

INDUCED RESPONSES TO HERBIVORY IN THE HAWAIIAN ENDEMIC
PRICKLY POPPY, *Argemone glauca*, AND THE MEXICAN POPPY, *Argemone
mexicana*

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ABSTRACT

Herbivory is an important factor in the evolutionary ecology of plants, and plants have many chemical and physical traits that reduce the negative effects herbivory has on plant fitness. Induced responses to herbivory is a now widely accepted ecological pattern, although most studies have examined responses in chemical defense traits, while physical defense traits and mechanisms of tolerance have received less attention. We studied the effects of removal of 50% of leaf area and of jasmonic acid application on the induction of leaf latex, prickle density, leaf toughness, as well as mechanisms of tolerance in two sister species, *Argemone glauca* and *Argemone mexicana*. Potentially important factors of variation in defense and tolerance were also investigated, including ontogeny and genetic variation within and between populations. *A. glauca* significantly increased prickle densities in response to treatments, while *A. mexicana* increased leaf toughness and showed a higher ability to compensate for biomass loss than *A. glauca*. No treatments induced changes in leaf latex volume. Genetic variation between populations was detected in physical defense traits at constitutive levels, but not in inducible responses. This study is the first to induce prickle densities in a plant using jasmonic acid as a form of simulated herbivory, and is the first to induce responses in defense traits in a Hawaiian endemic. This study also highlights the divergence of defenses in two closely related species. Additional studies using natural herbivores are needed to confirm that these responses provide defense against herbivory.

TABLE OF CONTENTS

TITLE PAGE	1
ACKNOWLEDGEMENTS	2
ABSTRACT	3
CHAPTER 1. A REVIEW OF THE INDUCTION OF PHYSICAL DEFENSE TRAITS IN RESPONSE TO DAMAGE	5
INTRODUCTION	5
PHYSICAL DEFENSE TRAITS.....	5
INDUCIBLE RESPONSES TO HERBIVORY	8
INDUCTION OF PHYSICAL DEFENSE TRAITS	9
INDUCED TOLERANCE IN RESPONSE TO HERBIVORY.....	13
CONCLUSIONS AND EXPERIMENTAL GOALS	14
CHAPTER 2. INDUCIBLE RESPONSES TO DAMAGE AND JASMONIC ACID IN TWO SISTER SPECIES OF ARGEMONE (PAPAVERACEAE)	16
ABSTRACT	16
INTRODUCTION	17
MATERIALS AND METHODS.....	24
<i>SAMPLING SUMMARY</i>	24
<i>EXPERIMENTAL DESIGN</i>	24
<i>STATISTICAL ANALYSES</i>	28
RESULTS.....	30
<i>EXPERIMENT ONE</i>	30
<i>EXPERIMENT TWO</i>	32
DISCUSSION	33
CHAPTER 3. FUTURE DIRECTIONS	38
INTRODUCTION	38
SPECIFICITY IN INDUCTION OF PHYSICAL TRAITS	39
BENEFITS TO INDUCED PHYSICAL DEFENSES.....	40
SIMULTANEOUS INDUCTION OF TOLERANCE AND PHYSICAL DEFENSE	41
INDUCED PHYSICAL RESPONSES AND ONTOGENY.....	41
ISLAND DEFENSE SYNDROME.....	42
CONCLUSIONS	43
TABLES AND FIGURES	44
LITERATURE CITED	50

CHAPTER 1. A REVIEW OF THE INDUCTION OF PHYSICAL DEFENSE TRAITS IN RESPONSE TO DAMAGE

INTRODUCTION

Herbivory, which is the consumption of plant tissue by animals (Schoonhoven and Dicke, 2005), has been postulated to be a major driving force in the evolution and diversification of plants (Ehrlich and Raven, 1964; Hairston *et al.*, 1960; Niklas, 1997). Indeed, plant-herbivore interactions have attracted considerable interest for many years (Mcnaughton, 1983; Karban and Myers, 1989; Herms and Mattson, 1992; Strauss *et al.*, 2002; Boege and Marquis, 2005; Howe and Schaller, 2008), and there is a considerable body of research on the chemical (alkaloids, cyanogenic glycosides, tannins, etc.) and physical (spinescence, pubescence, sclerophylly, etc.) attributes that render plants less preferred by herbivores and better able to reproduce than plants lacking those attributes (Karbon and Baldwin, 1997; Schoonhoven *et al.*, 2005). Yet, despite this extensive body of research, comparatively little is known about patterns of variation in physical defense traits compared with chemical defenses (Hanley *et al.*, 2007). In particular, very little is known about whether plants can alter expression of physical defenses in response to herbivory in a process called “induction”, and it is this topic that will be the focus of this review.

PHYSICAL DEFENSE TRAITS

Physical defenses are defined as any morphological or anatomical trait that confers a fitness advantage to the plant by directly deterring herbivores from feeding (Coley, 1983). Some physical defense traits are structural in nature (spines, thorns, prickles, cellulose, lignin), and some, such as latex, are not (Hanley *et al.*, 2007). These traits are united by their ability to physically decrease feeding behavior in vertebrate and invertebrate herbivores.

Trichomes are pubescent or hair-like outgrowths from the epidermis that form a dense boundary layer between plant tissue and the environment (Levin, 1973), and are found on plant leaves, stems, and fruits (Woodman and Fernandes, 1991). The morphology and density of trichomes vary considerably among plant species. They can be unicellular, multicellular, straight, hooked, glandular, or spiral shaped, and can release chemical defenses (Hanley *et al.*, 2007). Trichomes are effective defenses against insect feeding, maneuverability, and oviposition by creating a boundary between the insect and the leaf surface (Dalin *et al.*, 2008). Glandular trichomes that release chemical defenses are also effective against large mammalian herbivores (Pollard and Briggs, 1984). In addition to defense, trichomes perform ecophysiological functions (Bell and Bryan, 2008) such as mitigating water loss and heat gain (Levin, 1973; Woodman and Fernandes, 1991; Gutschick, 1999), and protection from UV radiation (Karabourniotis and Bornman, 2002; Melcher *et al.*, 1994; Manetas, 2003).

Plant spinescence is a collective term for spines, thorns, and prickles (Campbell, 1986). Spines are sharp petioles, midribs, veins, or stipules (Young and Okello, 1998); thorns are woody, sharp-pointed branches (Milewski *et al.*, 1991); and prickles are sharp outgrowths from the epidermis that rarely contain vasculature (Bazely *et al.*, 1991). Due

to their large size most forms of spinescence are recognized as a defense against larger herbivores, and a number of studies have confirmed that thorns, spines, and prickles are efficient in deterring mammalian herbivores (Milewski *et al.*, 1991; Gibson *et al.*, 1993; Milewski and Madden, 2006; Zinn *et al.*, 2007). In contrast to spines and thorns, prickles can protrude from the leaf lamina and cover stems leaves, and reproductive tissues, making them somewhat more similar in structure to trichomes than spines and thorns.

Latex is a very common means of defense among plants (Pickard, 2008; Agrawal and Konno, 2009), with over 20,000 flowering plant species containing latex (Agrawal and Konno, 2009), including 143 Hawaiian species (Barton, unpublished data). Latex is an aqueous slurry of compounds, many being toxic (terpenoids, phenolics, alkaloids, etc.), and may be white, yellow, orange, red, or brown in color (Pickard, 2007). Despite the toxicity of latex, it also serves as a physical defense (Becerra *et al.*, 2001). Latex is capable of trapping and asphyxiating herbivores (Dussourd and Hoyle, 2000), and can deter further feeding in insects by gumming up mouthparts (Dussourd and Eisner, 1987; Agrawal, 2004; Konno *et al.*, 2004). Separate from the vascular system, latex is contained in living cells (or files of cells) called laticifers (Hagel *et al.*, 2008; Agrawal and Konno, 2009). Latex is contained within these structures under considerable pressures, presumably due to osmotic water uptake (Pickard, 2008). When these structures are damaged by herbivory or by abiotic stress, latex is exuded in copious amounts and quickly coagulates, becoming sticky and eventually hardening to seal the wound site (Agrawal and Konno, 2009). Laticifers and its contents have been acknowledged to serve primarily as a means of defense against herbivory (Dussord and Eisner, 1987).

Leaf toughness is a measurement of how well a leaf is able to withstand biotic or abiotic damage, which can be maximized by the structural strengthening of cell walls with increased synthesis of cellulose, lignin, callose, as well as other substances (Hanley *et al.*, 2008). Although these defenses emerge from chemical synthesis, these carbon-based defenses (as opposed to secondary phenolic compounds) physically disrupt chewing behavior, and are thus considered physical defenses (Coley, 1983). This toughening of plant tissues negatively affects the ability of herbivorous insects to pierce or chew through plant tissue (Howlett *et al.*, 2001), and has been considered to be the most effective defense against herbivores (Kursar and Coley, 1991). The most appropriate way to measure leaf toughness has been the subject of considerable debate (Read and Sanson, 2003). However, a recent study that assessed different methods reports that leaf mass area (LMA= cm^2/g) and thickness correlate well with most other metrics and is suggested to be the ideal measure of toughness (Kitajima *et al.*, 2012).

INDUCIBLE RESPONSES TO HERBIVORY

Until relatively recently, plant defenses were generally thought to be constitutive, or always expressed in the plant at a fixed level (Karban and Baldwin, 1997). Constitutive defenses may increase or decrease at a relatively steady rate as a plant ages (Boege and Marquis, 2005) and may change in response to varying abiotic conditions (Macedo *et al.*, 2011). Within the past 40 years, however, it has been well documented that, just as plants are able to alter their phenotypes in response to abiotic conditions (light, nutrient, and water availabilities), plants are also very plastic in response to biotic factors, such as herbivory (Karban and Baldwin, 1997; Heil, 2010). Changes in plant

defense phenotypes following damage are called “inducible responses”, and these responses are so widespread and so important for understanding the evolution of plants as well as applied topics such as agricultural yield and transgenic crops, that there have now been over 12 books published on the topic (Crawley, 1983; Palo and Robbins, 1991; Karban and Baldwin, 1997; Agrawal *et al.*, 1999; Chadwick and Goode, 1999; Tollrian and Harvell, 1999; Cherry *et al.*, 2000; Herrera and Pellmyr, 2002; Schoonhoven and Dicke, 2005; Walters *et al.*, 2007; Schaller, 2008; Zobel, 2012). In general, inducible responses can be separated into two categories: 1) Changes in the expression of resistance traits, such as chemical toxins or physical deterrents that alter future interactions with herbivores; and 2) Changes in traits related to growth and reproduction that contribute to the ability of plants to recover from and mitigate the costs of damage, leading to plant tolerance of herbivory.

Inducible defenses are thought to be favored as a way to save costs in defense against herbivores that may only be a temporary threat to a plant (Obeso, 1997; Karban and Baldwin, 1997; Holeski *et al.*, 2010). When herbivores are not present, plants that have strong plasticity in defense traits can allocate resources to growth and reproduction, rather than defense, and increase defenses only when needed (Karbon and Baldwin, 1997; Obeso, 1997; Agrawal, 1999; Karban, 2011).

INDUCTION OF PHYSICAL DEFENSE TRAITS

Most studies of inducible defenses have focused on plant chemistry and far fewer studies have looked at the induction of physical defense traits (Howe and Schaller, 2008; Karban, 2011; Wu and Baldwin, 2010), despite the relative ease with which physical

defense can be measured and manipulated, making them ideal for the study of inducible responses (Young, 1987; Gomez and Zamora, 2002; Traw, 2003). Nonetheless, there have been several studies documenting induction of physical defenses, indicating that physical defenses are inducible.

Different forms of spinescence have been induced in a number of studies, many of which have responded to natural herbivory or after mechanical removal of leaves or shoots. Increased length, as well as increased numbers of thorns and spines in response to herbivory has been documented (Milewski *et al.*, 1991; Cooper *et al.*, 2003; Zhang, 2006; Hean and Ward, 2011). For example, Young (1987) found that thorns of *Acacia drepanolobium* trees were 27% longer on trees that were recently exposed to browsing goats than on trees that were protected from herbivory, and Gowda (1997) reported a higher production of spines in *Acacia tortilis* in response to mechanical pruning. Young and Okelo (1998) removed herbivores from *A. drepanolobium* plots and found that thorn length was reduced by 19% within 22 months of being protected from herbivores, and by 40% after being protected for 5 years, providing evidence that physical defenses also relax in response to lower herbivory (Karban and Baldwin, 1997).

While most studies on the inducibility of spinescence have resulted in significant increases in either the length, weight, or density of spines, thorns, and prickles (Young, 1987; Bazely *et al.*, 1991; Zhang *et al.*, 2006), some have failed to induce any responses. Gadd *et al.* (2001) found no induction in spine numbers in *A. drepanolobium* after mechanically removing plant biomass. Cooper *et al.* (2003) found that *Prosopis glandulosa* did not increase spine numbers when damaged, although spine increases were documented in 3 species of *Acacia* under the same damage treatments. In contrast, *A.*

drepanolobium and *P. glandulosa* were both found to tolerate herbivory by increasing growth rates in order to compensate for lost tissue (see *Induced Tolerance in Response to Herbivory* in this chapter).

