

Prickles, latex, and tolerance in the endemic Hawaiian prickly poppy (*Argemone glauca*): variation between populations, across ontogeny, and in response to abiotic factors

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Abstract Covariance among plant defense traits is predicted to occur both within and among plant species, potentially leading to characteristic defense syndromes. I examined patterns of variation in prickle density, latex exudation, and tolerance in order to assess whether traits varied between populations, across plant ontogeny, and as phenotypic plasticity in response to water and light limitation and physical damage using the endemic Hawaiian prickly poppy, *Argemone glauca*, as a model system. Plants produced copious latex, had extremely variable prickle densities, and were generally tolerant of 50 % defoliation. However, expression patterns differed among defense traits. Prickle density was consistent across ontogeny and was not induced by either water limitation or mechanical damage, but was significantly induced under high light conditions. In contrast, latex exudation increased significantly across ontogeny and was reduced by water limitation, but had no response to mechanical damage or light. Prickles, latex, and tolerance differed considerably between populations, suggesting different evolutionary histories for these populations. These disparate patterns indicate that latex and prickles are unlinked within *A. glauca*, potentially as a result of differences in their function, and providing little evidence that they jointly function as a defense syndrome. Moreover, this study provides the first description patterns of variation for multiple defense traits in an island endemic, and high levels of prickles, latex, and tolerance suggest that *A. glauca* is well defended against herbivores. Future

research in the field will provide additional insights into the functional ecology of these traits in *A. glauca*.

Keywords Island plant defense · Induced responses · Structural defenses · Genetic variation · Phenotypic plasticity

Introduction

Plant defense varies dramatically both within and among plant species, and one of the principal goals of plant ecological research is to characterize patterns within this variation (Agrawal 2007; Coley et al. 1985; Denno and McClure 1983). However, studies that focus on only one defense trait or only a single source of variation are limited in scope and cannot shed light on the range of trait variation or relationships among traits and factors. This is an important oversight because it is becoming increasingly clear that defense traits can co-vary within (Koricheva et al. 2004) and among species (Agrawal and Fishbein 2006; Moles et al. 2013), and there is ample evidence that abiotic and biotic factors affect plant defense in non-additive ways (Halpern et al. 2010; Hamback and Beckerman 2003).

Co-variance among plant defense traits occurs for different reasons within versus among plant species. Co-variance of traits among species is thought to result from the co-adaptation of multiple defense traits in response to the environment, leading to the emergence of identifiable “defense syndromes” (Agrawal and Fishbein 2006). Although there is some evidence for defense syndromes within particular phylogenetic groups (Agrawal and Fishbein 2006; da Silva and Batalha 2011; Travers-Martin and Muller 2008), a recent meta-analysis casts doubt on the generality of defense syndromes across plant families (Moles

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et al. 2013). In contrast, co-variance among defense traits within species is generally predicted to be negative, driven by trade-offs or allocational constraints in the simultaneous expression of multiple defense traits (Herms and Mattson 1992; Strauss et al. 2002). Again, despite the central role that trade-offs play in plant defense theories (Stamp 2003), the evidence is weak that these trade-offs are common or widespread (Koricheva et al. 2004; Leimu and Koricheva 2006).

In this study, I examined variation in prickle density, latex exudation, and tolerance to damage in order to assess whether traits varied among populations, across plant ontogeny, and as phenotypic plasticity in response to water and light limitation and as induction by physical damage, using the endemic Hawaiian prickly poppy (“pua kala”) as a model system, *Argemone glauca* Nutt. ex Prain (Papaveraceae). In general, physical defense traits like prickles and latex are less well studied compared to plant secondary chemistry (Hanley et al. 2007), and prickly poppies (genus *Argemone*) offer an ideal model system in which to examine whether physical defense traits show similar patterns of variation with respect to populations, ontogeny, and in response to abiotic factors (Hanley et al. 2007).

Argemone species are found in warm dry areas, particularly in the New World, although several species have become invasive pests throughout the world (Schwarzbach and Kadereit 1999). One species is endemic to the Hawaiian Islands, *A. glauca*, and molecular evidence suggests that *A. glauca* colonized Hawai'i from North America where its putative sister species, the Mexican poppy (*A. mexicana*) is native (Schwarzbach and Kadereit 1999). It is not clear how long *A. glauca* has been in Hawai'i, but it is clearly not a recent arrival (i.e. thousands of years), given its molecular divergence from *A. mexicana* (Schwarzbach and Kadereit 1999).

