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Kasey Barton

University of Hawai'i at Mānoa, kbarton@hawaii.edu

Aaron B. Shiels

USDA, APHIS, Wildlife Services, aaron.b.shiels@aphis.usda.gov

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Additive and non-additive responses of seedlings to simulated herbivory and drought

Kasey E. Barton¹  | Aaron B. Shiels² 

¹School of Life Sciences, University of Hawai'i at Mānoa, Honolulu, Hawaii

²USDA, National Wildlife Research Center, Fort Collins, Colorado

Correspondence

Kasey E. Barton, School of Life Sciences, University of Hawai'i at Mānoa, 3190 Maile Way, Room 101, Honolulu, HI 96822, USA.
Email: kbarton@hawaii.edu

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Abstract

Drought is a global threat, increasing in severity and frequency throughout tropical ecosystems. Although plants often face drought in conjunction with biotic stressors, such as herbivory or disease, experimental studies infrequently test the simultaneous effects of drought and biotic stress. Because multiple simultaneous stressors may have non-additive and complex effects on plant performance, it is difficult to predict plant responses to multiple threats from research examining one stress at a time. Using an experimental approach in the greenhouse, we investigated potential non-additivity in seedling growth and survival to simulated drought and herbivory across a phylogenetically diverse pool of ten Hawaiian plant species. Overall, seedlings showed limited tolerance, defined as similar growth and survival in stressed compared with control (non-stressed) plants, to simulated herbivory and drought, with the combined effects of both stressors to be generally additive and negative across species. Significant variation in stress tolerance was detected among species, and species variation was explained, at least in part, by functional traits such that species with larger root/shoot ratios and smaller seeds, tended to demonstrate greater herbivory and drought tolerance. Future research incorporating additional trait analysis and different stressors could shed light on mechanisms underlying seedling stress tolerance and clarify whether additivity, as detected in this study, extends across other combinations of stressors. Such work will provide needed insights into the regeneration of seedlings in tropical forests under threats of herbivory and climate change.

KEYWORDS

compensatory growth, drought tolerance, Hawaiian Islands, herbivory tolerance, interactive effects, press drought, pulse drought

1 | INTRODUCTION

Drought is increasing globally (IPCC 2014) and is one of the greatest climate change threats to both seasonal and aseasonal tropical ecosystems (Allen et al., 2017; Bonal, Burban, Stahl, Wagner, & Herault, 2016; McDowell et al., 2018; Schwalm et al., 2017). In addition to the direct effects of drought suppressing plant growth and

survival, there can be strong indirect effects due to increased pest and pathogen damage (Anderegg et al., 2015). Indirect effects often result in non-additivity between multiple stressors such that plants experiencing drought and herbivory simultaneously have lower performance than the summed effects of each stressor alone (Davidson, Gottschalk, & Johnson, 1999; Denton, Smith, Hamerlynck, & Sheley, 2018; Wallin & Raffa, 2001). Non-additive effects between

drought and herbivory may occur due to signaling cross talk underlying plant responses to multiple stressors (Atkinson & Urwin, 2012), or because drought alters the likelihood or intensity of herbivory on plants. In general, non-additive effects emerging from interactions among genotypes within species (Barton et al., 2015) and among species within communities (Ball, Hunter, Kominoski, Swan, & Bradford, 2008; Bernardo, Goad, Vitt, & Knight, 2019; Thompson, MacLennan, & Vinebrooke, 2018) are increasingly recognized to play important roles in community dynamics and environmental stress tolerance in plants and warrant further examination in tropical systems.

There is considerable evidence that drought-stressed plants have weakened resistance to herbivores, which could magnify the effects of herbivory on plants experiencing drought and lead to non-additivity between these combined stressors on plant performance. For example, secondary chemicals that deter herbivores may decline in production under drought, thereby making plants more attractive to herbivores (Gaylord et al., 2013; Mundim & Pringle, 2018; Netherer et al., 2015). Drought is also linked to insect outbreaks, due to the combined effects of drought-weakened tree resistance and direct effects on insect population dynamics (Anderegg et al., 2015; Raffa et al., 2008), although drought-induced insect outbreaks may be less common in tropical than temperate forests (Anderegg et al., 2015; Dyer, Carson, & Leigh, 2012). In addition to resistance traits that deter herbivores, plant defenses also include post-damage induced responses contributing to compensatory growth and recovery as part of herbivory tolerance (Fornoni, 2011; Strauss & Agrawal, 1999). How drought influences plant tolerance to herbivory remains poorly studied (Jamieson, Trowbridge, Raffa, & Lindroth, 2012), particularly in tropical plants, despite the prevalence of these simultaneous stressors in tropical forests.

Plant tolerance to herbivory is defined as the maintenance of fitness in damaged compared with undamaged control plants (Fornoni, 2011). Plants tolerate herbivory through increases in photosynthesis (Fang, Yuan, Wang, & Zhao, 2006; Thomson, Cunningham, Ball, & Nicotra, 2003), mobilization of stored reserves (Bosdorf, Schroder, Prati, & Auge, 2004; Latzel, Malikova, & Klimesova, 2011), and phenological shifts leading to early reproduction (Freeman, Brody, & Neefus, 2003). Herbivory tolerance at the seedling stage has been shown to increase with high light efficiency in photosynthesis (Barton, 2016) and from investment in storage tissues, such as cotyledons and roots (Armstrong & Westoby, 1993; Barton, 2013; Lurie, Barton, & Daehler, 2017). Herbivory tolerance is likely to be influenced by drought due to shared hormone signaling networks and underlying mechanistic traits. The two primary hormones mediating stress responses to drought and herbivory are abscisic acid and jasmonic acid, which are themselves involved in a positive feedback mechanism that results in "priming" of plants to respond more efficiently to subsequent episodes of stress (Avramova, 2019; Nguyen, Rieu, Mariani, & van Dam, 2016). Although this shared signaling network suggests that drought-stressed plants should have elevated induced defense (i.e., tolerance) following herbivory (Avramova, 2019), the dependence of both drought and herbivory

