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Consequences of Stress-induced Trait Plasticity in Cultivated Helianthus

Gillian Gomer
University of Central Florida

CONSEQUENCES OF STRESS-INDUCED TRAIT PLASTICITY IN
CULTIVATED *HELIANTHUS*

by

GILLIAN GOMER

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ABSTRACT

The Leaf Economics Spectrum (LES) describes the continuous range of leaf ecophysiological strategies that plants across the globe utilize to achieve a net-positive return on their resource investment. This spectrum is measured by traits such as leaf mass per area, leaf nitrogen, and leaf lifespan. Unsuitable leaf trait combinations are selected against in nature due to a net-loss on the return of initial resource investment, while some potentially extremely effective trait combinations may not be possible due to genetic constraints. Existing genetic variation in LES traits, however, suggests the spectrum may be less rigid than previously expected. To test this, we exploit phenotypic plasticity by subjecting cultivated sunflower, *Helianthus annuus*, to environmental stress to generate variability in LES traits. In a greenhouse experiment, treatment groups of Helianthus were exposed to six varying intensities each of four acute stress applications: nutrient limitation, high soil salinity, heat, and simulated herbivory. Leaves formed during the onset of stress and post-stress were sampled to measure leaf traits; changes in plant fitness were estimated through total plant biomass and the time of first flowering. Results from statistical analyses show a variety of significant effects that stress applications had on plant growth and LES traits. Our findings explore the possibility of achieving off-axis LES trait combinations through salt-induced trait plasticity, and if further studies are needed to reassess the rigidity of the spectrum.

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INTRODUCTION

Plant life strategies range from resource-acquisitive to resource-conservative, regardless of life history and growth form (Mason & Donovan, 2015). This leaf economics spectrum (LES) describes the continuous range of strategies plants utilize to achieve a net-positive return on their resource investment and is measured by leaf mass per area (LMA), leaf nitrogen (N), leaf lifespan (LL), leaf phosphorus (P), photosynthetic rate (A_{mass}), and respiration rate (R_{mass}) (Wright et al., 2004). To achieve sufficient returns on initial carbon investment, plants at one end of the LES may produce low-carbon-cost leaves with high N and A_{mass}, but sacrifice longevity: on the other end, plants may invest more carbon into each longer-lasting leaf, at the expense of low productivity per unit time through lower N and A_{mass} (Donovan et al., 2011). On a global scale, this spectrum characterizes global leaf trait variation in over 2,500 plant species and provides a useful framework for describing leaf economics across all vascular plants (Wright et al., 2004). In this vast sample, it is evident that the relationships among leaf traits derive from strong trade-offs that constrain trait covariation (Wright et al., 2004).

The ability for plants to alter ecophysiology can be beneficial, as they are highly confined to their local spatial environment and must effectively acclimate or adapt to changing conditions (Velikova et al., 2020). As such, leaf traits within a species can occupy different regions of the LES due to genetic variation, environmental plasticity, and ontogenetic trajectories (Xiong & Flexas, 2018). Unsuitable ecophysiological trait combinations are selected against in nature due to a net-loss on the return of initial resource investment, while some potentially extremely

effective trait combinations may not be possible due to genetic constraints (Figure 1) (Wright et al., 2004). Previous research has determined that there is heritable variation in multiple LES traits, including LMA and N, which suggests that a lack of genetic variation (one form of genetic constraint) has not constrained the evolution of the LES as initially expected (Donovan et al., 2011). Given this, the evolution of the LES is expected to be influenced more strongly by the action of natural selection than a lack of genetic variation, however, there are other forms of constraint that may shape the LES. One is pleiotropy, a form of genetic constraint where the same genes may influence multiple traits at once (Zhang & Wagner, 2013). This has been identified to play at least some role in the model system Arabidopsis (Vasseur et al., 2012). A related type of constraint is biophysical constraints, where the laws of physics preclude the construction of certain trait combinations (Pigliucci, 2007). Biophysical constraints are sometimes considered to be a form of genetic constraint, as there is no combination of available alleles that can produce a particular phenotype. Regardless, understanding whether particular trait combinations are precluded by selection or constraints is key to advancing ecophysiology. To investigate this, artificial selection has been proposed as an attempt to produce novel combinations of LES traits (Donovan et al., 2011). However, whereas artificial selection can be a lengthy process, an alternative approach involves exploiting phenotypic plasticity under environmental stress to generate variability in LES traits within a single generation.

In response to environmental stress, plants often undergo hormonal signaling cascades that elicit an observable change in plant physiology (Chapin, 1991). Phenotypic plasticity is a well-known facilitator of plant acclimation to variation in the environment, and critical for success in a sessile organism that must complete its life cycle in the location where it first establishes. Over plant ontogeny or in response to changing environmental conditions, an

individual can undergo physiological changes and produce successive cohorts of leaves with differences in LES traits (Mason et al., 2013). By imposing a variety of different stresses, it can be determined whether it is possible to produce outlier trait combinations off the largely rigid LES axis. Shifts along the main LES axis would confirm the ability of a plant to use phenotypic plasticity to adjust ecophysiological strategies and maintain fitness (Dudley & Schmitt, 1996). Off-axis shifts into areas of trait space that are expected to be impossible due to genetic constraints would prove that it is indeed possible to construct leaves with these trait combinations (Donovan et al., 2011). Additionally, off-axis shifts that are associated with lower growth and fitness would suggest that natural selection precludes the observed trait combinations in that portion of trait space. Each applied stress may affect LES traits differently (Chapin et al., 1987), and result in unique shifts. When compared in parallel, the relative influence of different forms of stress may assessed.

The genus *Helianthus* occupy habitats across North America and have a wide variety of life histories and leaf trait strategies to enable survival in diverse habitats (Mason & Donovan, 2015). The wild species that is the progenitor of cultivated sunflower, *Helianthus annuus,* occupies the largest range of any *Helianthus* species spanning at least half of the North American continent (Heiser, 1969; Kantar et al., 2015). a wide variety of environmental stresses. The wide variety of environmental stresses experienced by *Helianthus annuus* across its range makes this species a useful model for studying plant responses to the abiotic environment (Blackman et al., 2011; Hernandez et al., 2018). Furthermore, reports indicate that cultivated sunflower has likely retained much of the stress resistance exhibited by the progenitor wild *Helianthus annuus* (Tran et al. 2020; Wang et al., 2020).

METHODS

Plant Growth

For this study, we used a widely studied inbred oilseed line of cultivated *Helianthus annuus* RHA 801 retrieved from the USDA National Plant Germplasm System. A greenhouse experiment was conducted beginning in November 2014 in the University of Georgia Plant Biology greenhouses. Seeds were directly planted into 8-inch azalea pots containing three liters of a homogenized 3:1 mixture of sand and calcined clay (Turface). For each stress, twelve replicate plants per treatment level were planted and arranged in a randomized design, with stress treatments described in detail below. Plants were watered daily to field capacity and received ambient light plus supplemental metal halide lighting (16.5 mol m^{-2} d^{-1}) to ensure a 16-hour photoperiod. A combination of heaters and an evaporative cooling system were used to keep temperatures approximately 25°C during the day and 13°C at night. All pots other than those under a nutrient stress treatment received 1 tablespoon (16 grams) of slow-release Osmocote Plus 15-9-12 (7% NH_4, 8% NO_3) fertilizer. Plants were grown under these non-limiting conditions for two weeks before the onset of stress treatments.