To my knowledge, only two studies have investigated the induced responses of prickles to damage. Bazely *et al.* (1991) reported increased prickle numbers in response to herbivory in *Rubus fruticosus agg.*, in response to mechanical leaf removal, and Gibson *et al.* (1993) found increased prickle numbers after leaf removal and meristem damage. Clearly, additional tests are needed to investigate the induction of prickles.

Like spinescence, leaf toughness has also been found to increase in response to mechanical damage (Kudo, 1996; Liu *et al.*, 2010), and these responses likely vary with the degree on damage inflicted; Kudo (1996) found that removal of 75% leaf area resulted in higher increases in LMA than plants treated to 50% leaf area removal.

Of all physical defense traits, most studies have focused on the induction of trichomes (Dalin *et al.*, 2008- examples within), and these studies have greatly increased our knowledge of the specifics and intricacies of inducible responses to herbivory. Many induced responses have been found to be very specific to particular cues, with some plants responding to damage from certain herbivores, but not others (Traw and Dawson, 2002; Massey *et al.*, 2007). Traw and Dawson (2002) found that *Brassica nigra* (L.) Koch responded to damage from *Pieris rapae* with a 75% increase in trichome densities, while trichome densities failed to be induced after the same amount of damage was inflicted from two other herbivores. Dalin and Bjorkman (2003) found that herbivory by beetles induced higher densities of trichomes in *Salix cinerea* L than mechanical damage of the same degree of tissue removal. Indeed, removing plant tissue mechanically using

scissors or hole punchers (Pullin and Gilbert, 1989; Wright and Bonser, 1999; Abdala-Roberts and Para-Tabla, 2005; Holeski, 2007) may not fully replicate natural herbivory (Baldwin, 1990, 1996; Agrawal, 1999; Röse and Tumlinson, 2005), thus failing to induce a response in plants (Gadd *et al.*, 2001; Massey *et al.*, 2007; Wu and Baldwin, 2010). Specificity in plant induced responses is considered to be a critical ability for a plant to survive among herbivores (Agrawal 1999, Massey *et al.*, 2007, Reymond *et al.*, 2000), so it is not surprising that plants can discern herbivory from casual wounding caused by physical disturbance (Green and Ryan, 1972; Baldwin, 1996; Agrawal, 1999; Mithöfer *et al.*, 2005; Röse and Tumlinson, 2005).

Many induced defense mechanisms are mediated by the octadecanoid signaling pathway that results in the synthesis of the plant hormones, jasmonic acid and methyl jasmonate (Baldwin, 1988; Cipollini, 2010). While the use of natural herbivory or the use of herbivore saliva or oral secretions result in the most ideal test for the study of inducible responses in plant defense traits (Mithöfer *et al.*, 2005; Massey *et al.*, 2007), it is well agreed upon that treatment of plants with the defense hormone jasmonic acid is a suitable method of inducing responses similar to those induced by natural herbivores (Traw and Bergelson, 2003; Kleunen *et al.*, 2004; Cipollini, 2010; Kobayashi *et al.*, 2010; Kruidhof *et al.*, 2012). Spraying plants with jasmonic acid has resulted in the nine-fold increase in trichome densities in tomatoes (Boughton *et al.*, 2005). Kobayashi *et al.* (2010) found that increasing the concentration of methyl jasmonate from 0.2 mM to 0.4mM resulted in a proportionate increase in trichome densities. Agrawal (1999) found that the effects of damage and jasmonic acid on induced chemical defenses summed to the effect of natural herbivory, and van Dam and Baldwin (2001) found that the combination of both

(damage + jasmonic acid) has an even stronger effect than the sum of each treatment separately. In a study by Rasmann *et al.*, (2009), both jasmonic acid and natural herbivory by *Danaus plexippus* increased latex exudation in *Asclepias fascicularis* by 30%. Using jasmonic acid may be a critical tool in studying plant-herbivore interactions from the plant's perspective, especially when the herbivore is extinct, unknown, or difficult to work with in a laboratory setting.

INDUCED TOLERANCE IN RESPONSE TO HERBIVORY

In addition to the induction of chemical and physical defense traits, plants typically alter traits involved in primary metabolism, growth, and reproduction as part of their responses to herbivory (Fornoni, 2011; Strauss and Agrawal, 1999; Stowe *et al.*, 2000). While these changes typically do not alter future herbivory, they can contribute to the ability of plants to tolerate damage, and are thus part of plant defenses.

The induction of tolerance traits has been the focus of much less research than that of induced chemical and physical defense traits. Thus, while it has been shown that multiple traits may change in expression following herbivory, it remains unclear which changes are most common (Fornoni, 2011; Tiffin, 2000). Induced tolerance traits include: increases in photosynthesis (Thompson *et al.*, 2003), altered *root:shoot* ratios (Stevens *et al.*, 2008), mobilization of stored reserves (Latzel *et al.*, 2011), and shifts in phenology (Freeman *et al.*, 2003). Clearly, additional research is needed to establish which induced traits are most important for plant tolerance to herbivory, and because damage induces both chemical/physical defense traits simultaneously with tolerance traits

(Heil, 2010), it is important for studies to examine both kinds of responses at the same time.

CONCLUSIONS AND EXPERIMENTAL GOALS

Plants possess a wide array of physical defense traits in order to defend themselves against herbivores. While these traits are often effective in mitigating tissue loss, they are very expensive and require resources that could be allocated to growth and reproduction. Several plant species have been found to mitigate these costs by increasing the production of physical defense traits in response to herbivory. Due to the relatively small amount of research on the topic, as well as the disproportionate amount of attention paid to the induction of certain physical traits over others, it is very difficult to synthesize general patterns and conclusions about the induction of physical defense traits in response to herbivory. The effect of damage on the production of resistance traits may vary greatly depending on the species of herbivore, the type of artificial wounding, the extent of damage done, etc. Therefore, ecologists aiming to measure the inducibility of plant defense traits should do so using a wide array of natural and simulated herbivory in order to capture a broader response pattern. Additionally, studies should include measurements of tolerance, genetic variation in responses, and variability of these responses throughout plant ontogeny. Such integrated research will enrich our knowledge of plant and herbivore interactions, and how plant inducible responses to herbivory may have evolved.

While it has been predicted that plants endemic to the Hawaiian Islands lack defenses (Ziegler, 2002), many species have just as many trichomes as their continental relatives and are higher in leaf toughness (Funk *et al.*, 2010), more than 100 native plants

species exude latex (Barton, unpublished data), and a few also have prickles (Wagner *et al.*, 1999). *Argemone glauca* (Nutt. Ex Prain) Pope (Papaveraceae) is an island endemic that seems to be highly defended against herbivores (Ownbey, 1961). It exudes copious amounts of latex, is covered in dense, sharp prickles, and has very thick and glabrous leaves (Ownbey, 1961; Barton, in press). Native herbivores of *A. glauca* are most likely extinct and it has been speculated that prickles may be an ecophysiological adaptation to UV radiation in this species (Barton, in press). However, inducing responses in defense traits or in tolerance in this species may provide evidence that this species is well defended against a recently extinct herbivore. Indeed, Hawaii is rich in native and exotic insect herbivores and was once plentiful in native herbivorous birds (Ziegler, 2002).

The primary goal of my research is to provide a novel test of the induction of physical defenses using 2 species of *Argemone* as a model system. I investigated the responses of prickles, leaf toughness and latex to mechanical defoliation and jasmonic acid. To gain insights into variation in the induction of physical defenses in *Argemone*, I also tested how these responses varied genetically among maternal sibships and between populations on different islands, and across plant ontogeny. To my knowledge, this is the first test of induction by jasmonic acid of prickles, and the first examination of induced responses to damage in a native Hawaiian plant.

CHAPTER 2. INDUCIBLE RESPONSES TO DAMAGE AND JASMONIC ACID IN TWO SISTER SPECIES OF ARGEMONE (PAPAVERACEAE)

ABSTRACT

Plants have evolved a diverse array of mechanisms to reduce herbivory and the consequences of damage on plant performance, and some plants have a considerable ability to respond to herbivory, both with tolerance and increased defense. We investigated the effect of 50% defoliation and of jasmonic acid application on the induction of putative physical defense traits as well as mechanisms of tolerance in *Argemone mexicana* and in two populations and ontogenetic stages of *Argemone glauca*. *A. glauca* significantly increased prickle densities in response to treatments, *A. mexicana* increased leaf toughness and showed a higher ability to compensate for biomass loss than *A. glauca*. Leaf latex exudation did not change in response to treatments in either species. Variation between populations and ontogenetic stages were detected in physical defense traits at constitutive levels, but not in inducible responses. This study is the first to induce prickle densities in a plant using jasmonic acid as a form of simulated herbivory, and is the first to induce responses in defense traits in a Hawaiian endemic.

INTRODUCTION

Induced responses to herbivory are widespread and common, having been documented in hundreds of plant species (Karban and Baldwin, 1997; Dalin *et al.*, 2008). Induced responses may occur in deterrent traits such as the production of secondary compounds, leading to increases in the chemical resistance of plants following herbivory (Karban, 2011), and in traits related to growth and reproduction, contributing to the tolerance of plants to damage (Fornoni, 2011). Extensive research has examined induced responses to herbivory and documented that induced resistance may provide cost-savings that allow plants to allocate limited resources to growth and reproduction when defenses aren't needed (Strauss *et al.*, 2002; Cipollini *et al.*, 2003; Stamp, 2003; Orians *et al.*, 2011), and that tolerance can allow plants to quickly compensate for lost tissue and maintain fitness when herbivory cannot be avoided (Agrawal *et al.*, 1999; Strauss and Agrawal, 1999; Núñez-Farfán and Fornoni, 2007; Fornoni, 2011). Although induced responses appear to be a general response to herbivory, there are nonetheless examples of species that are not induced by herbivory (Gadd *et al.*, 1991; Ruiz *et al.*, 2002; Traw and Dawson, 2002; Dalin *et al.*, 2004; Valkama *et al.*, 2005).

Inducible response traits have been studied in the field and in laboratory settings by measuring changes in plant traits, or by measuring the performance of herbivores after plants have been damaged (Karban and Baldwin, 1997). Damage treatments have been applied to plants either by using natural herbivores (Baur *et al.*, 1991; Agrawal *et al.*, 1999), by mimicking herbivore damage mechanically (Traw and Bergelson, 2003; Pullin

and Gilbert, 1989), or more recently, by applying defense-related hormones such as jasmonic acid directly to plant tissues (Strauss and Agrawal, 1999; Boughton *et al.*, 2005; Traw, 2003). However, tests using simulated herbivory may not accurately represent how the plant responds to natural herbivory because plants have been found to respond to chemical cues present in the oral secretions and saliva of herbivores (Felton, 2008; Heil, 2009). Plants may then mount specific responses to particular herbivores defend themselves accordingly (Karban and Baldwin, 1997; Dalin and Bjorkman, 2003). Clean clipping with scissors is unlikely to release minimal internal cues, and thus the plant responses will likely be relatively weak (Karban and Baldwin, 1997). When natural herbivory is not possible, using a combination of mechanical wounding and defense hormones have proven to be a good alternative because the defense hormones can activate plant responses even in the absence of elicitor cues (Agrawal *et al.*, 1999; van Dam and Baldwin, 2001; van Kleunen *et al.*, 2004).

Increased defense in response to herbivory can't completely prevent damage and plants still suffer the consequences, which may take the identity of a lower fitness. Thus, the survival and fitness of a plant also depends on the capacity to overcome the effects of tissue removal (Boege and Marquis, 2005). Tolerance, or compensation, is the ability of plants to sustain tissue loss with little or no decrease in growth and fitness, compared to an undamaged plant (Strauss & Agrawal 1999). Like inducible defenses, tolerance protects a plant from the negative effects of herbivory and includes a multitude of mechanisms that are often increased in response to herbivory, and requires the re-allocation of resources and other costs. These mechanisms include increases in photosynthetic capacity (Thompson *et al.*, 2003; Johnson *et al.*, 2007), growth rates

(Mcnaughton, 1983; Stowe *et al.*, 2000), mobilization of stored reserves (Stevens *et al.*, 2008), and changes in physiology and phenology such as activation of dormant meristems and earlier flowering and fruiting (Fornoni, 2011).

While considerable research has examined induced defenses, the vast majority of this research has focused on chemical defense traits (Karban *et al.*, 1997; Chen, 2008; Agrawal, 2011). In contrast, little is known about the induction of physical defenses such as spinescence, leaf latex, and sclerophylly, despite the relative ease with which they can be measured and manipulated (Baur *et al.*, 1991; Gowda, 1997; Young and Okello, 1998; Agrawal, 1999; Boughton *et al.*, 2005).

Spinescence includes the structural defense traits spines, thorns, prickles and trichomes (Hanley *et al.*, 2007). Unlike spines and thorns, prickles and trichomes are emergences or outgrowths of epidermal tissue and rarely contain vasculature (Bell *et al.*, 2008) and may also perform similar ecophysiological functions such as mitigating water loss and heat gain (Levin, 1973; Gutschick, 1999; Press, 2002;), and may protect plants against UV radiation (Manetas, 2003; Jordan *et al.*, 2005).

