surveys revealed that *A. elliptica* was patchy at both sites. We selected high density *A. elliptica* plots (HD), where *A. elliptica* is dominant (≥ 2 mature plants per square meter), and low density *A. elliptica* (LD) plots, where *A. elliptica* had not previously invaded or was found in extremely low densities (~ 1 mature plant per 25 square meters). We refer to types of habitat experiencing different levels of invasion as ‘Ardisia Density’ in our analysis.

Stand structure of *Ardisia elliptica* in invaded areas

At both sites we selected fifteen 1 m x 1 m plots in areas where *Ardisia elliptica* invasion was prominent (high density plots). Plots were randomly selected using random compass bearings and random distances (1-50 m) from previous plots. We measured stem density of *A. elliptica* in each plot. Plants with basal diameter greater than 0.5 cm were pooled into a category “saplings and adults” and smaller plants were grouped into a “seedling” category. Height and DBH were measured on the largest *A. elliptica* adult in the plot. Height was measured using a metered pole to the nearest 0.05 m for plants less than 4 m tall and a digital clinometer for taller trees. DBH was measured to the nearest 0.1 cm. Canopy openness was used to describe the light environment and was measured using a spherical densiometer (Forest Densiometer Model-A). Four densiometer measurements were made per plot at 90 degree angles apart and averaged.
Species richness

At each site we selected fifteen 1 m x 1 m plots in both high density (HD) and low density (LD) *Ardisia elliptica* locations. These were the same plots used for assessment of stand structure. We counted the number of plant species present in each plot. Cotyledon stage seedlings were difficult to identify, so only juvenile and mature plants were counted. When juvenile and mature plant species could not be identified, we took photographs and recorded them as distinct morphotypes.

Soil feedback

To test whether soil biota and soil chemical properties impact *Ardisia elliptica* seedling growth and whether *A. elliptica* suppresses the growth of the two co-occurring species through soil-mediated processes, we conducted a soil feedback study in a shade house at Lyon Arboretum. In addition to *A. elliptica*, we used a common introduced ornamental tree, *Spathodea campanulata*, that has become invasive in low elevation mesic habitats, and an endemic perennial herb, *Bidens sandvicensis*, that is common in relatively disturbed mesic forest habitats between 150 – 900 m in elevation. We chose these species because they regularly occur in or adjacent to habitats where *A. elliptica* is present and because they could be grown from seeds. Soil was collected from eight high density (HD) and eight low density (LD) *A. elliptica* replicate plots at both Lyon and Hoomaluhia sites. Approximately 1 L of soil was removed at each plot. At HD plots, *A. elliptica* rhizosphere soil was collected by digging around the roots of *A. elliptica* and removing soil. At LD plots, we randomly collected bulk soil to a depth of 20 cm. Soil was sorted to remove rocks and debris larger than 1 cm diameter in the field. We sterilized half of the soil by autoclaving twice on successive days in aluminum trays loosely covered with aluminum foil (122 °C, 16 psi, 40 mins). For each plot we filled two 164 mL soil containers with soil (Ray Leach “Cone-tainers”; diameter 3.8 cm, height 21 cm) for each combination of sterilization (autoclaved vs. control) and species (*A. elliptica*, *S. campanulata*, and *B. sandvicensis*). These two cones were treated as subsamples of each plot replicate.
Seedling collection and germination was timed to correspond with soil preparation. *Ardisia elliptica* seedlings with two true leaves were collected from invaded field sites at Hoomaluhia. Seeds of *Spathodea campanulata* were collected from Waimanalo, Oahu from mature trees and germinated in a growth chamber on a 12 h day / 12 h night cycle at 25 °C and 20 °C respectively. Seedlings of *S. campanulata* had two true leaves at the beginning of the experiment. Seeds of *Bidens sandvicensis* were collected from Manoa Valley, Oahu and germinated at the Lyon Arboretum in a shade house. At the time of the experiment, seedlings of *B. sandvicensis* had between 2 and 6 true leaves. We only had enough seedlings of *B. sandvicensis* to conduct the experiment in soil for one site (Hoomaluhia). Prior to transplanting, initial shoot and root length of each seedling were measured to the nearest 0.1 cm. Shoot length was measured as distance from the root shoot boundary to the apical meristems. Root length was measured as the linear distance from the root shoot boundary to the end of the longest root. In mid-April 2012, a single seedling was transplanted in each cone by saturating the soil with water and then digging a 1 cm diameter hole to place the roots in and then backfilling. Cones were placed in trays and location were randomized within and across all trays. Plants were kept in the Lyon Arboretum shade house for the duration of the experiment and cones were re-randomized once a week. Harvesting occurred after 60 days for *A. elliptica* and *S. campanulata*. To prevent *B. sandvicensis* plants from becoming pot bound, harvest occurred after 30 days. Upon harvesting, soil was carefully removed from the roots using a water bath and final root and shoot length were measured to the nearest 0.1 cm. For each individual, root and shoot growth was measured as the difference between initial and final root and shoot lengths. Subsample cones for each plot replicate were averaged (eight replicates per *A. elliptica* density treatment per site for each sterilization treatment). In a small number of cones, the seedling did not survive and the remaining single cone was used as the plot measurement.