Like other poppy species, *A. glauca* primarily grows in dry forest and coastal habitats, and is most common on the island of Hawai'i, although it can also be found at low densities on the other main Hawaiian islands. Herbivore damage on leaves and stems is not common in *A. glauca*, although mango flower beetles (*Protaetia fusca*, Scarabaeidae) are often found feeding on petals (personal observation). Whether the beetle visits facilitate or interfere with pollination is not known. Although few studies have quantified herbivory in other *Argemone* species, limited insect folivory has also been reported for California prickly poppies, *A. corymbosa* and *A. munita* (Goeden and Ricker 1985). Whether non-native mammals in Hawai'i consume *A. glauca* is not known, although goats pose the most likely threat and are commonly found in the dry habitats where *A. glauca* grows. Historically, *A. glauca* likely encountered the now-extinct flightless ducks, which have been hypothesized to be the selective agents for the spinescence that

evolved in the endemic Hawaiian *Cyanea* (Givnish et al. 1994), although the diet of the ducks remains uncertain (James and Burney 1997).

As with all prickly poppies, *A. glauca* have two obvious defense traits, prickles and latex. The leaves, stems, bracts, and fruits are covered with prickles. In addition, *A. glauca* exudes a bright yellow latex upon damage to leaves, stems, buds, and fruits. Roots do not appear to contain latex or to exude latex upon damage, although they are bright yellow, suggesting the presence of the latex constituents (personal observation). The constituents and ecological functions of *A. glauca* latex have not previously been analyzed or investigated, although the use of *A. glauca* as a source of medicine by native Hawaiians and the view of locals that it is a poisonous plant (Baldwin 1979) provide circumstantial evidence that the latex contains secondary compounds. Possible insights into the latex of *A. glauca* may be inferred from the substantial body of research that has investigated the composition and function of latex in the putative sister species, *Argemone mexicana* L. (Schwarzbach and Kadereit 1999). The alkaloids of *A. mexicana* are in the protoberberine and protopine classes, and include dihydro-sanguinarine as the main constituent, composing approximately 87 % of the alkaloid content (Schwarzbach and Kadereit 1999; Singh et al. 2010; Verma et al. 2001). Extracts of *A. mexicana* leaves have broad toxicity, and have been shown to be fatal or deterrent to snails (Meléndez and Capriles 2002), bacteria and fungi (Osho and Adetunji 2010), Dipteran larvae (Sakthivadivel and Thilagavathy 2003), nematodes (Shaukat et al. 2002), and neighboring tomato plants (Shaukat et al. 2002). Accidental consumption by humans leads to illness and, in some cases, death (Verma et al. 2001).

The evolutionary and functional ecology of latex and prickles in *A. glauca* has not previously been investigated, and nothing is known about patterns of expression and important sources of variation. The primary goal of this study was to characterize the expression patterns of latex exudation and prickles in *A. glauca* in order to gain insights into an apparently well-defended island endemic. Potentially important sources of variation in latex and prickles were investigated, including plant ontogeny, population differences among islands, and abiotic factors (light, water availability, and mechanical damage). Furthermore, a third defense trait, tolerance to defoliation, was investigated. Defined as a maintenance of fitness in damaged plants comparable to that of undamaged control plants (Simms 2000), tolerance was measured as the production of inflorescences and biomass compensation in damaged versus undamaged plants, two important and commonly used metrics of tolerance (Tiffin 2000).

This study provides the first rigorous examination of multiple defense traits in an endemic island plant. Using

this unique island system, new insights can be gained into the evolution of plant defense, including intraspecific relationships among defense traits, population variation, and ontogenetic patterns in defense.

Materials and methods

Sample summary

Seeds were collected in summer 2010 from the islands of Maui and O'ahu. On Maui, mature capsules with seeds were collected from over 25 maternal plants throughout the Maui Nui Botanical Garden (MNBG). *Argemone glauca* at MNBG has a naturally regenerating population which receives no particular cultivation or care; seeds were collected from plants at least 2 m apart and often at much greater distances (<10s of meters). On O'ahu, *A. glauca* is uncommon, and seeds were collected from six maternal plants found in various private and commercial gardens around Honolulu. Seeds were combined from plants within islands to produce two genetic entities—a single population from each of Maui and O'ahu.

To break the dormancy typical of *Argemone* (Karlsson et al. 2003), seeds were soaked in tap-water for 48 h prior to sowing in germination flats filled with equal parts Promix BX (65–75 % Canadian sphagnum peat moss, perlite, dolomitic and calcitic limestone, macro- and micronutrients, and *Glomus intraradices* mycorrhizae inoculum) and black cinder. Seeds germinated in December 2010–February 2011, when they typically germinate in natural populations (Lilleeng-Rosenberger 2005). Between 14 February and 2 March, seedlings were transplanted at the two-leaf stage into pots filled with equal parts Promix BX and black cinder with a single initial application of slow-release fertilizer (Osmocote), and randomly assigned to the following four experiments: ontogeny, induction, tolerance, and water availability.