Latex is a milky, often toxic substance that is contained in living cells called laticifers (Hagel *et al.*, 2008). When these structures are damaged by herbivory or by abiotic stress, latex is exuded copiously and quickly coagulates, becoming sticky and eventually hardens to seal wound sites, as well as the mouthparts of herbivorous arthropods (Agrawal and Konno, 2009). Therefore, laticifers are considered to serve as both chemical and physical defenses because they may poison, as well as entrap, suffocate, and impede the movement of herbivorous insects (Southwood, 1986; Becerra, 1994; Huang *et al.*, 2003; Hagel *et al.*, 2008).

Sclerophylly, or the strengthening of cell walls by increased amounts of cellulose, lignin, silica, as well as other substances, impedes the ability of herbivorous insects to pierce or chew through plant tissue (Howlett *et al.* 2001), and has been suggested by some to be the most effective defense against herbivores (Kursar & Coley 1991). Also commonly known as leaf toughness (Kudo 1996), sclerophylly can be estimated in a number of ways, including the ratio between leaf dry mass and leaf area (LMA), which is highly correlated with toughness (Kudo 1996; Hanley *et al.* 2007; Kitajima *et al.*, 2012).

In general, physical defenses are thought to be very expensive to express because of the large investment in structural carbohydrates needed to construct them (Gibson *et al.*, 1993; Gomez and Zamora, 2002). It thus seems likely that plants would benefit from having physical defense traits be inducible in order to reduce construction costs until needed. On the other hand, because the induction of physical defenses requires the development of new tissues with higher densities of the physical defense traits (e.g. leaves with higher densities of prickles), there is an inherent time lag to physical defense induction, which may reduce its effectiveness at deterring herbivores in ecological time. Previous studies have revealed the induction of spines (Pisani, 1999; Gadd *et al.*, 2001; Takada *et al.*, 2001), thorns (Young, 1987; Gowda, 1997; Gomez, 2002), and trichomes (Dalin *et al.*, 2008- studies reviewed within), but relatively few studies have been conducted on prickles (Bazely *et al.*, 1991; Gibson *et al.*, 1992), latex exudation (van Zandt and Agrawal, 2004; Rasmann *et al.*, 2009), and sclerophylly (Liu *et al.*, 2010). Because physical defenses are found in a large diversity of plant species and have been shown to mediate interactions with herbivores ranging from large mammals to small

insects (Hanley *et al.*, 2007), it is important that we gain a better understanding of the inducibility of these traits.

The prickly poppies of the genus *Argemone* (*Papaveraceae*) are a great example of plants that display a suite of putative physical defense traits, including prickles, latex and a thick waxy cuticle giving leaves a glaucous appearance (Ownbey, 1961). *Argemone* consists of 30-32 species native to the Americas, with one species endemic to Hawaii, the Hawaiian prickly poppy, *Argemone glauca* (Nutt. Ex Prain) Pope (Schwarzbach and Kadereit, 1999). These annuals and short-lived perennials are found in warm, dry climates, and some are considered to be weedy pests throughout the world (Kingsbury, 1964; Holm *et al.*, 1977, 1979). Very little ecological research has examined expression patterns of prickles, latex and leaf toughness in *Argemone*, the only notable exception being work done on the Mexican poppies *Argemone Mexicana* L. and *Argemone ochroleuca* Sweet, because of their detrimental effects as worldwide invasive pests (Al-Hayyan, 2006; van der Westhuizen and Mpedi, 2011; Moussa *et al.*, 2012; Singh *et al.*, 2012). Latex of certain prickly poppies have been studied for their medicinal (Parsons and Cuthbertson, 1992) and antimicrobial (Reyes *et al.*, 2011) potential, and has been found to be fatal to livestock (Kingsbury, 1964), snails (Melendez, 2002), neighboring tomato plants (Shaukat, 2002), and bacteria and fungi (Reyes *et al.*, 2011; Osho, 2010). Consumption of seeds and tissue of *A. mexicana* has led to illness and even death in humans (Steyn, 1950; Verma, 2001). Only a few species of seed-eating beetles (*Curculionidae*) are considered to be effective at reducing populations of invasive prickly poppy species throughout the world (Goeden and Ricker, 1985; Westhuizen and Mpedi, 2011), and it has been suggested that no insect is able to

overcome the chemical and physical defenses that prickly poppies possess (Goeden and Ricker, 1985).

Recent research on *A. glauca*, has revealed that the expression of prickles and latex varies significantly between populations, across ontogeny, and due to phenotypic plasticity in response to water and light availability (Barton, in press). However, previous efforts to quantify induced responses to damage in *A. glauca* have provided mixed results (Barton, in press). Mechanical defoliation to simulate herbivory led to a significant decrease in latex in plants from a population on Oahu while plants from Maui responded by increasing latex. Prickles were not induced in either population. Tolerance via compensatory growth was found in response to mechanical defoliation, but plants from Oahu suffered from reduced flowering when damaged. Clearly, additional research is needed to elucidate the induced responses to damage in *A. glauca*.

The current study expands upon previous research by including a sister-species comparison of *A. glauca* with *A. mexicana* (Schwarzbach and Kadereit, 1999), as well as through the addition of hormonal signaling treatments in order to better simulate herbivory. In particular, this study addressed the following questions:

- 1) How do the patterns of expression in putative defense traits of an island endemic, *A. glauca*, differ from its sister species and worldwide pest, *A. mexicana* (Westhuizen and Mpedi, 2011)? Because island plants are generally predicted to have weakened defense due to the absence of some herbivore guilds on islands (Ziegler, 2002), I predict that the expression of prickles, latex and leaf toughness will be higher in *A. mexicana* than *A. glauca*.

- 2) Are prickles, latex and leaf toughness inducible in *A. glauca* and *A. mexicana*? Due to the cost-savings hypothesis (Herms and Mattson, 1992; Karban and Baldwin, 1997; Stamp, 2003), I predict to find that these defense traits are inducible in both species.
- 3) Are traits related to tolerance inducible in *A. glauca* and *A. mexicana*? Given the prediction that tolerance may be related to the establishment and spread of invasive plants (Rogers and Siemann, 2005; Raghu *et al.*, 2006), I predict that tolerance will be higher in *A. mexicana* than *A. glauca*.
- 4) Is there genetic variation within and between populations of *A. glauca* and *A. mexicana* in the expression of constitutive and inducible defense traits? In order for induction to evolve by natural selection, there must be genetic variation on which selection must act. Although not always examined, there is ample evidence that the induction of resistance and tolerance traits are genetically variable (Pilson, 2000; Agrawal *et al.*, 2002; Dobson, 2010; Fornoni, 2011). Thus, I predict to find genetic variation in all traits examined in both species.
- 5) Is there an ontogenetic pattern to the expression of structural defense traits in *A. glauca*? Because herbivory intensity and plant allocation priorities (among defense, growth and reproduction) generally change dramatically as plants develop, ontogenetic patterns in plant defense are common (Boege and Marquis, 2005; Barton & Koricheva, 2010). Although ontogenetic patterns have rarely been previously examined in prickles,

studies on thorns and spines have generally documented a decrease with plant age, which is thought to occur as plants outgrow the ground-dwelling herbivores that feed on young plants (Gowda and Palo, 2003; Boege and Marquis, 2005; Boege *et al.*, 2011). Because *A. glauca* is not likely to outgrow herbivores as they get older, I have no clear prediction for whether prickles should decrease or increase across ontogeny.

MATERIALS AND METHODS

SAMPLING SUMMARY

Seeds of *A. glauca* and *A. mexicana* were collected from the islands of Maui (2010) and Hawaii (2011). On Maui, seeds of *A. glauca* were collected throughout the Maui Nui Botanical Garden, where established and naturally regenerating populations receive no particular cultivation or care. Seeds of *A. mexicana* were collected from naturalized populations along Pulehu road in Kula. On Hawaii isle, *A. glauca* was collected from three distinct populations and *A. mexicana* was collected from a single population, all on the west side of the island. On both islands, seeds were collected from plants at least 2 m apart and often more than 10 m apart. Seeds were stored separately for each maternal sibship, which constitutes a single “genetic family.”

EXPERIMENTAL DESIGN

Seeds were soaked in tap water for 36 hours to facilitate germination (Lilleng-Rosenberger, 2005) and germinated in flats filled with equal parts Promix BX© (67-75% Canadian sphagnum peat moss, perlite, dolomitic and calcitic limestone, macro- and

micronutrients, *Glomus intraradices mycorrhizae inoculum*) and black cinder. A few genetic families of *A. glauca* experienced an early flush of seedling emergence, then tapered off to a few new seedlings per week, which continued for several months. *A. exicana* and other genetic families of *A. glauca* had poor germination rates. Seedlings with at least one true leaf were transplanted into gallon pots filled with equal parts Promix BX and black cinder and were supplemented with a single application of slow-release fertilizer (Osmocote©).

Experiments were conducted in an open-air grow area attached to the St John Plant Sciences Building on the UH-Manoa campus. Plants were exposed to full sun and precipitation, but were provided with supplementary water daily. Plant location was re-randomized every week in order to minimize the effects of environmental variation such as sun or wind exposure. Because of variation in the germination rates of *A. glauca* and *A. mexicana*, induction was examined in two separate experiments. The first experiment, conducted June 15-July 07 2012, focused on *A. glauca* and tested whether prickles, latex, leaf toughness and photosynthesis are inducible by mechanical defoliation and jasmonic acid application in seedlings versus juvenile plants. Seedlings were identified as plants with cotyledons (Hanley *et al.*, 2007- end of seedling phase) and were approximately two weeks of age. Juvenile plants were identified as older plants in which cotyledons are no longer present, but which are in the vegetative rosette stage before the onset of reproduction (Poethig, 1990). Plants from 19 genetic families sampled from Maui (n=15) and Hawaii (n=4) were examined, giving a total sample size of N=339.

The second experiment focused on the species comparison of *A. glauca* versus *A. mexicana* and included plants in the juvenile stage. All plants were from the island of

Hawaii (n=3 genetic families per species) and there was no overlap with genetic families used in experiment one. This experiment began ten days after experiment two was completed (July 17-August 01 2012). Replication per treatment group within genetic families ranged from 2-3 plants, giving a total sample size of N=67 plants.

For both experiments, plants were randomly assigned to the following four treatment groups: (1) **Control** (no damage), (2) **damage** (50% defoliation), (3) **JA** (jasmonic acid application), and (4) **dam + JA** (50% defoliation and jasmonic acid application). In damage groups, the distal half of each leaf and cotyledon was removed with scissors, leaving the newest fully expanded leaf undamaged in order to measure photosynthetic rates shortly following treatments. Excised leaf tissue was removed from pots in order to avoid any consequential nutrient input.

For plants receiving jasmonic acid application, leaves were sprayed with 0.5-mmol jasmonic acid solution, (Sigma Aldrich). Plants receiving jasmonic acid were temporarily transported downwind from the experiment site and sprayed until the lower surfaces of the leaves were saturated. The upper leaf surface of both *A. glauca* and *A. mexicana* proved difficult to saturate due to the presence of thick epicuticular waxes. Plants that were not receiving jasmonic acid treatments were sprayed with water until dripping to control for possible effects of spraying on induction.

Potential photosynthetic responses to simulated herbivory treatments were measured three days after treatments. Measurements were conducted at room temperature (approximately 70° Fahrenheit) and were taken on the newest fully expanded leaf (or the one left undamaged on damaged plants) of every plant using Walz Jr. PAM©. Three different parameters were taken: (1) photosynthetic light use efficiency, or the

amount of carbon gained per unit of light absorbed (also called potential quantum yield; Y_0), (2) photosynthetic capacity, the maximum photosynthetic electron transport rate under saturating light (ETR_m), and (3) the amount of excess light dissipated as heat via the xanthophyll cycle (non-photochemical quenching, NPQ). Photosynthetic readings were conducted during clear and sunny conditions between the hours of 9 A.M and 1 P.M. HST. Due to their small size, it was often impossible to measure photosynthesis on some of the seedlings.

The final harvest date occurred when each plant had developed at least two new leaves (14 days after treatment for Experiment one, 16 days for Experiment two). Because the next leaf to develop after treatment applications had generally already begun to expand at the time of treatments, the second leaf to expand following treatments was assayed for induction. Measurements were all made on this same leaf. Traits measured include the amount of latex exuded by the leaf (mg), fresh weight of leaf (g), total prickles count for upper (adaxial) and lower (abaxial) leaf surfaces, and leaf area (cm^2). Latex amount was quantified by cutting the distal tip of the leaf and collecting the exuded latex onto a filter paper of known weight. Because Papaveraceae is characterized by having articulated laticifers that likely obstruct latex from fully draining out of the leaf tip (Hagel *et al.*, 2008), latex was also collected on the same filter paper after removing the leaf at the leaf base from the stem. The filter paper was then enclosed in a pre-weighed plastic vial and immediately weighed. The difference between the filter paper with latex and the pre-weighed filter paper represents latex amount (mg). The excised leaf used to collect latex was then examined under 10x magnification to quantify prickles density. All prickles covering the adaxial and abaxial surfaces of the leaf were counted using a click

counter. Prickles found on the leaf edges were not counted. A digital photo was then taken of the leaf and a ruler for scale, and leaf area was quantified using ImageJ. Prickle density was calculated as the number of prickles/leaf area for both the adaxial and abaxial leaf surfaces. All aboveground tissue including shoot and newest fully expanded leaf were harvested and oven-dried at 60°C for several days before dry weights were measured to the nearest 0.01 mg. Leaf dry mass per unit area (LMA) was calculated and examined as a measure of leaf toughness.