Our stresses of interest were nutrient limitation, soil salinity, heat stress, and herbivory. Each stress was applied to the young plants as described below, and the plants were then permitted to continue growth until reproductive maturity and harvest. Nutrient limitation was imposed by varying fertilization regimes across six levels, specifically by adding either 0, 0.1, 0.25, 0.5, 0.75, or 1.0 tablespoons of slow-release Osmocote Plus 15-9-12 (7% NH_4, 8% NO_3) fertilizer levels. Soil salinity treatments were imposed across six levels by adding a liquid

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suspension of sodium chloride (NaCl) to pots to achieve soil NaCl concentrations of 0, 169, 833, 1666, 2778, and 8333 mg/kg. To impose heat stress, control plants were left in place while all other pots were moved to climate-controlled growth chambers with 16-hour photoperiod (Conviron, Winnipeg, Canada) set to a 38°C for five duration treatments: 6h, 24h, 48h, 72h, or 96h. Pots were watered to field capacity daily and returned to the main greenhouse bay at the end of their designated heat treatment duration. Simulated herbivory stress was imposed through a single application of jasmonic acid (CAS# 77026-92-7) in aqueous solution at six concentrations: 0, 0.13, 0.32, 1.22, 5.05, 16.38 mM.

Trait Collection

Each plant was sampled for leaf traits at three time points. The first time point was two weeks after the initiation of stressors, in order to capture mature leaves that were in the early stages of development during the onset of stress, and for which the onset of stress would have directly influenced leaf expansion. The second time point was four weeks after the initiation of stressors, in order to capture mature leaves whose development would have initiated well after plants had perceived stress conditions. The third time point was six weeks after the initiation of stressors, in order to capture mature leaves later in ontogeny just before the transition to reproduction. At each time point, one of the two leaves making up the most recently fully expanded leaf (MRFEL) pair was sampled to assess ecophysiological traits. All replicate plants were sampled at the first time point, while one half of replicate plants were sampled at each of the second and third time points. Sampled leaves were removed by cutting the petiole flush with the stem of the plant, and leaves were placed into resealable plastic bags in a cooler with ice for

transport to the laboratory. At this time, the paired leaf was tagged to track leaf lifespan (LL) three times per week until the leaf lost 100% of visible greenness.

Sampled leaves were brought to the laboratory within two hours of removal from the plant. There, leaves were weighed for fresh mass and leaf images were collected with a digital flatbed scanner. Chlorophyll content was then measured with a SPAD-502 chlorophyll meter (Konica-Minolta, Tokyo, Japan). The leaves were dried in a forced-air drying oven at 60°C for 48 hours, weighed for dry mass, then ground into a fine powder for further chemical analysis. Leaf carbon (C) and nitrogen content (N) were analyzed by Micro-Dumas Combustion (NA1500, Carlo Erba Strumentazione, Milan, Italy).

Leaf area was determined from scanned images using ImageJ (Schneider et al., 2012). Water content (W_m) was calculated as the mass of water present in the fresh leaf divided by the leaf dry mass (Shipley et al., 2006). Leaf mass per area (LMA) was calculated as the ratio of dry mass to leaf area. Leaf trichome density was determined by counting the number of trichomes present in a 1 cm^2 section in the center of the leaf under a dissecting microscope.

Each plant was monitored at least once every three days for the presence of the first open flower (anthesis), with the date of first flower recorded. Mortality was also recorded for any replicate plants that died before flowering. Once flowering began, self-pollination was facilitated by gently rubbing open capitula with paintbrushes. Plants were allowed to set seed and reach reproductive maturity, evidenced by the back of each capitula changing color from green to yellow. At this point, all plants were harvested, with shoot biomass separated into stems, leaves, and reproductive organs. Pots were gently emptied and rinsed to separate roots from soil, yielding root biomass. All organs were dried at 60°C for at least 48 hours until constant mass, and weighed to obtain individual organ dry mass, as well as total plant biomass and relative mass

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fractions. Mature seeds were separated from dried capitula, and both counted and weighed to obtain total seed mass.

Statistical Analysis

To determine whether acute stress applications of each level had a significant effect on total biomass and time to reproduction, as well as LES traits including N, SPAD, LMA, W_m, LL, one-way analysis of variance was conducted. After this, multiple regression was performed to model dose response relationships to visualize any changes in leaf traits and whole-plant traits across treatment levels. For each treatment and trait, model selection was conducted using Akaike Information Criterion (AIC). For plant-level traits (height at final harvest, total biomass, data of first flowering, etc.), treatment levels were modeled as either continuous linear predictors or second-order polynomials. For leaf-level traits measured at multiple harvests, treatment levels were again modeled as linear predictors or second-order polynomials, the harvest number was modeled as a categorical variable. Both plant-level and leaf-level relationships with each stress were visualized using ggplot in R.

RESULTS

Stress Effects on Fitness

The effects on plant fitness (total biomass and days to first flowering) were generally consistent across stress treatments and intensities (Figure 2). ANOVA results further demonstrated there were significant reductions in biomass when plants were subjected to nutrient deficiency ($p < 0.0001$), excess heat ($p = 0.0006$), simulated herbivory through application of jasmonic acid ($p = 0.0010$), and salt ($p < 0.0001$). There were only significant effects on time to reproduction caused by soil nutrient deficiency ($p = 0.0243$). Heat, simulated herbivory, and soil salinity had no significant effect on flowering time ($p = 0.1141$, $p = 0.0892$, $p = 0.6906$, respectively).

Stress Effects on LES Traits

Each stress had unique effects on LES and related traits, with stage of leaf development having varying relevance. Nutrient stress resulted in decreased leaf area, N, and chlorophyll content, while water content and LMA were not affected after harvest 1 (Figure 3; Table 1). There was a decrease in LL at intermediate stress intensities which then increased over controls at higher treatment levels. These shifts indicate that leaves shifted to a smaller size and more conservative LES strategy. Salt stress resulted in smaller leaves with decreased LMA and increased water content, with no strong effects on other traits (Figure 4; Table 2). These shifts indicate that leaves shifted to a smaller size and a potentially more acquisitive LES strategy. The

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effects of the chronic stresses, nutrient limitation and high salinity, did not depend strongly on the date of leaf harvest, with different leaf stages being affected similarly.

Both acute stresses, heat and simulated herbivory, showed effects dependent on leaf harvest date. Heat-shocked plants produced leaves with lower N and water content at the first stage, but these traits increased above baseline by the third stage (Figure 5; Table 3). Leaves harvested at the second stage from high heat intensity treatments had decreased leaf area compared to other harvests, indicating that heat stress strongly affected leaves formed just after heat stress exposure, but did not affect leaves already forming at the stress onset or leaves formed long after. Additionally, there were significant correlations between heat stress and SPAD and LMA after harvest 1 (Table 3). Plants that underwent applications of jasmonic acid to simulate herbivory also produced smaller leaves (Figure 6). Leaves that experienced more intense simulated herbivory showed a significant decrease in N and water content at the first stage (Table 4), while these effects reversed in later stages above baseline levels, suggesting a temporary shift in leaves becoming more resource-acquisitive upon herbivory stress. However, second-stage leaves had greatly lowered chlorophyll content (Table 4).

Ontogenetic and Stress-Induced LES Patterns

Our leaf trait data was plotted against over 2500 species in the Global Plant Trait Network (GLOPNET) dataset to see whether our study demonstrated any novel shifts into unoccupied trait space compared to what is currently documented (Wright, 2009). As *Helianthus annuus* produces leaves with short-lifespans and high rates of carbon returns to their low cost, the data generated from our study is located within the upper region of the spectrum. Unstressed

sunflowers had ontogenetic shifts in LES trait space that remained on-axis of the global spectrum, moving towards a slightly more conservative strategy at late stages (Figure 7). Understanding this baseline shift allows us to assess if the LES shifts seen as a result of applied stressors can be attributed to the treatment or if it is a result of the life-stage of the plant.