tolerance on non-structural carbohydrates and conflicting biomass allocation plasticity can lead to negative non-additivity. For example, non-structural carbohydrates function to maintain osmoregulation, thereby facilitating water uptake by roots under drought stress (O'Brien, Leuzinger, Philipson, Tay, & Hector, 2014), but in contrast, are quickly mobilized to shoot meristems to facilitate compensatory growth for herbivory tolerance (Myers & Kitajima, 2007; Willaume & Pages, 2011). Thus, drought-stressed plants are likely to be constrained in their capacity to mobilize non-structural carbohydrates aboveground for herbivory tolerance without compromising drought tolerance. Similarly, biomass allocation is typically very plastic in response to both drought and herbivory, although in different patterns. Drought-stressed plants typically prioritize growth belowground (Denton et al., 2018; Larson & Funk, 2016; Lucas, Bruna, & Nascimento, 2013), while herbivory-stressed plants typically prioritize growth aboveground, at least in response to aboveground herbivory (Barton, 2016; Stevens, Kruger, & Lindroth, 2008; Yoshizuka & Roach, 2011). Related storage and life-history traits, such as seed size and relative growth rate, have also been implicated in stress tolerance to both drought and herbivory (Gianoli & Salgado-Luarte, 2017; Green & Juniper, 2004; Krishnan, Barua, & Sankaran, 2019; Salgado-Luarte & Gianoli, 2017), highlighting the overlap in biotic and abiotic stress tolerance mechanisms. Non-additivity between drought and herbivory tolerance is therefore likely, although much less well-studied than that of drought and herbivory resistance, particularly for tropical plants.

At the population scale, plant resilience to combined drought and herbivory will depend on population dynamics and demographic processes. In particular, the migration of populations to track suitable climate, replacement of dying canopy trees, and in situ evolution to changing climate will all depend on successful seedling recruitment (Christmas, Breed, & Lowe, 2016; Maron, Baer, & Angert, 2014; Martinez-Vilalta & Lloret, 2016). In general, drought and herbivory are common sources of seedling mortality (Moles & Westoby, 2004) and play key roles in determining recruitment patterns and species distributions in the tropics (Baltzer & Davies, 2012; Gaviria, Turner, & Engelbrecht, 2017; Weissflog, Markesteijn, Lewis, Comita, & Engelbrecht, 2018). Because seedlings tend to be quite vulnerable to drought due to their limited carbohydrate reserves, shallow roots, and underdeveloped woody tissue (Comita & Engelbrecht, 2014; Gerhardt, 1996; Marod, Kutintara, Tanaka, & Nakashizuka, 2002), the regeneration niche may be more narrow with regard to aridity than that of the mature trees. In addition, herbivory tolerance is generally weaker in seedlings than older ontogenetic stages (Massad, 2013). Thus, investigating potential non-additivity in tolerance to herbivory and drought is particularly important at the seedling stage and could determine patterns of recruitment and species assemblages under climate change.

We tested seedling tolerance to combined herbivory and drought using an experimental approach on a diverse assemblage of Hawaiian plant species. Tropical islands, including the Hawaiian Islands, are biodiversity hot spots, with high rates of endemism (Kier et al., 2009). Tropical island floras are also among the most threatened globally,

TABLE 1 Species descriptions of 10 taxa included in the experiment. Seed mass (grams) data were obtained from populations used in the experiments (mean $n = 20$), with the exception of *Metrosideros polymorpha*, for which data were obtained from previous research

Species	Family	Distribution	Habitat	Growth form	Seed mass (G)
<i>Eragrostis grandis</i>	Poaceae	Endemic	Mesic, wet forest	Grass	0.00015
<i>Argemone glauca</i>	Papaveraceae	Endemic	Shrubland, coastal	Herb	0.00288
<i>Bidens torta</i>	Asteraceae	Endemic	Dry, mesic, wet forest	Herb	0.00141
<i>Jacquemontia sandwicensis</i>	Convolvulaceae	Endemic	Coastal	Prostrate Herb	0.00357
<i>Dodonaea viscosa</i>	Sapindaceae	Indigenous	Shrublands, dry forest	Shrub	0.00474
<i>Acacia koa</i>	Fabaceae	Endemic	Dry, mesic forest	Tree	0.1133
<i>Metrosideros polymorpha</i>	Myrtaceae	Endemic	Dry, mesic, wet forest	Tree	0.000057 (Drake, 1992)
<i>Myrsine lessertiana</i>	Primulaceae	Endemic	Mesic, wet forest	Tree	0.05313
<i>Nestegis sandwicensis</i>	Oleaceae	Endemic	Dry, mesic forest	Tree	0.4140
<i>Sophora chrysophylla</i>	Fabaceae	Endemic	Dry forest, alpine	Tree	0.06695