STATISTICAL ANALYSES

Statistical analyses for experiment one and two were conducted using SAS for Windows version 9.2 PROC MIXED (Cary, North Carolina). Residuals were examined for each variable, and data were log-transformed as needed to meet assumptions of normality and homoscedasticity. Type III sums of squares are reported for all analyses. Response variables analyzed in both experiments included: shoot biomass (g), leaf mass area (LMA, g/cm²), latex amount (g), adaxial prickle density (number of prickles/cm²), abaxial prickle density (number of prickles/cm²), photosynthetic light efficiency (Y_0), the maximum photosynthetic electron transport under saturating light (ETR_m), and non-photochemical quenching (NPQ). Photosynthetic parameters were first analyzed jointly with multivariate ANOVAs (MANOVA), and if significant, each variable was subsequently analyzed with a univariate ANOVA.

In experiment one, each variable was analyzed with a mixed-model ANCOVA that included the following factors: plant ontogenetic stage (seedling, juvenile), island population, genetic family nested within island, and treatment group (control, damage,

jasmonic acid, damage + jasmonic acid). In addition to plant ontogenetic stage, plant size was accounted for by including the number of leaves at harvest time as a covariate.

Genetic family was considered a random variable, and the significance of family and all interactions with family were tested by running the models with and without the random factor of interest, and then calculating the log-likelihood ratio statistics, which can be compared to a chi-square distribution with one degree of freedom (Littell *et al.*, 1996).

In experiment 2, data were analyzed with mixed-model ANCOVAs that included the following factors: plant species, genetic family nested within species, treatment group and leaf size as a covariate. Genetic family and interactions with genetic family were analyzed as random factors as in experiment one. For all analyses, significant effects of treatment group on defense traits would reveal induction of these traits. Tukey-adjusted least-square mean comparisons were used to identify patterns of induction. For example, a significant difference in prickles between control plants and those in the damage group would reveal the induction of prickles by mechanical defoliation. A significant difference between damage vs. damage + jasmonic acid groups would reveal the effect of jasmonic acid in the induction of traits over and above that caused by mechanical damage. Significant variation among genetic families and between islands would indicate genetic variation, and significant interactions between genetic factors (family and island) and treatment would reveal genetic variation in induction.

RESULTS

EXPERIMENT ONE

Overall, there were mixed results in the induction of physical defense traits in response to treatments (Table 1). Significant differences in prickles densities between treatment types were detected in both adaxial ($F_{3,299}=6.03$, $P=0.0005$) (Fig. 1A) and abaxial ($F_{3,308}=4.91$, $P=0.0024$) (Fig. 1B) leaf surfaces, while there was no differences in leaf latex exudation ($F_{3,289}$, $P=0.1428$) (Fig. 1C) or LMA ($F_{3,304}$, $P=0.2298$) (Fig. 1D) in response to treatments. Adaxial prickles densities increased significantly in response to damage + JA treatment (Fig. 1A): The combined application of 50% defoliation and jasmonic acid application resulted in the highest increased in prickles densities, 1.5x higher than control plants (Tukey-adjusted least-square mean comparison $P=0.0005$), and significantly higher than both damage (Tukey-adjusted least-square mean comparison $P=0.0041$) and JA (Tukey-adjusted least-square mean comparison $P=0.0174$) treatments. Abaxial prickles densities (Fig. 1B) were 1.31x higher in plants that received damage + JA treatment than control plants (Tukey-adjusted least-square mean comparison $P=0.0010$).

A. glauca did not fully compensate for loss in biomass (Fig. 1E). The application of jasmonic acid on plants appeared to have a negative effect on growth rates in *A.*

glauca: While differences in shoot biomass were due to 50% defoliation treatments (Fig. 1E), plants that received damage treatment were slightly better at compensating for lost tissue than plants that received damage + JA treatment. Plants that received JA treatment had a slightly lower biomass at harvest than control plants. There were significant differences in potential quantum yield (Y_0) ($F_{3,308}=6.42$, $P=0.0003$) (Fig. 1F);

JA treatment resulted in lower Y_0 values than damage treatment (Tukey-adjusted least-square mean comparison $P=0.0003$), damage + JA treatment (Tukey-adjusted least-square mean comparison $P=0.0066$), and marginally lower than control plants (Tukey-adjusted least-square mean comparison $P=0.0761$). However, differences were less than $0.0113 Y_0$, and were most likely not biologically significant. Readings in maximum photosynthetic electron transport rate (ETR_m) did not increase or decrease significantly in response to treatments in comparison with control plants, but JA treatment plants had a significantly lower ETR_m than damage + JA treatment plants (Tukey-adjusted least-square mean comparison $P=0.0015$) and marginally lower than damage treatments (Tukey-adjusted least-square mean comparison $P=0.0720$) (Fig. 1G). There was no difference in non-photochemical quenching of fluorescence (NPQ) between treatments ($F_{3,313}=0.80$, $P=0.4958$) (Fig. 1H).

Constitutive expression of physical defense traits varied considerably between Islands in *A. glauca* (Table 1, Island factor). There were significant differences in LMA both within ($\chi^2=4.5$, $P=0.0169$) and between islands ($F_{1,24.9}=8.97$, $P=0.0061$), with Maui plants having a 1.5x higher LMA than Hawaii plants. Leaf latex varied greatly between populations ($F_{1,27.8}=10.5$, $P=0.0037$) with Maui plants having 1.76x more leaf latex. There was no significant difference in prickly densities on adaxial ($F_{1,28.8}=0.30$, $P=0.5876$) (Fig. 2) or abaxial ($F_{1,30.5}=0.40$, $P=0.5295$) leaf surfaces between populations, but both varied significantly between genetic families ($\chi^2=45.6$, $P=<0.0001$; $\chi^2=46.3$, $P=0.006$). Additionally, Maui plants were 4.16x larger in shoot biomass than Hawaii plants ($F_{1,30.8}=32.05$, $P=<0.0001$), and had an average potential quantum yield (Y_0) 1.06x higher. There were no significant differences in ETR_m or NPQ between populations

(Table 1), but NPQ varied significantly among genetic families within populations ($\chi^2=19.4$, $P<0.0001$) of *A. glauca*.

Many physical defense traits increased with ontogeny (Table 1), including LMA ($F_{1,273}=88.46$, $P<0.0001$), leaf latex ($F_{1,25,2}=15.25$, $P=0.0006$), and abaxial leaf surface prickle density ($F_{1,300}=19.00$, $P<0.0001$). While constitutive levels in expression of adaxial leaf surface did not differ between ontogenetic stages ($F_{1,321}=0.71$, $P=0.4008$), there was a significant Age*Treatment interaction ($F_{3,299}=5.74$, $P=0.0008$), as well as a significant Isle*Age*Treatment interaction ($F_{3,299}=6.67$, $P=0.002$). Induction of adaxial leaf surface prickle densities were highly variable between ontogenetic stages between islands; In Hawaii island plants, with seedlings having higher densities after damage + JA treatment (mean prickle density= 16.79/cm²) than juveniles receiving the same treatment (mean prickle density= 7.13/cm²). On Maui, juvenile plants only increased prickle densities after damage + JA treatment, while seedlings increased densities in response to all treatments.

EXPERIMENT TWO

A. glauca and *A. mexicana* are differed greatly in the constitutive expression of physical defense traits (Table 2, Species). On average, *A. glauca* had a LMA 1.09x higher than *A. mexicana* ($F_{1,10,4}=10.19$, $P=0.0091$) (Fig. 2D), exuded 1.07x more latex ($F_{1,57}=17.81$, $P<0.0001$) (Fig. 2C), was 19.74x higher in adaxial leaf surface prickle density ($F_{1,4,22}=83.68$, $P=0.0006$) (Fig. 2A), and 2.65x higher in abaxial leaf surface prickle density ($F_{1,4,35}=11.71$, $P=0.0234$) (Fig. 2B). However, *A. mexicana* was, on average, 1.15x larger in shoot biomass than *A. glauca* ($F_{1,19,4}=11.36$, $P=0.003$) (Fig. 2E).

Both species responded to treatments with increases in physical defense traits but responses differed markedly (Table 2, Treatment). Both species responded to treatments with increases in adaxial leaf surface prickly densities ($F_{3,54.1}=5.20$, $P=0.0032$) (Fig. 2A) and slight increases in leaf latex exudation ($F_{3,57}=2.37$, $P=0.0803$) (Fig. 2C). After receiving JA treatment, *A. glauca* had an adaxial leaf surface prickly density 2.44x higher than control plants, but *A. glauca* did not respond to other treatments. Although *A. mexicana* increased prickly densities in response to treatments, differences were less than 1 prickly/cm² (Fig. 2A). Leaf latex exudation was highly variable in both species with no distinct pattern in responses (Fig. 2C). While increases in adaxial prickly density in *A. mexicana* were negligible (Fig. 2A), *A. mexicana* did respond to all treatments with an increase in LMA (Fig. 2D). LMA after damage treatments were 1.5x higher than control plants (Fig. 2D).

Differences in mechanisms of tolerance were determined between species (Table 2, treatment). *A. glauca* was not able to compensate for tissue loss after defoliation treatments (Fig. 2E), while *A. mexicana* compensated for some tissue loss after damage treatment, but not for damage + jasmonic acid treatment (Fig. 2E). Responses in photosynthetic mechanisms were mixed and weak in both species. *A. mexicana* showed a slight decrease in NPQ rates after damage and jasmonic acid treatments (Fig. 2H). *A. glauca* reduced its ETR_m after JA treatment (Fig. 2G).

DISCUSSION

This study provides one of the first tests of the simultaneous induction of several physical defense traits in a pair of sister species. Most importantly, it was demonstrated

that: (I) prickles are inducible by mechanical damage and jasmonic acid, and that these treatments may have additive effects when applied simultaneously to plants; (II) latex is not inducible in these *Argemone* species; (III) induction of tolerance traits was variable, differing among ontogenetic stages and populations, and as a consequence, that plants generally did not demonstrate tolerance via complete shoot compensation within the duration of the experiment.

The induction of physical defense traits has not been examined in great detail, and most work has concentrated on the induction of trichomes (Dalin *et al.*, 2008- examples within), or the induction of spines and thorns in *Acacia* (Young, 1987; Gadd *et al.*, 2001; Huntzinger *et al.*, 2004; Young *et al.*, 2003). The few previous studies on prickle induction detected increases following mechanical damage in *Rubus* (Bazely *et al.* 1991; Gibson *et al.*, 1993). However, because mechanical damage lacks the elicitors present in the saliva of herbivores, studies using only mechanical damage may underestimate induced responses to herbivory (Dalin *et al.*, 2008; Heil, 2010). This is the first study to test the induction of prickles with the application of jasmonic acid, and the first to induce prickles in *Argemone* species, further supporting the pivotal role jasmonic acid plays in recognition and responses to herbivory (Baldwin, 1988; Browse, 2009; Wu and Baldwin, 2010).

In the first experiment which focused on ontogenetic patterns in *A. glauca*, 50% defoliation and jasmonic acid treatments failed to induce responses in prickle densities and densities only increased when both treatments were combined, indicating that this combination of simulated herbivory produces a broader response than each treatment on its own. In general, because jasmonic acid is the key signaling molecule in herbivore-

induced responses (Browse 2009), without it, the plant response is weaker and also constrained by a loss of leaf area caused by damage (Cipollini and Sipe, 2001).

Examination of the induction of physical defenses in an island endemic, *A. glauca*, compared to its continental sister species, *A. mexicana*, provides a test of the hypothesis that defense against herbivores is weak in island plants because of the absence of some herbivore guilds on islands (Ziegler 2002). In contrast to this prediction, it was found that the constitutive expression of prickles, latex, and leaf toughness was significantly higher in the island species compared to the continental species. Moreover, induction of prickles was significantly higher in *A. glauca* than in *A. mexicana*. *A. mexicana* increased leaf toughness in response to damage and was much better at compensating for lost body mass than *A. glauca*, supporting our hypothesis that this worldwide pest would have a comparatively high level of tolerance to herbivory. Neither species responded to simulated herbivory by augmenting latex exudation, suggesting that laticifers do not change in size after herbivory. However, further research on the induction of chemical constituents of latex in both species is needed before excluding latex as an inducible response to herbivory in *Argemone* species.