**Statistics**

For stand structure measurements (height, diameter at breast height, canopy openness, stem density), one-way ANOVA’s were conducted to assess whether there
were differences between sites (Lyon vs. Hoomaluhia). Because stand structure measurements did not meet equality of variance assumptions, Welch’s ANOVA’s were used. For species richness comparisons, the effect of site and *Ardisia elliptica* density (high density vs. low density areas) were investigated using a two-way ANOVA. The interaction between site and *A. elliptica* density was included to determine if the effect of *A. elliptica* invasion was different between Lyon and Hoomaluhia. For the growth experiments, we ran separate analyses of growth measurements for each species. For *A. elliptica* and *Spathodea campanulata* we tested the effects of site, *A. elliptica* density, sterilization, and their interactions on root and shoot growth. For *Bidens sandvicensis*, we tested the effect of *A. elliptica* density, sterilization and the interaction on root and shoot growth. We did not test a site factor for *B. sandvicensis* because we only grew seedlings in soil from one site due to a limited number of available seedlings. All statistics analyses were done using SAS software, Version 9.4 for Windows (2013). Hypothesis tests and Type III ANOVA tables were generated using *Proc GLM*. Normality of variables and equality of variances were confirmed using normal quantile-quantile plots of residuals and scatter plots of predicted values versus residuals. Post hoc Tukey’s adjusted least square mean analysis examined pair-wise differences between significant effects with more than two levels.

**Results**

*Stand structure of Ardisia elliptica in invaded areas*

Mean sapling and adult stem density was significantly lower at Lyon than at Hoomaluhia (6.2 ± 1.2 saplings and adults m\(^{-2}\), 11.7 ± 2.3 saplings and adults m\(^{-2}\), mean ± standard error, respectively; \(F_1 = 4.66, P = 0.043\)). Seedling density was significantly lower at Lyon than at Hoomaluhia (14.2 ± 3.1 seedlings m\(^{-2}\), 296.0 ± 62.3 seedlings m\(^{-2}\), respectively; \(F_1 = 20.41, P < 0.001\)). Percent canopy openness was significantly higher at Lyon than at Hoomaluhia (8.7 ± 0.8 %, 1.5 ± 0.4 %, respectively; \(F_1 = 70.23, P < 0.001\)). Height and diameter at breast height (DBH) was not related to site (Table 5.1). Across sites mean height was 4.0 ± 0.4 m and mean DBH was 2.8 ± 0.3 cm.
Species richness

Mean species richness was significantly higher in low density (LD) *Ardisia elliptica* plots, compared with high density (HD) *A. elliptica* plots (6.3 species m$^{-2}$ (5.5, 7.1), 3.2 species m$^{-2}$ (2.5, 3.9), mean (95 % confidence limits); $F_{1,51} = 35.68$, $P < 0.001$; Fig. 5.1). The effect of *A. elliptica* density did not differ between sites as evidenced by the non-significant interaction term (Table 5.2). The main effect of site was also not significant (Table 5.2).

