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Assessment of leaf defense hypotheses at the intraspecific scale in three *Helianthus* (Asteraceae) species¹

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Abstract. Interactions between plants and insects have shaped biodiversity at multiple ecological and evolutionary scales in both diverse taxonomic groups. Antagonistic interactions (such as herbivory) can impose strong selective pressures on plants, resulting in increased defense levels or diverse defense strategies. The nature and extent of plant defenses can vary both within and between species, potentially reflecting trade-offs between defense and growth strategy that are associated with environmental resources, known as the resource availability hypothesis. Global interspecific patterns may or may not reflect mechanisms acting within species at more restricted geographic scales. Here, we ask whether there is evidence for associations between growth strategies and defense levels, resource availability, and herbivore damage within three *Helianthus* species (*H. giganteus*, *H. grosseserratus*, and *H. maximiliani*) in a restricted region of the Upper Midwest in the USA. We measure growth traits, leaf defense traits, and leaf herbivore damage levels in wild sunflower populations to assess patterns across populations and in two common gardens to assess patterns within populations. We estimate associations between growth traits and defense traits, defense traits and environmental resources, and herbivore damage and environmental resources. Overall, we find that slower growth strategies are associated with increased levels of defenses, though these higher defense levels are not associated with lower-resource environments in wild populations, and some of these patterns are detected in the commons. We also find that herbivore damage levels are not associated with these resource levels. We conclude that defenses in these species are related to growth strategy even at this intraspecific scale and are largely in line with macroevolutionary patterns across the genus. Both defense levels and herbivore damage are not strongly related to resource availability, which may reflect limited amounts of resource variation in this restricted region and follow prediction within a general framework for intraspecific defense trait associations.

Key words: herbivory, intraspecific variation, resource availability hypothesis

The interactions between insects and plants have helped to shape biodiversity both within and among species, potentially contributing to the extraordinarily high levels of species richness in both groups (Ehrlich and Raven 1964; Farrell *et al.*

1992; Janz 2011). Antagonistic plant–herbivore interactions have been a focus for the study of potential reciprocal adaptation and coevolution driving biodiversity (reviewed in Futuyama and Agrawal 2009). Plant defenses against herbivore pressures are numerous, including structural traits such as the immense diversity of trichomes (including glandular and nonglandular), which can serve as a major form of defense against herbivore attack (Esau 1953; Levin 1973; Wagner 1991; Werker 2000; Wagner *et al.* 2004), or general scleromorphy, which reduces palatability/digestibility (Hanley *et al.* 2007). Defense traits have been linked to either reduced herbivore damage or reduced herbivore presence, but associations are not always detectable and vary broadly (*e.g.*, McKey *et al.* 1978; Agrawal 2005; Carmona *et al.* 2011). Studies spanning multiple taxonomic scales, functional and experimental assays, and community ecological work are necessary to more fully understand what drives the diversity and levels of plant defenses, including how environmental context affects plant strategies (Agrawal 2011).

Variation in levels and types of plant defenses can be attributed to multiple factors and may exist at

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different scales (Stamp 2003). The resource availability hypothesis is perhaps the most prominent theory explaining how abiotic environmental factors can affect plant defenses against herbivores. This hypothesis states that plants in resource-rich environments have faster growth (and regrowth) rates and lower investment in antiherbivore defenses since resources are plentiful to facilitate regrowth (Coley *et al.* 1985). In resource-poor environments, plants are expected to have slower growth rates and higher investment in antiherbivore defenses as the reinvestment of resources lost to herbivory in slow-growing plants is more costly, and any loss of biomass represents a greater proportion of investment than in faster-growing plants (Coley *et al.* 1985). Herbivore damage levels are therefore also higher in high-resource environments (due to the lack of defenses).

At the interspecific level, the resource availability hypothesis is supported by observational and experimental studies documenting the predicted patterns and trade-offs (*e.g.*, Coley 1987; Endara and Coley 2011). Examining multiple species within lineages, predicted associations along environmental gradients are supported in a phylogenetic context across oak species on the global scale (Pearse and Hipp 2012) and congeneric salt marsh species pairs from high and low latitudes (Pennings *et al.* 2007). However, other meta-analyses do not find strong support for these ideas, including a lack of consistent latitudinal associations with defenses and herbivory (Moles *et al.* 2011).

Beyond the conflicting evidence at this scale, interspecific patterns are not always detectable within species, where patterns can be highly dependent on spatial and temporal variation (Bazzaz *et al.* 1987). There can be substantial intraspecific variation in plant traits, especially among populations found along environmental gradients (Albert *et al.* 2010). The extent and sources of intraspecific variation are also highly variable, ranging from phenotypic plasticity and physiology (detectable among individuals) to microevolutionary differences (detectable among populations) (Westerband *et al.* 2021). In terms of the resource availability hypothesis, different drivers may be affecting plant defenses at the *interspecific* versus intraspecific scales (Hahn and Maron 2016). Across species, wide ranges in resources and the general use of species mean trait values can result in the predicted negative relationship

between resource and defenses, whereas selective pressures within species (that represent more restricted portions of the resource continuum) may vary, with low-resource species exhibiting predicted patterns and high-resource species demonstrating opposite trends driven by biotic environment rather than abiotic (Hahn and Maron 2016). The scale of study therefore matters, where analyses across populations may indicate local adaptation and those among individuals within a population may reflect physiological trade-offs.

Evidence for intraspecific predictions associated with the resource availability hypothesis also varies. Multiple studies use latitudinal gradients as proxies for resource availability and examine associations across populations, with overall evidence for lower damage, increased palatability, and lower damage at higher latitudes (Pennings *et al.* 2001, 2007; Pennings and Silliman 2005; Woods *et al.* 2012; Nunes *et al.* 2016), whereas others test for clinal patterns with more specific environmental factors (such as temperature) (Abdala-Roberts *et al.* 2016; Hahn *et al.* 2019). These studies exhibit nuance beyond the straightforward expectations of the resource availability hypothesis, and results from reviews and meta-analyses at the intraspecific level also stress the lack of clear patterns and context-dependent nature of these patterns (Rasmann *et al.* 2014; Hahn and Maron 2016).

The genus *Helianthus* L. (Asteraceae; sunflowers) is a model system in plant ecology and evolution because of taxonomic richness (some 50 species), morphologic diversity (stark differences in growth form, life history, secondary metabolites, reproductive morphology, *etc.*), agricultural importance, sequenced genome, well-studied examples of hybridization, and interactions with insects via both pollination and herbivory (Seiler and Gulya 2004). *Helianthus* has substantial diversity in secondary metabolites, which have previously been used in systematic studies, as well as multiple types of trichomes used as both physical and chemical defenses (Seaman 1982; Spring *et al.* 1987; Spring 1991; Aschenbrenner *et al.* 2013; Brentan Silva *et al.* 2017). At the interspecific scale, Mason *et al.* (2016) used a phylogenetic comparative approach to assess herbivore defenses across 28 species of diploid *Helianthus*. They found that degree of defense was related to leaf economic strategy, with higher defenses in resource-conservative species, but that

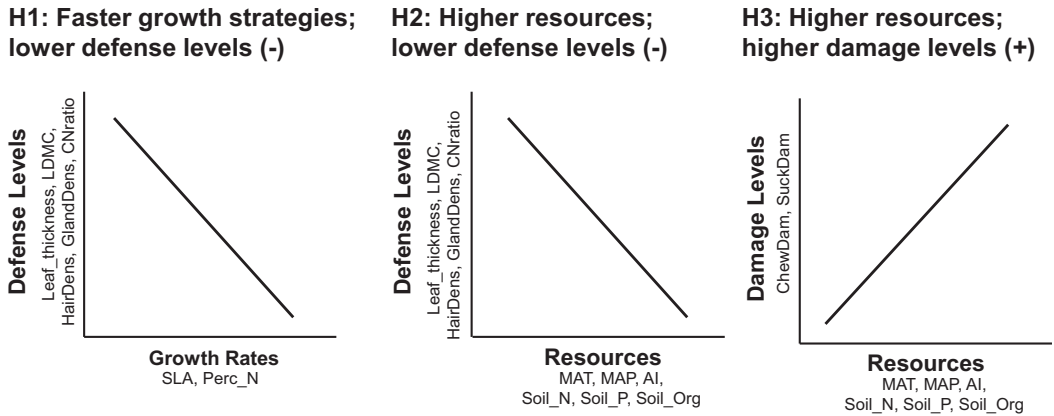


FIG. 1. Conceptual diagram of hypothesized predictions relating defense levels and growth rates, defense levels and resources, and damage levels and resources.

this was not necessarily tied to environmental resources. At the intraspecific scale, a study on the common sunflower *H. annuus* using seeds collected from a latitudinal gradient grown in a greenhouse setting, populations from lower latitudes had elevated resistance to herbivory and slower growth strategies (Beaton 2020). Whether or not these patterns hold true at a more restricted geographic scale associated with specific environmental factors and in perennial species, to our knowledge, remains untested.

Here, we ask whether there is evidence for the resource availability hypothesis within species in three closely related sunflower species using observational evidence from wild populations at the regional scale and evidence from two common gardens established using commercially available wild-collected seeds. The use of multiple species, wild populations, and common gardens are well suited to test intraspecific predictions related to the resource availability hypothesis (Hahn and Maron 2016). Specifically, we hypothesize that (H1) high levels of leaf defenses are associated with slower growth strategy both across populations and within common gardens; (H2) high levels of leaf defenses are associated with resource-poor environments; and (H3) levels of leaf herbivore damage are lower in resource-poor environments (Fig. 1).

Materials and Methods. **STUDY SPECIES.** We investigated three closely related species of herbaceous perennial *Helianthus* found in Wisconsin and into Minnesota: the giant sunflower *H. giganteus* L., the sawtooth sunflower *H. grosseserratus* M. Martens, and Maximilian's sunflower *H.*

maximiliani Schrad. All three *Helianthus* species have broad distributions across North America, with *H. giganteus* largely found in the Midwest and Northeast of the USA; *H. grosseserratus* in the Midwest, Great Plains, and farther south; and *H. maximiliani* largely overlapping the range of the other two. Additionally, there have been documented cases of hybridization between these species either under natural conditions or via experimental crosses, largely made in the Midwest (Long 1955, 1959, 1960, 1961).

WILD STUDY SITES AND COMMON GARDENS. We identified wild populations of all three species across Wisconsin and into nearby northeastern Minnesota using locations found in the Consortium of Wisconsin Herbaria and the community science database iNaturalist. We sampled a total of 20 populations (between 5 and 7 populations per species) between July and September 2020 (Fig. 2, Appendix 1). Populations included roadside stands, preserved prairies, and restored prairies.

We established two common gardens in Eau Claire, WI using commercially available seeds: Jeffers (44.8565°N, 91.5235°W) and Cornell (44.8399°N, 91.4925°W) (Fig. 2, overlapping orange points). Although located a short distance apart (approximately 3 km), the two sites differ in soil nutrients (see environmental data methods and results below). We obtained wild-collected seeds from Prairie Moon Nursery. *Helianthus giganteus* seeds were sourced from Wisconsin, *H. grosseserratus* from Illinois, and *H. maximiliani* from North Dakota. We exposed seeds to a 30-day wet/cold

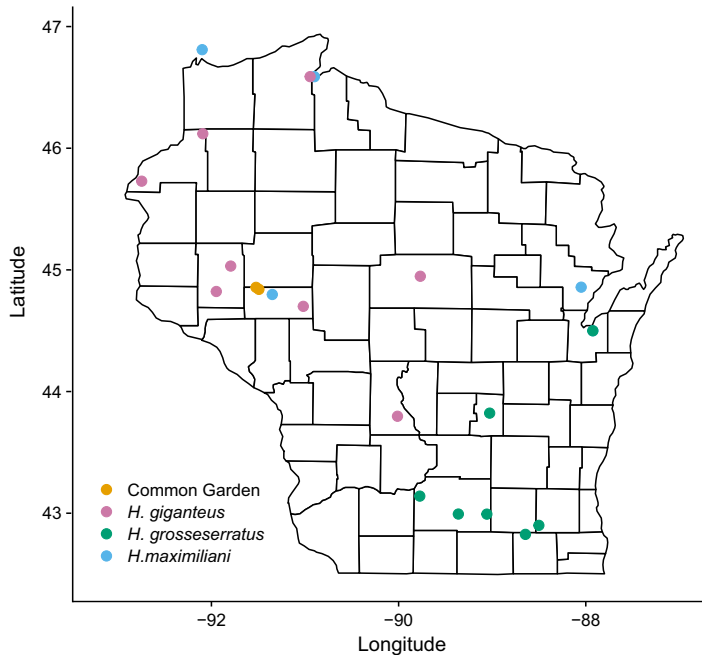


FIG. 2. Map of sampling locations. Pink = *Helianthus giganteus*, green = *H. grosseserratus*, light blue = *H. maximiliani*, orange = common gardens (overlapping).

treatment before germination. In late April 2020, we germinated seeds in petri dishes, transferred seedlings to peat pellets (Jiffy), and established them in the University of Wisconsin–Eau Claire greenhouse. We watered seedlings, rotated trays daily, and used a standing rotating fan to simulate wind conditions until transplantation into common gardens.

We tractor-tilled the sites used for common gardens and planted seedlings during the first week of June. We randomly assigned locations to 75 seedlings of each species in each of the two common gardens. We planted seedlings 90 cm apart in rows 1 m apart. Because of unusually hot and dry conditions, we hand-watered the common garden plants for approximately 3 wk after transplantation. We performed routine maintenance on the common gardens, including hand-removal of weeds and installation of deer fencing around the gardens to deter large vertebrate predation.