Ontogeny experiment: design and statistical analyses

Plants used to examine early ontogenetic patterns in prickly density and latex exudation were transplanted into 540-mL pots and placed on benches in a shaded grow area attached to the St. John Plant Sciences Building on the UH-Manoa campus. Plants were exposed to ambient temperatures and humidity, but, because they were under a concrete canopy, they were shielded from precipitation, and light levels were reduced. Plants were watered daily, and placement on benches were re-randomized weekly. Beginning on 16 March, plants were analyzed and harvested weekly for 6 weeks. At each sampling period, 4–6 plants per population were assayed, with the exception of the final harvest

when there were no remaining plants from O'ahu to sample. The total sample sizes were $n = 22$ plants from O'ahu plants and $n = 34$ plants from Maui.

At each sampling period, prickly density and latex exudation was quantified for each plant using the youngest fully expanded leaf. Latex was quantified by cutting the distal tip of the leaf and collecting the exuded latex on a pre-weighed 1-cm² filter paper. Because Papaveraceae is characterized by having articulated laticifers which may not fully drain at the leaf tip (Hagel et al. 2008), latex was also collected on the same filter paper after excising the leaf at the leaf base from the stem and collecting the latex exuded from the leaf base (not the stem). The filter paper was then enclosed in a pre-weighed plastic vial and put on ice until it could be weighed, within a few hours. The difference between the filter paper with latex and the pre-weighed filter paper represents latex amount (mg).

The excised leaf was then examined using a dissecting microscope to quantify prickly density. All prickles covering the abaxial and adaxial sides of the leaf were counted. Prickles were consistently found along the leaf edges, and so, for this study, I focused on prickly density across the leaf lamina, excluding prickles on leaf edges. Following quantification of the prickles, a digital photo was taken of the leaf and analyzed using ImageJ (Rasband 1997–2011) to quantify leaf area (cm²). Prickly density was calculated as the number of prickles/leaf area for both the abaxial and adaxial sides of the leaves. Due to experimental error, prickly density data are missing for the first harvest.

Following latex and prickly quantification, remaining shoot tissue and roots were harvested and oven-dried. The leaf sampled for latex and prickles was also dried. Dried tissues were weighed, and biomass data were used for trade-off analyses.

Data were analyzed using SAS v.9.2 (Cary, NC, USA). Residuals were examined, and latex amount was log-transformed to meet assumptions of normality and homoscedasticity. Because abaxial and adaxial prickly densities are highly correlated ($r = 0.7300$, $n = 45$, $P < 0.0001$), a single measure of density was obtained by summing the total number of prickles (abaxial + adaxial) divided by the leaf area. A two-factor ANOVA using PROC GLM was conducted for each of latex amount and total prickly density to examine how population, time, and their interaction affected expression patterns. Due to imbalances in replication across groups, type III sums of squares are reported for all analyses.

Induction experiment: design and statistical analyses

Plants used to examine the induction of latex and prickles by mechanical damage were transplanted into 4.4-L pots and placed on benches on an exposed grow area of the

St. John Plant Sciences building. In contrast to the covered area used for the ontogeny study, this growth area is completely open, and plants were exposed to ambient temperatures, humidity, precipitation, and light. Plants were watered daily, and placement on benches was re-randomized weekly.

On 24 March, approximately 4 weeks following transplantation when plants were at the 5–6 leaf stage, plants assigned to the damage treatment received 50 % defoliation by removing approximately the distal half of all leaves with scissors. There were no herbivores observed on any plants due to the absence of insects in the grow-area. One week later, when a second new leaf had completed expansion, damaged plants and undamaged control plants were processed to quantify latex amount and prickle density on that youngest new leaf, and all tissues were harvested, dried, and weighed. A second 50 % damage treatment was imposed on 10 April, approximately 6.5 weeks following transplantation when plants were at the 9–11 leaf stage. One week later, damaged and control plants were processed and harvested as previously described. For each population, there were 3–7 replicate plants per treatment \times time level, giving a total sample size of $n = 18$ plants from O'ahu and $n = 24$ plants from Maui.

Data were analyzed using PROC GLM to test whether population, mechanical damage, time of damage, and the interactions between population \times damage, time \times damage, and population \times time \times damage affected latex exudation and total prickle density. Latex amount was log-transformed.