Stress-induced LES patterns occupy the same region of trait space on the global scale as unstressed sunflowers and produce similar shift trajectories that closely align with the GLOPNET LES axis, except those in salt treatments. A deficiency in nutrients had the greatest and most linear LES shift of the four stresses, increasing LMA and LL as time progressed. Though more variability was present across harvest date, exposure to heat induced plants to produce slightly more conservative leaves, as did applications of jasmonic acid. Salt was unique in producing occupied trait space outside of the main spectrum when comparing LL to N (Figure 8).

DISCUSSION

Stress Effects on Fitness

We observed detrimental effects on whole plant fitness by nutrient deficiency, heat exposure, high salinity, and simulated herbivory. Decreases in biomass generally scaled with intensity of each stress. Allocating resources to defense responses or storage comes at a cost of growth investments and likely explains why biomass sharply declines for simulated herbivory and nutrient stress (Chapin et al. 1987). Heat stress can impede plant growth and overall biomass, particularly of roots, as it decreases levels of nutrient-uptake (Giri et al., 2017). While our results demonstrated that salt decreased biomass at moderate levels, at high levels there was a large range of uncertainty regarding biomass effects at high levels due to low survivorship. Previous research demonstrated that in response to salinity, there is a threshold value of when plants begin to respond negatively from no initial reaction, and this slope remains negative and linear when considering yield (Dalton et al., 1997; Dalton et al., 2000). It should be noted that providing plants with adequate nutrients via fertilizer when saline conditions are not severe, may actually increase yield and plant growth (Hu & Schmidhalter, 2005).

Reproduction was delayed in plants subjected to nutrient limitation. Growth rates are known to decrease in plants that use resources for non-growth functions, particularly stress responses (Chapin et al. 1991). Though our results do not provide significant support that non-nutrient stresses affected flowering time, literature suggests there are known relationships between stress and time to reproduction. Previous research suggests that cereal crops under heat stress, due to an unknown mechanism, have hastened flowering when stressed under long days,

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but shorter days delayed flowering (Kazan & Lyons, 2016). Simulated herbivory via applications of jasmonic acid is also known to increase time to reproduction in *Arabidopsis* (Kazan & Lyons, 2016). Salt stress, however, does not often promote early flowering: this is thought to hint at an underlying mechanism that some plants have developed to handle seasonal water stress (Takeno, 2016). In wheat specifically, salt stress is known to decrease time to reproduction (Läuchli & Grattan, 2007).

Stress Effects on LES Traits

In nutrient-stressed plants, there was a slight increase in LMA with high levels of deficiency, which supports previous findings across 100 species (Poorter et al., 2009). Leaf lifespan underwent little change while it was expected to increase (Chapin et al. 1987). As the fertilizer withheld from plants supplied predominately macronutrients like nitrogen, leaf nitrogen content decreased as expected. Our results indicate a clear, well-documented shift towards a resource-conservative strategy when *Helianthus annuus* is under nutrient stress. Other affected leaf traits like chlorophyll content and leaf area match previous findings by responding negatively to increasing intensity of nutrient stress (Ciompi et al. 1996; Poorter et al., 2009).

Salt treatments were responsible for a reduction in leaf area, further supporting previous findings (Munns et al., 1988; Jabeen & Ahmad, 2012). LMA, while shown to decrease in this study, increased in a separate study that also evaluated salinity's effect on *Helianthus annuus* (Temme et al., 2020). Notably, although saline soil can disrupt nutrient uptake by plants and result in lower plant N, this decrease is not typically found in leaves (Hu and Schmidhalter, 1998). In response to the oxidative stress caused by salt, reactive oxygen species (ROS) are

formed which can increase lipid peroxidation and electrolyte leakage when in high amounts (Nazar et al., 2011; Gunes et al., 2007). Cellular metabolism is negatively impacted as a result (Gunes et al., 2007). It has been demonstrated that, in response, plants up-regulate glutathione (GSH), which is partly composed of nitrogen, to address and combat the oxidative stress (Nazar et al., 2011; Szalai et al., 2009). Nitrogen, as a result, may remain more constant in leaves than within other plant organs following salt stress due to an oxidative stress adaptation. Here, high salinity also resulted in an increased uptake of water, likely to offset dehydration in cells and address diminished rates of photosynthesis (Sultana et al., 2001). Additionally, photosynthesis is disrupted by salt stress because of the H^+ electrochemical gradient being affected by excessive Na^+ and Cl^- ion accumulation in plant tissues following salt stress (Sultana et al., 2001). As solute regulation is affected by saline conditions, changes in turgor pressure and osmotic adjustment reduces water potential and increases the intake of water (Sultana et al., 2001). The unchanging N, alongside significant increases in water content and slight reductions in SPAD provides support that the underlying oxidative stress response was strong enough to alter leaf economics, seen by lowered LL and LMA.

Heat applications caused sunflowers to produce leaves with lower leaf area and chlorophyll content, aligning with previous literature (Rawson & Hindmarsh, 1982; Akladious, 2014; Hayat et al., 2009). As these effects were only noted at the time of Harvest 2, this may suggest a delayed, temporary resource reallocation to help the plant handle the heat stress (Hayat et al., 2009). We saw an initial decrease in leaf N and water content; however, this trend reversed over Harvests 2 and 3 which led to a final increasing trend over increasing stress intensity. A similar effect has been noted in *Brassica juncea* L. for both traits, and for water in *Helianthus annuus* L. (Hayat et al., 2009; Akladious, 2014). Similar to the effects of salt on leaf water

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content, cell water potential is negatively affected (Hussain et al., 2010). Leaf N, necessary for chlorophyll and Rubisco production, was previously found to be more scarce in heat-stressed sunflower leaves (Akladious et al., 2014). Our results contradict this after the first sample point, and suggest a recovery in leaf N occurred following the early effects of the acute heat stress. Despite opposite findings in other systems (De la Haba et al., 2014), LMA slightly decreased here, scaling with increasing stress levels at all time points in this study. This, in conjunction with the changing leaf N, suggests our sunflowers that underwent heat stress produced more conservative leaves after a brief recovery period, while the literature provides evidence for an opposing shift towards resource-acquisitive leaves. This discrepancy is likely driven by differences in the timing of stress onset as well as plant ontogeny, and further research should be considered to generate more clarifying data on LES shifts due to heat stress.

Plants that underwent simulated herbivory via jasmonic acid produced leaves with varying leaf traits across intensities and sampled time points. In this study, JA was chosen to avoid removing leaves that might influence LES shifts through compensation of lost leaf area; however, few studies explore LES trait effects due to JA applications, and instead manually defoliate or utilize live herbivores in their studies (Moriondo et al., 2003; Venâncio et al., 2016). Because of this and the complex nature of plant-herbivore interactions, it is difficult to directly compare all our results to previous works. Leaf water content decreased with increasing JA intensities at the first harvest, and in later harvests, increased with increasing stress. Leaf area and chlorophyll content decreased most intensely during harvest two. We found harvest-dependent effects of JA on LL but an especially strong decrease in harvest two, which suggests a delayed response that agrees with the literature (Moriondo et al., 2003). From this, it can be concluded that newly formed leaves post-acute simulated herbivory stress were constructed

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differently with different lifespans than previous leaves. N is known to increase or decrease in response to herbivory: here, we see both effects depending on stage (Casotti & Bradley, 1991; Hartley, 1998). Building upon what is currently known, our data most closely supports the interpretation that simulated herbivory triggered an overall shift towards a more conservative LES strategy, which resulted in decreased growth and likely overall fitness.