Ardisia elliptica growth

Roots of *Ardisia elliptica* grew on average 5.6 cm over 60 days. Over the same time period there was a net loss in shoot length of 0.2 cm. There were no significant interactions between site, sterilization, and *A. elliptica* plot density for root or shoot growth (Table 5.3), with the exception that root growth had a significant interaction of site and sterilization ($F_{1,55} = 34.02$, $P < 0.008$). Tukey’s test indicated that mean root growth only significantly differed at the Hoomaluhia sites, with roots growing 7.0 cm (6.0, 8.1) (mean; 95 % confidence limit) in the autoclaved treatments compared to 4.4 cm (3.4, 5.5) in control treatments ($P = 0.006$; Fig. 5.2). There was no significant difference in root growth between sites ($F_{1,55} = 0.10$, $P = 0.750$), but there was for shoot growth ($F_{1,55} = 4.90$, $P = 0.031$). Shoot length remained the same at Lyon over the course of the experiment but decreased by 0.4 cm (0.6, 0.2) at Hoomaluhia. Sterilization of soil positively affected *A. elliptica* root growth ($F_{1,55} = 19.48$, $P = 0.042$) but not shoot growth ($F_{1,55} = 0.44$, $P = 0.306$). The main effect of *A. elliptica* plot density was not significant for either root or shoot growth (Table 5.3).

Spathodea campanulata growth

Roots of *Spathodea campanulata* grew on average 11.4 cm, while shoots grew an average of 2.2 cm over 60 days. There were no significant two- or three-way interaction effects in the model (Table 5.4). The main effect of site was significant for root length ($F_{1,56} = 8.4$, $P = 0.005$). Roots grew 12.8 cm (11.4, 14.2) (mean; 95 % confidence limit) at Hoomaluhia and 10.0 cm (8.6, 11.3) at Lyon. The main effect of sterilization was significant ($F_{1,56} = 31.37$, $P < 0.001$). Roots grew 5.5 cm (3.5, 7.4
longer in unsterilized soil compared to sterilized soil. None of the main effects of site, sterilization, or *A. elliptica* plot density had significant effects on shoot length (Table 5.4).

**Bidens sandvicensis growth**

Roots of *Bidens sandvicensis* grew on average 8.2 cm while shoots grew an average of 0.1 cm over 30 days. The main effects of sterilization, *A. elliptica* plot density, and their interaction were not significant in the model (Table 5.5).