with climate change and herbivory by invasive animals identified as key sources of native plant declines (Caujape-Castells et al., 2010; Harter et al., 2015). Drought is projected to increase in frequency and duration on the Hawaiian Islands, and, with simultaneous increases in temperatures, is leading to significantly greater water stress for Hawaiian plants (Chu, Chen, & Schroeder, 2010; Cook, Smerdon, Seager, & Coats, 2014; Frazier & Giambelluca, 2017; Timm, Giambelluca, & Diaz, 2015). Introduced mammals reduce Hawaiian seedling recruitment directly through consumption of flowers, seeds, and seedlings (Pender, Shiels, Bialic-Murphy, & Mosher, 2013; Shiels & Drake, 2011; Shiels, Pitt, Sugihara, & Witmer, 2014), as well as indirectly through disturbance to the plant-growth substrate (Cole, Litton, Koontz, & Loh, 2012; Murphy, Inman-Narahari, Ostertag, & Litton, 2014). Non-native insects may similarly threaten seedling recruitment indirectly through damage to flowers or through failure to pollinate native plants (Aslan, Shiels, Haines, & Liang, 2019; Aslan, Zavaleta, Tershy, Croll, & Robichaux, 2014). Invasive slugs and snails also target native seedlings directly for consumption in Hawaiian forests (Joe & Daehler, 2008; Shiels, Ennis, & Shiels, 2014). While it has been predicted that island plants have weaker anti-herbivore defenses than continental plants due to the disharmonic herbivore communities on islands and the absence of some native herbivore guilds (Bowen & VanVuren, 1997; Ziegler, 2002), comparative research on Hawaiian native versus non-native plant defenses and herbivory levels has been inconclusive (Funk & Throop, 2010; Hoan, Ormond, & Barton, 2014; Peñuelas et al., 2010; Sardans, Llusia, Niinemets, Owen, & Peñuelas, 2010). Considering the extinction of a highly diverse native bird community consisting of many grazing species, it is likely that the native Hawaiian flora evolved under reasonably high herbivore pressure (Baldwin, 1947; Black, Hunter, Woog, Marshall, & Bowler, 1998; Givnish, Sytsma, Smith, & Hahn, 1994; Hess, Banko, Miller, & Laniawe, 2014).

We quantified Hawaiian seedling tolerance to simulated drought and herbivory within a controlled experimental context in order to assess fitness consequences of consistent levels of damage and drought applied across a diverse species pool. Because seedling establishment depends on survival and growth, we consider these

to be key fitness metrics for seedling stress tolerance (Barton & Hanley, 2013). Herbivory was simulated through the mechanical removal of shoot biomass, in addition to an exogenous jasmonate application to further ensure that the full induced defense response was stimulated, providing a measure of the capacity for these species to tolerate herbivory at the seedling stage. While artificial damage does not reflect the likelihood of natural herbivory, by achieving consistent damage across species, it provides the most unbiased measure of herbivory tolerance (Tiffin & Inouye, 2000). Our goal was to assess patterns within species as well as compare responses across species in order to begin to develop a general framework for seedling stress tolerance in the Hawaiian flora. Specifically, we examined the following predictions:

- (i) There will be non-additivity between the effects of drought and herbivory on seedling survival and growth, detected as significant statistical interactions due to greater effects of combined herbivory and drought on seedling survival and growth than what would be expected based on summed seedling responses to the individual stress treatments.
- (ii) Seedling tolerance to drought and herbivory will vary among species, and be positively linked to storage tissues, including root/shoot ratio and seed size.

2 | METHODS

To test for potential non-additive effects of simulated herbivory and drought on seedling performance, a greenhouse experiment was conducted at the Pope Greenhouses on the University of Hawaii at Manoa campus. Ten native species, wide-ranging in life forms and phylogenetic diversity (Table 1), were grown from seeds collected from at least 10 maternal plants in natural populations within 12 months of the experiment. Focal species include a grass (*Eragrostis grandis*), three herbaceous species from different habitat types (*Argemone glauca*, *Bidens torta*, and *Jacquemontia sandwicensis*),

a widespread shrub (*Dodonaea viscosa*), Hawaii's most abundant and widespread canopy tree species (*Acacia koa*, *Metrosideros polymorpha*), and three other common tree species (*Myrsine lessertiana*, *Nestegis sandwicensis*, and *Sophora chrysophylla*). Seed mass was measured to the nearest 0.1 mg for 20 individual seeds prior to germination to obtain species mean seed mass, except for *M. polymorpha*, which were too small to be detectable on our laboratory balances. For *M. polymorpha*, mean seed mass data were referenced from a previously published study (Drake, 1992). Seeds were germinated in the laboratory, following scarification or gibberellic acid application as needed to break dormancy, and were moved to the greenhouse as true leaves emerged. Due to space constraints and variation among species in their phenology, which influenced the timing of seed collections, species were not tested simultaneously, but were grown sequentially between June 2014 and April 2015. Because day length and temperature have very weak seasonality in Manoa where the greenhouses are located (Giambelluca et al., 2013), this rolling experimental design is unlikely to have introduced substantial abiotic variability to the experiments.

Seedlings were transplanted at the 1 or 2 true leaf stage into 1-gallon (4.4 L) pots filled with equal parts by volume Pro-Mix BX (65%–75% Canadian sphagnum peat moss, perlite, dolomitic and calcitic limestone, macro- and micronutrients, *Glomus intraradices* mycorrhizae inoculum) and black cinder, and treated with a single application of slow-release fertilizer (Osmocote ©). All seedlings were watered daily for one week to allow seedlings to recover from transplant shock. The full factorial experiment included 3 factors: drought, herbivory, and harvest time. Seedlings were randomly assigned to the treatment group at the time of transplant.