LEAF MEASUREMENTS. We measured leaf traits, leaf damage, and trichome densities on plants from the common gardens and wild populations (Table 1). The leaf traits assessed are related to either growth strategy or putatively related to resistance or palatability. Although we are unaware of bioassays directly relating these traits to defense in sunflowers, there is evidence from other *Helianthus*

species that these traits are under selection (Whitney *et al.* 2006) and they are also commonly used measures of leaf palatability (Hanley *et al.* 2007). We assessed all living plants from the common gardens in late July and haphazardly selected 10 plants (located at least 1 m apart) from each of the natural populations (see Appendix 1 for sampling dates). We harvested two recently fully expanded leaves per plant—we used the first to measure leaf investment traits and the second to estimate trichome densities and C:N ratio. For leaf traits, we measured the area of the leaf using a CI-202 portable laser leaf area meter (CID Bio-Science, Camas, WA) and the fresh mass of the leaf (without the petiole) using a microbalance. We estimated the leaf thickness (mm) using a digital micrometer (Mitutoyo, Kawasaki, Japan). We placed leaves in a drying oven for at least 48 hr before measuring leaf dry mass. We calculated specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$) as leaf area/leaf dry mass and leaf dry matter content (LDMC, leaf dry mass/leaf fresh mass).

For the second leaf sample, we used cork borers to extract leaf disks, which were then dried for at least 48 hr. We estimated the densities of glandular (GlandDens) and nonglandular (HairDens) trichomes on the abaxial side of a single dried leaf disk. We counted trichomes using a Leica s9i

Table 1. Descriptions of variables and abbreviations used in this study for plant traits, herbivore damage, and environmental variables.

Variable	Description	Units
Plant traits		
SLA	Specific leaf area	cm ² g ⁻¹
% N	Leaf nitrogen content	%
Leaf thickness	Thickness of leaf blade	mm
LDMC	Leaf dry matter content (dry mass/fresh mass)	
HairDens	Nonglandular trichome density	cm ⁻²
GlandDens	Glandular trichome density	cm ⁻²
CNratio	Leaf carbon:nitrogen ratio	
Herbivore damage		
ChewDam	Leaf chewing damage	%
SuckDam	Leaf vascular tissue damage	%
Environmental variables		
MAT	Mean annual temperature	°C
MAP	Mean annual precipitation	mm
Aridity index (AI)	MAP/mean annual reference evapotranspiration	
Soil P	Soil phosphorus content	parts per million (ppm)
Soil N	Soil nitrogen content	ppm
Soil org	Soil organic matter	%

stereomicroscope (Leica, Wetzlar, Germany) under $5.5 \times$ magnification with a $1 \text{ cm} \times 1 \text{ cm}$ reticle and converted these raw values to densities (measuring a $0.18 \text{ cm} \times 0.18 \text{ cm}$ area, 0.0325 cm^2). To estimate leaf C:N ratio and leaf N content, we weighed 3–4 mg of material from another leaf disk for processing at the University of New Mexico Center for Stable Isotopes.

To assess herbivore damage, for each plant, we scored the three oldest nonsenesced leaves for insect damage as percentage of leaf area caused by different types of insect herbivores and calculated as a damage index D (see Whitney *et al.* 2006 for details). We constructed composite damage indices for leaf chewers (ChewDam: Orthoptera, Lepidoptera, Diptera) and for leaf vascular tissue feeders (SuckDam: Hemiptera, Homoptera) by summing D scores for each of the component taxa.

ENVIRONMENTAL DATA. To characterize the climate of each wild population site, we downloaded historical climate data from WorldClim at 30-sec resolution (Fick and Hijmans 2017). We chose to analyze mean annual temperature and mean annual precipitation, as these two variables are commonly analyzed in the literature and broadly capture the variation across the region. We used the CGIAR Consortium for Spatial Information database to download aridity index (AI) values (at 30 arc seconds) for each of our populations (Zomer *et al.* 2022) (<http://www.cgiar-cis.org>). Here, higher values correspond to higher humidity

and lower values correspond to higher aridity. To obtain information on soil nutrient content, we revisited sites in August–September 2021 and collected a surface soil sample from each wild population and three samples from across each common garden. Soil samples were processed at Midwest Laboratories (Omaha, NE) for soil texture, the “S1AN” soil nutrient package. We chose to analyze the effects of mean annual temperature (MAT), mean annual precipitation (MAP), soil P content, soil N content, and soil % organic matter on the plant defense traits (Table 1). In this study area, we would generally characterize “resource-rich” environments as those with higher levels of precipitation (MAP and AI), warmer temperatures, and higher levels of soil N, P, and organic matter (Fig. 1).

STATISTICAL ANALYSES. We conducted all analyses in R v4.0.2 (R Core Development Team 2016). To assess overall relationships between leaf defenses and growth strategy across populations, we standardized major axis regressions (SMAR) across populations separately for each species with the population mean values for each defense trait as the response variables and the population mean values for each growth trait as predictors using the `sma()` function in the R package `smatr` (Warton *et al.* 2018). We chose to use SMAR because these analyses allow for uncertainty in the estimation of both x and y variables, and we chose to use population means to examine patterns across populations (rather than within). For the

common gardens, we ran similar SMARs but did not take common garden averages; instead, both response and predictor variables were individual plant measurements, to assess patterns across individuals. We also included common garden (Jeffers *versus* Cornell) as “groups” and used a likelihood ratio test to determine whether the SMA lines shared a common slope between gardens and to estimate the confidence intervals for the slope (where the null hypothesis is that the slopes are the same between common gardens). We examined multivariate trait space by performing principal components analysis (PCA) on standardized traits separately for each species in both the wild populations and common gardens using the `princomp()` function.

To test for relationships between both leaf defense traits and herbivore damage with environmental variables, we ran univariate linear mixed effects models separately for each species with each trait as a response variable and each environmental variable as a predictor with site as a random effect using the `lme()` function in the R package `nlme` (Pinheiro *et al.* 2015). We used site as a random effect to account for the fact that multiple individuals were measured per population. We also assessed differences between the common gardens in resource traits, defense traits, and herbivore damage levels using Welch two-sample *t* tests using the `t.test()` function, separately for each species.

Results. VARIATION IN TRAITS AND ENVIRONMENTS. We found substantial variation both within and among the three species in terms of growth traits, defense traits, and amount of leaf damage experienced both in the wild populations and in the common gardens (Appendix 2; Figs. S1, S2). For one example characterizing trait variation, in the wild populations, values for SLA ($\text{cm}^2 \text{g}^{-1}$) are within the global range and close to the median value for herbaceous plants (Poorter *et al.* 2009), with *H. giganteus* having higher values (mean = 185.9, SD = 59.1) and *H. grosseserratus* and *H. maximiliani* having similar values (mean = 109.8, SD = 17.2; mean = 107.0, SD = 21.5, respectively). Common garden mean SLA in *H. giganteus* was similar to that in wild populations (mean = 175.7, SD = 40.2) but was higher in *H. grosseserratus* and *H. maximiliani* (mean = 163.5, SD = 31.3; mean = 157.5, SD = 49.8, respectively). See Appendix 2 for trait data summaries for all traits and Figs. S1 and S2 for visualization of trait data.

Wild sites and common gardens generally had environmental characteristics associated with high resources. Mean annual temperature ranged from 3.8°C to 8.0°C, with *H. grosseserratus* tending to occupy warmer areas (Fig. S3). Mean annual precipitation ranged from 739 mm to 846 mm and AI ranged from 0.720 to 0.868. Soil P ranged from 2.0 parts per million (ppm) to 83.0 ppm (with one high value for *H. maximiliani*), soil N ranged from 1.0 ppm to 57.0 ppm (with one high value for *H. giganteus*), and soil organic matter content ranged from 0.60% to 19.1%. Climatic values for the two common gardens were essentially the same, but Jeffers had higher levels of soil P than Cornell (51.3 ppm *versus* 16.3 ppm, averaged across three samples per garden). See Appendix 2 for full environmental data summaries and Fig. S3 for full visualization of environmental data.

LEAF DEFENSES AND GROWTH STRATEGIES. We tested for a total of 30 possible associations between defense traits and growth traits (10 for each species) in each setting (wild populations and common gardens). Higher values for all five defense traits correspond to increased defenses, whereas higher values for the two growth traits correspond to faster growth strategies, so we expected negative relationships (fast growth traits with low defenses and vice versa). We found evidence for six significant negative associations in the wild populations, including negative associations between SLA and leaf thickness, LDMC, and glandular trichome densities in *H. grosseserratus* and negative associations between leaf N and leaf C:N ratio in all three species (Fig. 3, Appendix 3). We found two significant positive associations: higher glandular trichome density with faster growth strategy in *H. giganteus* (SLA) and *H. maximiliani* (leaf N) (Fig. 3, Appendix 3). See Appendix 3 for full statistical results.

We found six significant negative associations between growth and defense traits at the Cornell common garden, where SLA and LDMC were negatively associated across all three species, SLA was negatively associated with leaf thickness in *H. grosseserratus*, and leaf N and C:N ratio were negatively associated in *H. giganteus* and *H. maximiliani* (Table 2). In contrast, there was only one negative association at the Jeffers common garden, where leaf N and C:N ratio were negatively related in *H. grosseserratus* (Table 2). Furthermore, 19 of the 30 associations had significantly different

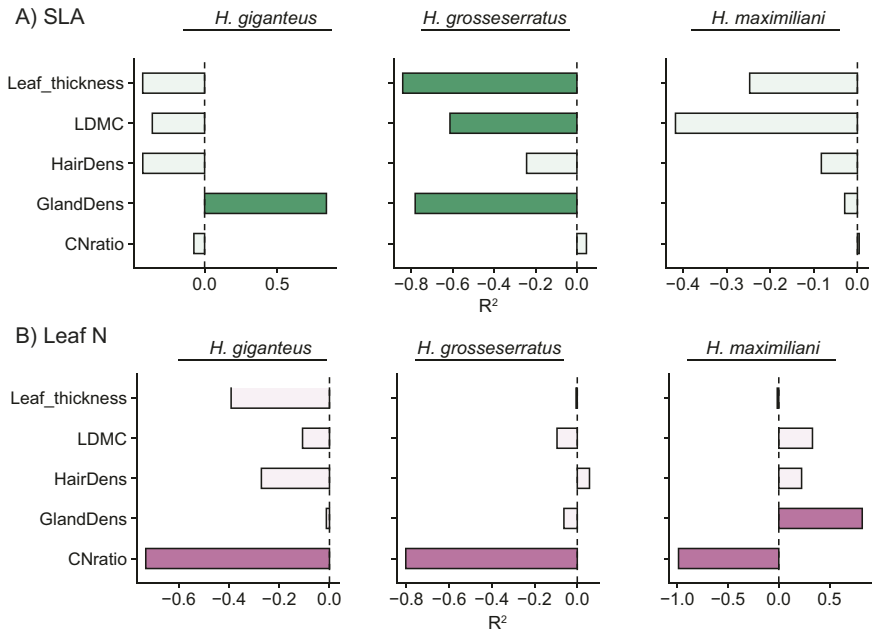


FIG. 3. Associations between defense traits and growth traits (A) specific leaf area (SLA, green) and (B) leaf N (pink) for each species. Length of bar corresponds to the marginal R^2 value; bars to the left of the dashed zero line indicate a negative association and bars to the right of the dashed zero line indicate a positive association. Associations significant at $P < 0.05$ are deeply shaded.

slopes between the common gardens, and most of the time the relationship was stronger at Cornell than at Jeffers. Thirteen of these differences were cases where the direction of the relationship was the same (11 both negative, 2 both positive), but the slope differed, and in 12 of these 13 cases, the slope was steeper at Cornell (Appendix 4). In the remaining six significant slope differences between common gardens, the signs of the slopes differed. See Appendix 4 for full statistical results.

We used PCAs to examine multivariate trait space in defense and growth traits in both the wild populations and common gardens. In the wild populations, growth traits (SLA and leaf N) were sometimes orthogonal, loading onto principal component (PC)1 versus PC2 (Fig. 4A). SLA generally traded off with defense traits like leaf thickness, LDMC, and hair density along PC1, whereas leaf N generally traded off with C:N ratio along PC2 (Fig. 4A). Glandular trichome densities did not consistently fit into these patterns in *H. giganteus* or *H. maximiliani*, whereas patterns in the latter species were less clear overall (Fig. 4A). In the common gardens, growth traits and defense traits largely traded off along PC1 in all three species, whereas trichomes loaded onto PC2 in different

ways (Fig. 4B). For instance, in *H. giganteus*, nonglandular trichome density and leaf thickness loaded heavily onto PC2, whereas glandular trichome density loaded onto PC2 in *H. grosseserratus*, and both trichome types loaded onto PC2 in *H. maximiliani* (Fig. 4B). See Appendix 5 for details of the PCAs, including loadings and percentage of variation explained by each axis.