**Discussion**

*Ardisia elliptica*-invaded habitats were characterized by dense thickets, relatively closed canopy, and many recruiting seedlings on the forest floor, probably contributing to dominance. Species richness in sites invaded by *A. elliptica* was lower than in un-invaded sites, suggesting that *A. elliptica* is suppressing growth of other species. However, we found little evidence that *A. elliptica*’s dominance was explained by positive soil feedbacks in *A. elliptica* rhizosphere soil, despite plant-soil feedbacks being suggested as a general mechanism promoting invasion success (Klironomos 2002). Furthermore, we found no evidence that *A. elliptica* rhizosphere soil was negatively impacting two co-occurring species, *Spathodea campanulata* and *Bidens sandvicensis*. Though we did not measure chemical properties directly, preliminary soil chemical analyses from a separate study suggest that chemical properties (pH, total phenolics, organic carbon and total organic nitrogen) in habitats invaded and not invaded by *A. elliptica* are similar (Inderjit unpublished data). Based on these preliminary data and our measures of impacts of *A. elliptica* rhizosphere soil on seedling performance, we suspect that *A. elliptica* is not producing novel allelopathic compounds or changing soil chemistry in a way that benefits its own growth and suppresses co-occurring species (i.e. a lack of rhizochemical dominance; Daneshgar and Jose 2009). A lack of invader rhizosphere soil impact has been observed in at least one other study (Del Fabbro, Güsewell & Prati 2014), suggesting that invasion success may be attributed to other factors beyond soil-mediated mechanisms in certain species.
Contrary to our prediction, we did not observe performance benefits of soil biota in *Ardisia elliptica*. Rather *A. elliptica* experienced increased root growth in sterilized compared to unsterilized treatments at one site (Fig. 5.2). We also found that soil biota had no significant impacts on root and shoot growth in *Bidens sandvicensis* and had a positive impact on root growth in *Spathodea campanulata*. One explanation for these observations is that each species responds differently to aspects of the soil biota, whether it be the negative effects (e.g. soil borne herbivores, enemies, and pathogens) or the positive effects (e.g. mycorrhizal fungi, nitrogen fixing bacteria). Another possible explanation is that because *A. elliptica* and *B. sandvicensis* had germinated in soil outside the lab prior to the experiment, it may have already been exposed to beneficial soil biota (such as root mycorrhizae) which would have accompanied them when they were transplanted into sterilized and unsterilized soils. Thus any positive impacts of biota existing in the soil we collected would not be detected by comparing sterilization treatments. We observed positive impacts of sterilization for *A. elliptica* growth (Fig. 5.2) and a lack of impact for *B. sandvicensis* growth, supporting the idea that these species may have already benefited from soil biota at the start of the experiment. The only species that could not have had exposure to beneficial organisms prior to the experiment was *S. campanulata*, because it was germinated in sterile laboratory conditions. Thus, the reduction of *S. campanulata* root growth observed in the sterilization treatment reflects a true positive impact of soil biota from our sites. This explanation of species differences is only valid if the three species associate with beneficial root organisms, though this is common in most plants (Pringle et al. 2009). Arbuscular mycorrhizal fungi have been observed in roots of mature plants of *S. campanulata* (Uma et al. 2012; Esoeyang et al. 2014) and *B. sandvicensis* (Gemma, Koske & Habte 2002). To our knowledge there is no record of such associations in *A. elliptica*.

*Ardisia elliptica* seedlings in our experiment exhibited root growth but very little shoot growth. As plants grow, biomass allocation to roots and shoots can be associated with limiting resources as plants compensate for environmental conditions (Wilson 1988). Thus one explanation for root as opposed to shoot growth may be that plants are compensating for a lack of available soil resources (e.g. water or macronutrients).
Plants were watered regularly in the shade house so water is unlikely to be a limiting resource. Nutrient limitation is more likely since the soil we collected may have had low levels of nutrients prior to the experiment, or nutrients may have been rapidly used by seedlings in the cones.

There are several additional reasons why Ardisia elliptica may be a successful species in Hawaii. First, A. elliptica could be a superior competitor compared to other species in the environment. For example, high rates of photosynthetic capacity compared to neighboring species has been shown for A. elliptica in invaded regions in China (Zhao & Chen 2011). Second, some invasive species may be successful invaders because they reproduce quickly and have high fecundity (Daneshgar & Jose 2009). During our sampling, mature A. elliptica had numerous drupes, high germination in the understory, and seeds that were dispersed by frugivorous birds (pers. obs.), which may allow it to disperse to un-invaded regions rapidly compared to other co-existing species. Third, it is possible that A. elliptica may be undergoing enemy release. Release from specialist enemies has been suggested as a driver of invasion in invaded habitats in Florida (Koop 2003). Though we did not quantitatively measure herbivory in seedlings of A. elliptica, we observed that seedlings generally had < 1 % foliar tissue loss in our 1 m² plots (M. Lurie pers. obs.), indicating high herbivore resistance or escape from herbivory. Measurements of herbivory rates in the native range could clarify if enemy release is occurring.