The drought factor includes three levels, including a control group watered daily to 100% field capacity ("100FC"), and two simulated drought treatments, to mimic a persistent decrease in precipitation ("70FC press drought"), or a period of no precipitation ("pulse drought"). These drought treatments simulate annual decreases in precipitation as well as more consecutive days without rainfall, both of which are predicted for the Hawaiian Islands (Frazier & Giambelluca, 2017; Timm et al., 2015). The 70FC group received water three times a week to maintain the pots at 70% FC. At each watering event, 20 pots were randomly selected and weighed. The mean mass of these pots was compared with that of well-watered pots at 100% FC, and the amount of water necessary to bring them to 70% FC was measured and applied by hand to each pot (Sack, 2004). The 70FC drought treatment was applied throughout the experiment, starting one week after transplant. For our soil media, the 100FC and 70FC treatments correspond to soil matric potentials of -2.6 and -4.7 kPa, respectively, as determined with a soil-water characteristic curve following the Tempe pressure cell method (Westerband, Bialic-Murphy, Weisenberger, & Barton, 2020). Plants in the pulse-drought treatment received no water for a duration of up to 28 days. For species that wilted before 28 days, the pulse drought was ended as soon as 50% of the plants had severely wilted (Engelbrecht & Kursar, 2003). At the end of the pulse drought, each pot was weighed so that gravimetric water

content (% FC) could be calculated. Following the end of the pulse drought, plants in this treatment group were watered daily throughout the rest of the experiment.

The herbivory factor included a damage treatment fully crossed with the drought treatment (applied to half of the plants in each of the three drought treatment groups). One week following the end of the pulse-drought treatment, herbivory was simulated by clipping the stem to remove 50% of leaves and the apical meristem, which approximates the herbivory by crabs, birds, mollusks, and rodents that these species most commonly experience at the seedling stage. Because our goal was to activate the induced responses underlying herbivory tolerance, which are known to be mediated by the jasmonate signal transduction pathway (Atkinson & Urwin, 2012), we also treated damaged plants with an exogenous application of jasmonic acid by spraying the plants with a 0.5 mM jasmonic acid solution until tissues were saturated and dripping (Rasmann, Johnson, & Agrawal, 2009). Control plants were sprayed with distilled water at the same time.

For the harvest factor, plants were harvested at three different times, allowing us to investigate the immediate- and long-term effects of drought and herbivory on seedling performance. The first group ("pre-harvest") were harvested immediately prior to the herbivory treatments, one week following the end of the pulse drought. Half of the remaining plants were harvested two ("early harvest") and five ("late harvest") weeks after the herbivory treatment. Harvested plants were separated into shoots and roots, oven-dried at 60°C for 7 days, and weighed to the nearest 0.1 mg.

Tolerance was quantified on the basis of survival and growth (total biomass) in response to the drought and herbivory treatments. Biomass data were log-transformed as needed to meet assumptions of normality for analysis. Because species varied considerably in size, leading to heteroscedasticity, and because species trials were not run concurrently, we analyzed each species separately. For a few species, germination occurred over time, leading to temporal blocks, and for these species, block was included in the analyses as a random factor. All analyses were done using SAS for Windows 9.4, procedures "logistic," "mixed," and "reg."

Survival was analyzed using logistic regression with the main factors of drought and herbivory as well as their interaction. To investigate plasticity in root/shoot ratio in response to the drought treatments, data collected at the pre-harvest were analyzed in separate models with a single fixed effect of drought. Growth variables from the main experiment were analyzed with ANOVA models including the fixed factors drought (3 levels), herbivory (2 levels), and harvest time (2 levels), and block as a random factor (where appropriate). Two-way interactions were included for the fixed factors, and the three-way interaction was included in the final model if significant. Due to imbalances in sample sizes, type III sums of squares are reported, and degrees of freedom were calculated using the Kenward–Roger method (Kenward & Roger, 1997). When main effects and interactions were significant, Tukey-adjusted least-square mean comparisons were made.

To examine trends across species, mean species responses to the herbivory and drought treatments were analyzed using the approach

TABLE 2 Mortality effects across species throughout the experiment

Species	N	Drought	Herbivory	Drought × Herbivory
<i>Acacia koa</i>	268	1.00	0.31	0.01
<i>Argemone glauca</i>	183	0.43	0.04	0.11
<i>Bidens torta</i>	121	15.94**	0.64	0.08
<i>Dodonaea viscosa</i>	203	0.01	0.01	0.01
<i>Eragrostis grandis</i>	209	0.01	0.01	0.01
<i>Jacquemontia sandwicensis</i>	203	0.01	0.01	0.01
<i>Metrosideros polymorpha</i>	176	26.52***	0.01	1.19
<i>Myrsine lessertiana</i>	135	0.01	0	0.01
<i>Nestegis sandwicensis</i>	184	0.01	0	0
<i>Sophora chrysophylla</i>	170	0.08	0.01	0.46

Note: Tests of drought, herbivory, and their interaction are reported as Wald chi-square joint tests, and significant effects are shown as follows: ***, $p < .0001$; **, $p < .01$; and *, $p < .05$.

of meta-analysis with the “metafor” package in R version 3.5.1 (Viechtbauer, 2014). The use of meta-analysis allows us to examine seedling responses to herbivory and drought across species using effect sizes, which are generally more informative than comparing test significance, particularly when species vary in sample sizes (Nakagawa & Cuthill, 2007), and the use of meta-analysis to synthesize results across sites or species within studies is not uncommon (Agrawal et al., 2005; Fajardo & Siefert, 2018; Gurevitch, 2013). Log-response ratios for total biomass were calculated from means, standard deviations, and sample sizes for the control and treatment groups of each species as follows: $\ln(M_S/M_C)$, where M_S is the mean biomass of stressed groups (herbivory, pulse drought, 70FC press drought, pulse drought + herbivory, and 70FC press drought + herbivory), and M_C is the mean biomass of control groups, so that positive response ratios indicate over-compensation (greater growth in stressed than control groups) and negative response ratios indicate under-compensation (lower growth in stressed than control groups). Mixed-model analyses using the *rma.mv* function tested for variation in tolerance (a) to the three types of stressors (herbivory, pulse drought, 70FC press drought); (b) to single versus multiple stressors; (c) over time (early versus late harvest); (d) between woody versus herbaceous species; and (e) among the 10 focal species.