Tolerance experiment: design and statistical analyses

Plants used to examine tolerance of mechanical damage were transplanted into 8.8-L deep pots (30.5 cm deep) and placed on benches in the covered grow area of St. John. Plants were watered daily and re-randomized in placement weekly.

Plants were randomly assigned to four treatment groups: D1 plants received 50 % mechanical defoliation on 24 March at the 5–6 leaf stage; D2 plants received 50 % mechanical defoliation on 14 April at the 9–11 leaf stage; D3 plants received 50 % mechanical defoliation on 10 May at the 11–14 leaf stage; and C plants which received no defoliation. Plants damaged at D1 and D2 were in the juvenile ontogenetic stages, while most plants had initiated bolting and flower development by the time the D3 defoliation treatment was imposed. No natural herbivory was observed on any plant.

On July 20–22, aboveground tissues (leaves, stems and reproductive parts) were harvested, oven-dried, and weighed. For each population, there were 6–10 replicate

plants per treatment group, giving a total sample size of $n = 26$ plants from O'ahu and $n = 36$ plants from Maui.

Effects of defoliation treatment and population, as well as their interaction, were tested using PROC GLM. Shoot biomass (stems + leaves) was analyzed as a measure of compensation, and reproductive biomass (buds + flowers + immature capsules) was analyzed as a measure of fitness. Reproductive biomass was log-transformed to meet the assumption of normality.

Water availability experiment: design and statistical analyses

Plants used to examine the effects of water availability on latex exudation and prickle density were transplanted into 4.4-L pots and placed on benches in the covered grow area of St. John. Plants were randomly assigned to one of three watering regimes: control plants receiving daily watering (C); plants watered only once a week during the early juvenile ontogenetic stage between 28 March and 26 April, and then watered daily throughout the remainder of the experiment (JD); and plants watered daily during the juvenile stage and then watered once a week from 26 April–26 May (MD). Plants were re-randomized on benches weekly.

Latex was quantified at two harvest times: April 27–29 and May 24–26 in order to assess how early versus late water limitation affects latex exudation. Due to some mortality of plants receiving limited watering, replication within populations was low (1–5 plants per treatment group per harvest for each population), and so all plants were combined into a single analysis, omitting population effects. Prickle densities were quantified only for plants in harvest 1.

Differences in latex among treatment groups were tested separately at harvest 1 (C vs. JD) and harvest 2 (C vs. JD vs. MD) using PROC GLM; prickle density effects were tested only at harvest 1. Least-square mean comparisons were used to determine how the treatment groups differed in harvest 2.

Light effects

To examine whether light availability influenced the expression of latex and prickles, comparisons were made of similarly sized plants growing under the covered (shaded) canopy versus exposed area with no canopy. Specifically, undamaged control plants from the “induction experiment” growing in the exposed area were statistically compared to fully watered control plants from the “water availability experiment” growing in the shaded area. Photosynthetically active radiation was quantified with a Minolta SPAD meter, revealing dramatic differences in light between the shaded area (daily maximum 130 $\mu\text{mol PAR}/\text{m}^2/\text{s}^2$), and the sunny

area (daily maximum 1,994 $\mu\text{mol PAR}/\text{m}^2/\text{s}^2$). Prickle density and latex exudation were compared using PROC GLM in which the only factor tested was grow area (shaded vs. sunny site).

Results

Ontogeny experiment

Across all plants, prickly density was highly variable, ranging from 0.63 to 10.87 prickles/ cm^2 on the abaxial leaf side, and from 0.53 to 13.15 prickles/ cm^2 on the adaxial side. Total prickly density measured on the youngest fully expanded leaf remained constant over the 5 weeks surveyed ($F_{4,36} = 1.55$, $P = 0.2073$), but differed significantly between populations ($F_{1,36} = 11.85$, $P = 0.0015$), with O'ahu plants having approximately 1.5 \times higher densities than Maui plants when averaged across all weeks (Fig. 1a). Differences between populations were variable over time,