**Conclusion**

Plant-soil feedbacks have generally been recognized as a mechanism of dominance for some non-native species through novel weapons, changes in soil chemical properties, and interactions with soil biota. Understanding factors that contribute to invasion success and impacts on surrounding species is an important goal of invasion biology. We observed impacts of a small invasive tree in Hawaii, Ardisia elliptica, on plant species richness and assessed whether plant-soil feedbacks are a possible mechanism of invasion success. We observed declines in the number of species present in habitats invaded by A. elliptica. We found no evidence of positive soil
feedbacks promoting *A. elliptica* growth in its own rhizosphere soil or through soil biota. However, these results were inconclusive because seedlings, which were collected directly from the field, may have been exposed to soil mutualists from the *A. elliptica* rhizosphere before the start of the experiment. We also found no evidence that soil taken from beneath *A. elliptica* suppresses growth of two co-occurring species. This suggests that *A. elliptica* does not have strong allelopathic effects on its neighbors.
Table 5.1. Results of separate Welch’s one way ANOVAs showing whether site (Lyon vs. Hoomaluhia) was associated with differences in stand structure variables (sapling and adult density, seedling density, height, diameter at breast height (DBH), and percent canopy openness). Significant differences in stand structure variables between sites are bolded (α = 0.05 level).

<table>
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<th>Variable</th>
<th>F (df = 1)</th>
<th>P Value</th>
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<tr>
<td>Sapling and Adult Density</td>
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<td>Seedling Density</td>
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<td>DBH</td>
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<tr>
<td>Canopy Openness</td>
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**Table 5.2.** Results of plant species richness surveys using a 2 factor ANOVA with species richness as dependent variable and site (Hoomaluhia vs. Lyon), *Ardisia elliptica* plot density (high density vs. low density), and their interaction as independent variables. Significant factors that associated with species richness are bolded (α = 0.05 level).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F Value</th>
<th>P Value</th>
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<td>Site</td>
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<td>Error</td>
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**Table 5.3.** Results of soil feedback study for *Ardisia elliptica* using a 3 factor ANOVA with *A. elliptica* root and shoot growth as dependent variables. Independent variables site (Hoomaluhia vs. Lyon), *A. elliptica* plot density (high density vs. low density), sterilization (autoclaved vs. control) and their interactions as independent variables. Significant factors associated with root or shoot growth are bolded (α = 0.05 level).

<table>
<thead>
<tr>
<th>Source</th>
<th>Root Growth</th>
<th>Shoot Growth</th>
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Table 5.4. Results of soil feedback study for *Spathodea campanulata* using a 3 factor ANOVA with *S. campanulata* root and shoot growth as dependent variables. Independent variables included were site (Hoomaluhia vs. Lyon), *Ardisia elliptica* plot density (high density vs. low density), sterilization (autoclaved vs. control) and their interactions as independent variables. Significant factors associated with root or shoot growth are bolded (α = 0.05 level).

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<td>F Value</td>
<td>P Value</td>
<td>df</td>
<td>MS</td>
<td>F Value</td>
<td>P Value</td>
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Table 5.5. Results of soil feedback study for *Bidens sandvicensis* using a 2 way ANOVA with *B. sandvicensis* root and shoot growth as a dependent variable. Independent variables are *Ardisia elliptica* plot density (high density vs. low density), sterilization (autoclaved vs. control). Significant factors associated with root or shoot growth are bolded (α = 0.05 level).

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Figure 5.1. Mean plant species richness in *Ardisia elliptica* high density and low density plots at Lyon Arboretum and Hoomaluhia Botanical Garden. Error bars represent 95% confidence limits.
Figure 5.2. Root growth for *Ardisia elliptica* at two sites, Lyon Arboretum and Hoomaluhia Botanical Garden, in sterilized (autoclaved) and control soil treatments. Error bars represent 95% confidence limits.
Acknowledgements

We thank the Harold T. Lyon Arboretum and Hoomaluhia Botanical Garden for support with field access and collections. Shade house space was generously provided by the Lyon Arboretum. Seana Walsh provided invaluable help in the field and the lab. Mashuri Waite provided plant material and green house assistance. Don Drake provided tools for forest stand structure assessments.
Herbivory and plant invasion in Hawaii