Because species varied significantly in tolerance to herbivory and drought (see Results), we ran correlation analyses to test whether species traits (mean seed mass or root/shoot biomass ratio quantified at the pre-harvest) are potential mechanisms underlying variation in stress tolerance (log-response ratios).

3 | RESULTS

Mortality rates were low in most species, typically below 10% in treatment and controls (Table S1). Exceptions include *B. torta* and *M. polymorpha*, which had significantly greater mortality for seedlings in drought (44%–52% and 65%–95%, respectively) than control watering conditions (5% and 14%, respectively; Table 2, Table S1). Herbivory did not influence mortality in any species, nor did we

detect interactive effects between drought and herbivory on mortality (Table 2).

Drought altered biomass allocation, detected at the “pre-harvest” in only four species: *A. koa*, *B. torta*, *E. grandis*, and *N. sandwicensis* (Table 3, Figure 1). In these four species, root/shoot ratios were higher in plants in the drought groups compared with that of control plants.

From the early and late harvest data, we found that both herbivory and drought significantly reduced total plant biomass in most species (Table 4, Figure 2, Figures S1–S3). Only *A. glauca* fully tolerated herbivory, and full drought tolerance was only detected in *M. polymorpha*, with similar total biomass in drought and control groups for those seedlings that survived drought (Table 4). Not surprisingly, plants grew between harvest times, leading to significant main effects of harvest on total biomass in all species but *M. polymorpha* (Table 4). Overall, there is limited evidence that drought and herbivory have non-additive effects on seedling biomass (Table 4), with only one marginally significant two-way interaction in *D. viscosa* (herbivory*drought, $p = .0734$) and one significant three-way interaction detected in *B. torta* (herbivory*drought*harvest, $p = .0158$). Specifically, in *D. viscosa*, negative effects of herbivory on total biomass were stronger for plants subjected to the 4-week pulse drought than for plants subjected to the 70FC press drought (Figure 2). Similarly, in *B. torta*, herbivory suppressed biomass more in pulse drought than 70FC drought, but only in the early harvest, leading to a significant three-way interaction (Table 4, Figure S1).

Significant two-way interactions between harvest time and drought or harvest time and herbivory reveal that tolerance shifts during early seedling development (Table 4). For some species, tolerance increased over time, resulting in greater negative treatment effects at the early than late harvest (Figure S1). For *A. koa*, this meant that by the late harvest, seedlings had acclimated to the drought treatments, showing no evidence of drought suppressing growth (Figure S1). For *B. torta* and *J. sandwicensis*, herbivory reduced total plant biomass at the early harvest, but by the late harvest, the damaged seedlings had recovered to achieve similar sizes as the control

TABLE 3 Effects of drought on root/shoot biomass ratio assessed from plants harvested on the day before the herbivory treatment ("pre-harvest")

Species	N	Root/Shoot ratio
<i>Acacia koa</i>	35	3.92 *
<i>Argemone glauca</i>	33	1.49
<i>Bidens torta</i>	12	4.44 *
<i>Dodonaea viscosa</i>	44	1.54
<i>Eragrostis grandis</i>	41	6.52 **
<i>Jacquemontia sandwicensis</i>	38	0.25
<i>Metrosideros polymorpha</i>	19	0.77
<i>Myrsine lessertiana</i>	24	1.21
<i>Nestegis sandwicensis</i>	28	7.72 **
<i>Sophora chrysophylla</i>	27	1.77

Note: Reported are type III *F*-tests, and significant effects are shown as follows: **, $p < .01$; and *, $p < .05$.

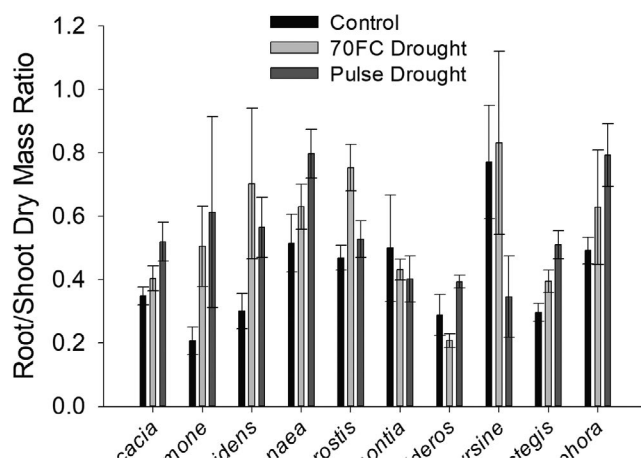


FIGURE 1 Root/shoot ratio calculated from dry mass harvested on the final day of the pulse drought, before herbivory treatments were applied. Drought was imposed as a press-drought treatment in which plants received measured water to maintain a 70% field capacity soil water content throughout the experimental period ("70FC") or as a pulse drought during which plants received no water for a four-week period ("Pulse Drought"). Seedlings were harvested 2 ("Early Harvest") or 5 ("Late Harvest") weeks following the herbivory treatment. Bars are means \pm 1 standard error ($n = 4$ –12)

plants (Figure S1). In contrast, some species showed stronger negative effects of drought and herbivory in the late compared with early harvest (Table 4, Figure S2), revealing weakening tolerance over time. For *E. grandis* and *S. chrysophylla*, drought effects increased over time (particularly for seedlings in the 70FC press drought), while *M. polymorpha* and *M. lessertiana* revealed increasingly negative effects of the herbivory treatment over time (Figure S2). Finally, for some species (*A. glauca* and *N. sandwicensis*), tolerance was relatively stable over time, as indicated by the lack of significant interactions between harvest time and either drought or herbivory (Table 4, Figure S3).