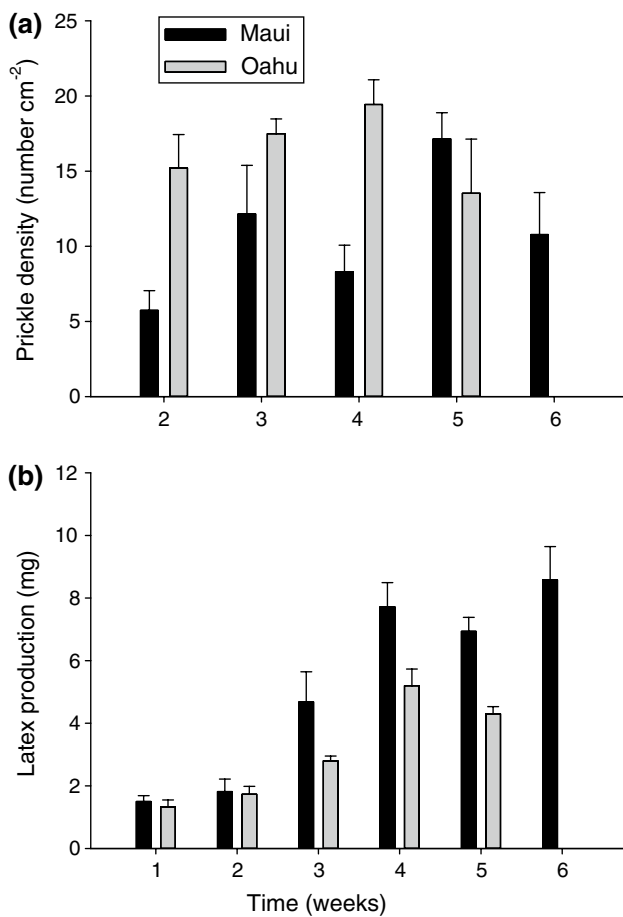


Fig. 1 Ontogenetic patterns in **a** prickly density and **b** amount of latex exudation in two populations of the Hawaiian prickly poppy (*Argemone glauca*) in Hawai'i. Bars means + 1SE ($n = 3-6$)

leading to a significant population \times time interaction ($F_{4,36} = 4.14$, $P = 0.0128$).

Latex amount significantly increased during the 6 weeks surveyed ($F_{5,42} = 27.97$, $P < 0.0001$), and was nearly 2 \times higher in Maui plants compared to O'ahu plants on average across the entire 6 weeks ($F_{1,42} = 5.66$, $P = 0.0220$; Fig. 1b). Differences between populations were consistent over time (population \times time interaction not significant: $F_{4,42} = 0.67$, $P = 0.6182$).

Induction experiment

Mechanical damage failed to induce higher levels of prickly density ($F_{1,35} = 0.25$, $P = 0.6209$; Fig. 2a), and this was consistent for both populations and at both times (no interactions significant, $P > 0.1325$). Although there was no overall effect of damage on latex exudation ($F_{1,35} = 0.85$, $P = 0.3615$), there is evidence that populations differed in their responses to the first damage treatment (population \times time \times damage interaction: $F_{2,35} = 2.97$,

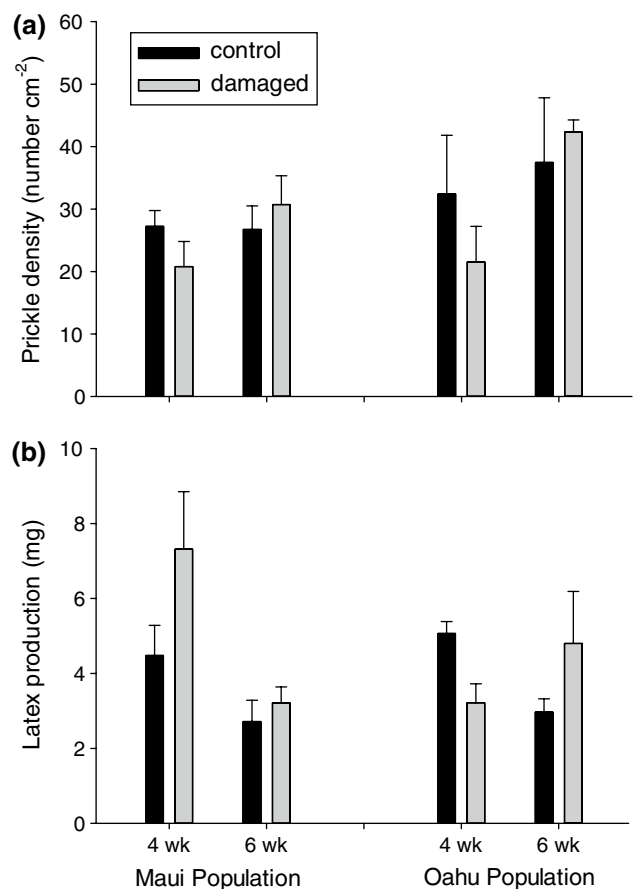


Fig. 2 Differences in **a** prickly density and **b** amount of latex exudation in plants defoliated at 4 and 6 weeks of age. Bars means + 1SE ($n = 3-7$)

$P = 0.0641$; Fig. 2b). Specifically, at 4 weeks of age (time 1), plants from Maui tended to increase latex in response to damage while plants from O'ahu tended to decrease latex in response to damage (Fig. 2b). There is no evidence for induction of latex in response to the damage at 6 weeks of age (time 2; Fig. 2b).