Ontogenetic and Stress-Induced LES Patterns

On-axis LES shifts due to ontogeny and stress indicate existing phenotypic plasticity adaptations within *Helianthus* to changing environments (Dudley & Schmitt, 1996). Whereas nutrient, salt and herbivory stresses incited fairly coordinated shifts with multiple traits changing together, heat stress resulted in less coordinated shifts. In the natural world, plants may not acclimate to heat stress solely through LES changes; heat is often accompanied by drought and potentially high insolation. Instead, plants may have a more complex physiological adaptation to these combinations of abiotic stresses.

Salt applications at all intensities induced LES shifts horizontally, with large changes in both LMA and LL without a substantial corresponding change in leaf N – challenging the rigidity of the LES and suggesting that leaf construction cost and length of persistence may be able to decouple from leaf productivity per unit time. Common salinity stress responses include compatible solute accumulation and cell wall stiffening (Verslues et al., 2006), and it is possible that the specific salinity stress response employed by sunflowers facilitates the putatively observed decoupling. Increased soil salinity was observed to increase water content, suggesting the accumulation of compatible solutes, while soil salinity was observed to decrease chlorophyll

content. This may suggest that the lack of change in leaf N masks a re-allocation of nitrogen from photosynthesis to other nitrogen-intensive metabolic functions like the production of nitrogen-containing compatible solutes (e.g., proline, glycinebetaine) or stress response enzymes (Albert et al., 2012; Ashraf & Foolad, 2007; Versules et al., 2006). If this is the case, then leaf N is not a good predictor of leaf productivity, an effect noted in plants with high levels of nitrogen-containing compounds, like coffee (Martin et al., 2017).

CONCLUSION

Our study aimed to explore abiotic and biotic stress effects on cultivated sunflowers, and to the extent these stresses result in leaf economic strategies outside the confines of a globally accepted model. Our data indicate that inducing phenotypic plasticity to adjust ecophysiological strategies was sufficient to test aspects of the rigidity of the LES model. While nutrient, heat, and herbivory stress each resulted in shifts qualitatively similar to ontogenetic effects through the production of more conservative leaves, our results suggest salt stress may be sufficient to generate off-axis LES strategies in *Helianthus annuus*.

This work provides a foundation for future studies to more closely and comprehensively examine the rigidity of the LES and consequences for phenotypic plasticity that results in shifts outside the range of typical LES trait combinations. Building on our exploratory research, it would be highly beneficial to further clarify the role of nitrogen under salt stress, and whether photosynthetic productivity is altered when leaf construction and lifespan shift in response to salinity. Additional research is needed to determine if other unexplored abiotic or biotic stresses can also result in trait combinations into unoccupied portions of trait space, and whether these directly contribute to reduced fitness. Testing pairwise combinations of stressors would offer unique insights into whether LES shifts are additive or multiplicative in plants, as well as opening a window to seeing relative strength of effects when experienced collectively.

Using stress to observe changes in leaf construction allows us to interrogate how plants maintain leaf economics under varying conditions. By making these observations through the

LES lens, we can learn what leaf traits to target for breeding purposes and ultimately explore

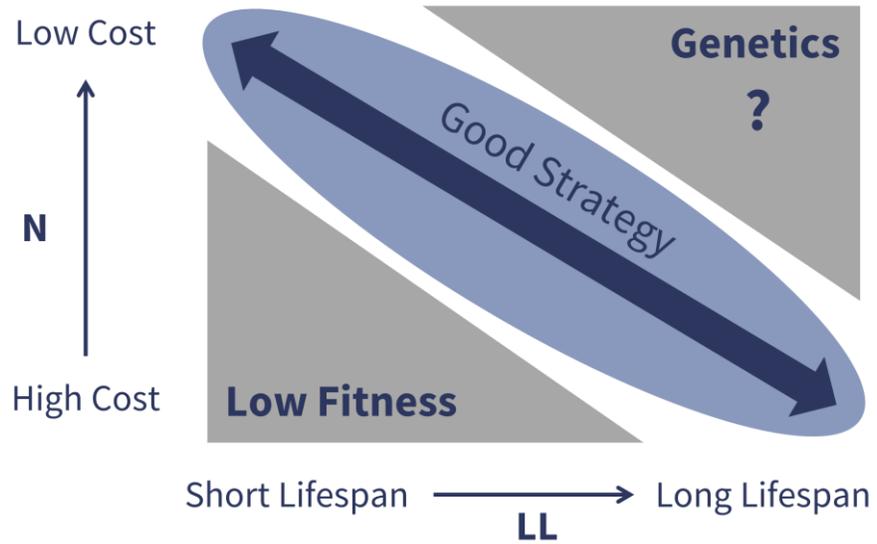improving plant resistance to harsh conditions.