Synthesizing species-level responses to herbivory and drought, we detected that overall, seedlings tolerated a single stressor (pulse drought, press drought, or herbivory) significantly better than they tolerated simultaneous herbivory and drought ($Q_M = 112.30$, $df = 1$, $p < .0001$; Figure 3). Moreover, although tolerance increased significantly over time overall, revealing compensation for stress between the early and late harvests ($Q_M = 7.21$, $df = 1$, $p = .0073$), compensation varied among the different stress treatments, resulting in a significant interaction between stress and time using log-likelihood model comparison ($\chi^2 = 16.24$, $df = 4$, $p = .0027$). Species tended to recover from pulse-drought treatments (alone or combined with herbivory) better than the ongoing 70FC press drought, which was maintained throughout the experimental period (Figure 3). Significant variation in seedling stress tolerance was detected among focal species ($Q_M = 104.83$, $df = 9$, $p < .0001$), ranging across species from 70% to 30% reduction in growth for stressed compared with control seedlings (Figure S4). This species variation did not reflect consistent differences between woody versus herbaceous species ($Q_M = 1.29$, $df = 1$, $p = .2558$).

We detected only a few marginally significant correlations between seed mass or the post-drought root/shoot ratio and seedling tolerance at the species level (Table S2). Herbivory tolerance at the early harvest was marginally greater in small-seeded species ($r = 0.616$, $p = .0578$), although this relationship was largely driven by the very low tolerance of the large-seeded *N. sandwicensis* (Figure 4a) as analysis run after removing *N. sandwicensis* detected no correlation between seed size and herbivory tolerance ($r = 0.406$, $p = .2777$). Consistent with predictions, species with a greater root/shoot ratio tended to demonstrate greater short-term tolerance to the 70FC press drought ($r = 0.595$, $p = .0697$, Figure 4b).

4 | DISCUSSION

In contrast to our prediction, the effects of simulated drought and herbivory on Hawaiian seedling performance were largely additive. Non-additive effects occur when one stressor alters the response of plants to the second stressor, usually by magnifying the combined effect beyond what would be predicted from summing the effects of each stress alone (Bernardo et al., 2019). Such synergistic effects can make it difficult to predict plant resilience under simultaneous stressors from studies examining plant responses to individual stressors. We had predicted that drought would constrain plant tolerance to subsequent herbivory, leading to non-additivity and synergistic effects of herbivory and drought on seedling survival and growth, given the widespread evidence that drought magnifies the effects of herbivory on plant fitness (Anderegg et al., 2015; Jamieson et al., 2012), via induction of a shared signaling network that mediates plant responses (Atkinson & Urwin, 2012; Nguyen et al., 2016) and trade-offs in allocation of stored carbohydrates (Myers & Kitajima, 2007; O'Brien et al., 2014). However, while seedlings experiencing combined drought and herbivory grew significantly less

TABLE 4 Treatment effects of drought and herbivory on total dry biomass (g) from early (2 weeks) and late (5 weeks) harvests across species

Species	N	Drought	Herbivory	Harvest	Drought × Herbivory	Drought × Harvest	Herbivory × Harvest	3-way int
<i>Acacia koa</i>	203	13.28 ***	33.72 ***	29.29 ***	2.22	3.0	1.01	
<i>Argemone glauca</i>	131	20.94 ***	0.99	69.42 ***	0.12	2.07	0.01	
<i>Bidens torta</i>	52	24.61 ***	8.11 **	20.25 ***	1.45	0.45	5.2 *	4.51 *
<i>Dodonaea viscosa</i>	155	30.03 ***	46.41 ***	84.49 ***	2.66	1.42	0.06	
<i>Eragrostis grandis</i>	167	34.78 ***	19.51 ***	54.46 ***	0.27	7.11 **	0	
<i>Jacquemontia sandwicensis</i>	160	14.58 ***	39.42 ***	108.42 ***	0.1	2.1	4.53 *	
<i>Metrosideros polymorpha</i>	48	2.99	13.1 ***	0.73	0.31	0.63	10.49 **	
<i>Myrsine lessertiana</i>	93	16.93 ***	62.2 ***	75.45 ***	2.17	1.73	3.23	
<i>Nestegis sandwicensis</i>	139	16.30 ***	25.39 ***	14.16 ***	0.32	0.03	1.03	
<i>Sophora chrysophylla</i>	124	5.06 **	22.09 ***	26.13 ***	1.57	4.34 *	0.07	

Note: Reported are type III F-tests, and significant effects are shown as follows: ***, $p < .0001$; **, $p < .01$; and *, $p < .05$. Three-way interactions were removed from models when not significant.