Although there have been previous studies comparing defense traits in island vs. continental species (Funk and Throop, 2010), this is the first study to examine induced defenses in island vs. continental plants, and provides clear evidence against the island defense hypothesis. Of course, because Hawaii includes some native herbivores, such as the now-extinct flightless geese (Givnish *et al.* 1994) and many native insects, it is an oversimplification to assume defense has been lost in island plants due to a decrease in herbivore selection pressure. Unfortunately, because most native herbivores are now

engangered or extinct, it will be difficult to properly assess the hypothesis through measures of herbivore intensity and fitness effects on plants. However, the larvae of *Sirocalodes wickhami* are known to mine the leaves of several species of *Argemone*, including the mexican poppies, *A. mexicana* and *A. ochroleuca* (van der Westhuizen and Mpedi, 2011), and could possibly be used as a natural herbivore for further induction studies.

This study revealed much variation in the patterns of expression in putative defense traits between islands in *A. glauca*. On Maui, induction of prickles densities in both age classes and on both leaf surfaces demonstrated a synergistic pattern to induction. Hawaii plants, however, did not increase densities on lower leaf surfaces, and only seedlings increased densities on the upper surface after simulated herbivory. A possible explanation for the different responses between islands is that Maui seedlings and juveniles might experience occasional herbivory and respond accordingly, while Hawaii populations do not benefit from inducing prickles densities. A caveat to this explanation is that we collected seeds from three distinct populations on Hawaii and from only one population on Maui, which may have added much variation in patterns of induction for Hawaii plants.

Our results clearly reveal significant differences in constitutive expression of traits both between populations and across age classes, suggesting that there is genetic variation in the expression of physical defense traits in which natural selection could act upon. However, we did not demonstrate variation in plasticity of morphological and photosynthetic traits between populations, thus implying that the degree of induction of certain traits are genetically fixed, and can't be selected for.

In closing, we provide a strong example of strikingly different responses to simulated herbivory in two closely related species, and add to the limited amount of literature on the induction of physical defense traits in response to jasmonic acid application. We also demonstrated the benefits of including measurements of genetic variation between populations, ontogeny, and tolerance in studies of induced responses. Further evidence is needed to confirm that these responses to damage and hormonal application do in fact minimize damage from herbivores and increase the fitness of *A. glauca* and *A. mexicana*. This study captured the complexity of expression in defense traits in two well-defended plant species, and sheds light on how two sister species may become divergent in defense syndromes as a consequence of contrasting herbivore assemblages. Lastly, this study contributes to the limited literature on plant defenses on islands.

CHAPTER 3. FUTURE DIRECTIONS

INTRODUCTION

Inducible responses to herbivory have been of great interest to ecologists for the last 40 years and are now widely accepted ecological processes. However, many aspects of induced responses remain poorly understood and not well examined, and there are still many unanswered questions. In particular, very little is known about the induction of physical defense traits. My study is the first to simultaneously examine the inducibility of prickles and tolerance traits. Most notably, I detected the induction of higher prickle densities in response to the defense hormone, jasmonic acid in *A. glauca*. Two other physical defense traits, latex and leaf toughness, failed to respond to damage treatments, and tolerance traits (photosynthesis and growth) showed weak responses.

Patterns in the induction of tolerance and defense traits differed between *A. glauca* and its sister species, *A. mexicana*, and in contrast to predictions based on the island plant defense hypothesis (Carlquist 1980; Ziegler, 2002), I found that the expression and inducibility of prickles were higher in the island endemic, *A. glauca*, than in the continental invasive, *A. mexicana*. This research highlights key sources of variation in the physical defense traits of plants and provides a good example for future studies simultaneously examining the induction of physical defenses and tolerance in response to herbivory. Nonetheless, there remain several important aspects of the induction of physical defense traits that require further examination, including:

- i. Specificity in the induction of physical defense traits (i.e. does the identity of the herbivore or damage type matter?)
- ii. Demonstration that induced physical defenses improve plant fitness and/or reduce future herbivore attacks.
- iii. Examination of whether the simultaneous induction of physical defense traits and tolerance traits contribute to plant fitness in the presence of herbivory. (i.e. Does the examination of “defense syndromes” offer new insights into plant-herbivore interactions and the role of physical defenses?)
- iv. More precise characterization of the ontogenetic patterns in the induction of physical defense traits.
- v. Tests of the island plant defense, with specific focus on physical defense traits and induction.

SPECIFICITY IN INDUCTION OF PHYSICAL TRAITS

Factors in the salivary glands, ovipositors, and other bodily fluids of herbivores may induce physiological defense responses against herbivores, in addition to the responses caused by tissue loss (Karban and Baldwin, 1997). In *A. glauca*, the variation in induction of prickles in response to different treatments in both experiments highlights the intricacies of damage detection and response. Although our treatments successfully increased prickle densities in our study species, induction in other species has been found to be dependent on the degree of damage sustained (the amount of tissue removed) (Cooper *et al.*, 2003; Abdala-Roberts and Para-Tabla, 2005), and well as the duration

(repeated damage events have been found to produce higher responses) (Massey *et al.*, 2007). This suggests that priming may be a critical aspect of inducible responses. That is, perhaps plants are primed for a response during the first attack, but it takes subsequent attacks in order for a plant to respond (Karban and Baldwin, 1997).

BENEFITS TO INDUCED PHYSICAL DEFENSES

While many studies have shown the intricacies of induction on a wide array of plant life, more is needed in order to confirm that these physical responses actually act as an effective defense, and do not serve alternative functions. To prove that inducible responses actually act as defense, they must meet the following criteria (Karban and Baldwin, 1997): (1) the response needs to result in the decrease in the preference or the performance of the herbivore, (2) and the benefits of the reduced herbivory for plant fitness must outweigh the cost of producing such expensive defenses. Furthermore because more than one type of physical trait may be induced in response to damage treatments, it is important to evaluate the separate and combined benefits of each trait could be extremely difficult to accomplish. Nonetheless, additional studies should aim to distinguish the effects of certain physical defenses from those of other defense induced at the same time (Wu and Baldwin, 2010). For example, does increasing prickly densities have different effects from inducing chemical defenses in a plant, or do they work synergistically?

SIMULTANEOUS INDUCTION OF TOLERANCE AND PHYSICAL DEFENSE

Tolerance plays a large role in plant responses to herbivory. *A. glauca* showed no signs of compensation, and *A. mexicana* was better able to compensate for the loss of tissue. Additionally, we did not find evidence that either plant compensated by up regulating photosynthetic rates. However, plants can compensate for lost tissue in many ways, including by re-allocating root carbon reserves from aboveground reproduction and growth. Furthermore, Just as plants may use a combination of physical and chemical defenses in response to herbivory, they may also use a combination of different tolerance traits in order to compensate for damage. Thus future studies should aim to include multiple measurements of tolerance alongside other response traits (defense) in order to elucidate on the benefits of each type singularly or collectively.

INDUCED PHYSICAL RESPONSES AND ONTOGENY

Despite the importance of ontogeny in plant defense theory (Barton and Koricheva, 2010), few studies have looked at inducible responses at all ontogenetic stages of study plants. To understand the impact of herbivores of plants, and how plants respond to herbivory, it has proven to be highly beneficial and perhaps necessary to look at all life stages of a plant. The role of ontogeny should be incorporated as an important aspect of inducible defenses, as it will offer new perspectives in understanding the impacts of and the patterns of herbivory throughout a plant's life.

Herbivory often comes in different forms as a plant ages from a seedling to an adult, and some ontogenetic stages may be subject to much higher levels of herbivory than others (Barton and Koricheva, 2010). Therefore, the expression of physical defenses

against herbivores is thought to reflect these changes in herbivore pressure. Indeed, variation among ontogenetic stages has been found in spines (Gowda and Palo, 2003), trichomes (Traw and Feeny, 2008), and leaf toughness (Loney *et al.*, 2006). While the ontogenetic patterns in the inducible physical defenses traits has not been well documented, it is thought that fast growing stages of ontogeny, such as the seedling and juvenile stages, should have a higher ability to induce physical defense traits in response to herbivory (Herms and Mattson, 1992). In contrast to slower growing, long-lived plants, faster growing plants allocate most of their resources to growth and reproduction, and should have a lower capacity to produce constitutive defense traits (Mattson *et al.*, 1988; Herms and Mattson, 1992). Thus, faster growing plants are likely to rely in physical defense traits to be inducible, as inducible defenses accommodates the need to grow and reproduce quickly. Furthermore, increased expression of physical defense traits is restricted to new growth. That is, a plant is unable to produce more defenses such as prickles on an older leaf, and can only produce higher defenses in leafs formed after damage. Slower growing plants do not produce leaves fast enough for induction to be effective, so inducible defenses are likely to be prevalent in fast growing plants, and the seedling and juvenile stages rather than adult plants.

ISLAND DEFENSE SYNDROME

Lastly, plant defense syndromes in island endemics are virtually unknown, and many landmark publications on Hawaiian biota suggest that island endemics have lost their defenses against herbivores (Carlquist, 1970; Ziegler, 2002). Although many islands lack large herbivorous mammals, many islands either are, or have been rich in

native insect diversity (Zimmerman and Liebherr, 1948; Zimmerman, 1970; Rubinoff 2008), and it is likely that Hawaiian plants evolved closely with insect herbivores. Therefore, we suggest that more ecologists investigate the interactions between endemic herbivorous insects and the plants they feed upon.

CONCLUSIONS

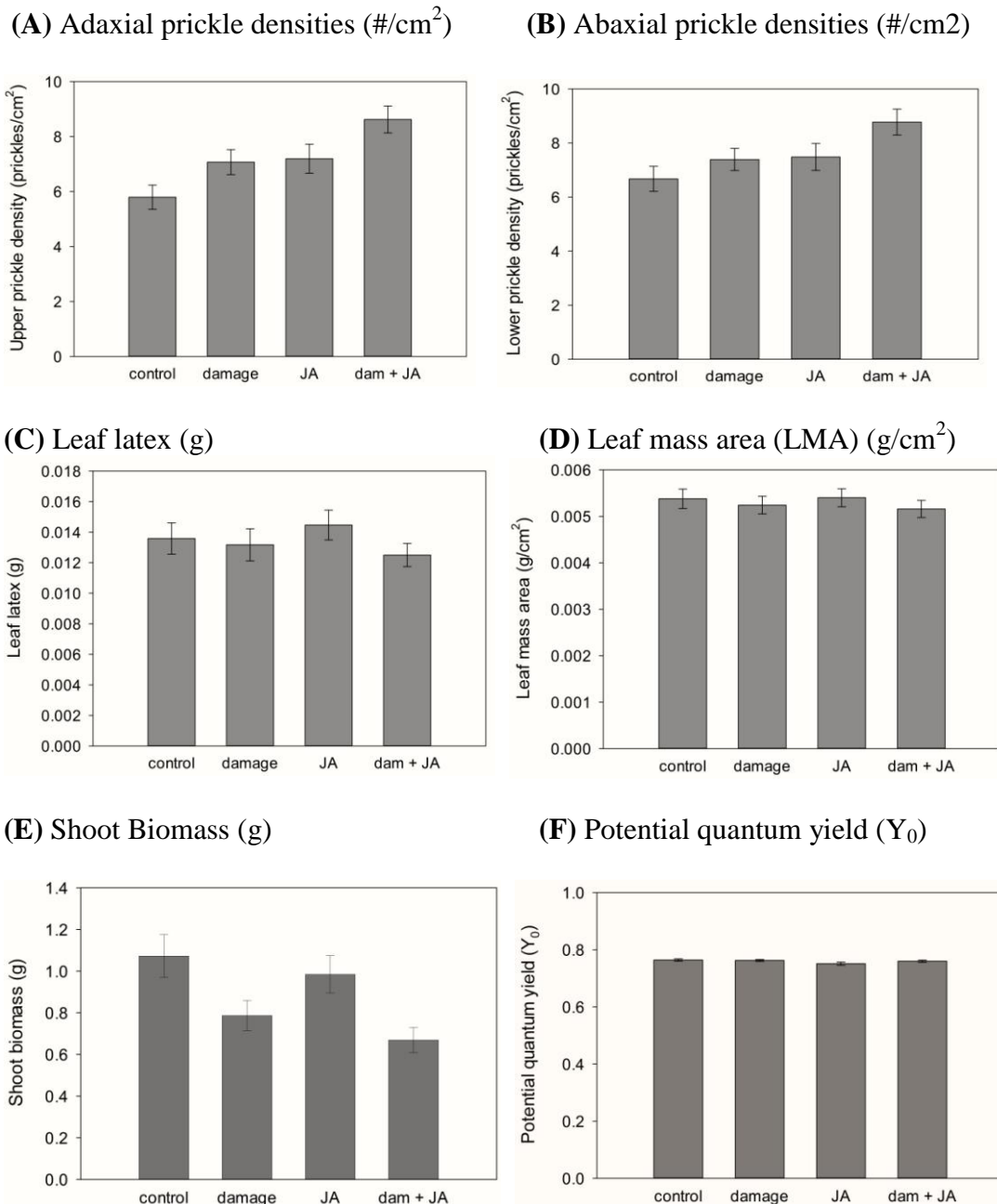
In closing, this study adds to the growing body of knowledge on the inducible responses of physical defense traits. We still know very little about and have not thoroughly documented the specifics of damage detection and the following response, how these responses vary with ontogeny, the costs and benefits of these traits in natural environments, whether these physical defenses act singularly or synergistically, how they evolve, and how they affect both plants and animals at a population level. Further studies are strongly encouraged to take a multidimensional approach to examining inducing responses, and should include as many as these factors as possible, as doing so will contribute to a more comprehensive understanding of plant defense and inducible responses to herbivory.