FIGURES

Figure 1: The Leaf Economics Spectrum
The Leaf Economics Spectrum demonstrates how a maximized return on investment results in a range of leaf traits, limited by natural selection and genetic constraints.
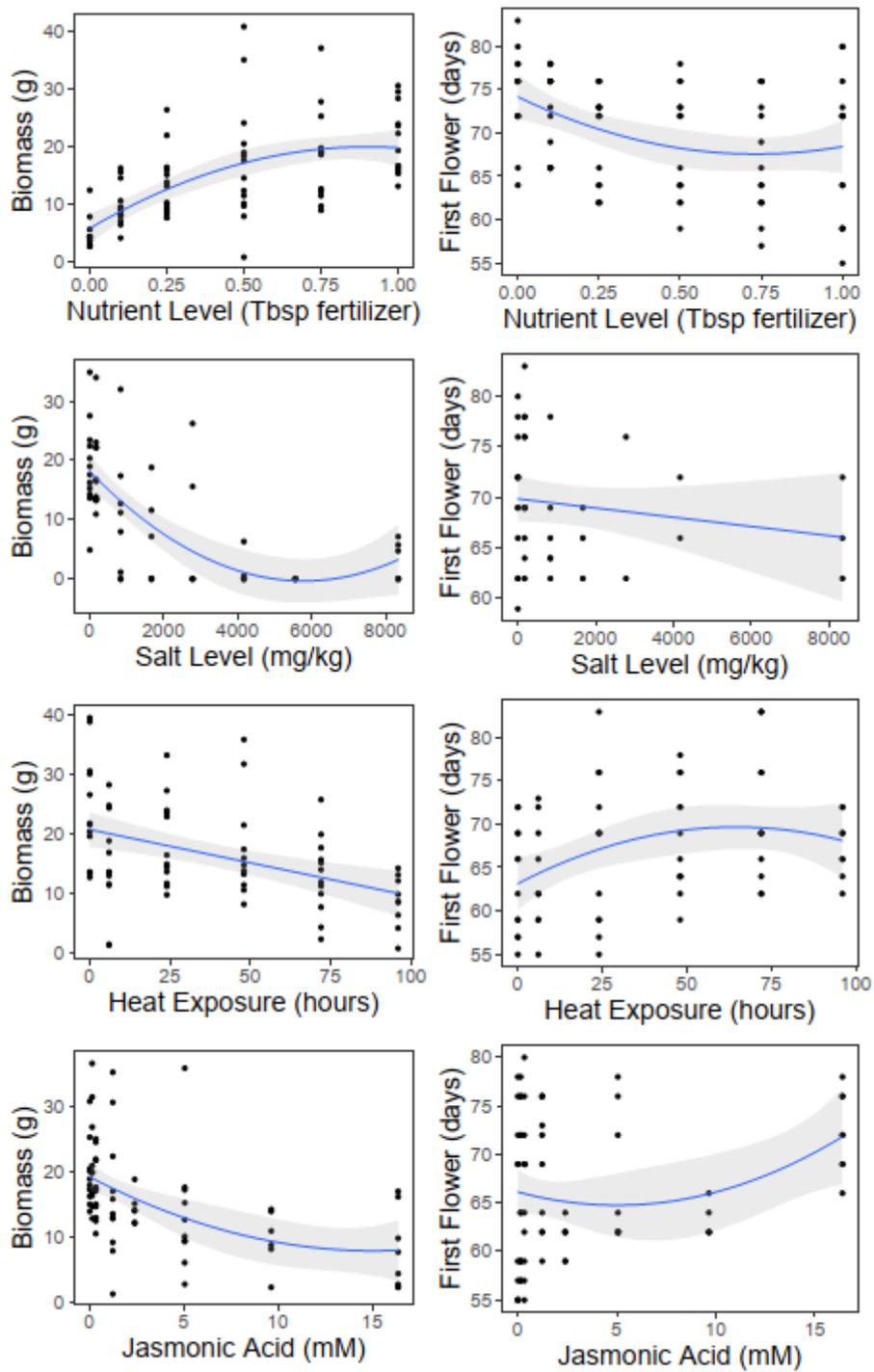
Figure 2: Stress effects on fitness
Biomass decreased in response to each stress, then slightly increased at high levels of salinity. The time to first flowering increased for all but salt stresses.
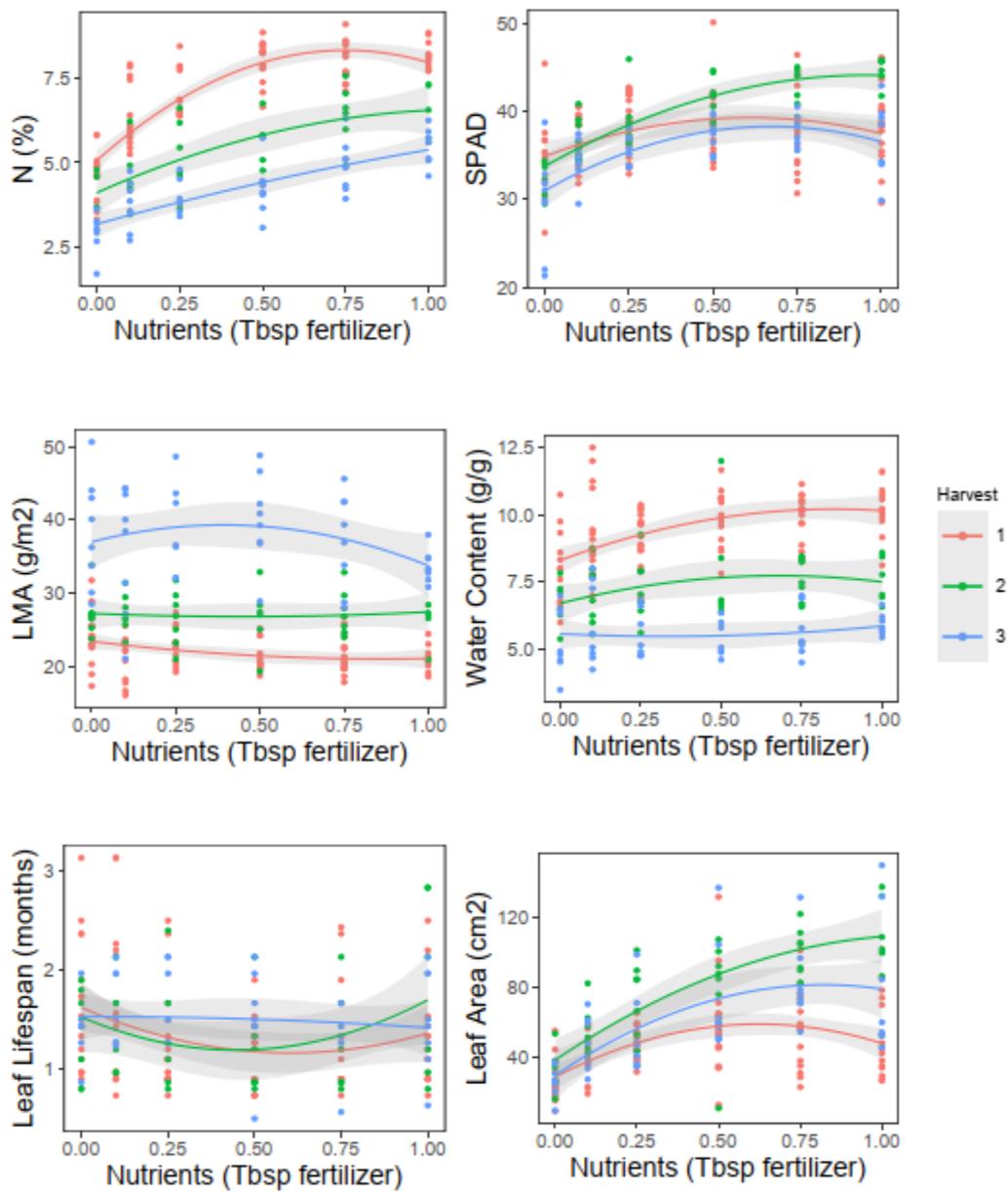
Figure 3: Nutrient stress effects on LES traits
Applying nutrient stress to sunflowers resulted in leaves with lower nitrogen, chlorophyll, and leaf area across Harvests 1-3. There were no major changes in LMA or water content across treatment levels and Harvest times. Leaf lifespan initially decreased then increased across Harvests 1 and 2.
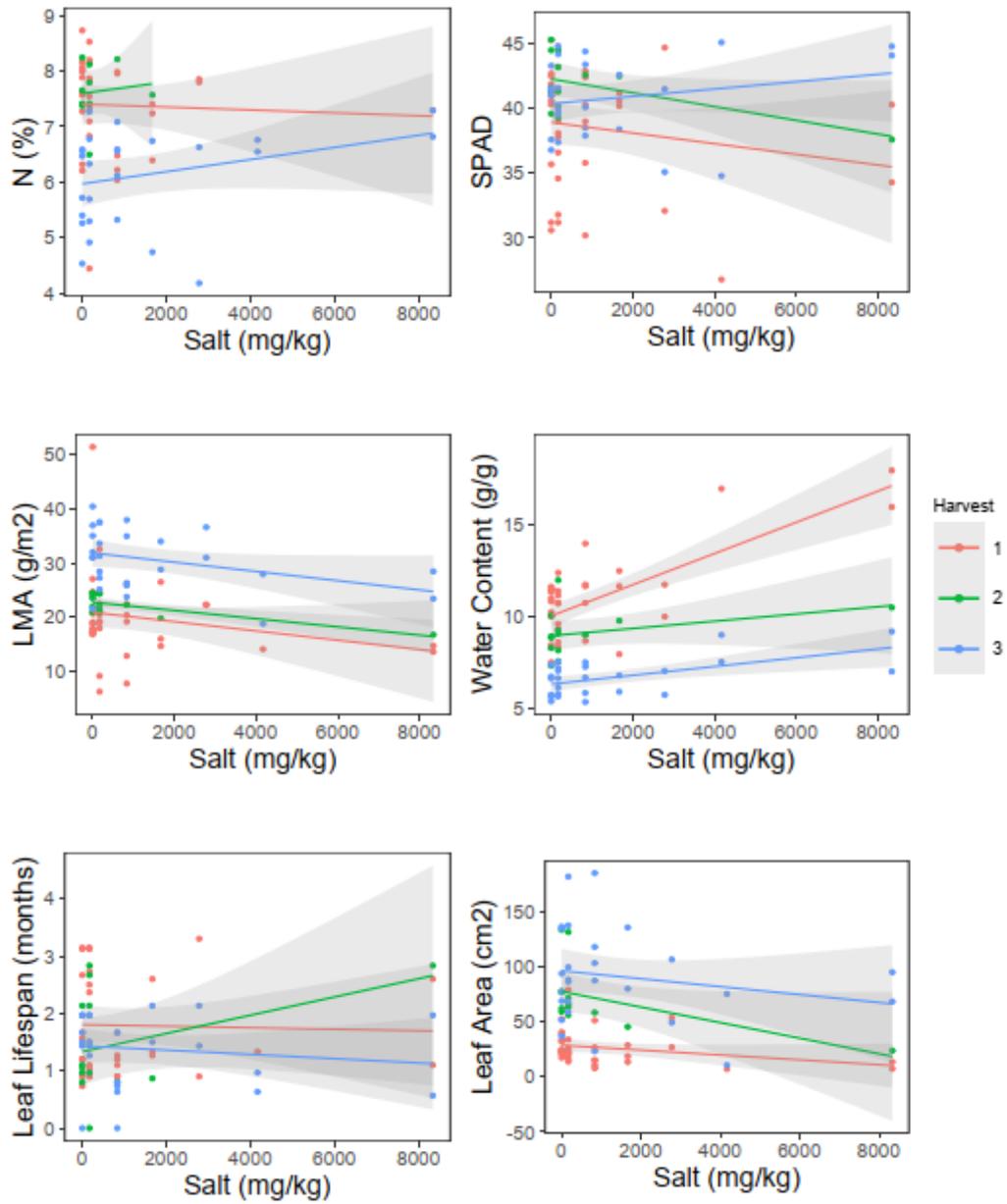
Figure 4: Salt stress effects on LES traits
Applying salt stress to sunflowers resulted in leaves with increased water content and lower LMA across all Harvest times. While leaf lifespan and leaf area decreased in Harvests 1 and 3, it increased in Harvest 2. Similarly, nitrogen content increased outside Harvest 1, while chlorophyll content decreased outside of Harvest 3.
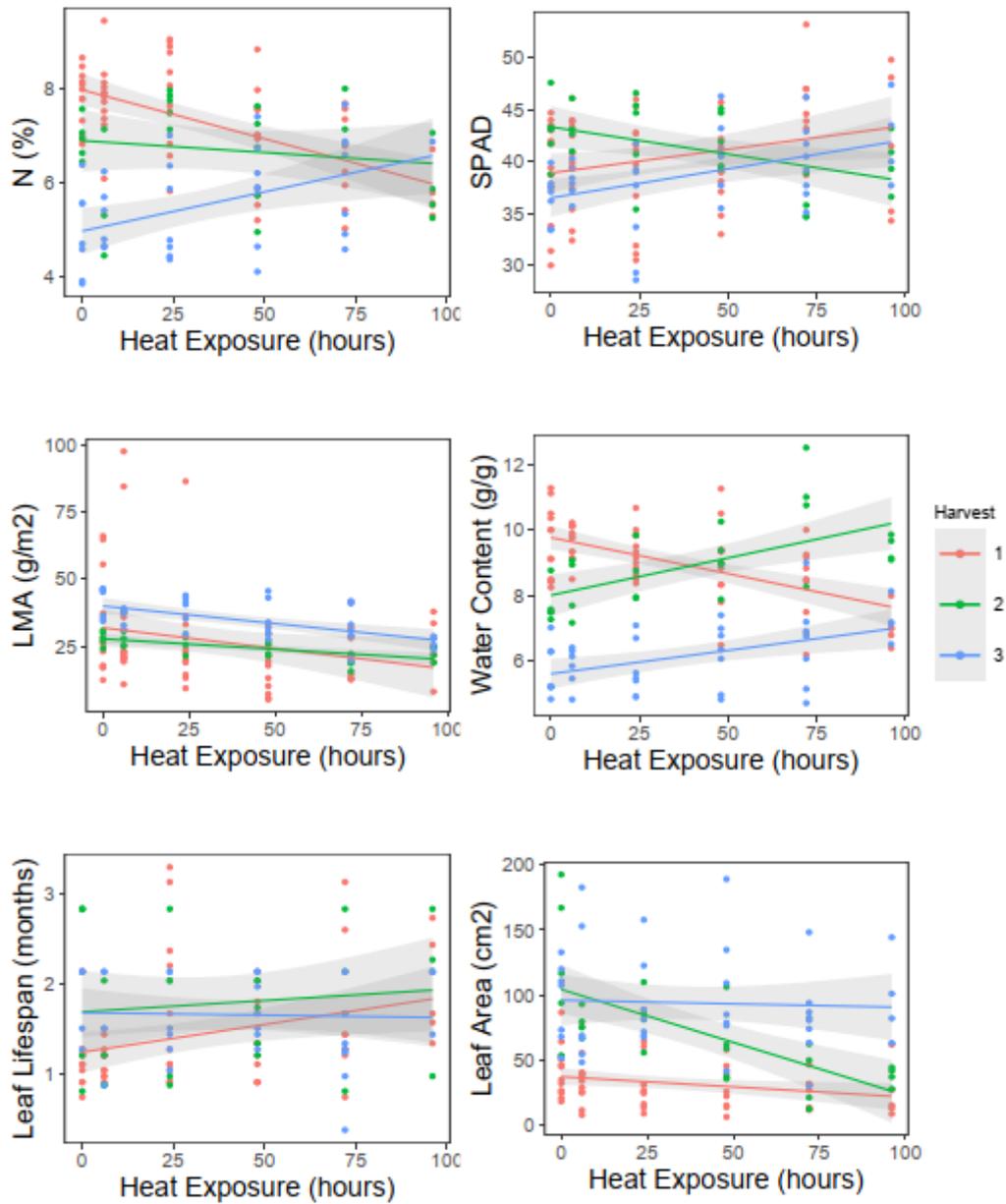
Figure 5: Heat stress effects on LES traits
Applying heat stress to sunflowers resulted in leaves with lower LMA across all Harvest times. In leaves sampled during Harvests 1 and 3, leaf area slightly decreased and chlorophyll increased, while leaves at Harvest 2 had opposite effects in each. Water content initially decreased but then increased in Harvests 2 and 3. Nitrogen effects decreased at early Harvest times and increased at later dates. Leaf lifespan slightly increased at early Harvests.
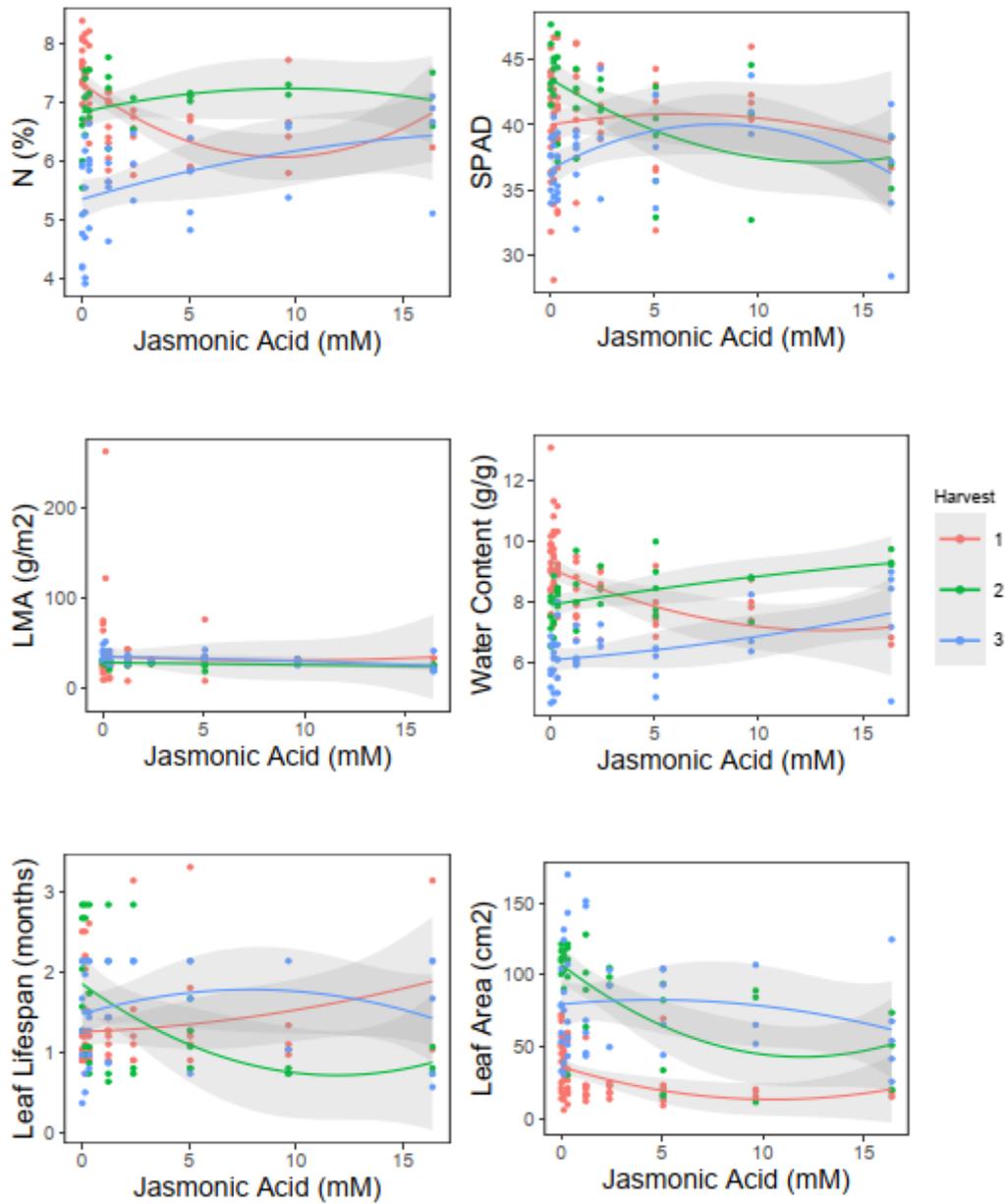
Figure 6: Simulated herbivory stress effects on LES traits
Applying herbivory stress to sunflowers resulted in leaves with increased nitrogen and water content across Harvests 2 and 3, but was decreased in Harvest 1. Chlorophyll content initially increased then decreased in Harvests 1 and 3, but was the opposite for Harvest 2. There were no major shifts in LMA (why does the y-axis include an outlier?). Leaf lifespan increased in Harvest 1, decreased initially then slightly increased at Harvest 2, and increased only at intermediate treatments in Harvest 3. Leaf area decreased then increased slightly in Harvests 1 and 2, but had opposite effects in Harvest 3.