The first portion of my dissertation set out to determine the role of herbivory in plant invasion success. Herbivory is broadly acknowledged in the literature as influencing invasions either through high herbivory preventing establishment and spread of introduced species leading to invasion failure (Elton 1958) or conversely through reductions of herbivory in the introduced compared to native ranges leading to invasion success (Keane & Crawley 2002). Previous studies have found evidence for and against these hypotheses (Maron & Vila 2001), suggesting that the role of herbivory in invasions is complex. Because the invasion process can be viewed as a series of stages with progressively fewer species moving from one stage to the next (Williamson & Fitter 1996; Lockwood et al. 2007), I decided to frame my investigation around the hypothesis that herbivory may act as a post-establishment invasion barrier. Using a multi-species approach comparing invasive and non-invasive species, I expected that introduced species that were non-invasive would have higher rates of herbivore damage and fewer defensive traits, possibly accounting for their invasion failure. Conversely I expected that invasive species would have lower rates of herbivore damage and more defensive traits, possibly accounting for their invasion success. To test these hypotheses, I compared herbivore resistance (a plants ability to deter herbivory) and tolerance of herbivore damage, in a wide range of woody plant species in Hawaii. Below I will discuss specific results and what I have learned about invasion success or failure.

One of my research questions focused on whether or not invasive species experienced less herbivory (higher resistance to herbivores) compared to non-invasive species. In Chapter 2, I showed that herbivores were not more likely to consume non-invasive than invasive plants in laboratory comparisons of seedling acceptability to two generalist herbivores (the slug Laevicaulis alte and the grasshopper Conocephalus saltator) in no-choice feeding trials. Though my results could be herbivore specific, using drastically different types of generalist herbivores (slug vs. grasshopper),
suggests that the result I observed is not an artifact of herbivore identity. I next investigated herbivory in a field setting. In Chapter 3, I demonstrated that there was no difference in mean foliar herbivory based on invasion category (5 invasive and 4 non-invasive species) two to five months following planting in a field common garden experiment. My results add to a growing consensus that herbivore resistance may not be a good indicator of how well non-native plants invade post-establishment (Liu et al. 2007; Matter et al. 2012). Furthermore, species in the common garden experiment generally experienced low levels of mean foliar herbivory (< 2 %), consistent with the lower range found for seedlings and juveniles of woody invasive species in tropical forests (~ 1-20 %: de la Cruz & Dirzo 1987; Dawson, Burslem & Hulme 2009). Though I didn’t test the same subsets of non-native species each experiment, I generally observed much higher levels of plant consumption in feeding trials compared to the common garden experiment. This probably indicates that the consumption rates experienced by species in the no-choice feeding trials represent maximum possible resistance to herbivores, while herbivory rates in the field also reflect herbivore preference since herbivores in the field may be presented with a wide variety of food choices.

My next research question addressed the influence of tolerance to herbivory as a driver of invasion success. Given the low levels of mean foliar herbivory I observed in the field (Chapter 3), tolerance might not be expected to provide a selective advantage in the field. However, individual plants did experience foliar damage as high as 100 %. Thus, tolerance could still play an important role in population dynamics. In Chapter 4, I conducted simulated herbivory experiments in seedlings of 8 invasive and 8 non-invasive species. I removed 50% of leaf tissue and measured growth after two weeks. In general plants did not grow to similar levels as controls (under-compensation) and invasive species did not tolerate damage more than non-invasive species, suggesting that highly invasive species do not characteristically have high tolerance to herbivory.