LEAF DEFENSES AND ENVIRONMENTAL VARIABLES. In line with the resource availability hypothesis, we expected negative relationships between defense traits and environmental variables; however, there were only a few detectable relationships between defenses and environment in these plant populations. Of the 90 associations tested (three species \times five traits \times six environmental variables), only six were significant (five positive and one negative) (Table 3, Appendix 6). Mean annual temperature was positively associated with nonglandular trichome density in *H. giganteus* ($\beta = 719.056$, $t = 3.075$, d.f. = 6, $P = 0.022$, marginal $R^2 = 0.414$). Mean annual precipitation was positively associated with both leaf thickness ($\beta = 0.001$, $t = 3.303$, d.f. = 5, $P = 0.021$, marginal $R^2 = 0.233$) and leaf dry matter content in *H. grosseserratus* ($\beta < 0.001$, $t = 3.409$, d.f. = 5, $P = 0.019$,

Table 2. Common garden standardized major axis regressions results. Includes slope estimate, R^2 values, and associated P -values for all combinations of growth and defense traits across all three species of *Helianthus* and two common gardens. Relationships significant at $P < 0.05$ are in bold. See Appendix 4 for full statistical results; see Table 1 for abbreviations.

Species	Growth	Defense	Cornell			Jeffers		
			Slope estimate	R^2	P -value	Slope estimate	R^2	P -value
<i>H. giganteus</i>	SLA	Leaf_thickness	-0.003	0.022	0.249	-0.001	0.012	0.372
		LDMC	-0.001	0.253	<0.001	0.001	0.002	0.743
		HairDens	-85.362	0.007	0.513	-40.910	0.000	0.864
		GlandDens	5.144	0.034	0.151	-3.846	0.017	0.289
		CNratio	0.083	0.030	0.634	-0.108	0.214	0.178
	% N	Leaf_thickness	-0.234	0.127	0.312	0.044	0.379	0.058
		LDMC	0.056	0.023	0.679	-0.019	0.315	0.091
		HairDens	5,245.916	0.023	0.673	1,209.158	0.011	0.776
		GlandDens	299.941	0.091	0.396	104.679	0.001	0.934
		CNratio	-5.438	0.593	0.009	-1.784	0.274	0.120
<i>H. grosseserratus</i>	SLA	Leaf_thickness	-0.003	0.075	0.040	-0.002	0.017	0.311
		LDMC	-0.002	0.355	<0.001	-0.001	0.003	0.669
		HairDens	123.779	0.000	0.944	-107.999	0.010	0.447
		GlandDens	35.769	0.000	0.954	-16.315	0.052	0.072
		CNratio	0.088	0.000	0.974	-0.062	0.000	0.968
	% N	Leaf_thickness	-0.093	0.014	0.746	0.052	0.031	0.625
		LDMC	0.066	0.138	0.291	-0.031	0.140	0.287
		HairDens	4,344.692	0.003	0.873	-1,589.846	0.207	0.186
		GlandDens	-1,889.686	0.217	0.174	-480.823	0.011	0.772
		CNratio	3.433	0.004	0.871	-2.294	0.904	<0.001
<i>H. maximiliani</i>	SLA	Leaf_thickness	-0.003	0.056	0.110	0.001	0.001	0.850
		LDMC	-0.002	0.565	<0.001	-0.001	0.010	0.397
		HairDens	-9.706	0.000	0.983	-18.751	0.006	0.524
		GlandDens	-27.416	0.019	0.355	14.846	0.006	0.504
		CNratio	-0.106	0.411	0.046	-0.034	0.169	0.238
	% N	Leaf_thickness	-0.130	0.232	0.158	-0.022	0.294	0.105
		LDMC	0.047	0.025	0.663	-0.018	0.155	0.260
		HairDens	-160.069	0.064	0.481	-174.725	0.002	0.903
		GlandDens	665.909	0.018	0.715	526.957	0.000	0.989
		CNratio	-4.695	0.433	0.039	-1.030	0.099	0.376

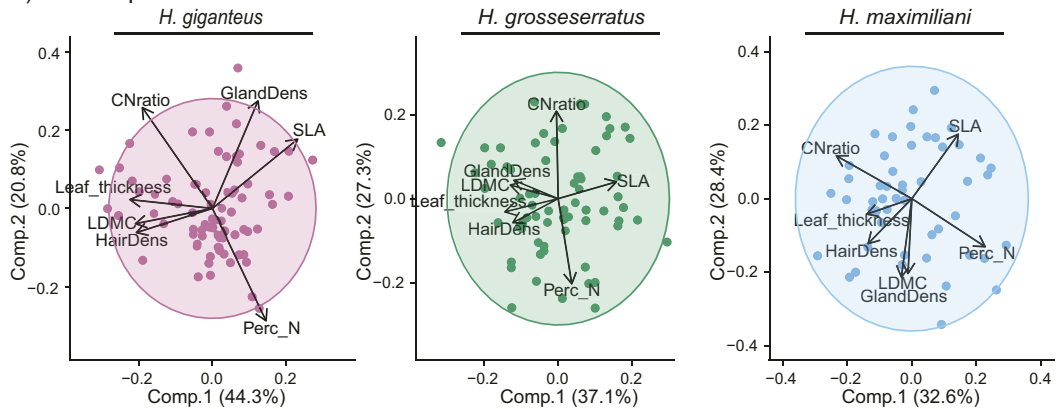
marginal $R^2 = 0.255$). Aridity index was positively related to nonglandular trichome density in *H. grosseserratus* ($\beta = 105798$, $t = 3.248$, d.f. = 5, $P = 0.023$, marginal $R^2 = 0.313$). In terms of soil variables, in *H. grosseserratus*, soil N was negatively related to leaf C:N ratio ($\beta = -0.199$, $t = -4.201$, d.f. = 5, $P = 0.008$, marginal $R^2 = 0.457$), whereas soil organic matter was positively related to glandular trichome density ($\beta = 68.757$, $P = 0.024$, $t = 3.191$, d.f. = 5, marginal $R^2 = 0.306$). See Appendix 6 for full statistical results.

HERBIVORE DAMAGE AND ENVIRONMENTAL VARIABLES. We expected higher levels of herbivore damage in higher-resource environments (positive relationships)—of 36 potential associations, we found 8 significant ones. We detected positive associations between leaf chewing damage and MAT, MAP, AI, and soil organic matter, all in *H. grosseserratus*

(Fig. 5, Appendix 7). With sucking damage, we found positive associations with soil P in *H. maximiliani*, positive associations with soil N in *H. giganteus* and *H. maximiliani*, and with soil organic matter in *H. giganteus*. See Appendix 7 for full statistical results.

TRAIT COMPARISONS BETWEEN COMMON GARDENS. We compared trait values between the two common gardens as a preliminary means of examining trait differences in low- versus high-resource environments, where Cornell has naturally lower soil P levels and is thus considered a lower-resource site, and Jeffers has naturally higher soil P levels and is therefore considered a higher-resource site (Fig. S3; Appendix 2). In line with the resource availability hypothesis, we expected higher values for resource traits, lower values for defense traits, and higher levels of herbivore damage at Jeffers

A) Wild Populations



B) Common Gardens

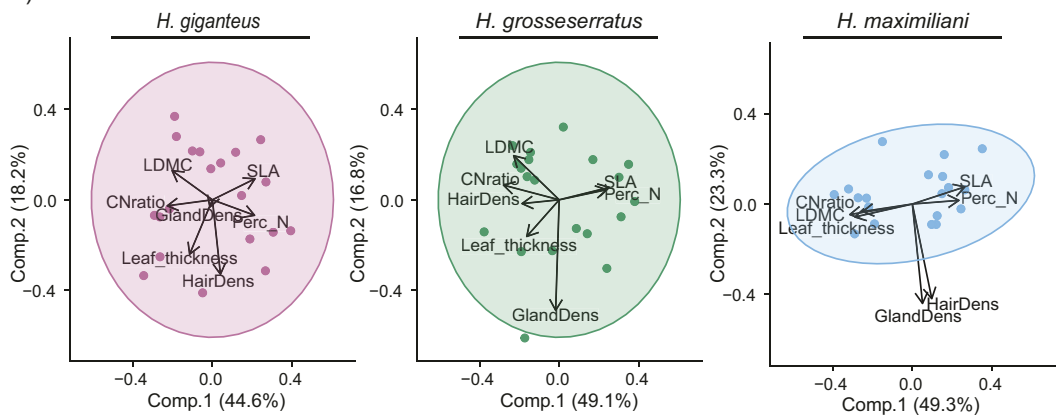


FIG. 4. Principal components analysis results for defense and growth traits, analyzed separately for each species in both the (A) wild populations and (B) common gardens. Ellipses are 95% confidence intervals. Arrows point to direction of increasing values for the labeled trait variable. Pink = *Helianthus giganteus*, green = *H. grosseserratus*, light blue = *H. maximiliani*.

compared with Cornell. In all three species, both resource-related traits (SLA and leaf N) were higher at Jeffers than at Cornell (Fig. 6). Of the 15 potential differences in defense traits, 10 were significant, and in all 10 the defense trait values were lower at Jeffers than at Cornell (Fig. 6). Only non-glandular trichome density was not significantly different between gardens in any of the species. Finally, of the six potential differences in herbivore damage levels (ChewDam and SuckDam), only one was significant, where chewing damage was higher at Jeffers than at Cornell (Fig. 6). See Appendix 8 for full statistical results.

Discussion. We asked whether interspecific patterns between leaf defenses and growth strategy, leaf defenses and environmental resources,

and herbivore damage and environmental resources were also detectable at the intraspecific scale in three species of *Helianthus* within a single geographic region. We found evidence for associations between lower defense levels and faster growth strategies (negative relationships) in support of the first prediction of the resource availability hypothesis, but these were largely unrelated to environmental variables.

HIGH LEVELS OF DEFENSE ARE GENERALLY ASSOCIATED WITH SLOWER GROWTH STRATEGY AMONG POPULATIONS. Overall, we found associations between slower growth strategies and increased levels of defenses, in line with expectations of the resource defense hypothesis (Coley *et al.* 1985). These associations were detectable across wild populations (within

Table 3. Defense trait–environment relationship linear mixed effect modeling results for three *Helianthus* species. Numbers are marginal R^2 values from the model; + or – in parentheses refer to positive or negative relationship. Relationships significant at $P < 0.05$ are in bold. See Table 1 for description of abbreviations and Appendix 6 for full statistical results.

Trait	Species	MAT	MAP	AI	Soil P	Soil N	Soil organic matter
Leaf thickness	<i>H. giganteus</i>	(+) 0.150	(+) 0.073	(–) 0.020	(–) 0.010	(–) 0.017	(–) 0.000
	<i>H. grosseserratus</i>	(+) 0.167	(+) 0.233	(+) 0.190	(–) 0.009	(+) 0.001	(+) 0.175
	<i>H. maximiliani</i>	(+) 0.087	(+) 0.091	(+) 0.008	(+) 0.001	(+) 0.009	(–) 0.216
LDMC	<i>H. giganteus</i>	(+) 0.079	(+) 0.028	(+) 0.000	(–) 0.089	(–) 0.018	(–) 0.033
	<i>H. grosseserratus</i>	(+) 0.165	(+) 0.255	(+) 0.141	(–) 0.092	(–) 0.000	(+) 0.201
	<i>H. maximiliani</i>	(+) 0.075	(+) 0.050	(–) 0.028	(+) 0.343	(+) 0.317	(–) 0.046
HairDens	<i>H. giganteus</i>	(+) 0.414	(+) 0.062	(–) 0.159	(–) 0.116	(+) 0.071	(+) 0.124
	<i>H. grosseserratus</i>	(+) 0.226	(+) 0.202	(+) 0.313	(–) 0.002	(+) 0.030	(+) 0.025
	<i>H. maximiliani</i>	(–) 0.009	(–) 0.020	(+) 0.016	(+) 0.000	(+) 0.001	(–) 0.018
GlandDens	<i>H. giganteus</i>	(–) 0.059	(+) 0.012	(+) 0.093	(–) 0.028	(–) 0.000	(+) 0.036
	<i>H. grosseserratus</i>	(+) 0.049	(+) 0.089	(+) 0.106	(+) 0.000	(+) 0.016	(+) 0.306
	<i>H. maximiliani</i>	(–) 0.037	(+) 0.050	(+) 0.204	(+) 0.062	(+) 0.105	(–) 0.008
CNratio	<i>H. giganteus</i>	(+) 0.030	(+) 0.078	(+) 0.006	(–) 0.001	(–) 0.055	(+) 0.000
	<i>H. grosseserratus</i>	(–) 0.407	(–) 0.247	(–) 0.317	(–) 0.228	(–) 0.457	(–) 0.034
	<i>H. maximiliani</i>	(+) 0.082	(–) 0.095	(–) 0.185	(–) 0.209	(–) 0.245	(–) 0.009

species, where of the eight detectable associations between leaf defense traits and growth traits, six were in the expected direction and two were opposite to that [both associated with glandular trichome density] (Fig. 3, Appendix 3). Principal components analyses in the wild populations also reflected trade-offs between SLA and defense traits along one axis and leaf N and leaf C:N ratio along a second axis (Fig. 4, Appendix 5). These trade-offs across populations are potentially indicative of local adaptation, though population-level differences may also be driven by phenotypic plasticity in the field associated with environmental differences, herbivore pressure, or other biotic interactions. Herbivore-induced plasticity in floral traits, for example, is highly species specific and reflects the interactions among all interacting members of the floral community (Rusman *et al.* 2019).

Although not explicitly defined by the resource availability hypothesis, we also tested for associations between defenses and growth across individuals in each of our common gardens to ask whether intraspecific variation within a population follows the same patterns as among populations. In the common gardens, we found few significant associations, but all of these were in the expected directions (Table 2, Appendix 4). At this scale, growth–defense patterns are more likely the result of physiological trade-offs rather than adaptive variation. Similar trade-offs between growth and defense were found across individuals within populations of *Pinus pinaster* grown in common garden settings, though the strength of these relationships

varied among populations (indicative of genetic differences) (Vázquez-González *et al.* 2020).