Figure 7: Ontogenetic shifts in LES trait space
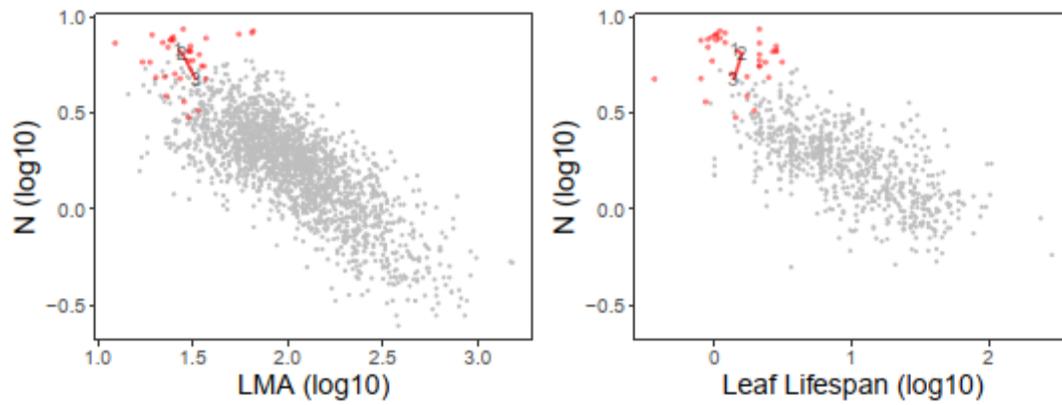*Helianthus annuus* leaves occupy LES trait space that indicate low cost and low returns on carbon investment. Over 3 Harvest points, unstressed leaves naturally shift towards more conservative leaf strategies, with slightly higher cost and returns.