Tolerance experiment

Plants receiving 50 % defoliation were able to fully compensate in shoot biomass regardless of the timing of defoliation ($F_{3,53} = 1.20$, $P = 0.3198$). However, reproductive biomass was significantly affected by defoliation ($F_{3,53} = 2.64$, $P = 0.0588$), with no O'ahu plants damaged at the mature stage (D3) producing any reproductive tissues at the time of harvest (Fig. 3). Reproductive biomass was similar across treatment groups for Maui plants (Fig. 3), and this difference between O'ahu and Maui plants in their reproductive biomass led to a marginally significant interaction effect between defoliation and population ($F_{3,53} = 2.47$, $P = 0.0716$). Shoot biomass was

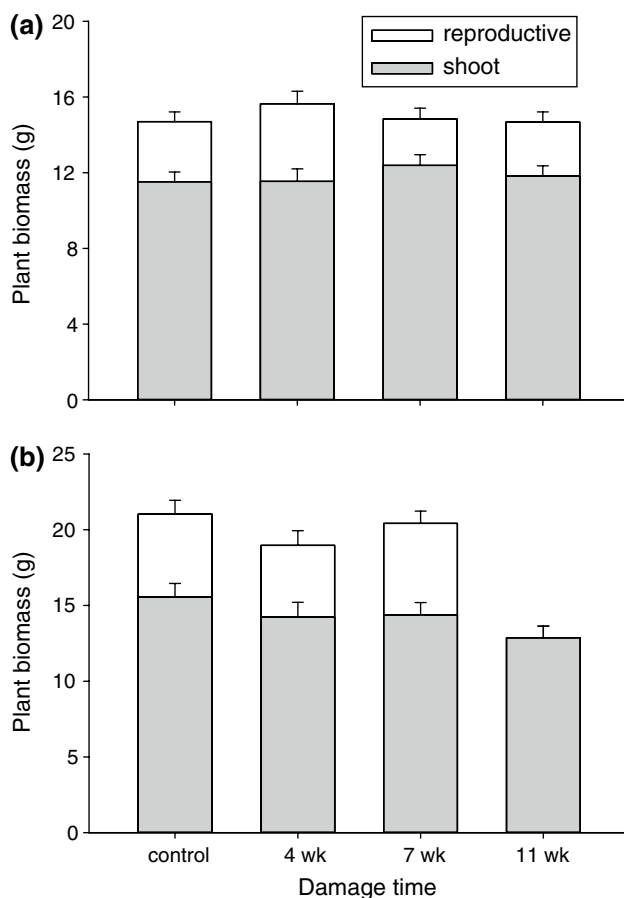


Fig. 3 Effects of defoliation on shoot and reproductive biomass in plants from **a** Maui and **b** Oahu. Bars mean + 1SE ($n = 6-10$)

significantly higher for O'ahu plants compared to Maui plants ($F_{1,53} = 23.46$, $P < 0.0001$), and this was consistent across treatment groups (population \times damage interaction not significant: $F_{3,53} = 1.70$, $P = 0.1783$).

Water availability experiment

Reducing water availability had no effect on latex exudation at the juvenile stage (harvest 1: $F_{1,10} = 0.01$, $P = 0.9910$; Fig. 4). However, by harvest 2, water limitation was observed to significantly reduce the relative investment in latex compared to fully watered control plants (latex per leaf weight: $F_{2,19} = 6.68$, $P = 0.0063$). Although total latex exudation was similar among the three treatment groups ($F_{2,19} = 2.22$, $P = 0.1364$), plants receiving limited water during the juvenile stage (JD) had significantly larger leaves than control plants (Tukey-adjusted least-square mean comparison $P = 0.0642$), leading to relatively lower latex amount per leaf mass (Tukey-adjusted least-square mean comparison $P = 0.0469$). Plants receiving limited water during the mature stage also had lower relative latex amount per leaf mass than control plants (Tukey-adjusted least-square mean comparison $P = 0.0065$), despite having similar-sized leaves (Tukey-adjusted least-square mean comparison $P = 0.8095$).

Water availability had no effect on prickly density ($F_{1,9} = 0.01$, $P = 0.9074$).