In both settings, aimed at detecting associations across or within populations, we found similar patterns between growth and defense. Hahn *et al.* (2021) compared defense–growth correlations in populations of *Monarda fistulosa* at multiple scales, including between high- and low-resource environments (Wisconsin and North Dakota) and among populations in Wisconsin. They found that scale mattered where they generally detected negative associations between regions but positive associations among populations found in Wisconsin (the high-resource region) where our study was also conducted. We found generally negative associations or failed to detect associations among populations. Hahn *et al.* (2021) also found neutral correlations within populations in common garden settings, in line with no significant associations in our gardens.

In multivariate trait space, our wild populations and common gardens differed in terms of relationships between the resource-associated traits. We found that SLA and leaf N load more or less congruently along the same PC axis in the common gardens, as expected from the leaf economics spectrum (Wright *et al.* 2004), but largely orthogonally in the wild populations (Fig. 4). This goes against the ideas of Zhou *et al.* (2022), who defined the SLA–leaf N relationships as coincident coordination, with the expectation of no relationship within species. These differences may reflect physiological trade-offs in the common

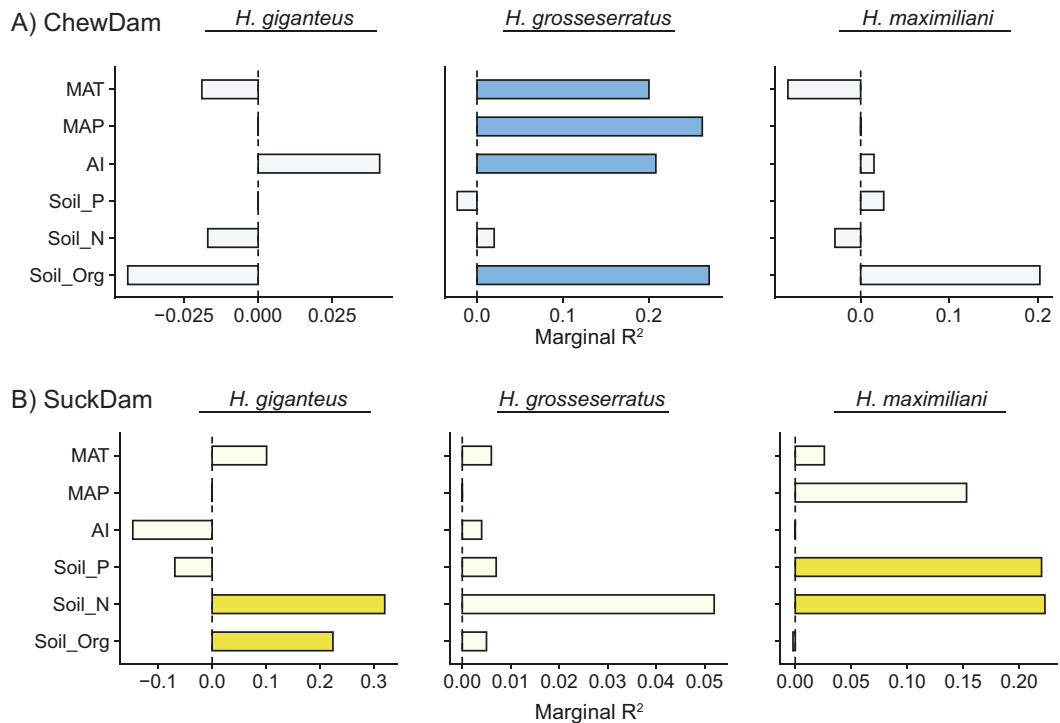


FIG. 5. Associations between environmental variables and damage traits (A) ChewDam (blue) and (B) SuckDam (yellow) for each species. Length of bar corresponds to the marginal R^2 value; bars to the left of the zero line indicate a negative association and bars to the right of the zero dashed line indicate a positive association. Associations significant at $P < 0.05$ are deeply shaded.

gardens and potentially different adaptive drivers in the wild populations, and may also be driving differences in other relationships between traits.

These intraspecific patterns may also vary from species to species or across time (Bazzaz *et al.* 1987). Among our species, some patterns differed. For instance, across the wild populations glandular trichome density had relationships with SLA in opposite directions in *H. giganteus* and *H. grosseserratus* and no detectable relationship in *H. maximiliani* (Fig. 3). Likewise, although major PC axes were similar across species, individual traits are loaded onto the PC axes in different ways in our three species (Fig. 3, Appendix 5). These differences may reflect slightly different strategies among species in terms of defenses, in line with interspecific predictions and findings (Mason *et al.* 2016). We note that the three *Helianthus* studied here are perennials, where temporal variation across years could also affect ontogenetic allocation of resources (Boege and Marquis 2005; Mason and Donovan 2015), and we do not know the age of the plants measured.

We examined largely mechanical defenses or trichomes that contain secondary metabolites, rather than analyzing the specific composition of these metabolites, which are common and diverse across *Helianthus*. Additional associations between growth strategies and chemical defenses may differ from associations with the traits analyzed here (Aschenbrenner *et al.* 2013; Mason *et al.* 2016; Brentan Silva *et al.* 2017). However, we expect findings similar to those of Mason *et al.* (2016), where there were strong relationships between leaf economics spectrum traits and secondary metabolites across *Helianthus*.

INCREASED LEAF DEFENSES ARE NOT ASSOCIATED WITH ENVIRONMENTAL RESOURCES. In line with the resource availability hypothesis, we expected lower levels of leaf defenses in resource-rich environments (Coley 1987; Endara and Coley 2011). In our study area, we would generally characterize resource-rich environments as those with higher levels of precipitation (MAP and AI), warmer temperatures, and higher levels of soil N, P, and

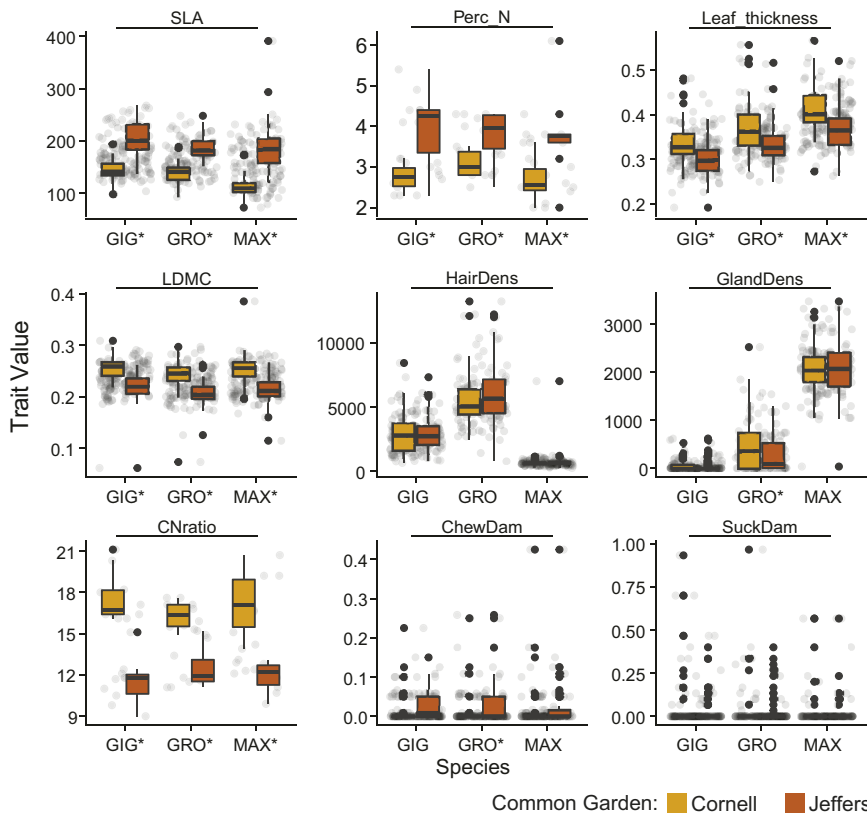


FIG. 6. Comparisons between plant traits at common gardens. Box plots represent the mean, quarterlies, and outliers with jittered data points in gray. Trait values are on the y -axis, with the three different species (GIG = *Helianthus giganteus*, GRO = *H. grosseserratus*, MAX = *H. maximiliani*) on the x -axis, and colors indicate the common garden (light orange = Cornell, left; dark orange = Jeffers, right). Asterisks next to the species abbreviation denote significant differences between gardens ($P < 0.05$).

organic matter. We found only a handful of detectable relationships between leaf defenses and environmental variables in the wild populations, and these were generally in the direction opposite to the resource availability hypothesis predictions; we found higher defenses in resource-rich areas (Fig. 5, Appendix 6). These patterns were fairly consistent across the three focal species, though glandular trichome density in *H. giganteus* was positively associated with SLA, whereas this trait was not associated with any growth traits in the other species (Fig. 2). These findings opposite to the expectations of the interspecific resource availability hypothesis are, however, in line with the intraspecific framework of Hahn and Maron (2016), which predicts that positive relationships between resource and defenses in high-resource environments are driven by herbivore pressure rather than by physiological stresses. Mason *et al.* (2016) also did not find support for this prediction

at the macroevolutionary scale across the genus. We did find that the common garden with the higher resources (in terms of soil P only) had higher levels of defense traits in most cases (Fig. 6, Appendix 8).

Across a latitudinal gradient, *H. annuus* populations from lower latitudes (higher resources) had increased herbivore resistance and slower growth, in line with predictions (Beaton 2020). It may be that these intraspecific patterns are only detectable across a larger breadth of climatic conditions, and that the location of our study, largely in Wisconsin and into Minnesota, may result in both reduced and different gradients of stressors in plants. For instance, MAT ranged from 6.9°C to 24.4°C across the latitudinal gradient of Beaton (2020), and only 3.8°C to 8.0°C at our sites, whereas MAP ranged from 73.7 mm to 1443.0 mm in the *H. annuus* study and only 739 mm to 846 mm in our study (Fig. S3; Appendix 2). We also did not find

strong associations between soil nutrients and defense traits in the wild populations, which have been detected at (relatively) finer spatial scales within species (Sanczuk *et al.* 2021), despite the variation in soils across our sites (Fig. S3). Our common garden sites, however, had some extreme levels of soil P compared with the wild populations, with the Jeffers site having nearly four times higher P levels than Cornell and the wild populations. These high levels of P may remove any constraint imposed by nutrients in the framework of Hahn and Maron (2016).

HERBIVORE DAMAGE LEVELS ARE SOMETIMES ASSOCIATED WITH HIGHER RESOURCE LEVELS. We expected higher levels of herbivore damage in locations with more plentiful resources. Both leaf chewing damage (inflicted by Orthoptera, Lepidoptera, and Diptera) in *H. grosseserratus* and sucking damage (inflicted by Hemiptera and Homoptera) in *H. giganteus* and *H. maximiliani* had some positive associations with aspects of temperature, moisture, and soil nutrients (Fig. 5). In the common gardens, only chewing damage in *H. grosseserratus* differed between the two locations, with higher damage levels at the higher-resource site (Jeffers), in line with expectations for both the interspecific and intraspecific resource availability hypotheses (Fig. 6, Appendix 8) (Coley 1985; Hahn and Maron 2016). The overall lack of strong patterns here could be linked to the lack of patterns between defense traits and resources across these environmental gradients. Both hypothesized relationships hinge on the idea that it is less costly for plants with access to plentiful resources to replace damaged tissue, so it is not as essential to invest in protection (Coley *et al.* 1985; Endara and Coley 2011). This reduced investment should reduce damage, so the lack of patterns in defense traits would logically lead to few patterns with damage traits.

Evidence for higher levels of herbivore damage associated with higher resources is inconsistent. For instance, studies report higher damage levels in *Salvia nubicola* found at lower *versus* higher altitudes (but with different overall strategies in different populations) (Dostálek *et al.* 2016), a lack of differences in damage along a latitudinal gradient within *Acacia falcata* (Andrew and Hughes 2005), and increased damage at drier sites (opposite to the expectation) in 13 tropical tree species (Muehleisen *et al.* 2020). Our results are consistent with the lack of strong

detectable relationships between herbivore damage levels and environmental resources.

DIFFERENCES BETWEEN COMMON GARDENS STRESS THE IMPORTANCE OF ECOLOGICAL SCALE. There was stronger evidence for growth–defense trade-offs in the low-resource common garden (Cornell) than in the high-resource garden (Jeffers) (Table 2, Appendix 4), where nutrient stress may have resulted in this pattern because growth is constrained by resources at Cornell, whereas those at Jeffers were not subject to this constraint. This is in line with both predictions and observations made in the intraspecific defense framework of Hahn and Maron (2016). Jeffers plants had more resource-acquisitive trait values, lower defenses, and (in only one case) more damage than Cornell plants, consistent with predictions related to resource availability. Of interest, the only marked difference between these sites was soil P levels, though nutrients alone can markedly alter defense strategies. Across genotypes of the tall goldenrod *Solidago altissima*, those with artificially higher nutrient levels had lower constitutive plant defenses, but increased induced resistance after experimental exposure to herbivory (Burghardt 2016). Differences between constitutive and induced defenses could also be important, as they may represent different responses to herbivore pressures (energetic trade-offs *versus* plastic responses) (Sun *et al.* 2023). Although we did not measure induced defenses in this study, experimental treatments could be applied to observe if this pattern can be replicated in sunflowers.