Figure 8: Stress-induced shifts in LES trait space

Stressed *Helianthus annuus* across all Harvest times and treatment levels occupies the same LES trait space as when unstressed. When subjected to nutrient stress, sunflowers produced leaves with LES trait shifts mimicking ontogenetic shifts across LMA, with an initially elevated N content. Except when highly fertilized, nutrient-stressed leaves initially suffered in leaf lifespan to either recover or slightly improve by Harvest 3. Across all treatment levels, nitrogen content did not change, while LMA increased, and leaf lifespan slightly decreased over time. Heat and jasmonic acid treatments produced noise in LES shifts, with a general shift towards slightly more conservative strategies.
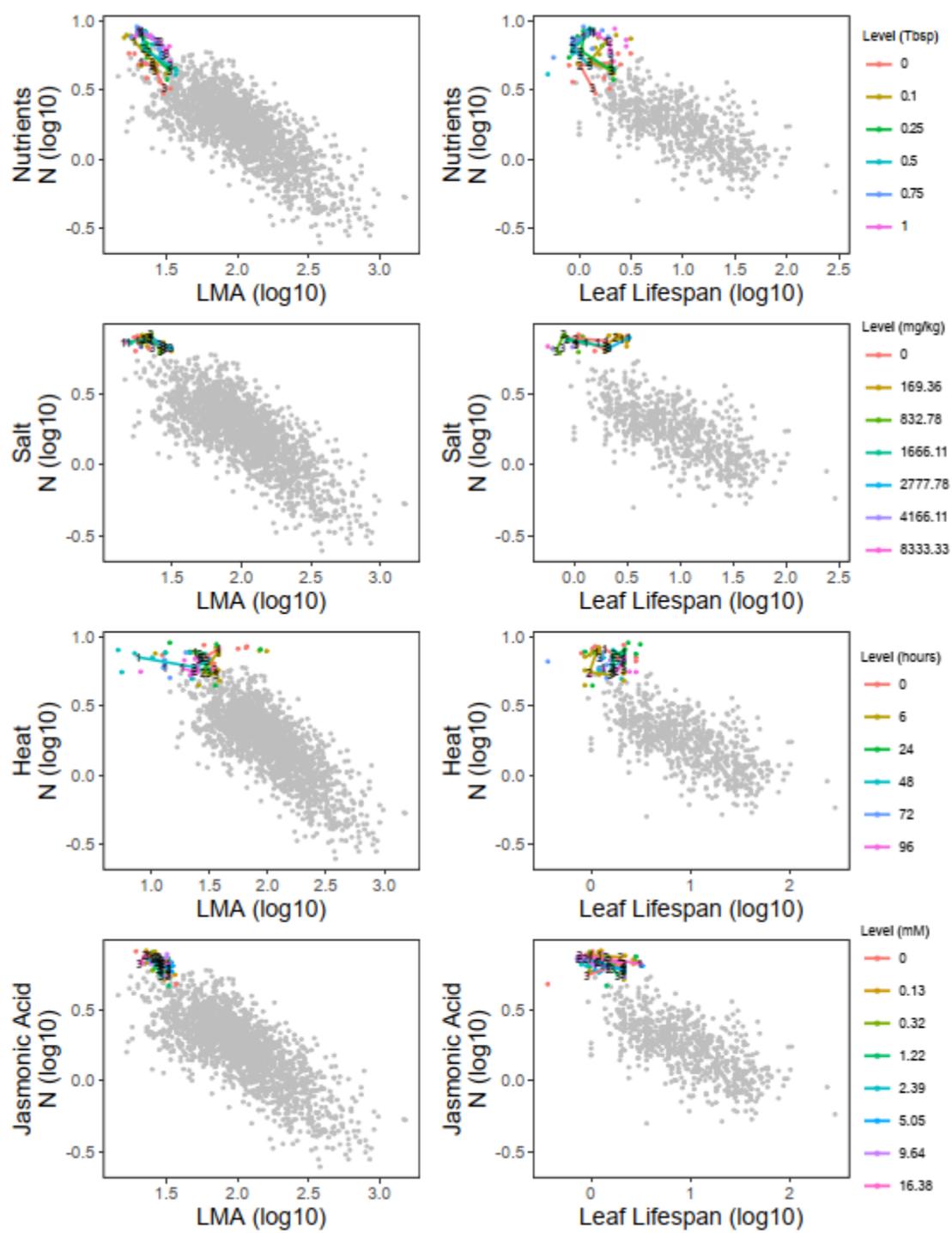
TABLES