Despite the lack of significant differences in resistance traits between invasive and non-invasive species, I did observe species specific differences within each invasion category. This raises several important questions. First, how do the invasive species that have higher levels of herbivory persist in the environment? For example,
Alstonia macrophylla (invasive: Apocynaceae) had nearly 100 % of seedlings consumed in slug feeding trials and Justicia betonica (invasive: Acanthaceae) had the highest mean damage rate observed in the common garden experiment. Invasive plants may be able to tolerate herbivory by reducing the impact herbivory has on the fitness of the plant once damage has occurred. This seems possible for A. macrophylla which nearly completely compensated for 50 % foliar loss in simulated herbivory trials and had the highest tolerance capacity for any of the invasive species I investigated (Chapter 4). However, the cotyledons of A. macrophylla were completely consumed when a feeding encounter occurred with a slug (Chapter 2) resulting in mortality. It may be that high tolerance contributes to plant performance in cases where < 100 % of aboveground tissue is removed or when plants become larger. High propagule pressure has also been suggested as a mechanism to overcome invasion barriers, especially associated with establishment (Eschtruth & Battles 2009; Simberloff 2009). For example, highly acceptable species may be able to overcome biotic resistance through repeated introductions that make seedlings more likely to find safe sites. This seems possible for J. betonica, which is commonly used as a landscape plant around Oahu. This species also has high reproductive output and high growth rate (pers. obs.) which could also be relevant for increasing the probability of finding and taking advantage of safe sites.

Second, why due non-invasive species with lower susceptibility to herbivory still fail to invade? In Chapter 2, I observed Tabebuia aurea (non-invasive: Bignoniaceae) and Tamarindus indica (non-invasive: Fabaceae) having zero seedlings consumed by slugs and Enterolobium cyclocarpum (non-invasive: Fabaceae) experiencing zero damage by grasshoppers. In Chapter 3, Kigelia africana (non-invasive: Bignoniaceae) and Tecoma capensis (non-invasive: Bignoniaceae) had the lowest foliar damage rates (< 1 %) in the field. Because high resistance in these species does not appear to confer invasion success, other barriers are likely preventing significant population spread. Seedlings may have structural or chemical traits that confer resistance to generalist herbivores but lack vigor in terms of plant performance to compete with neighboring plants. In addition, the plants may lack mutualists needed to facilitate population growth. For example K. africana has large woody fruits which are consumed and passed through the digestive system of large mammals (e.g. elephants and giraffes) in the
native range (Theuerkauf et al. 2000). In Hawaii, large herbivores are not present to aid in dispersal, which may hinder spread beyond cultivated settings and into surrounding habitats. Furthermore, genetic constraints and other forms of biotic resistance due to competitors and/or pathogens could cause failure of these species to invade.

**Plant-soil feedback and the success of invasive *Ardisia elliptica***

Plant-soil feedbacks have generally been recognized as important mechanisms of dominance for some invasive species by promoting their own growth and suppressing recruitment and growth of co-occurring resident species through novel weapons (Callaway et al. 2008), changes in soil chemical properties (Weidenhamer & Callaway 2010), and interactions with soil biota (Reinhart & Callaway 2006). I investigated whether these soil-mediated processes could explain the invasion success of *Ardisia elliptica* in Hawaii (Chapter 5). After showing that sites dominated by *A. elliptica* had a lower species richness compared to sites where *A. elliptica* had not yet invaded, I conducted greenhouse soil feedback experiments to determine if *A. elliptica* positively impacts its own growth and suppresses growth of two co-occurring species. I found no evidence of positive soil feedbacks promoting *A. elliptica* growth in its own rhizosphere soil through soil biota. However, these results were inconclusive because seedlings, which were collected directly from the field, may have been exposed to soil mutualists from *A. elliptica* rhizosphere before the start of the experiment. I also found no evidence that soil taken from beneath *A. elliptica* suppresses growth of two co-occurring species. This suggests that *A. elliptica* does not have strong allelopathic effects on its neighbors.