Conclusions. Overall, we found that faster growth strategies were associated with lower defense levels against herbivores within three *Helianthus* species, but that defense traits were not linked to the availability of resources in their growth environments. Evidence for trade-offs between defense and growth across populations may indicate local adaptation, whereas those in the common gardens within populations point to physiological constraints, which were stronger in the lower-resource garden. Thus, the main predictions of the resource availability hypothesis were not detectable at this regional scale but are in line with the intraspecific framework of Hahn and Maron (2016). Our findings within closely related species across Wisconsin were largely consistent with patterns found across *Helianthus* species at the macroevolutionary scale (Mason *et al.* 2016). These

consistencies suggest that similar evolutionary or ecological processes may be acting in similar ways across geographic and phylogenetic scales. Results here provide a solid basis for understanding the complexities in this system and future work related to specific secondary metabolites, the ontogeny of defenses, and insights into intraspecific variation at both broad and restricted spatial scales.

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Appendix 1

Sampling sites for wild populations of *Helianthus* and two common garden sites. All voucher specimens are deposited at the University of Wisconsin–Eau Claire herbarium (UWEC).

Site identification	Site name	Species	Collection date	Latitude	Longitude	Site description	Voucher number
GIG1	Colfax	<i>H. giganteus</i>	July 8, 2020	45.0314°N	91.7938°W	Roadside	UWEC06711
GIG2	Coon Fork Dam	<i>H. giganteus</i>	July 15, 2020	44.7003°N	91.0183°W	Mesic woods, near dam	UWEC06712
GIG3	Fish Creek	<i>H. giganteus</i>	July 19, 2020	46.5882°N	90.9455°W	Mesic woods, trailside	UWEC06713
GIG4	Red Cedar	<i>H. giganteus</i>	July 20, 2020	44.8224°N	91.9478°W	Mesic woods, trailside	UWEC06714
GIG5	Blaine	<i>H. giganteus</i>	July 22, 2020	46.1190°N	92.0920°W	Roadside	UWEC06715
GIG6	Fish Lake Wildlife Area	<i>H. giganteus</i>	July 22, 2020	45.7290°N	92.7451°W	Marshy area	UWEC06716
GIG7	Marathon	<i>H. giganteus</i>	August 12, 2020	44.9476°N	89.7680°W	Roadside	UWEC06717
GIG9	Lemonweir	<i>H. giganteus</i>	September 14, 2020	43.7969°N	90.0118°W	Roadside	UWEC06718
GRO2	Baird Creek	<i>H. grosseserratus</i>	August 5, 2020	44.4995°N	87.9249°W	Roadside	UWEC06719
GRO3	Green Lake	<i>H. grosseserratus</i>	August 5, 2020	43.8226°N	89.0271°W	Prairie	UWEC06720
GRO4	Drumlin Prairie	<i>H. grosseserratus</i>	August 19, 2020	42.9925°N	89.0570°W	Prairie	UWEC06721
GRO5	Waubesa Wetland	<i>H. grosseserratus</i>	August 19, 2020	42.9931°N	89.3616°W	Roadside	UWEC06722
GRO6	Black Earth	<i>H. grosseserratus</i>	August 19, 2020	43.1405°N	89.7737°W	Prairie	UWEC06730
GRO7	Scuppernong Prairie	<i>H. grosseserratus</i>	September 13, 2020	42.9002°N	88.5013°W	Prairie	UWEC06728
GRO8	Young Prairie	<i>H. grosseserratus</i>	September 13, 2020	42.8260°N	88.6448°W	Prairie	UWEC06729
MAX1	Ashland	<i>H. maximiliani</i>	July 19, 2020	46.5879°N	90.8998°W	Restored area	UWEC06723
MAX2	Baird Creek	<i>H. maximiliani</i>	August 5, 2020	44.4995°N	87.9249°W	Prairie	UWEC06724
MAX3	Chester Park	<i>H. maximiliani</i>	August 10, 2020	46.8098°N	92.0986°W	Restored area	UWEC06725
MAX4	Stiles Landing	<i>H. maximiliani</i>	August 12, 2020	44.8579°N	88.0492°W	Roadside	UWEC06726
MAX5	Mason Prairie	<i>H. maximiliani</i>	August 17, 2020	44.7976°N	91.3495°W	Restored area	UWEC06727
CG1	Jeffers	Common garden		44.856°N	91.524°W	Roadside	
CG2	Cornell	Common garden		44.840°N	91.525°W	Roadside	

Appendix 2

Trait and environmental variable summaries. Means, standard deviations, and minimum and maximum values for each of the trait and environmental variables for wild populations and common gardens for each species of *Helianthus*. See Table 1 for description of abbreviations and Figs. S1 and S2 for visualizations.

Species-level data

Species	Variable	Wild populations				Common gardens			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
<i>H. giganteus</i>	SLA	185.94	59.11	102.72	397.14	175.66	40.17	97.94	267.50
	% N	2.65	0.51	1.70	4.20	3.39	0.94	2.30	5.40
	Leaf thickness	0.29	0.05	0.19	0.51	0.32	0.05	0.19	0.48
	LDMC	0.29	0.04	0.20	0.40	0.24	0.03	0.06	0.31
	HairDens	806.54	940.40	61.54	4,984.62	2,916.70	1,409.38	646.15	8,430.77
	GlandDens	279.62	543.59	0.00	2,461.54	49.00	108.97	0.00	615.38
	CNratio	17.04	2.75	12.20	25.80	14.56	3.55	9.00	21.10
	ChewDam	0.10	0.12	0.00	0.75	0.02	0.04	0.00	0.23
	SuckDam	0.02	0.08	0.00	0.47	0.04	0.14	0.00	0.93
	MAT	5.84	0.93	4.60	7.10	0.04	0.14	0.00	0.93
	MAP	794.12	29.40	739.00	822.00	0.04	0.14	0.00	0.93
	AI	0.80	0.04	0.72	0.87	0.04	0.14	0.00	0.93
	Soil_P	17.62	6.23	10.00	25.00	0.04	0.14	0.00	0.93
	Soil_N	11.38	18.68	2.00	57.00	0.04	0.14	0.00	0.93
Soil_org	3.09	2.33	0.60	7.80	0.04	0.14	0.00	0.93	
<i>H. grosseserratus</i>	SLA	109.76	17.24	77.35	158.62	163.48	31.27	92.60	247.97
	% N	2.75	0.58	1.80	4.70	3.42	0.60	2.50	4.30
	Leaf thickness	0.41	0.06	0.30	0.60	0.35	0.05	0.25	0.56
	LDMC	0.30	0.02	0.25	0.35	0.22	0.03	0.07	0.30
	HairDens	9,894.51	3,518.41	3,015.38	17,723.08	5,717.23	2,228.73	769.23	13,230.77
	GlandDens	700.66	745.95	0.00	2,707.69	383.47	486.12	0.00	2,523.08
	CNratio	16.89	3.05	11.20	22.60	14.40	2.34	11.10	17.60
	ChewDam	0.10	0.11	0.00	0.50	0.02	0.05	0.00	0.26
	SuckDam	0.01	0.03	0.00	0.23	0.03	0.11	0.00	0.97
	MAT	7.57	0.50	6.60	8.00	0.03	0.11	0.00	0.97
	MAP	812.57	29.84	758.00	846.00	0.03	0.11	0.00	0.97
	AI	0.76	0.02	0.73	0.79	0.03	0.11	0.00	0.97
	Soil_P	12.57	13.66	2.00	41.00	0.03	0.11	0.00	0.97
	Soil_N	11.14	11.33	1.00	30.00	0.03	0.11	0.00	0.97
Soil_org	8.49	6.60	2.10	19.10	0.03	0.11	0.00	0.97	

Appendix 2

Continued.

Species		Wild populations				Common gardens			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
<i>H. maximiliani</i>	SLA	107.01	21.48	77.21	162.91	157.50	49.75	72.33	391.16
	% N	2.23	0.55	1.40	3.70	3.22	0.97	2.00	6.10
	Leaf_thickness	0.43	0.08	0.28	0.56	0.39	0.05	0.26	0.57
	LDMC	0.28	0.03	0.23	0.35	0.23	0.03	0.11	0.39
	HairDens	795.69	257.43	338.46	1,753.85	672.27	571.70	246.15	7,015.38
	GlandDens	2,724.31	734.98	1,261.54	4,123.08	2,056.67	543.58	30.77	3,476.92
	CNratio	19.72	3.83	12.10	27.00	14.51	3.17	9.90	20.70
	ChewDam	0.04	0.05	0.00	0.23	0.02	0.06	0.00	0.43
	SuckDam	0.04	0.16	0.00	0.93	0.02	0.06	0.00	0.57
	MAT	5.58	1.26	3.80	6.60	0.02	0.09	0.00	
	MAP	771.80	23.20	746.00	806.00				
	AI	0.78	0.03	0.73	0.82				
	Soil_P	26.40	31.93	8.00	83.00				
	Soil_N	5.40	8.73	1.00	21.00				
	Soil_org	3.54	1.11	1.80	4.80				

Common garden environmental data

Variable	Cornell				Jeffers			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
MAT	6.6				6.5			
MAP	790				794			
AI	0.7335				0.7489			
Soil_P	16.3	3.21	14	20	51.3	15.5	45	69
Soil_N	2.0	1.0	1	3	3.7	1.5	2	5
Soil_org	1.77	0.06	1.7	1.8	1.6	0.46	1.2	2.1

Appendix 3

Defense-growth standardized major axis regression results for wild populations of *Helianthus*; models run separately for each species on population means, with growth traits SLA and % N as response variables and defense traits leaf thickness, LDMC, HairDens, GlandDens, and CNratio as predictors (see Table 1 for description of abbreviations). Values indicate the elevation (intercept) estimates and 95% confidence intervals (CIs), slope estimates and 95% confidence intervals, and R^2 and P values.

Species	Growth	Defense	Elevation (estimate, 95% CI)	Slope (estimate, 95% CI)	R^2	P
<i>H. giganteus</i>	SLA	Leaf thickness	0.446 (0.321, 0.571)	-0.001 (-0.002, 0.000)	0.425	0.080
		LDMC	0.389 (0.302, 0.475)	-0.001 (-0.001, 0.000)	0.359	0.116
		HairDens	3,849.4 (1,449.5, 6,249.4)	-16.4 (-32.9, -8.1)	0.425	0.080
		GlandDens	-1,124.1 (-1,710.3, -537.9)	7.5 (5.1, 11.2)	0.837	0.001
		CNratio	22.79 (16.967, 28.614)	-0.031 (-0.073, -0.013)	0.074	0.515
	% N	Leaf thickness	0.725 (0.382, 1.069)	-0.165 (-0.338, -0.081)	0.391	0.097
		LDMC	0.571 (0.299, 0.842)	-0.108 (-0.25, -0.046)	0.107	0.428
		HairDens	9,205.6 (1,999.2, 16,411.9)	-3,168.0 (-6,865.7, -1,461.8)	0.271	0.186
		GlandDens	4,154.2 (276.5, 8,031.9)	-1,461.4 (-3,510.0, -608.5)	0.012	0.793
		CNratio	32.9 (24.7, 41.2)	-5.988 (9.845, -3.642)	0.731	0.007
<i>H. grosseserratus</i>	SLA	Leaf thickness	0.72 (0.576, 0.864)	-0.003 (-0.004, -0.002)	0.842	0.004
		LDMC	0.409 (0.332, 0.485)	-0.001 (-0.002, 0.000)	0.613	0.037
		HairDens	30,146.7 (9,695.8, 50,597.6)	-184.5 (-445.5, -76.4)	0.243	0.261
		GlandDens	4,970.9 (2,657.5, 7,284.4)	-38.9 (-65.1, -23.3)	0.781	0.008
		CNratio	-2.821 (-25.214, 19.571)	0.18 (0.068, 0.472)	0.046	0.644
	% N	Leaf thickness	0.645 (0.367, 0.923)	-0.086 (-0.23, -0.032)	0.006	0.873
		LDMC	0.383 (0.294, 0.473)	-0.029 (-0.075, -0.011)	0.095	0.502
		HairDens	-5,472.0 (-22,968.4, 12,024.3)	5,584.9 (2,135.8, 14,604.0)	0.057	0.605
		GlandDens	3,940.8 (262.8, 7,618.8)	-1,177.6 (-3,072.8, -451.3)	0.063	0.588
		CNratio	31.844 (24.117, 39.571)	-5.435 (-8.873, -3.329)	0.803	0.006
<i>H. maximiliani</i>	SLA	Leaf thickness	0.863 (0.158, 1.568)	-0.004 (-0.014, -0.001)	0.247	0.394
		LDMC	0.453 (0.21, 0.695)	-0.002 (-0.005, -0.001)	0.418	0.239
		HairDens	1,413.1 (309.1, 2,517.0)	-5.769 (-21.829, -1.525)	0.083	0.639
		GlandDens	5,765.5 (161.9, 11,369.0)	-28.4 (-110.2, -7.3)	0.029	0.784
		CNratio	2.63 (-29.324, 34.583)	0.16 (0.041, 0.626)	0.004	0.919
	% N	Leaf thickness	0.811 (0.095, 1.527)	-0.172 (-0.67, -0.044)	0.015	0.843
		LDMC	0.131 (-0.098, 0.361)	0.067 (0.02, 0.223)	0.332	0.310
		HairDens	252.5 (-642.4, 1,147.4)	243.2 (69.1, 855.9)	0.225	0.420
		GlandDens	48.5 (-2,055.9, 2,152.9)	1,197.7 (586.8, 2,444.6)	0.822	0.034
		CNratio	34.761 (31.627, 37.895)	-6.732 (-8.257, -5.488)	0.987	0.001