TABLES AND FIGURES

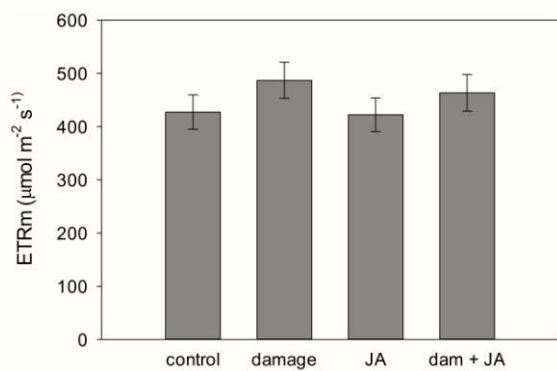
Table 1. Summary of the mixed ANOVA results for experiment one. Response variables include photosynthetic light efficiency (Y_0), maximum photosynthetic electron transport rate under saturating light (ETR_m), and non-photochemical quenching (NPQ). Significance is given as *** (P<0.0001), ** (P<0.001), * (P<0.05), + (P<0.07). Fixed factors are tested with F-test statistics, and random variables are tested using log-likelihood ratio statistics, which can be compared to a chi-square distribution with one degree of freedom.

Variable	n	Isle (F)	Age (F)	Treatment (F)	A*T (F)	Family (χ^2)	Covleaf (F)	Significant Interactions (F)
Shoot biomass (g)	339	32.05***	121.06***	2.20	1.59	8.4**	241.64***	
LMA (g/cm ²)	339	8.97**	88.46***	1.44	0.81	4.5*	141.75***	
Latex (mg)	338	10.05**	15.25**	1.82	3.15	0	63.81***	Isle x Age x Treat* Age x Fam ($\chi^2=4^*$)
Upper Prickle Density (g/cm ²)	339	0.30	0.71	6.03**	5.74**	45.6***	3.80+	Isle x Age x Treat**
Lower Prickle Density (g/cm ²)	339	0.40	19.0***	4.91**	0.82	6.3**	19.36***	
Y	341	47.34***	2.15	6.42**	4.94**	0.9	6.53*	Isle x Treat** Isle x Age*** Isle x Age x Treat**
ETR _m	341	6.05*	2049.0***	4.85**	1.80	0	3.9*	Isle x Treat+
NPQ	341	1.15	3.11	0.80	0.21	19.5***	3.79+	

Figure 1. Experiment One. The effects of damage and spraying with jasmonic acid on mean (A) adaxial leaf surface prickle density (# prickles/cm²), (B) abaxial leaf surface prickle density (# prickles/cm²), (C) leaf latex exudation (g), (D) leaf mass area (g/cm²), (E) shoot biomass (g), (F) potential quantum yield (Y₀), (G) the maximum photosynthetic electron transport rate under saturating light (ETR_m), (H) and the amount of excess light dissipated as heat via the xanthophyll cycle (non-photochemical quenching, NPQ) of *Argemone glauca*. Bars are means + S.E.



(G) ETRm ($\mu\text{mol m}^{-2}\text{s}^{-1}$)



(H) Non-photochemical quenching (NPQ)

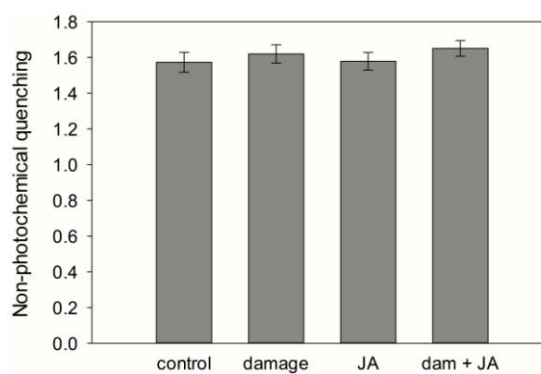
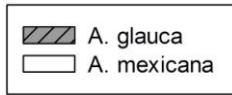


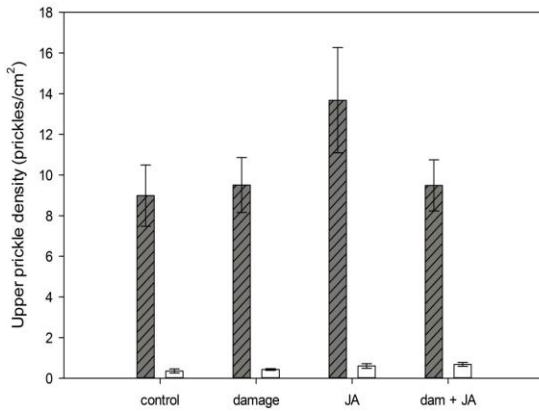
Table 2. Summary of the mixed ANOVA results for experiment two. Response variables include photosynthetic light efficiency (Y_0), maximum photosynthetic electron transport rate under saturating light (ETR_m), and non-photochemical quenching (NPQ). Significance is given as *** (P<0.0001), ** (P<0.001), * (P<0.05), + (P<0.07). Fixed factors are tested with F-test statistics, and random variables are tested using log-likelihood ratio statistics, which can be compared to a chi-square distribution with one degree of freedom.

Variable	n	Species (F)	Treatment (F)	S*T (F)	Covleaf (F)	Family (χ^2)
Shoot biomass (g)	67	11.36**	11.91**	0.31	72.02**	0
LMA (g/cm ²)	67	10.19**	1.10	3.65	12.21**	0
Latex (mg)	66	18.81***	2.37	0.39	36.37***	0
Upper Prickle Density (g/cm ²)	67	83.68**	5.20**	1.15	6.18*	9.2**
Lower Prickle Density (g/cm ²)	67	11.71*	1.76	1.99	0.68	7.9**
Y	67	0.19	1.32	1.41	5.46*	0
ETR _m	67	2.13	2.06	0.64	0.09	0
NPQ	67	21.62**	2.92*	1.87	3.03	0

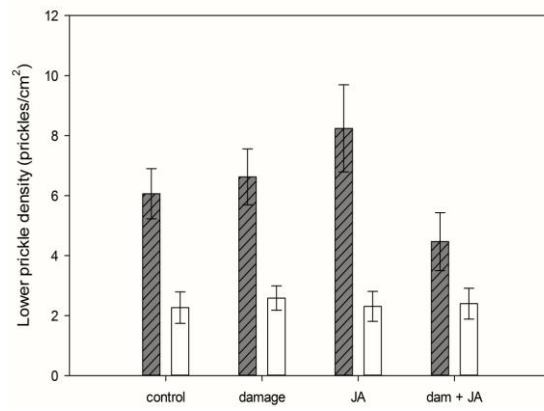
Figure 2. Experiment Two. The effects of damage and spraying with jasmonic acid on mean (A) adaxial leaf surface prickle density (# prickles/cm²), (B) abaxial leaf surface prickle density (# prickles/cm²), (C) leaf latex exudation (g), (D) leaf mass area (g/cm²), (E) shoot biomass (g), (F) potential quantum yield (Y₀), (G) the maximum photosynthetic electron transport rate under saturating light (ETR_m), (H) and the amount of excess light dissipated as heat via the xanthophyll cycle (non-photochemical quenching, NPQ) of *Argemone glauca* and *Argemone mexicana*. Bars are means + S.E.



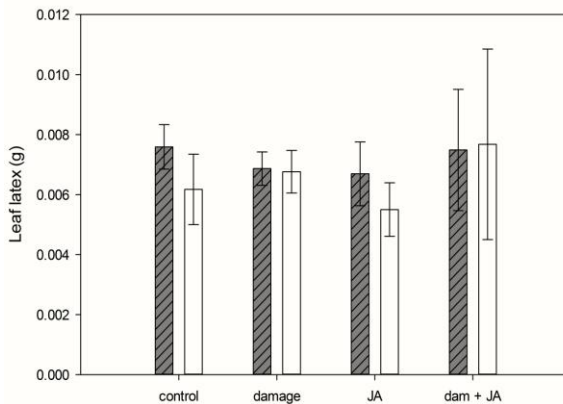
(A) Adaxial prickle density



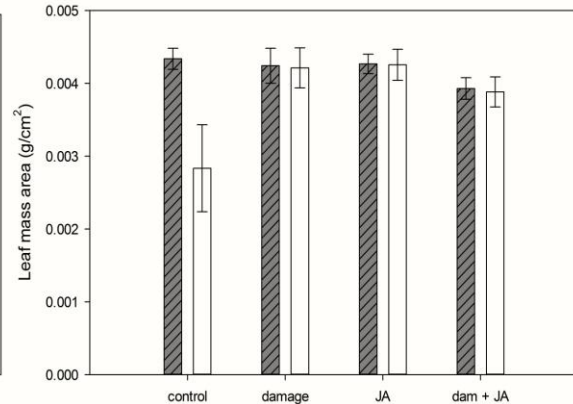
(B) Abaxial prickle density

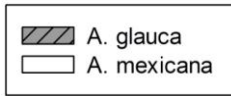


(C) Leaf latex exuded (g)

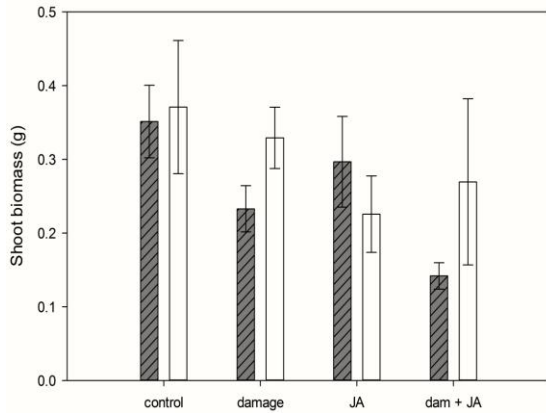


(D) Leaf mass area (LMA)

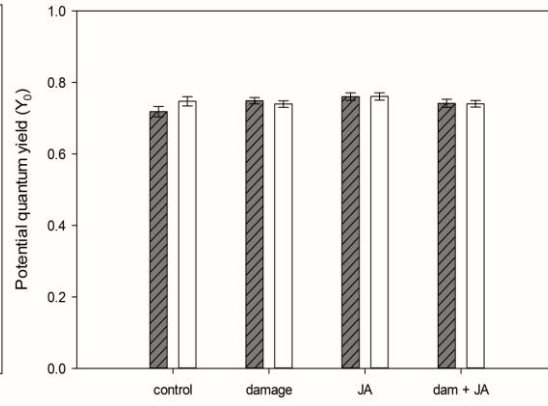




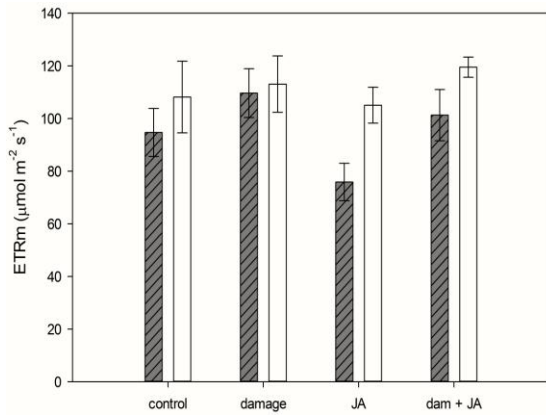
(E) Shoot Biomass (g)



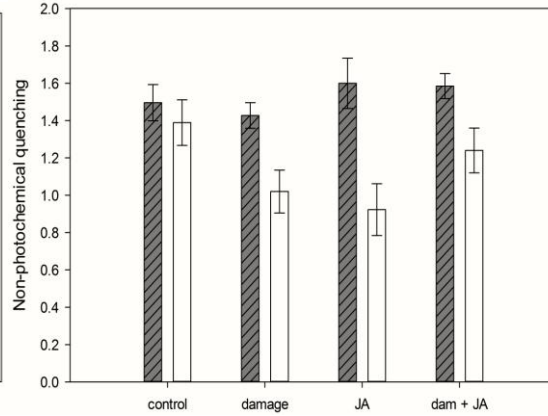
(F) Potential quantum yield (Y_0)



(G) ETRm ($\mu\text{mol m}^{-2} \text{s}^{-1}$)



(H) Non-photochemical quenching (NPQ)



LITERATURE CITED

- Abdala-Roberts, L. and Parra-Tabla, V., 2005. Artificial Defoliation Induces Trichome Production in the Tropical Shrub *Cnidoscolus aconitifolius* (Euphorbiaceae). *Biotropica* 37, 251–257.
- Abrahamson, W.G., 1975. Reproductive strategies in dewberries. *Ecology* 56, 721-726.
- Agrawal, A.A., 1999. Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. *Ecology* 80, 1713–1723.
- Agrawal, A.A., 2004. Resistance and susceptibility of milkweed: competition, root herbivory, and plant genetic variation. *Ecology* 85, 2118–2133.
- Agrawal, A.A., 2005. Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research* 7, 651–667.
- Agrawal, A.A., Conner, J.K., Johnson, M.T., Wallsgrave, R., 2002. Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. *Evolution* 56, 2206-2213.
- Agrawal, A.A., Konno, K., 2009. Latex: A model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. *Annual review of ecology, evolution, and systematics* 40, 311-331.
- Agrawal, A.A., 2011. Current trends in the evolutionary ecology of plant defense. *Functional Ecology* 25, 420–432.
- Agrawal, A.A., Tuzun, S., Bent, E., 1999. *Induced Plant Defenses against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture*. APS: St. Paul.
- Baldwin, R.E., 1979. *Hawaii's poisonous plants*. Petroglyph Press, Hilo.
- Baldwin, I.T., 1988. The alkaloidal responses of wild tobacco to real and simulated herbivory. *Oecologia* 77, 378-381.
- Baldwin, I.T., 1990. Herbivory simulation in ecological research. *Trends in Ecology and Evolution* 5, 91-93.
- Baldwin, I.T., 1996. Methyljasmonate-induced nicotine production in *Nicotiana attenuata*: inducing defenses in the field without wounding. *Entomological Experimental Applications* 80, 213-220.