Light effects

High light significantly increased prickly density ($F_{1,25} = 5.79$, $P = 0.0238$; Fig. 5), but had no discernible effect on latex ($F_{1,25} = 1.48$, $P = 0.2352$). These patterns were found in both populations (no significant light \times population interactions).

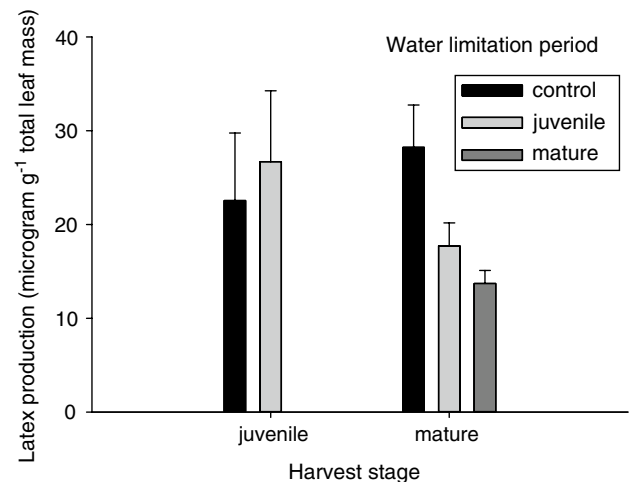


Fig. 4 Effects of watering regime on latex exudation collected during the juvenile and mature plant stages. Bars mean + 1SE ($n = 7-8$)

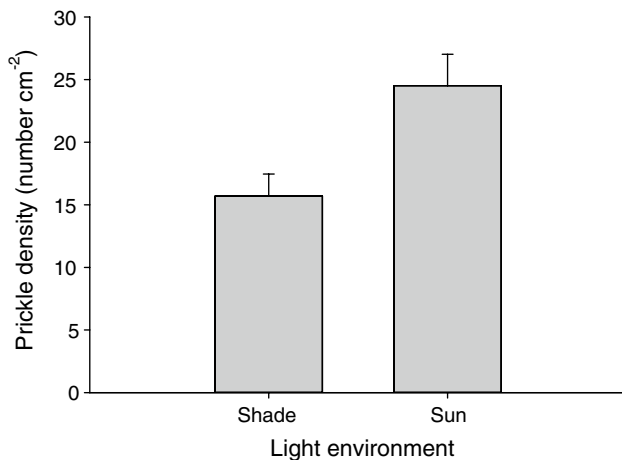


Fig. 5 Effects of light environment on leaf prickle density. Bars means + 1SE ($n = 10$ – 19)

Discussion

Argemone glauca expresses highly variable levels of latex exudation, prickle densities, and tolerance to damage, and the traits appear to respond in different ways to the potential sources of variation. Prickle density was consistent across ontogeny and was not induced by either water limitation or mechanical damage, but was significantly induced under high light conditions. In contrast, latex exudation increased significantly across ontogeny and was reduced by water limitation, but had no response to mechanical damage or light. Prickles, latex, and tolerance differed considerably between populations, suggesting different evolutionary histories for these populations. These disparate patterns indicate that latex and prickles are unlinked within *A. glauca*, potentially as a result of differences in their function, and providing little evidence that they jointly function as a “defense syndrome” (Agrawal and Fishbein 2006).

Although field experiments are needed to prove that these traits do in fact function in anti-herbivore defense, the undisputed roles of latex (Agrawal and Konno 2009), prickles (Hanley et al. 2007), and tolerance (Fornoni 2011) in defense strongly suggest that they can be considered part of the Hawaiian poppy defense suite. However, in addition to likely roles in defense, the light study suggests that prickles may also provide ecophysiological benefits similar to trichomes, such as photoprotection via reflectance or the maintenance of water balance by increasing the boundary layer, thereby reducing transpiration. The anatomical similarity of prickles and trichomes, both being epidermal outgrowth on leaves and stems (Bell and Bryan 2008), may also argue for their functional similarity. Although recent evidence suggests that trichomes may actually have weak or negligible effects on transpirational loss of water (Benz and Martin 2006; Schreuder et al. 2001; Skelton et al.

2012), they have been clearly demonstrated to reflect light, reducing photoinhibition and light stress (Jordan et al. 2005; Liakopoulos et al. 2006). Neither of these functions have been previously investigated for prickles. Experiments testing whether prickles reduce photo-damage and maintain water balance under high light are needed to ascertain whether these functions can be ascribed to prickles in the Hawaiian poppy.