Appendix 4

Defense-growth standardized major axis regression modeling results for common gardens; models run separately for each species of *Helianthus* on individual trait values, with growth traits SLA and % N as response variables and defense traits leaf thickness, LDMC, HairDens, GlandDens, and CNratio as predictors (see Table 1 for description of abbreviations). Values for each garden (Cornell and Jeffers) indicate the elevation (intercept) estimates and 95% confidence intervals (CIs), slope estimates and 95% CIs, and R^2 and P values. Comparisons indicate results from the likelihood ratio (LR) statistic, associated P value

Species	Growth	Defense	Elevation (estimate, 95% CI)	Slope (estimate, 95% CI)	R^2	P	
<i>H. giganteus</i>	LA	Leaf thickness	0.711 (0.615, 0.807)	-0.003 (-0.003, -0.002)	0.022	0.249	
		LDMC	0.405 (0.371, 0.438)	-0.001 (-0.001, -0.001)	0.253	<0.001	
	HairDens	GlandDens	15,173.1 (11,996.5, 18,349.7)	-85.4 (-109.9, -66.3)	0.000	0.500	
		CNratio	-686.4 (-875, -497.7)	5.1 (4, 6.6)	0.000	0.200	
	% N	Leaf thickness	5.239 (-4.856, 15.334)	0.083 (0.04, 0.174)	0.030	0.634	
		LDMC	0.99 (0.494, 1.486)	-0.234 (-0.473, -0.116)	0.127	0.312	
	HairDens	GlandDens	0.096 (-0.03, 0.223)	0.056 (0.027, 0.118)	0.023	0.679	
		CNratio	-11,681.7 (-23,435.3, 72)	5,245.9 (2,510.2, 10,963.3)	0.000	0.700	
	<i>H. grosseserratus</i>	SLA	Leaf thickness	-757.1 (-1,404.6, -109.7)	299.9 (146.8, 613)	0.100	0.400
			LDMC	32,565 (24,73, 40.4)	-5.438 (-8.958, -3.301)	0.593	0.009
HairDens		GlandDens	0.85 (0.724, 0.976)	-0.003 (-0.005, -0.003)	0.075	0.040	
		CNratio	0.505 (0.447, 0.562)	-0.002 (-0.002, -0.002)	0.355	<0.001	
% N		Leaf thickness	-11,651.7 (-16,326.4, -6,976.9)	123.8 (94.8, 161.7)	0.000	0.900	
		LDMC	-4,435.1 (-5,786, -3,084.2)	35.8 (27.4, 46.7)	0.000	1.000	
HairDens		GlandDens	4.954 (-4.393, 14.301)	0.088 (0.042, 0.185)	0.000	0.974	
		CNratio	0.661 (0.427, 0.896)	-0.093 (-0.196, -0.045)	0.014	0.746	
SLA		Leaf thickness	0.036 (-0.118, 0.19)	0.066 (0.033, 0.132)	0.138	0.291	
		LDMC	-7,166.3 (-18,141.5, 3,809)	4,344.7 (2,065.7, 9,138)	0.000	0.900	
HairDens	GlandDens	6,186.4 (1,965.1, 10,407.6)	-1,889.7 (-3,692.9, -967)	0.200	0.200		
	CNratio	5.767 (-2,904, 14,438)	3,433 (1,632, 7,22)	0.004	0.871		
% N	Leaf thickness	0.686 (0.602, 0.771)	-0.003 (-0.003, -0.002)	0.056	0.110		
	LDMC	0.437 (0.401, 0.474)	-0.002 (-0.002, -0.001)	0.565	<0.001		
HairDens	GlandDens	1,663.8 (1,330.1, 1,997.6)	-9.7 (-13, -7.2)	0.000	1.000		
	CNratio	5,057.9 (4,126.8, 5,988.9)	-27.4 (-36.8, -20.4)	0.000	0.400		
SLA	Leaf thickness	29,738 (21.7, 37,776)	-0.106 (-0.191, -0.058)	0.411	0.046		
	LDMC	0.741 (0.49, 0.992)	-0.13 (-0.252, -0.067)	0.232	0.158		
HairDens	GlandDens	0.127 (0.023, 0.231)	0.047 (0.023, 0.099)	0.025	0.663		
	CNratio	961.2 (617.5, 1,304.8)	-160.1 (-330.1, -77.6)	0.100	0.500		
% N	Leaf thickness	157.9 (-1,310.2, 1,626.1)	665.9 (318, 1,394.3)	0.000	0.700		
	LDMC	29,598 (21,795, 37.4)	-4.695 (-8.392, -2.626)	0.000	0.433		

Cornell

Appendix 4

Continued.

Species	Growth	Defense	Jeffers			R ²	P
			Elevation (estimate, 95% CI)	Slope (estimate, 95% CI)	R ²		
<i>H. giganteus</i>	SLA	Leaf thickness	0.554 (0.491, 0.617)	-0.001 (-0.002, -0.001)	0.012	0.372	
		LDMC	0.047 (0.004, 0.09)	0.001 (0.001, 0.001)	0.002	0.743	
	HairDens	HairDens	11,314.3 (9,225, 13,403.5)	-40.9 (-52.1, -32.1)	0.000	0.864	
		GlandDens	830.9 (636.5, 1,025.3)	-3.8 (-4.9, -3)	0.017	0.289	
	% N	CNratio	31.935 (17.118, 46.752)	-0.108 (-0.211, -0.055)	0.214	0.178	
		Leaf thickness	0.107 (-0.01, 0.225)	0.044 (0.024, 0.081)	0.379	0.058	
	LDMC	Leaf thickness	0.303 (0.25, 0.357)	-0.019 (-0.036, -0.01)	0.315	0.091	
		HairDens	-1,583.9 (-5,693.8, 2,526)	1,209.2 (576.2, 2,537.2)	0.011	0.776	
	GlandDens	HairDens	-390 (-748.6, -31.5)	104.7 (49.7, 220.3)	0.001	0.934	
		CNratio	18.69 (13.555, 23.824)	-1.784 (-3.41, -0.933)	0.274	0.120	
<i>H. grosseserratus</i>	SLA	Leaf thickness	0.703 (0.607, 0.798)	-0.002 (-0.003, -0.002)	0.017	0.311	
		LDMC	0.387 (0.34, 0.434)	-0.001 (-0.001, -0.001)	0.003	0.669	
	HairDens	HairDens	26,146.8 (20,946.7, 31,346.9)	-108 (-139, -83.9)	0.010	0.447	
		GlandDens	3,319.8 (2,552.3, 4,087.4)	-16.3 (-20.9, -12.7)	0.052	0.072	
	CNratio	GlandDens	23,896 (14,437, 33,355)	-0.062 (-0.132, -0.03)	0.000	0.968	
		Leaf thickness	0.145 (-0.015, 0.306)	0.052 (0.025, 0.109)	0.031	0.625	
	LDMC	Leaf thickness	0.321 (0.232, 0.41)	-0.031 (-0.062, -0.015)	0.140	0.287	
		HairDens	10,965.5 (6,549.2, 15,381.9)	-1,589.8 (-3,118.5, -810.5)	0.207	0.186	
	GlandDens	HairDens	2,192.5 (692.4, 3,692.6)	-480.8 (-1,008.8, -229.2)	0.011	0.772	
		CNratio	21,084 (18,873, 23,294)	-2,294 (-2,946, -1,786)	0.904	0.000	
<i>H. maximiliani</i>	SLA	Leaf thickness	0.166 (0.116, 0.215)	0.001 (0.001, 0.001)	0.001	0.850	
		LDMC	0.325 (0.298, 0.352)	-0.001 (-0.001, 0)	0.010	0.397	
	HairDens	HairDens	4,225.1 (3,370.5, 5,079.7)	-18.8 (-23.7, -14.9)	0.006	0.524	
		GlandDens	-691.1 (-1,367.4, -14.7)	14.8 (11.8, 18.7)	0.006	0.504	
	CNratio	GlandDens	17,864 (13,354, 22,375)	-0.034 (-0.068, -0.017)	0.169	0.238	
		Leaf thickness	0.435 (0.377, 0.493)	-0.022 (-0.041, -0.011)	0.294	0.105	
	% N	LDMC	0.283 (0.228, 0.337)	-0.018 (-0.037, -0.009)	0.155	0.26	
		HairDens	1,271.4 (699.8, 1,843)	-174.7 (-367.7, -83)	0.002	0.903	
	GlandDens	GlandDens	36.7 (-1,692.5, 1,765.9)	527 (250.3, 1,109.5)	0.000	0.989	
		CNratio	15,813 (12,648, 18,978)	-1.03 (-2.099, -0.505)	0.099	0.376	

Appendix 4

Continued.

Species	Growth	Defense	Comparison				LR	P
			Slope (estimate, 95% CI)	Variance (slope)	Lambda	d.f.		
<i>H. giganteus</i>	SLA	Leaf thickness	-0.002 (-0.002, -0.001)	0.000	0.000	1	17.315	<0.001
		LDMC	-0.001 (-0.001, -0.001)	0.000	0.000	1	1.59	0.207
		HairDens	-58.092 (-69.912, -48.202)	26.282	3,374.662	1	16.824	<0.001
		GlandDens	4.424 (3.716, 5.264)	0.149	19.569	1	2.74	0.098
		CNratio	-0.096 (-0.156, -0.059)	0.001	0.009	1	0.283	0.594
		Leaf thickness	0.089 (0.046, 0.187)	0	0.008	1	11.673	0.001
		LDMC	-0.03 (-0.054, -0.018)	0.000	0.001	1	4.872	0.027
		HairDens	2,528.791 (1,323.693, 4,822.008)	395,500.635	6,394,784	1	7.543	0.006
		GlandDens	181.336 (101.208, 321.427)	1,978.293	32,882.71	1	4.145	0.042
		CNratio	-3.712 (-5.99, -2.041)	0.528	13.776	1	7.14	0.008
<i>H. grosseserratus</i>	SLA	Leaf thickness	-0.003 (-0.003, -0.002)	0.000	0.000	1	9.262	0.002
		LDMC	-0.001 (-0.002, -0.001)	0.000	0.000	1	15.328	<0.001
		HairDens	-115.188 (-138.334, -95.889)	113.804	13,268.36	1	0.535	0.464
		GlandDens	-23.401 (-28.44, -19.235)	4.605	547.622	1	17.632	<0.001
		CNratio	-0.074 (-0.125, -0.044)	0	0.005	1	0.429	0.513
		Leaf thickness	0.07 (0.041, 0.119)	0	0.005	1	1.284	0.257
		LDMC	-0.045 (-0.076, -0.026)	0	0.002	1	2.424	0.119
		HairDens	-2,489.103 (-4,423.705, -1,437.668)	350,681.145	6,195,633	1	4.021	0.045
		GlandDens	-1,025.11 (-1,878.235, -539.144)	60,065.035	1,050,850	1	7.217	0.007
		CNratio	-2.374 (-3.069, -1.896)	0.062	5.636	1	1.093	0.296
<i>H. maximiliani</i>	SLA	Leaf thickness	-0.001 (-0.002, -0.001)	0.000	0.000	1	20.152	<0.001
		LDMC	-0.001 (-0.001, -0.001)	0.000	0.000	1	38.834	<0.001
		HairDens	-14.606 (-17.743, -12.1)	1.817	213.342	1	11.674	0.001
		GlandDens	-18.794 (-22.652, -15.498)	2.989	353.197	1	10.276	0.001
		CNratio	-0.066 (-0.113, -0.037)	0	0.004	1	6.108	0.013
		Leaf thickness	-0.051 (-0.109, -0.024)	0	0.003	1	13.249	<0.001
		LDMC	-0.029 (-0.05, -0.016)	0.000	0.001	1	3.509	0.061
		HairDens	-167.002 (-278.001, -100.386)	1,684.119	27,889.83	1	0.03	0.863
		GlandDens	592.984 (353.119, 995.32)	21,784.352	351,630.3	1	0.207	0.649
		CNratio	-2.613 (-4.752, -1.285)	0.341	6.83	1	10.043	0.002

Appendix 5

Growth and defense trait principal components analysis results. Analyses performed separately for each species of *Helianthus* for the wild populations and common gardens. Values are loadings for each principal component and proportion of variance (Prop of var) explained by each principal component. See Table 1 for description of abbreviations.