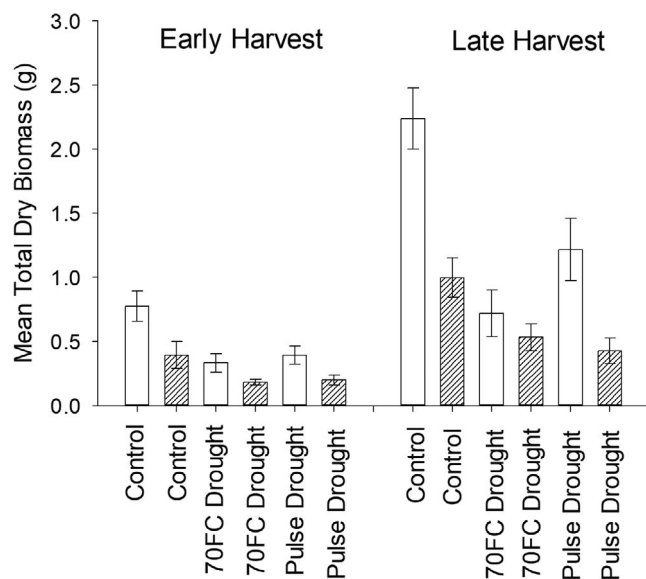


FIGURE 2 Mean total dry biomass for *Dodonaea viscosa*, demonstrating marginally significant non-additivity in effects of simulated herbivory (hatched bars) and simulated drought treatments on seedling growth. Drought was imposed as a press-drought treatment in which plants received measured water to maintain a 70% field capacity soil water content throughout the experimental period ("70FC") or as a pulse drought during which plants received no water for a four-week period ("Pulse Drought"). Seedlings were harvested 2 ("Early Harvest") or 5 ("Late Harvest") weeks following the herbivory treatment. Bars are means \pm 1 standard error ($n = 4$ –12)

than seedlings experiencing only drought or herbivory, the combined effects were largely additive. Perhaps our results differ from most previous studies investigating interactions between herbivory and drought because most of those studies detected non-additivity mediated through resistance traits, such as secondary chemicals (Gaylord et al., 2013; Netherer et al., 2015). Our focus on tolerance metrics (survival and compensatory growth) highlights how drought

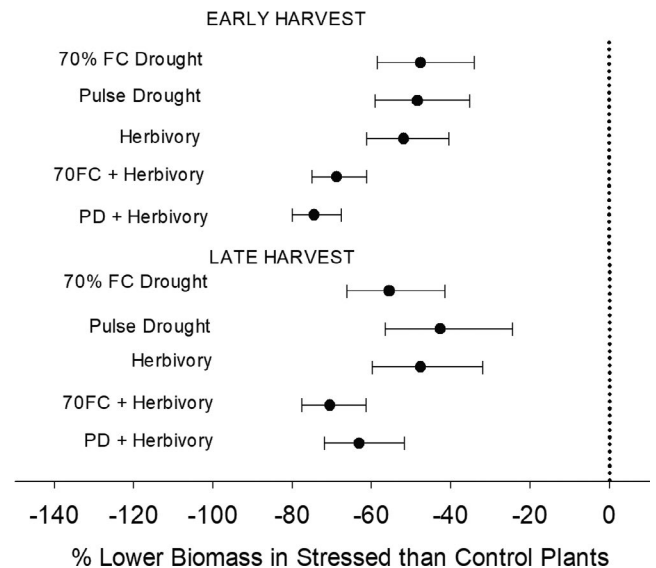


FIGURE 3 Percent reduction in total biomass of stressed versus control plants subjected to individual (70% FC press drought, pulse drought, and herbivory) and combined (70% FC or pulse drought plus herbivory) stress treatments and harvested 2 weeks ("Early") or 5 weeks ("Late") following herbivory treatments. Calculated from log-response ratios, with bias-corrected 95% confidence intervals. Sample sizes are 9–10 species

may alter plant–herbivore interactions differently via resistance versus tolerance defense mechanisms.

For the few species in which non-additivity was detected, we found that the more extreme pulse drought reduced seedling herbivory tolerance compared with seedlings exposed to the 70FC press-drought or control water conditions. That the intensity of drought matters for understanding non-additives in biotic \times abiotic stress tolerance underscores the importance of implementing more complex drought stress treatments that coincide with projected shifts in precipitation frequency, duration, and intensity (Beier et al., 2012; Hoover, Duniway, & Belnap, 2015; Smith, 2011). For example, in