In contrast to prickles, there is no evidence presented here that latex benefits the Hawaiian poppy via an ecophysiological role. Latex amount showed no plasticity in response to light, and was reduced under water limitation. Because laticifers are anatomically associated with the plant vascular system (Pickard 2008), it is not surprising that reduced water availability leads to a reduction in latex amount. However, it is unlikely that this reduction can be considered a functional attribute of the plant. In a recent review of latex in plants, all functional hypotheses, except defense, were rejected due to a lack of supportive evidence (Agrawal and Konno 2009). Further research on the chemical constituents of *A. glauca* latex would help clarify whether latex functions in defense or other ecophysiological roles, and reveal whether plasticity in latex amount extends to plasticity in the chemical composition of latex.

Interesting differences were observed between the populations from Maui versus O’ahu. Plants from O’ahu were larger, had higher densities of prickles, but produced less latex. Induction of prickles was not found for either population, but O’ahu plants had less latex when damaged while plants from Maui produced more latex in response to damage at the juvenile stage. Finally, although both populations tolerated damage in terms of shoot biomass, O’ahu plants damaged at the onset of bolting failed to develop any reproductive biomass, demonstrating lower tolerance to damage in O’ahu versus Maui plants. Whether these significant population differences reflect different herbivore selection pressure in Maui versus O’ahu requires further research quantifying damage in the two populations. However, because plants were grown from seeds collected in gardens, and the original source of the garden plants is not known, it will be difficult to conclusively identify whether population differences are due to local differences in the O’ahu versus Maui gardens or whether they are due to inherited differences from original source populations. However, the strong differences observed between populations for all three defense traits points to the presence of genetic variation which is important for putting these results into the context of the evolutionary ecology of plant defense.

This study revealed an important role of ontogeny in *A. glauca* defense, as has been found for many other species (Barton and Koricheva 2010). While latex amount increases significantly during early ontogeny, prickle density remains consistent across early ontogeny. Whether

latex increases due to the development of larger leaves in older plants (unpublished data), or whether latex defense has been selected for in older ontogenetic stages, is not known. Anatomical studies and field measures of selection coefficients would provide insights into these two possible mechanisms. The consistent expression of prickles across ontogeny is an interesting and unexpected pattern, given the general tendency for spines and thorns to decrease as plants age (Cooper and Ginnett 1998; Cooper and Owensmith 1986; Gómez and Zamora 2002; Gowda 1996, 1997; Pisani and Distel 1998). Ontogenetic declines observed in spines and thorns are generally thought to reflect the greater need for structural defense against ground-dwelling mammals in juvenile plants, followed by a decrease as plants outgrow these herbivores. Because *A. glauca* is a herb, it is not possible to outgrow ground-dwelling herbivores (such as the extinct flightless birds that may have fed on it historically, or the now-present goats), and so it seems likely that prickles would be favorable at all ontogenetic stages. Because ontogenetic patterns in prickles have not been examined in any other prickly poppy species, it remains unclear whether this is a general ontogenetic trajectory for all *Argemone*. Furthermore, this result highlights how research focused explicitly on prickles is needed to determine how the functional ecology of prickles differs from that of spines and thorns.

In conclusion, this study reveals that prickles, latex, and tolerance to damage differ in their patterns of variation, providing a compelling case study for the examination of structural defense traits in the context of defense syndromes. Moreover, these results can shed light on the prediction that island plants have weak defenses due to the absence of some herbivore guilds, such as herbivorous mammals, mollusks, and reptiles in Hawai'i compared to mainland communities (Carlquist 1970; Ziegler 2002). In contrast to this prediction, *A. glauca* expresses high levels of latex, prickles, and tolerance to damage, suggesting that this island endemic is very well defended against herbivores. After having colonized Hawai'i, presumably from Mexico given its close relation to *A. mexicana* (Schwarzbach and Kadereit 1999), the poppy most likely encountered herbivory by native geese (Givnish et al. 1994) and insects. Due to the recent extinction of the native geese and much of the native insect fauna, *A. glauca*'s current natural enemies are likely to be non-native mammals (goats, pigs) and non-native insects. Whether prickles and latex provided defense against now-extinct native herbivores is impossible to test, but it is possible to investigate whether prickles and latex are under current selection by novel interactions with non-native herbivores. Future field studies examining interactions between *A. glauca* and its new natural enemies will shed light on the current "evolution in action" of prickles, latex, and tolerance in this island endemic. Furthermore, there is evidence

that prickles may perform ecophysiological functions for *A. glauca* under high light, circumstantially supporting the idea that plant traits can have multiple functions and be selected for by various factors (Agrawal et al. 2009).

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