Setting	Species	Trait	Component 1	Component 2	Component 3	Component 4	Component 5	Component 6	Component 7	
Wild populations	<i>H. giganteus</i>	SLA	0.452	0.346	0.148			0.262	0.120	0.752
		% N	0.284	-0.561	0.448			-0.156	-0.597	0.152
		Leaf thickness	-0.437		0.381	0.255		-0.646	0.279	0.320
		LDMC	-0.408		0.193	-0.826		0.207		0.248
		HairDens	-0.403	-0.121	0.413	0.454		0.665		
		GlandDens	0.242	0.537	0.627	-0.182				-0.471
		CNratio	-0.371	0.504	-0.178				-0.740	0.148
		Prop of var	0.443	0.208	0.117	0.086		0.068	0.043	0.034
		SLA	0.516	0.131	0.127			0.472	0.684	
		% N	0.120	-0.663	-0.217					0.697
		Leaf thickness	-0.472	-0.103	0.379	-0.434		-0.340	0.531	0.193
		LDMC	-0.424	0.106	-0.352	0.719		0.382	0.382	0.120
		HairDens	-0.402	-0.187	0.572	0.253		0.611	-0.196	
Common gardens	<i>H. grosseserratus</i>	GlandDens	-0.393	0.143	-0.565	-0.480		0.523		
		CNratio		0.682	0.152				-0.243	0.672
		Prop of var	0.371	0.273	0.117	0.099		0.073	0.045	0.023
		SLA	0.353	0.432		0.502		0.212	0.616	
		% N	0.558	-0.322	0.149			-0.251		0.702
		Leaf thickness	-0.341	-0.104	0.735	-0.325		-0.110	0.461	
		LDMC		-0.524	-0.532	-0.214		0.241	0.573	
		HairDens	-0.333	-0.301		0.636		-0.614	0.107	
		GlandDens		-0.501	0.349	0.426		0.622	-0.237	
		CNratio	-0.574	0.287	-0.140			0.242		0.703
		Prop of var	0.326	0.284	0.149	0.131		0.071	0.031	0.008
		SLA	0.481	0.209		0.166		0.476	0.447	0.512
		% N		-0.154	-0.107	0.111		-0.601	-0.368	0.479
Leaf thickness	-0.257	-0.541	-0.107	0.746		0.265	0.265	0.378		
LDMC	-0.455	0.290	-0.181	-0.204		-0.462	0.528	0.114		
HairDens		-0.740		-0.600		0.101	0.243			
GlandDens			0.955			-0.253	0.129			
CNratio	-0.509		0.142			0.351	-0.491	0.593		
Prop of var	0.446	0.182	0.150	0.101		0.071	0.029	0.022		

Appendix 5

Continued.

Setting	Species	Trait	Component 1	Component 2	Component 3	Component 4	Component 5	Component 6	Component 7
	<i>H. grosseserratus</i>	SLA	0.450		0.267	0.113	0.410	0.675	0.286
		% N	0.427		-0.380	0.377	-0.528		0.487
		Leaf thickness	-0.291	-0.290	-0.673	0.395	0.434	0.183	
		LDMC	-0.409	0.349	-0.215	-0.218	-0.450	0.642	
		HairDens	-0.334		0.496	0.762	-0.205		-0.112
		GlandDens		-0.874	0.135	-0.213	-0.293	0.237	0.170
		CNratio	-0.501	0.116	0.143	-0.124	0.188	-0.172	0.797
		Prop of var	0.491	0.167	0.134	0.089	0.057	0.045	0.016
		SLA	0.427	0.123	0.584		0.362	0.216	0.527
		%N	0.379		-0.723	-0.373	0.134	0.131	0.398
	<i>H. maximiliani</i>	Leaf thickness	-0.404		0.276	-0.849	0.182	0.722	0.138
		LDMC	-0.468		-0.190	0.294	0.358	0.368	-0.170
		HairDens	0.158	-0.678	0.137		-0.579	-0.367	0.730
		GlandDens		-0.712			0.574	-0.376	0.010
		CNratio	-0.509			0.181	-0.157		
		Prop of var	0.493	0.233	0.113	0.071	0.049	0.031	

Appendix 6

Defense-environment linear mixed effect modeling results. Models run separately for each species of *Helianthus*, with defense traits leaf thickness, LDMC, HairDens, GlandDens, and CNratio as response variables and environmental variables MAT, MAP, AI, Soil_P, Soil_N, and Soil_org as predictors (see Table 1 for description of abbreviations). Values indicate the β slopes, SE is the standard error of the value estimate, d.f. = degrees of freedom, t value = t statistic, associated P value and marginal R^2 values from ANOVA testing.

Trait	Species	Environmental variables	Value	SE	d.f.	t	P	Marginal R^2
Leaf thickness	<i>H. giganteus</i>	MAT	0.025	0.017	6	1.518	0.180	0.150
		MAP	0.001	0.001	6	0.978	0.366	0.073
		AI	-0.195	0.400	6	-0.487	0.644	0.020
		Soil_P	-0.001	0.003	6	-0.343	0.743	0.010
		Soil_N	0.000	0.001	6	-0.448	0.670	0.017
	<i>H. grosseserratus</i>	Soil_org	0.000	0.008	6	-0.012	0.991	0.000
		MAT	0.058	0.026	5	2.248	0.074	0.167
		MAP	0.001	0.000	5	3.303	0.021	0.233
		AI	1.492	0.583	5	2.560	0.051	0.190
		Soil_P	-0.001	0.001	5	-0.394	0.710	0.009
<i>H. maximiliani</i>	Soil_N	0.000	0.002	5	0.098	0.925	0.001	
	Soil_org	0.004	0.002	5	2.355	0.065	0.175	
	MAT	0.024	0.029	3	0.816	0.474	0.087	
	MAP	0.001	0.002	3	0.834	0.465	0.091	
	AI	0.273	1.206	3	0.226	0.836	0.008	
	Soil_P	0.000	0.001	3	0.074	0.946	0.001	
	Soil_N	0.001	0.005	3	0.239	0.826	0.009	
	Soil_org	-0.041	0.028	3	-1.469	0.238	0.216	
	MAT	0.012	0.012	6	1.041	0.338	0.079	
	MAP	0.000	0.000	6	0.592	0.575	0.028	
LDMC	<i>H. giganteus</i>	AI	0.013	0.266	6	0.051	0.961	0.000
		Soil_P	-0.002	0.002	6	-1.116	0.307	0.089
		Soil_N	0.000	0.001	6	-0.472	0.654	0.018
		Soil_org	-0.003	0.005	6	-0.644	0.543	0.033
		MAT	0.019	0.009	5	2.090	0.091	0.165
	<i>H. grosseserratus</i>	MAP	0.000	0.000	5	3.409	0.019	0.255
		AI	0.425	0.232	5	1.838	0.126	0.141
		Soil_P	-0.001	0.000	5	-1.353	0.234	0.092
		Soil_N	0.000	0.001	5	-0.051	0.962	0.000
		Soil_org	0.000	0.000	5			

Appendix 6

Trait	Species	Environmental variables	Value	SE	d.f.	<i>t</i>	<i>P</i>	Marginal <i>R</i> ²
Continued.	<i>H. maximiliani</i>	Soil_org	0.002	0.001	5	2.526	0.053	0.201
		MAT	0.009	0.012	3	0.746	0.510	0.075
		MAP	0.000	0.001	3	0.598	0.592	0.050
		AI	-0.203	0.462	3	-0.440	0.690	0.028
		Soil_P	0.001	0.000	3	2.188	0.117	0.343
		Soil_N	0.002	0.001	3	2.018	0.137	0.317
	<i>H. giganteus</i>	Soil_org	-0.008	0.014	3	-0.573	0.607	0.046
		MAT	719.056	233.838	6	3.075	0.022	0.414
		MAP	9.258	11.281	6	0.821	0.443	0.062
		AI	-9,647.340	6,756.118	6	-1.428	0.203	0.159
		Soil_P	-59.514	50.586	6	-1.176	0.284	0.116
		Soil_N	15.669	17.591	6	0.891	0.407	0.071
<i>H. grosseratus</i>	Soil_org	164.465	134.234	6	1.225	0.266	0.124	
	MAT	3,744.815	1,655.971	5	2.261	0.073	0.226	
	MAP	59.401	29.076	5	2.043	0.097	0.202	
	AI	105,798.300	32,570.510	5	3.248	0.023	0.313	
	Soil_P	-12.047	85.869	5	-0.140	0.894	0.002	
	Soil_N	62.086	99.909	5	0.621	0.562	0.030	
<i>H. maximiliani</i>	Soil_org	98.550	172.534	5	0.571	0.593	0.025	
	MAT	-21.608	44.187	3	-0.489	0.658	0.009	
	MAP	-1.710	2.285	3	-0.748	0.509	0.020	
	AI	1,068.527	1,607.318	3	0.665	0.554	0.016	
	Soil_P	0.075	1.808	3	0.041	0.970	0.000	
	Soil_N	0.936	6.589	3	0.142	0.896	0.001	
<i>H. giganteus</i>	Soil_org	-34.000	48.048	3	-0.708	0.530	0.018	
	MAT	-158.870	160.525	6	-0.990	0.361	0.059	
	MAP	2.275	5.409	6	0.421	0.689	0.012	
	AI	4,149.742	3,185.056	6	1.303	0.240	0.093	
	Soil_P	-16.573	24.988	6	-0.663	0.532	0.028	
	Soil_N	-0.206	8.634	6	-0.024	0.982	0.000	
<i>H. grosseratus</i>	Soil_org	50.093	66.149	6	0.757	0.478	0.036	
	MAT	381.027	466.457	5	0.817	0.451	0.049	
	MAP	8.539	7.374	5	1.158	0.299	0.089	
	AI	13,532.050	10,491.510	5	1.290	0.254	0.106	
	Soil_P	0.577	18.140	5	0.032	0.976	0.000	
	Soil_N	9.545	21.444	5	0.445	0.675	0.016	
GlandDens	<i>H. giganteus</i>							
Continued.	<i>H. maximiliani</i>	Soil_org	0.002	0.001	5	2.526	0.053	0.201
		MAT	0.009	0.012	3	0.746	0.510	0.075
		MAP	0.000	0.001	3	0.598	0.592	0.050
		AI	-0.203	0.462	3	-0.440	0.690	0.028
		Soil_P	0.001	0.000	3	2.188	0.117	0.343
		Soil_N	0.002	0.001	3	2.018	0.137	0.317
	<i>H. giganteus</i>	Soil_org	-0.008	0.014	3	-0.573	0.607	0.046
		MAT	719.056	233.838	6	3.075	0.022	0.414
		MAP	9.258	11.281	6	0.821	0.443	0.062
		AI	-9,647.340	6,756.118	6	-1.428	0.203	0.159
		Soil_P	-59.514	50.586	6	-1.176	0.284	0.116
		Soil_N	15.669	17.591	6	0.891	0.407	0.071
<i>H. grosseratus</i>	Soil_org	164.465	134.234	6	1.225	0.266	0.124	
	MAT	3,744.815	1,655.971	5	2.261	0.073	0.226	
	MAP	59.401	29.076	5	2.043	0.097	0.202	
	AI	105,798.300	32,570.510	5	3.248	0.023	0.313	
	Soil_P	-12.047	85.869	5	-0.140	0.894	0.002	
	Soil_N	62.086	99.909	5	0.621	0.562	0.030	
<i>H. maximiliani</i>	Soil_org	98.550	172.534	5	0.571	0.593	0.025	
	MAT	-21.608	44.187	3	-0.489	0.658	0.009	
	MAP	-1.710	2.285	3	-0.748	0.509	0.020	
	AI	1,068.527	1,607.318	3	0.665	0.554	0.016	
	Soil_P	0.075	1.808	3	0.041	0.970	0.000	
	Soil_N	0.936	6.589	3	0.142	0.896	0.001	
<i>H. giganteus</i>	Soil_org	-34.000	48.048	3	-0.708	0.530	0.018	
	MAT	-158.870	160.525	6	-0.990	0.361	0.059	
	MAP	2.275	5.409	6	0.421	0.689	0.012	
	AI	4,149.742	3,185.056	6	1.303	0.240	0.093	
	Soil_P	-16.573	24.988	6	-0.663	0.532	0.028	
	Soil_N	-0.206	8.634	6	-0.024	0.982	0.000	
<i>H. grosseratus</i>	Soil_org	50.093	66.149	6	0.757	0.478	0.036	
	MAT	381.027	466.457	5	0.817	0.451	0.049	
	MAP	8.539	7.374	5	1.158	0.299	0.089	
	AI	13,532.050	10,491.510	5	1.290	0.254	0.106	
	Soil_P	0.577	18.140	5	0.032	0.976	0.000	
	Soil_N	9.545	21.444	5	0.445	0.675	0.016	

Appendix 6

Trait	Species	Environmental variables	Value	SE	d.f.	<i>t</i>	<i>P</i>	Marginal <i>R</i> ²
Continued.	<i>H. maximiliani</i>	Soil_org	68.757	21.549	5	3.191	0.024	0.306
		MAT	-136.419	212.016	3	-0.643	0.566	0.037
		MAP	8.489	11.240	3	0.755	0.505	0.050
		AI	11,374.490	5,366.561	3	2.120	0.124	0.204
		Soil_P	6.886	7.972	3	0.864	0.451	0.062
		Soil_N	32.263	26.714	3	1.208	0.314	0.105
		Soil_org	-71.319	252.334	3	-0.283	0.796	0.008
		MAT	0.479	0.682	6	0.703	0.508	0.030
		MAP	0.029	0.019	6	1.489	0.187	0.078
		AI	4.240	14.680	6	0.289	0.782	0.006
<i>H. giganteus</i>	Soil_P	-0.011	0.106	6	-0.106	0.919	0.001	
	Soil_N	-0.033	0.033	6	-1.000	0.356	0.055	
	Soil_org	0.022	0.284	6	0.079	0.939	0.000	
	MAT	-3.529	1.662	5	-2.123	0.087	0.407	
	MAP	-0.041	0.034	5	-1.212	0.280	0.247	
	AI	-70.850	46.057	5	-1.538	0.185	0.317	
	Soil_P	-0.085	0.075	5	-1.131	0.309	0.228	
	Soil_N	-0.199	0.047	5	-4.201	0.008	0.457	
	Soil_org	-0.061	0.171	5	-0.356	0.737	0.034	
	MAT	0.796	1.185	3	0.671	0.550	0.082	
<i>H. grosseratus</i>	MAP	-0.047	0.063	3	-0.735	0.515	0.095	
	AI	-47.421	39.017	3	-1.215	0.311	0.185	
	Soil_P	-0.054	0.039	3	-1.372	0.264	0.209	
	Soil_N	-0.218	0.133	3	-1.640	0.199	0.245	
	Soil_org	-0.280	1.428	3	-0.196	0.857	0.009	
	<i>H. maximiliani</i>	Soil_org	68.757	21.549	5	3.191	0.024	0.306
		MAT	-136.419	212.016	3	-0.643	0.566	0.037
		MAP	8.489	11.240	3	0.755	0.505	0.050
		AI	11,374.490	5,366.561	3	2.120	0.124	0.204
		Soil_P	6.886	7.972	3	0.864	0.451	0.062
Soil_N		32.263	26.714	3	1.208	0.314	0.105	
Soil_org		-71.319	252.334	3	-0.283	0.796	0.008	
MAT		0.479	0.682	6	0.703	0.508	0.030	
MAP		0.029	0.019	6	1.489	0.187	0.078	
AI		4.240	14.680	6	0.289	0.782	0.006	

Continued.