- Barton, K.E. Not all island plants are defenseless: Prickles, latex and tolerance in the Hawaiian prickly poppy (*Argemone glauca*). In Press.
- Barton, K.E., Koricheva J., 2010. The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. *American Naturalist* 175, 481-493.
- Baur, R., Binder, S., Benz, G., 1991. Nonglandular leaf trichomes as short-term inducible defense of the grey alder, *Alnus incana*, against the chrysomelid beetle, *Agelastica alni*. *Oecologia* 87, 219-226.
- Bazely, D.R., Myers, J.H., Silva, K.B., 1991. The response of numbers of bramble prickles to herbivory and depressed resource availability. *Oikos* 61, 327-336.
- Becerra, J.X., Venable, D.L., Evans, P.H., Bowers, W.S., 2001. Interactions between chemical and mechanical defenses in the plant genus *Bursera* and their implications for herbivores. *American Zoologist* 41, 865-876.
- Bell, A.D., Bryan, A., 2008. *Plant Form: An illustrated guide to flowering plant morphology*. Timber Press, London.
- Belsky, A.J., 1986. Does herbivory benefit plants? A review of the evidence. *The American Naturalist* 127, 870-892.
- Boege, K., Marquis, R.J., 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in Ecology and Evolution* 20, 441-448.
- Boege, K., Barton, K.E., Dirzo, R., 2011. Influence of tree ontogeny on plant-herbivore interactions. *Size- and Age-Related Changes in Tree Structure and Function*, 193-214.
- Boughton, A.J., Hoover, G.W., Felton. 2005. Methyl jasmonate application induces increased densities of glandular trichomes on tomato, *Lycopersicon esculentum*. *Journal of Chemical Ecology* 31: 2211-2216.
- Browse, J., 2009. Jasmonate passes muster: a receptor and targets for the defense hormone. *Annual Review of Plant Biology* 60, 183-205.
- Campbell, B.M., 1986. Plant spinescence and herbivory in a nutrient poor ecosystem. *Oikos* 47, 168-172.
- Carlquist, S., 1970. *Hawaii: a natural history*. Pacific tropical botanical garden, Lawai, Kauai, Hawaii. Printers, Inc., Honolulu.
- Chadwick, D., Goode, J., 1999. *Insect-plant Interactions and Induced Plant Defence*. Wiley, Chichester.

- Chen, M.S., 2008. Inducible direct plant defense against insect herbivores: A review. *Insect Science* 15, 101–114.
- Cherry, J.H., Locy, R.D., Rychter, A., 2000. *Plant Tolerance to Abiotic Stresses in Agriculture: Role of Genetic Engineering*. Kluwer Academic: Dordrecht.
- Cipollini, D.F., 2010. Constitutive expression of methyl jasmonate-inducible response delays reproduction and constrains fitness responses to nutrients in *Arabidopsis thaliana*. *Evolutionary Ecology* 24, 59-68.
- Cipollini, D.F., Purrington, C.B., Bergelson, J., 2003. Costs of induced responses in plants. *Basic and applied ecology* 4, 79-85.
- Cipollini, D.F., Sipe, M.L., 2001. Jasmonic acid treatment and mammalian herbivory differentially affect chemical defenses and growth of wild mustard (*Brassica kaber*). *Chemoecology* 11, 137-143.
- Coley, P.D., 1983. Herbivory and Defensive Characteristics of Tree Species in a Lowland Tropical Forest. *Ecological Monographs* 53, 209–234.
- Cooper, S.M., Owen-Smith, N., 1986. Effects of plant spinescence on large mammalian herbivores. *Oecologia* 68, 446-455.
- Cooper, S.M., Owens, M.K., Spalinger, D.E., Ginnett, T.F., 2003. The architecture of shrubs after defoliation and the subsequent feeding behavior of browsers. *Oikos* 100, 387-393.
- Crawley, M.J., 1983. *Herbivory. The dynamics of animal-plant interactions*. Blackwell Scientific Publications, Oxford.
- Dalin, P., Björkman, C., 2003. Adult beetle grazing induces willow trichome defence against subsequent larval feeding. *Oecologia* 134, 112-118.
- Dalin, P., Ågren, J., Björkman, C., Huttunen, P., Kärkkäinen, K., 2008. Leaf trichome formation and plant resistance to herbivory. *Induced Plant Resistance to Herbivory*, 89-105.
- Dussourd, D.E., Eisner, T., 1987. Vein-cutting behavior: insect counterploy to the latex defense of plants. *Science* 237, 898-901.
- Dussourd, D.E., Hoyle, A.M., 2000. Poisoned plusiines: toxicity of milkweed latex and cardenolides to some generalist caterpillars. *Chemoecology* 10, 11-16.
- Ehrlich, P.R., Raven, P.H., 1964. *Butterflies and Plants: A Study in Coevolution*. *Evolution* 18, 586-608.

- Farmer, E.E., Ryan, C.A., 1992. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. *The plant cell* 4, 129-134.
- Felton, G.W., 2008. Caterpillar secretions and induced plant responses. *Induced Plant Resistance to Herbivory*, 369-387
- Fordyce, J.A., Agrawal, A.A., 2002. The role of plant trichomes and caterpillar group size on growth and defence of the pipevine swallowtail *Battus philenor*. *Journal of Animal Ecology* 70, 997-1005.
- Fornoni, J., 2011. Ecological and evolutionary implications of plant tolerance to herbivory. *Functional Ecology* 25, 399-407.
- Freeman, R.S., Brody, A.K., Neefus, C.D., 2003. Flowering phenology and compensation for herbivory in *Ipomopsis aggregate*. *Oecologia* 136, 394-401.
- Funk, J.L., Throop, H.L., 2010. Enemy release and plant invasion: patterns of defensive traits and leaf damage in Hawaii. *Oecologia* 162, 815-823.
- Gadd, M.E., Young, T.P., Palmer, T.M., 2001. Effects of simulated shoot and leaf herbivory on vegetative growth and plant defense in *Acacia drepanolobium*. *Oikos* 92, 515-521.
- Gibson, R., Basely, D.R., Shore, J.S., 1993. Responses of brambles, *Rubus vestitus*, to herbivory. *Oecologia* 95, 454-457.
- Givnish, T.J., Sytsma, K.J., Smith, J.F., Hahn, W.J., 1994. Thorn-like prickles and heterophylly in *Cyanea*: adaptations to extinct avian browsers on Hawaii?. *Proceedings of the National Academy of Sciences* 91, 2810-2814.
- Goeden, R.D., Ricker, D.W., 1985. Prickly poppies, *Argemone corymbosa* and *A. munita*, in southern California—native weeds attacked by few insects. *Annals of the entomological society of America* 78, 214-216.
- Gomez, J.M., and Zamora, R., 2002. Thorns as induced mechanical defense in a long-lived shrub (*Hormathophylla spinosa*, Cruciferae). *Ecology* 83, 885-890.
- Gowda, J.H., 1997. Physical and chemical response of juvenile *Acacia tortilis* trees to browsing. Experimental evidence. *Functional Ecology* 11, 106-111.
- Gowda, J.H., Palo, R.T., 2003. Age - related changes in defensive traits of *Acacia tortilis* Hayne. *African Journal of Ecology* 41, 218-223.

- Green, T.R., Ryan, C.A., 1972. Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. *Science* 175, 776-777.
- Gutschick, V. P., 1999. Biotic and abiotic consequences of differences in leaf structure. *New Phytologist* 143, 3-18.
- Hagel, J.M., Lamont, B.B., Fairbanks, M.M., Rafferty, C.M., 2007. Plant structural traits and their role in anti-herbivore defense. *Perspectives in plant ecology, evolution, and systematics* 8, 157-178.
- Hagel, J.M., Yeung, E.C., Facchini, P.J., 2008. Got milk? The secret life of laticifers. *Trends in Plant Science* 13, 631-639.
- Haines, W.P., Heddle, M.L., Welton, P., Rubinoff, D., 2009. A recent outbreak of the hawaiian koa moth, *Scotorythra paludicola* (Lepidoptera: Geometridae), and a review of outbreaks between 1892 and 2003. *Pacific Science* 63, 349-369.
- Hairston, N.G., Smith, F.E., Slobodkin, L.B., 1960. Community Structure, Population Control, and Competition. *The American Naturalist* 94, 421-425.
- Hanley, M.E., Lamont, B.B., Fairbanks, M.M., Rafferty, C.M., 2007. Plant structural traits and their role in anti-herbivore defense. *Perspectives in Plant Ecology, Evolution and Systematics* 8, 157-178.
- Hean, J.W., Ward, D., 2011. Fire and herbivory are not substitutable: evidence from regrowth patterns and changes in physical and chemical defences in *Acacia* seedlings. *Journal of Vegetation Science* 23, 13-23.
- Heil, M., 2010. Plastic defence expression in plants. *Evolutionary Ecology* 24, 555-569.
- Herms, D.A., Mattson, W.J., 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, 283-335.
- Herrera, C.M., Pellmyr, O., 2002. *Plant-animal Interactions: An Evolutionary Approach*. Blackwell Science, Oxford.
- Holeski, L.M., 2007. Within and between generation phenotypic plasticity in trichome density of *Mimulus guttatus*. *Journal of evolutionary biology* 20, 2092-2100.
- Holeski, L.M., Chase-Alonge, R., Kelly, J.K., 2010. The genetics of phenotypic plasticity in plant defense: trichome production in *Mimulus guttatus*. *The American Naturalist* 175, 391-400.
- Holm, L., Pancho J.V., Herberger, J.P., Pluckett, D.L., 1979. *A geographical atlas of world weeds*. John Wiley and Sons, New York.

- Holm, L., Pluckett, D.L., Pancho, J.V., Herberger, J.P., 1977. The worlds worst weeds. University Press of Hawaii, Honolulu.
- Howe, G.A., Schaller, A., 2008. Direct defenses in plants and their induction by wounding and insect herbivores. *Induced plant resistance to herbivores*, 7-29.
- Howlett, B.E., Davidson, D.W., 2001. Herbivory on planted dipterocarp seedlings in secondary logged forests and primary forests of Sabah, Malaysia. *Journal of Tropical Ecology* 17, 285-302.
- Huntzinger, M., Karban, R., Young, T.P., Palmer, T.M., 2004. Relaxation of induced indirect defenses of acacias following exclusion of mammalian herbivores. *Ecology* 85, 609-614.
- Karban, R., Agrawal, A.A., Mangel, M., 1997. The benefits of induced defenses against herbivores. *Ecology* 78, 1351-1355.
- Karban, R., and Baldwin, I.T., 1997. *Induced responses to herbivory*. University of Chicago Press, Chicago.
- Karban, R., 2011. The ecology and evolution of induced resistance against herbivores. *Functional Ecology* 25, 339-347.
- Karban, R., Myers, J.H., 1989. Induced Plant Responses to Herbivory. *Annual Review of Ecology and Systematics* 20, 331-348.
- Karabourniotis, G., Bornman, J.F., 2002. Penetration of UV - A, UV - B and blue light through the leaf trichome layers of two xeromorphic plants, olive and oak, measured by optical fibre microprobes. *Physiologia plantarum* 105, 655-661.
- Karlsson, L.M., Tamdado, T., Milberg, P., 2003. Seed dormancy pattern of the annuals *Argemone ochroleuca* and *A. mexicana* (Papaveraceae). *Flora* 198, 329-339.
- Kingsbury, J.M., 1964. *Poisonous plants of the United States and Canada*. Prentice Hall, Englewood Cliffs.
- Kitajima, K., Llorens, A.M., Stefanescu, C., Timchenko, M.V., Lucas, P.W., Wright, S.J., 2012. How cellulose-based leaf toughness and lamina density contribute to long leaf lifespans of shade-tolerant species. *New Phytologist* 195, 640-652.
- Kleunen, M.V., Ramponia, G., Schmid, B., 2004. Effects of herbivory simulated by clipping and jasmonic acid on *Solidago canadensis*. *Oecologia* 152, 677-683.