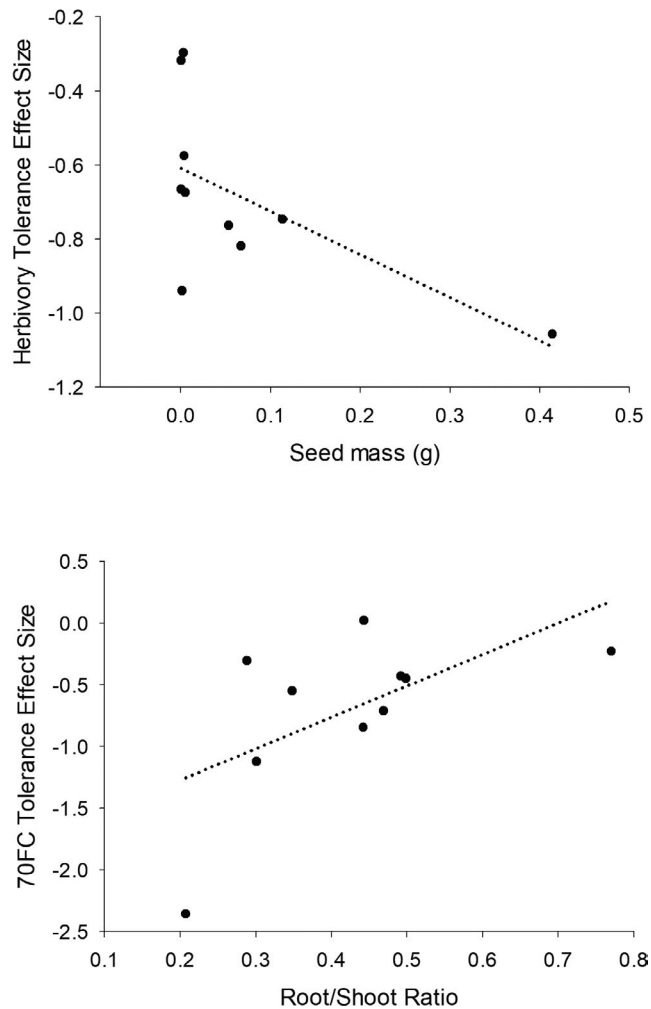


FIGURE 4 Pearson correlations with best-fit line between tolerance and species mean traits at the early harvest, in which tolerance is quantified as log-response ratio effect sizes: $LRR = \ln(M_S/M_C)$, where M_S is the mean biomass of stressed groups (herbivory, pulse drought, 70FC press drought, pulse drought + herbivory, and 70FC press drought + herbivory), and M_C is the mean biomass of control groups. Species is the unit of replication, and sample sizes are 9–10 species. Marginally significant correlations were detected between seed mass and herbivory tolerance (a) and root/shoot ratio and 70% FC press-drought tolerance (b)

a previous study on one of the same focal species examined here, *Metrosideros polymorpha* seedlings exposed to a range of simulated drought treatments that varied in intensity and duration demonstrated complex responses in plasticity of photosynthetic parameters to the drought treatments (Westerband et al., 2019). Specifically, although *M. polymorpha* mortality was greatest under the longest drought treatment, growth under drought was maximized in surviving seedlings with higher chlorophyll content and greater stomatal conductance (Westerband et al., 2019). Complex watering treatments are necessary to characterize plant responses to more realistic climate change scenarios and are particularly needed for tests of simultaneous multiple stressors such as drought and herbivory.

As predicted, we observed significant variation among species in their stress tolerance, although no species demonstrated full tolerance to either herbivory or drought stress within the experimental timeframe. Considering the phylogenetic diversity represented (10 species from 10 plant families) and the range in growth form and habitat types, variation across species is not surprising. Consistent with other studies (Denton et al., 2018; Eziz et al., 2017; Larson & Funk, 2016; Lucas et al., 2013), seedling drought induced a shift in root/shoot ratio in some species, and drought tolerance was positively linked to root/shoot ratio across species. In the four species demonstrating phenotypic plasticity, root/shoot ratios were higher in plants in the drought groups compared with that of control plants, as predicted by adaptive plasticity of organs to maximize uptake of limited resources (Denton et al., 2018; Larson & Funk, 2016; Lucas et al., 2013). Greater investment in roots enhances drought tolerance through greater access to water as well as greater storage, likely leading to enhanced non-structural carbohydrate reserves that can function in osmoregulation to maintain water uptake (O'Brien et al., 2014).

Seed mass was not found to be linked to drought tolerance across species, but did relate to herbivory tolerance. In contrast to our prediction, small-seeded species tended to have greater herbivory tolerance than large-seeded species, although this relationship is likely driven by a single outlier, the very low tolerance of the large-seeded *N. sandwicensis*. The presumed function of seed mass for seedling tolerance is in the provision of stored non-structural carbohydrates (NSC) for re-growth or osmoregulation. However, seed mass is not always a good predictor of seedling tolerance (Lucas et al., 2013; Myers & Kitajima, 2007), because of potential mismatches between seed mass and NSC content and because of the high relative growth rates of small-seeded species (O'Brien, Philipson, Tay, & Hector, 2013). Because we did not examine physiological responses to the experimental treatments, it remains largely unclear what drives the observed variation in drought and herbivory tolerance across species. Previous work on Hawaiian seedlings have revealed chlorophyll content, stomatal conductance, and non-photochemical quenching to function in drought and herbivory tolerance (Barton, 2016; Westerband et al., 2019), and future work is needed to determine plant ecophysiological responses to the combined stressors (Mundim & Pringle, 2018).

Despite variation across species, tolerance to herbivory and drought alone and in combination was generally quite low in this study. Considering the projected increases in aridity (Chu et al., 2010; Cook et al., 2014; Frazier & Giambelluca, 2017; Timm et al., 2015) for the Hawaiian Islands, as well as the high abundances of invasive herbivores that target seedlings (Joe & Daehler, 2008; Shiels, Ennis, et al., 2014; Shiels, Pitt, et al., 2014), low seedling tolerance among the native plants to these stressors could limit future population regeneration. Particularly worrisome are the very high mortality rates of Hawaii's most abundant and widespread keystone tree species, *M. polymorpha*, to simulated drought, and the trend for some species such as *N. sandwicensis* to have weaker tolerance to herbivory and drought over time.

These patterns corroborate earlier studies showing similarly high mortality of *M. polymorpha* to extreme experimental drought (Westerband et al., 2019), and increasingly weaker tolerance to simulated herbivory in several native Campanulaceae species (Barton, 2016). Future studies considering population variation in seedling drought tolerance will provide additional insights into whether these results apply throughout the species' distributions, especially for widespread species such as *M. polymorpha*, which demonstrate local adaptation in seedling drought tolerance on Oahu (Barton, Jones, Edwards, Shiels, & Knight, 2020). Conservation of this unique endemic flora may become increasingly threatened as drought increases and invasive herbivory continues, highlighting the continued need for intensive management to foster native seedling recruitment. Moreover, these results likely extend to other tropical island systems where climate change and invasive herbivory are implicated in native plant declines (Caujape-Castells et al., 2010; Harter et al., 2015) and where similar experimental studies are needed to determine plant responses to their combined effects in order to develop effective conservation plans.

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DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.rv15dv45j> (Barton & Shiels, 2020).

ORCID

Kasey E. Barton  <https://orcid.org/0000-0002-4549-4150>

Aaron B. Shiels  <https://orcid.org/0000-0002-6774-4560>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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