Appendix 7

Damage–environment linear mixed effect modeling results. Models run separately for each species of *Helianthus*, with damage traits ChewDam and SuckDam as response variables and environmental variables MAT, MAP, AI, Soil_P, Soil_N, and Soil_org as predictors (see Table 1 for description of abbreviations). Values indicate the β slopes; SE is the standard error of the value estimate, d.f. = degrees of freedom, $t = t$ statistic, associated P and marginal R^2 values from ANOVA testing.

Trait	Species	Environmental variables	Value	SE	d.f.	t	P	Marginal R^2
ChewDam	<i>H. giganteus</i>	MAT	−0.020	0.025	6	−0.795	0.457	0.019
		MAP	0.000	0.001	6	0.080	0.939	0.000
		AI	0.606	0.489	6	1.240	0.261	0.041
		Soil_P	0.000	0.004	6	−0.047	0.964	0.000
		Soil_N	−0.001	0.001	6	−0.738	0.488	0.017
	<i>H. grosesseratus</i>	Soil_org	−0.012	0.009	6	−1.296	0.242	0.044
		MAT	0.104	0.040	5	2.606	0.048	0.200
		MAP	0.002	0.001	5	3.753	0.013	0.262
		AI	2.570	0.944	5	2.722	0.042	0.208
		Soil_P	−0.001	0.002	5	−0.610	0.568	0.023
	<i>H. maximiliani</i>	Soil_N	0.001	0.003	5	0.565	0.597	0.020
		Soil_org	0.009	0.002	5	3.945	0.011	0.270
		MAT	−0.013	0.011	3	−1.224	0.308	0.082
		MAP	0.000	0.001	3	−0.020	0.986	0.000
		AI	0.219	0.482	3	0.453	0.681	0.015
SuckDam	<i>H. giganteus</i>	Soil_P	0.000	0.000	3	−0.602	0.590	0.026
		Soil_N	−0.001	0.002	3	−0.647	0.564	0.029
		Soil_org	0.023	0.007	3	3.092	0.054	0.202
		MAT	0.028	0.017	6	1.700	0.140	0.101
		MAP	0.000	0.001	6	−0.085	0.935	0.000
	<i>H. grosesseratus</i>	AI	−0.710	0.311	6	−2.286	0.062	0.147
		Soil_P	−0.004	0.003	6	−1.322	0.234	0.069
		Soil_N	0.002	0.000	6	6.091	0.001	0.320
		Soil_org	0.017	0.005	6	3.697	0.010	0.224
		MAT	0.006	0.010	5	0.569	0.594	0.006
	<i>H. maximiliani</i>	MAP	0.000	0.000	5	−0.019	0.985	0.000
		AI	0.107	0.249	5	0.430	0.685	0.004
		Soil_P	0.000	0.000	5	0.587	0.582	0.007
		Soil_N	0.001	0.000	5	1.941	0.110	0.052
		Soil_org	0.000	0.001	5	−0.519	0.626	0.005
<i>H. maximiliani</i>	MAT	0.025	0.037	3	0.667	0.553	0.026	
	MAP	0.003	0.001	3	2.596	0.081	0.153	
	AI	−0.084	1.481	3	−0.057	0.958	0.000	
	Soil_P	0.003	0.001	3	3.715	0.034	0.220	
	Soil_N	0.010	0.003	3	3.753	0.033	0.223	
		Soil_org	−0.009	0.044	3	−0.192	0.860	0.002

Appendix 8

Comparison of traits of *Helianthus* species between common gardens. $t = t$ statistic, where positive values indicate higher trait values at Cornell and negative values indicate higher trait values at Jeffers; d.f. = degrees of freedom; PCI_low and CI_high are the confidence intervals for the mean under the alternative hypothesis, and Cornell and Jeffers means are the mean trait values at the respective common garden. Abbreviations and units follow Table 1.

Species	Trait	t	d.f.	P	CI_low	CI_high	Cornell mean	Jeffers mean
<i>H. giganteus</i>	SLA	-13.622	108.3	<0.001	-69.671	-51.971	143.867	204.689
	% N	-4.071	11.2	0.002	-1.955	-0.585	2.750	4.020
	Leaf thickness	4.846	127.2	<0.001	0.022	0.051	0.337	0.301
	LDMC	9.346	120.6	<0.001	0.029	0.045	0.256	0.219
	HairDens	-0.199	128.5	0.843	-532.040	434.787	2,891.841	2,940.468
	GlandDens	0.580	125.9	0.563	-26.144	47.832	54.545	43.701
	CNratio	7.872	17.9	<0.001	4.464	7.716	17.610	11.520
	ChewDam	-1.736	133.9	0.085	-0.022	0.001	0.012	0.022
	SuckDam	1.138	92.8	0.258	-0.020	0.074	0.058	0.032
	<i>H. grosseserratus</i>	SLA	-13.743	112.4	<0.001	-55.623	-41.606	137.953
% N		-3.048	12.6	0.010	-1.164	-0.196	3.080	3.760
Leaf thickness		4.016	108.6	<0.001	0.019	0.056	0.368	0.331
LDMC		7.748	102.5	<0.001	0.028	0.047	0.243	0.206
HairDens		-1.461	117.4	0.147	-1,377.715	208.063	5,412.732	5,997.558
GlandDens		2.551	95.7	0.012	49.761	398.876	500.265	275.946
CNratio		6.880	15.9	<0.001	2.684	5.076	16.340	12.460
ChewDam		-2.738	115.1	0.007	-0.037	-0.006	0.008	0.029
SuckDam		-0.652	123.5	0.515	-0.048	0.024	0.023	0.035
<i>H. maximiliani</i>		SLA	-13.821	108.5	<0.001	-85.573	-64.107	111.727
	% N	-3.187	12.9	0.007	-1.897	-0.363	2.660	3.790
	Leaf thickness	5.657	124.8	<0.001	0.030	0.063	0.413	0.366
	LDMC	9.327	126.7	<0.001	0.032	0.050	0.255	0.214
	HairDens	-1.275	82.0	0.206	-298.700	65.417	610.177	726.819
	GlandDens	-0.514	134.9	0.608	-227.140	133.399	2,031.716	2,078.586
	CNratio	6.609	12.7	<0.001	3.496	6.904	17.110	11.910
	ChewDam	-0.970	143.7	0.334	-0.028	0.009	0.014	0.023
	SuckDam	0.339	128.8	0.735	-0.025	0.036	0.026	0.021

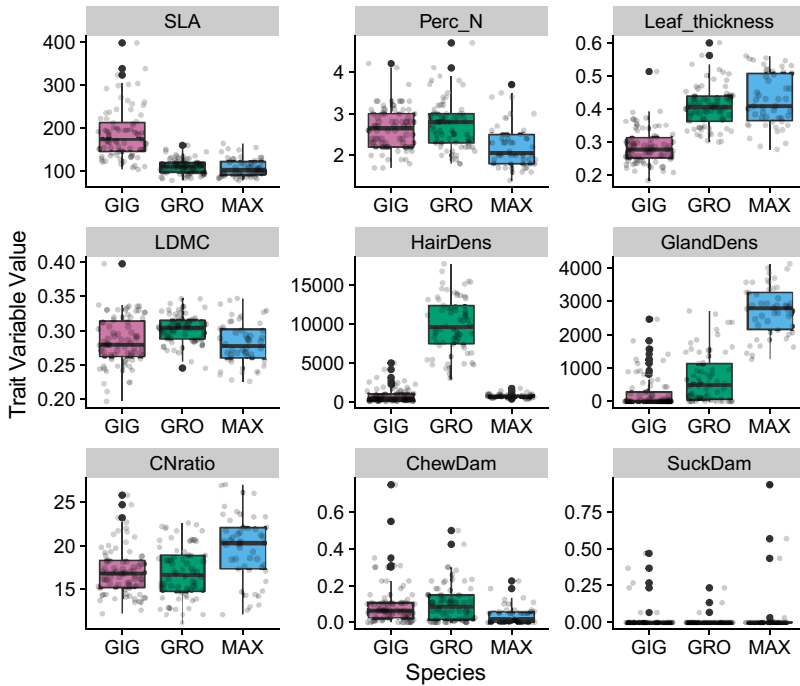


FIG. S1. Summaries for trait values associated with growth, defense, and herbivore damage in the wild populations. Boxplots display the medians, quartiles, upper and lower maxima, and outliers. Pink = *H. giganteus*, green = *H. grosseserratus*, blue = *H. maximiliani*.

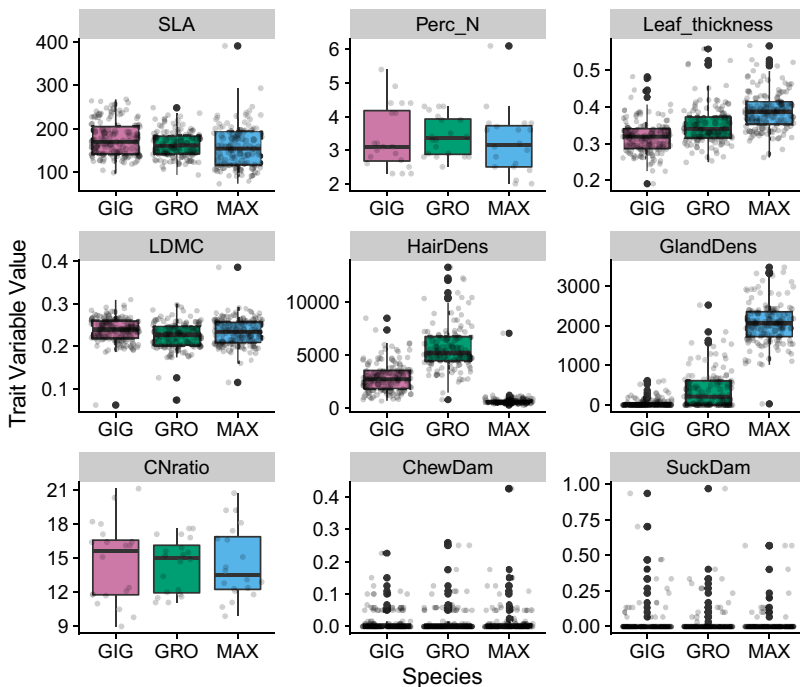


FIG. S2. Summaries for trait values associated with growth, defense, and herbivore damage in the common gardens. Boxplots display the medians, quartiles, upper and lower maxima, and outliers. Pink = *H. giganteus*, green = *H. grosseserratus*, blue = *H. maximiliani*.

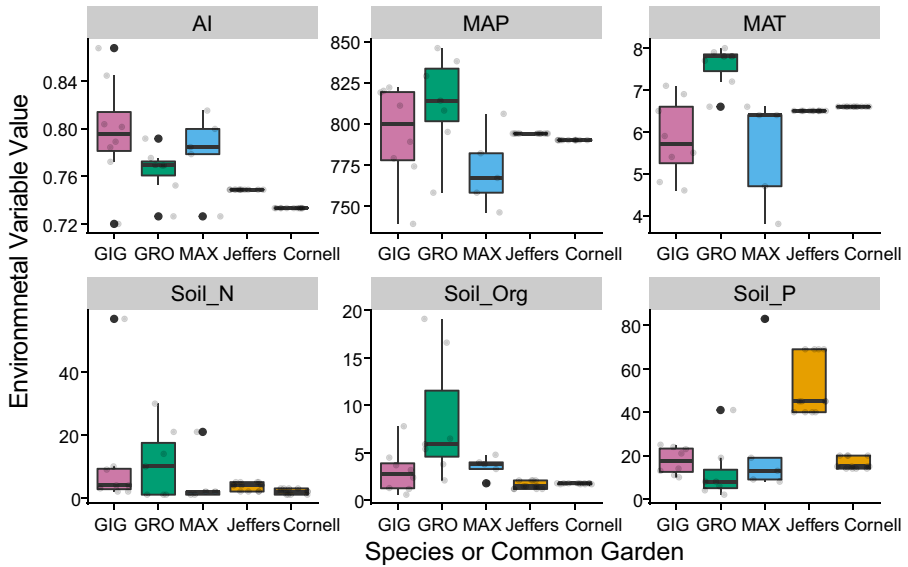


FIG. S3. Summaries for environmental variables at each site. Boxplots display the medians, quartiles, upper and lower maxima, and outliers. Pink = *H. giganteus*, green = *H. grosseserratus*, blue = *H. maximiliani*, orange = common garden (Jeffers and Cornell).