- Kobayashi, H., Yanaka, M., Ikeda, T.M., 2010. Exogenous methyl jasmonate alters trichome density on leaf surfaces of rhodes grass (*Chloris gayana* Kunth). *Journal of Plant Growth Regulation* 29, 506-511.
- Konno, K., Hirayama, C., Nakamura, M., Tateishi, K., Tamura, Y., Hattori, M., Kohno, K., 2004. Papain protects papaya trees from herbivorous insects: role of cysteine proteases in latex. *The Plant Journal* 37, 370–378.
- Kruidhof, H.M., Allison, J.D., Hare, J.D., 2012. Abiotic induction affects the costs and benefits of inducible herbivore defenses in *Datura wrightii*. *Journal of Chemical Ecology* 38, 1215-1224.
- Kudo, G., 1996. Herbivory pattern and induced responses to simulated herbivory in *Quercus mongolica* var. *grosseserrata*. *Ecological Research* 11, 283-289.
- Kursar, T.A., Coley, P.D., 1991. Nitrogen Content and Expansion Rate of Young Leaves of Rain Forest Species: Implications for Herbivory. *Biotropica* 23, 141-150.
- Latzel, V., Malikova, L., Klimesova, J., 2011. Compensatory growth of *Euphorbia peplus* regenerating from a bud bank. *Botany-Botanique* 89, 313-321.
- Levin, D.A., 1973. The Role of Trichomes in Plant Defense. *The Quarterly Review of Biology* 48, 3-15.
- Lilleeng-Rosenberger, K.E., 2005. Growing Hawai'i's Native Plants: A Simple Step-by-step Approach for Every Species. Mutual Pub., Honolulu.
- Liu, Z., Cai, Y., Fang, Y., Jing, J., Li, K., 2010. Induced response in *Schima superba*: Effects of early-season herbivory on leaf traits and subsequent insect attack. *African Journal of Biotechnology* 9, 8731-8738.
- Loney, P.E., McArthur, C., Potts, B.M., Jordan, G.J., 2006. How does ontogeny in a *Eucalyptus* species affect patterns of herbivory by Brushtail Possums?. *Functional Ecology* 20, 982-988.
- Macedo, A. F., Leal-Costa, M.V., Tavares, E.S., Lage, C.L.S., Esquibel, M.A., 2011. The effect of light quality on leaf production and development of in vitro-cultured plants of *Alternanthera brasiliana* Kuntze. *Environmental and Experimental Botany* 70, 43-50.
- Manetas, Y., 2003. The importance of being hairy: the adverse effects of hair removal on stem photosynthesis of *Verbascum speciosum* are due to solar UV-B radiation. *New Phytologist* 158, 503–508.

- Massey, F.P., Roland Ennos, A., Hartley, S.E., 2007. Herbivore specific induction of silica-based plant defences. *Oecologia* 152, 677-683.
- Mattson, W. J., Lawrence, R. K., Haack, R. A., 1988. Defensive strategies of woody plants against different insect-feeding guilds in relation to plant ecological strategies and intimacy of association with insects. Springer, 3-38.
- McNaughton, S.J., 1983. Compensatory plant growth as a response to herbivory. *Oikos* 40, 329-336.
- Melcher, P.J., Goldstein, G., Meinzer, F.C., Minyard, B., Giambelluca, T.W., Loope, L.L., 1994. Determinants of thermal balance in the Hawaiian giant rosette plant, *Argyroxiphium sandwicense*. *Oecologia* 98, 412-418.
- Meléndez, P.A., Capriles, V.A., 2002. Molluscicidal activity of plants from Puerto Rico. *Annals of Tropical Medicine and Parasitology* 96, 209-218.
- Milewski, A.V., Madden, D., 2006. Interactions between large African browsers and thorny *Acacia* on a wildlife ranch in Kenya. *African Journal of Ecology* 44, 515-522.
- Milewski, A.V., Young, T.P., Madden, D., 1991. Thorns as induced defenses: experimental evidence. *Oecologia* 86, 70-75.
- Mithöfer, A., Wanner, G., Boland, W., 2005. Effects of feeding *Spodoptera littoralis* on lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. *American society of plant biologists* 137, 1160-1168.
- Niklas, K.J., 1997. *The Evolutionary Biology of Plants*. University of Chicago, Chicago.
- Nykänen, H., Koricheva, J., 2004. Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. *Oikos* 104, 247-268.
- Obeso, J.R., 1997. Costs of reproduction in *Ilex aquafolium*: effects at tree branch and leaf levels. *Journal of Ecology* 85, 159-166.
- Osho, A., Adetunji, T., 2010. Antimicrobial activity of the essential oil of *Argemone mexicana* Linn. *Journal of medicinal plants research* 4, 19-22.
- Orians C.M., Thorn, A., Gomez, S., 2011. Herbivore-induced resource sequestration in plants: why bother? *Oecologia* 167, 1-9.
- Ownbey, G.B., 1961. The genus *Argemone* in South America and Hawaii. *Brittonia* 13, 91-109.

- Palo, R.T., Robbins, C.T., 1991. Plant Defenses against Mammalian Herbivory. CRC, Boca Raton.
- Parsons, W.T., Cuthbertson, E.G., 1992. Noxious Weeds of Australia. Inkata Press, Madison.
- Pisani, J.M., Distel, R.A., 1999. Production of Phenols and Spines in Response to Shoot Damage in *Prosopis caldenia* and *Prosopis flexuosa*. *Journal of Chemical Ecology* 25, 1141-1150.
- Pickard, W.F., 2007. Laticifers and secretory ducts: two other tube systems in plants. *New Phytologist* 177, 877-888.
- Poethig, R.S., 1990. Phase change and the regulation of shoot morphogenesis in plants. *Science* 250, 923-930.
- Pollard, A. J., Briggs, D., 1984. Genecological Studies of *Urtica dioica* L. III. Stinging Hairs and Plant-Herbivore Interactions. *New phytologist* 97, 507-522.
- Read, J., Sanson, G.D., 2003. Characterizing sclerophylly: the mechanical properties of a diverse range of leaf types. *New Phytologist* 160, 81-99.
- Pullin, A.S., Gilbert, J.E., 1989. The stinging nettle, *Urtica dioica*, increases trichome density after herbivore and mechanical damage. *Oikos*, 275-280.
- Rasmann, S., Johnson, M.D., Agrawal, A.A., 2009. Induced responses to herbivory and jasmonate in three milkweed species. *Journal of chemical ecology* 35, 1326-1334.
- Reyes, F.D., Peña, C.J., Canales, M., Jiménez, M., Meráz, S., Hernandez, T., 2011. Antimicrobial activity of *Argemone ochroleuca* Sweet (Chicalote). *BLACPMA* 10, 139- 146
- Reymond P., Weber, H., Damond, M., Farmer, E.E., 2000. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *Plant Cell* 12, 707-719.
- Röse, U.S.R., Tumlinson, J.H., 2005. Systemic induction of volatile release in cotton: How specific is the signal to herbivory? *Planta* 222, 327-335.
- Rubinoff, D. (2008). Phylogeography and ecology of an endemic radiation of Hawaiian aquatic case-bearing moths (*Hyposmocoma*: *Cosmopterigidae*). *Philosophical Transactions of the Royal Society B: Biological Sciences* 363, 3459-3465.

- Schaller, A., 2008. *Induced Plant Resistance to Herbivory*. Springer, Berlin.
- Schoonhoven, L.M., Dicke, M., 2005. *Insect-plant Biology*. Oxford UP, Oxford.
- Schwarzbach, A.E., Kadereit, J.W., 1999. Phylogeny of prickly poppies, *Argemone* (Papaveraceae), and the evolution of morphological and alkaloid characters based on ITS nrDNA sequence variation. *Plant Systematics and Evolution* 218, 257-279.
- Shaukat, S.S., Siddiqui, I.A., Khan, G.H., Zaki, M.J., 2002. Nematicidal and allelopathic potential of *Argemone mexicana*, a tropical weed. *Plant and Soil* 245, 239-247.
- Stamp, N., 2003. Out of the Quagmire of Plant Defense Hypotheses. *The Quarterly Review of Biology* 78, 23-55.
- Stevens, M.T., Kruger E.L., Lindroth R.L., 2008. Variation in tolerance to herbivory is mediated by differences in biomass allocation in aspen. *Functional Ecology* 22, 40-47.
- Strauss, S.Y., Agrawal, A.A. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution* 14, 179-185.
- Strauss S.Y., Rudgers, J.A., Lau, J.A., Irwin, R.E., 2002. Direct and ecological costs of resistance to herbivory. *Trends in ecology and evolution* 17, 278-285.
- Stowe, K.A., Marquis, R.J., Hochwender, C.G., Simms, E.L., 2000. The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology and Systematics*, 565-595.
- Thomson, V.P., Cunningham, S.A., Ball, M.C., Nicotra, A.B., 2003. Compensation for herbivory by *Cucumis sativus* through increased photosynthetic capacity and efficiency. *Oecologia* 134, 167-175.
- Tiffin, P., 2000. Mechanisms of tolerance to herbivore damage: what do we know?. *Evolutionary Ecology* 14, 523-536.
- Tollrian, R., Harvell, C.D., 1999. *The Ecology and Evolution of Inducible Defenses*. Princeton UP, Princeton.
- Traw, M.B., Bergelson, J., 2003. Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*. *American Society of Plant Biologists* 133, 1367-1375.
- Traw, M.B., Dawson, T.E., 2002. Differential induction of trichomes by three herbivores of black mustard. *Oecologia* 131, 526-532.

- Traw, M.B., Feeny, P., 2008. Glucosinolates and trichomes track tissue value in two sympatric mustards. *Ecology* 89, 763-772.
- Truman, W., Bennett, M.H., Kubigsteltig, I., Turnbull, C., Grant, M., 2007. *Arabidopsis* systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. *Plant Biology* 104, 1075-1080.
- van der Westhuizen, L., Mpedi, P., 2011. The initiation of a biological control programme against *Argemone mexicana* and *Argemone ochroleuca* sweet subsp. *ochroleuca* (Papaveraceae) in South Africa. *African Entomology* 19, 223-229.
- van Dam, N.M., Baldwin, I.T., 2001. Competition mediates costs of jasmonate-induced defences, nitrogen acquisition and transgenerational plasticity in *Nicotiana attenuata*. *Functional Ecology* 15, 406-415.
- Verma, S.K., Dev, G., Tyagi, A.K., Goomber, S., Jain, G.V., 2001. *Argemone mexicana* poisoning autopsy findings of two cases. *Forensic science international* 115, 135-141.
- Wagner, W.L., Herbst, D.R., Sohmer, S.H., 1999. *Manual of the Flowering Plants of Hawai'i*. University of Hawai'i, Honolulu.
- Walters, D., Newton, A.C., Lyon, G., 2007. *Induced Resistance for Plant Defence: A Sustainable Approach to Crop Protection*. Blackwell Pub, Oxford.
- Wu, J., Baldwin, I.T., 2010. New insights into plant responses to the attack from insect herbivores. *Annual review of genetics* 44, 1-24.
- Woodman, R.L., Fernandes, G.W., 1991. Differential mechanical defense: herbivory, evapotranspiration, and leaf-hairs. *Oikos*, 11-19.
- Wright, P.J., Bonser, R., 1999. An investigation into induced plant defences. *Journal of Biological Education* 33, 217-219.
- Wu, J., Baldwin, I.T., 2010. New insights into plant responses to the attack from insect herbivores. *Annual review of genetics* 44, 1-24.
- Young, T.P., 1987. Increased thorn length in *Acacia drepanolobium*—an induced response to browsing. *Oecologia* 71, 436-438.
- Young, T.P., Okello, B.D., 1998. Relaxation of an induced defense after exclusion of herbivores: spines on *Acacia drepanolobium*. *Oecologia* 115, 508-513.
- Young, T.P., Stanton, M.L., Christian, C.E., 2003. Effects of natural and simulated

- herbivory on spine lengths of *Acacia drepanolobium* in Kenya. *Oikos* 101, 171-179.
- Zangerl, A.R., Rutledge, C.E., 1996. The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *The American Naturalist* 147, 599-608.
- Zhang, Z., Wang, S.P., Nyren, P., Jiang, G.M., 2006. Morphological and reproductive response of *Caragana microphylla* to different stocking rates. *Journal of arid environments* 67, 671-677.
- Ziegler, A.C., 2002. Hawaiian natural history, ecology and evolution. University of Hawaii Press, Honolulu.
- Zimmerman, E.C., 1970. Adaptive radiation in Hawaii with special reference to insects. *Biotropica*, 32-38.
- Zimmerman, E.C., Liebherr, J.K., 1948. Insects of Hawaii: a manual of the insects of the Hawaiian Islands, including an enumeration of the species and notes on their origin, distribution, hosts, parasites, etc (Vol. 16). University of Hawaii Press, Honolulu.
- Zinn, A.D., Ward, D., Kirkman, K., 2007. Inducible defences in *Acacia sieberiana* in response to giraffe browsing. *African Journal of Range and Forage Science* 24, 123-129.
- Zobel, B.J., 2012. Mechanisms of Woody Plant Defenses against Insects Search for Pattern. [S.l.]: Springer, New York.