**General conclusions**

I began my dissertation with the goal of discovering factors that influence plant invasion success and failure. However, my experiments showed that for several species of woody invaders in Hawaii, there was no general difference in herbivory or herbivore resistance between invasive and non-invasive species. I was also unable to explain
dominance of a non-native tree, *Ardisia elliptica*, based on plant-soil feedbacks. While these null results were contrary to my expectations, they are still important ecological findings because they present future researchers with additional information to construct hypotheses about plant invasions post-establishment. This continues to be a critically important field of study because introduced plants have huge ecological and economic impacts, and developing a comprehensive understanding of why non-native plants become successful invaders can help us to reduce or prevent their entry into novel communities.
APPENDICES

Appendix 2.1. Model specification for the ‘step-down’ mixed effects analysis for slug seedling acceptability trials. The dependent variable is the number of seedlings consumed by day six. The independent variables are categorized as either fixed or random effects. Variables included in each model (1.1-1.6) are denoted with an (X). Models used for specific hypothesis testing are listed in Appendix 2.2.

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<th>Effect Type</th>
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<th>Model 1.3</th>
<th>Model 1.4</th>
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<td>-</td>
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Appendix 2.2. Summary of mixed model analysis hypothesis testing used in slug feeding trials. To test whether each variable has an effect on the seedling acceptability, the reference model was compared to the nested model using maximum likelihood (ML) or restricted maximum likelihood (ReML) and a subsequent likelihood ratio test was performed. Model specification for nested and reference models can be found in Appendix 2.1. Significance testing used a likelihood ratio test.

<table>
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**Appendix 2.3.** Model specification for the ‘step-down’ mixed effects analysis for grasshopper seedling acceptability trials. The dependent variable is the number of seedlings consumed by day six. The independent variables are categorized as either fixed or random effects. Variables included in each model (2.1-2.5) are denoted with an (X). Models used for specific hypothesis testing are listed in Appendix 2.4.

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**Appendix 2.4.** Summary of mixed model analysis hypothesis testing used in grasshopper feeding trials. To test whether each variable has an effect on the seedling acceptability, the reference model was compared to the nested model using maximum likelihood (ML) or restricted maximum likelihood (ReML) and a subsequent likelihood ratio test was performed. Model specification for nested and reference models can be found in Appendix 2.3. Significance testing used a likelihood ratio test.

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**Appendix 4.1.** Model selection summary for linear mixed models to predict above- and belowground biomass at the end of the two week experiment. The table shows the step down approach from a loaded model (1.1) to the best fit model (1.4). Model selection was the same for above- and belowground biomass. Models used for specific hypothesis testing are listed in Appendix 4.2.

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Appendix 4.2. Summary of mixed model analysis hypothesis testing for above- and belowground growth. Model specification for nested and reference models can be found in Appendix 4.1. Maximum likelihood (ML) or restricted maximum likelihood estimation methods are noted for each effect.

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Appendix 4.3. Model selection summary for linear mixed models to predict tolerance indices. The table shows the step down approach from a loaded model (2.1) to the best fit model (2.6). Models used for specific hypothesis testing are listed in Appendix 4.4.

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<td>x</td>
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<td>-</td>
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<tr>
<td>Random</td>
<td>Family</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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</table>
Appendix 4.4. Summary of mixed model analysis hypothesis testing for above- and belowground growth. Model specification for nested and reference models can be found in Appendix 4.3. Maximum likelihood (ML) or restricted maximum likelihood estimation methods are noted for each effect.

<table>
<thead>
<tr>
<th>Effect Type</th>
<th>Variable</th>
<th>Nested Model</th>
<th>Reference Model</th>
<th>Estimation Method</th>
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<td>Fixed</td>
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<td>2.1</td>
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<td>Cotyledon Mass Fraction</td>
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<td></td>
<td>Relative Growth Rate</td>
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<td>2.4</td>
<td>ML</td>
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<td>Root to Shoot Ratio</td>
<td>2.5</td>
<td>2.4</td>
<td>ML</td>
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<td></td>
<td>Invasion Category</td>
<td>2.6</td>
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<td>2.7</td>
<td>2.6</td>
<td>ReML</td>
</tr>
</tbody>
</table>
LITERATURE CITED


SAS system for Windows. (2013)