Table 1: One-way Analyses of Variance for nutrient stress and LES traits

LES Traits	Harvest 1	Harvest 2	Harvest 3
N	< 0.0001	0.0005	< 0.0001
SPAD	0.0147	< 0.0001	0.0002
LMA	0.0069	0.9972	0.4789
W_m	< 0.0001	0.5757	0.6803
LL	0.1048	0.3492	0.5722

Significant ($p < 0.05$, red) or nonsignificant (black) p-values for each one-way analysis of variance between nutrient stress and LES traits performed at each harvest time. Significant p-value indicates that levels of stress differed in mean trait value.

Table 2: One-way Analyses of Variance for salt stress and LES traits

LES Traits	Harvest 1	Harvest 2	Harvest 3
N	0.7178	0.7068	0.4634
SPAD	0.2591	0.4506	0.6324
LMA	0.0876	0.0441	0.3697
W_m	0.0020	0.7857	0.0487
LL	0.6293	0.1361	0.0271

Significant ($p<0.05$, red) or nonsignificant (black) p-values for each one-way analysis of variance between salt stress and LES traits performed at each harvest time. Significant p-value indicates that levels of stress differed in mean trait value.

Table 3: One-way Analyses of Variance for heat stress and LES traits

LES Traits	Harvest 1	Harvest 2	Harvest 3
N	< 0.0001	0.0190	0.1755
SPAD	0.0575	0.0249	0.0249
LMA	0.2411	0.0010	0.0050
W_m	< 0.0001	0.0053	0.0918
LL	0.0774	0.6310	0.4033

Significant (p<0.05, red) or nonsignificant (black) p-values for each one-way analysis of variance between heat stress and LES traits performed at each harvest time. Significant p-value indicates that levels of stress differed in mean trait value.

Table 4: One-way Analyses of Variance for simulated herbivory stress and LES traits

LES Traits	Harvest 1	Harvest 2	Harvest 3
N	< 0.0001	0.1536	0.0780
SPAD	0.5622	0.0390	0.3031
LMA	0.7798	0.0827	0.0205
W_m	0.0018	0.0585	0.0525
LL	0.4308	0.2168	0.8466

Significant ($p<0.05$, red) or nonsignificant (black) p-values for each one-way analysis of variance between simulated herbivory stress and LES traits performed at each harvest time. Significant p-value indicates that levels of stress differed in mean trait value.

LIST OF REFERENCES

Akladious, S. A. (2014). Influence of thiourea application on some physiological and molecular criteria of sunflower (Helianthus annuus L.) plants under conditions of heat stress. Protoplasma, *251*(3), 625-638. doi:10.1007/s00709-013-0563-2

Albert, B., Le Cahérec, F., Niogret, M.F., Faes, P., Avice, J.C., Leport, L., Bouchereau, A. (2012). Nitrogen availability impacts oilseed rape (Brassica napus L.) plant water status and proline production efficiency under water-limited conditions. Planta *236*, 659–676. https://doi.org/10.1007/s00425-012-1636-8

Ashraf, M. & Foolad, M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany *59*(2), 206-216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Blackman BK, Michaels SD, Rieseberg LH. (2011). Connecting the sun to flowering in sunflower adaptation. Molecular Ecology *20*(17), 3503-3512.

Casotti, G., & Bradley, J. S. (1991). Leaf nitrogen and its effects on the rate of herbivory on selected eucalypts in the jarrah forest. Forest Ecology and Management, *41*(3), 167-177. doi:https://doi.org/10.1016/0378-1127(91)90101-Z

Chapin, F. S. (1991). Integrated responses of plants to stress. *BioScience, 41*(1), 29-36. doi:10.2307/1311538

Chapin, F. S., Bloom, A. J., Field, C. B., & Waring, R. H. (1987). Plant responses to multiple environmental factors. *BioScience, 37*(1), 49-57. doi:10.2307/1310177

Dalton, F. N., Maggio, A., & Piccinni, G. (1997). Effect of root temperature on plant response

    functions for tomato: comparison of static and dynamic salinity stress indices. Plant and

    Soil, *192(*2), 307-319. doi:10.1023/A:1004263505595

Dalton, F. N., Maggio, A., & Piccinni, G. (2000). Simulation of shoot chloride accumulation:

    separation of physical and biochemical processes governing plant salt tolerance. Plant

    and Soil, *219*(1), 1-11. doi:10.1023/A:1004334805471

De la Haba, P., De la Mata, L., Molina, E., Agüera, E. (2014). High temperature promotes early

    senescence in primary leaves of sunflower (Helianthus annuus L.) plants. Canadian

    Journal of Plant Science, *94*(4), 659-669. doi:10.4141/cjps2013-276

Donovan, L. A., Maherali, H., Caruso, C. M., Huber, H., & de Kroon, H. (2011). The evolution

    of the worldwide leaf economics spectrum. *Trends in Ecology & Evolution, 26*(2), 88-95.

    doi:https://doi.org/10.1016/j.tree.2010.11.011

Dudley, S. A., & Schmitt, J. (1996). Testing the adaptive plasticity hypothesis: density-

    dependent selection on manipulated stem length in Impatiens capensis. *The American

    Naturalist, 147*(3), 445-465. Retrieved from http://www.jstor.org/stable/2463217

Giri, A., Heckathorn, S., Mishra, S., & Krause, C. (2017). Heat stress decreases levels of

    nutrient-uptake and -assimilation proteins in tomato roots. *Plants (Basel,

    Switzerland)*, *6*(1), 6. https://doi.org/10.3390/plants6010006

Gunes, A., Inal, A., Alpaslan, M., Eraslan, G., Bagci, E. G., Cicek, N. (2007). Salicylic acid

    induced changes on some physiological parameters symptomatic for oxidative stress and

    mineral nutrition in maize (Zea mays L.) grown under salinity. *Journal of Plant

    Physiology*, *164*, 728-736. doi: https://doi.org/10.1016/j.jplph.2005.12.009

Hartley, S. E. (1998). The chemical composition of plant galls: are levels of nutrients and

    secondary compounds controlled by the gall-former? Oecologia, *113*(4), 492-501.

    doi:10.1007/s004420050401

Hayat, S., Masood, A., Yusuf, M., Fariduddin, Q., & Ahmad, A. (2009). Growth of Indian

    mustard (Brassica juncea L.) in response to salicylic acid under high-temperature stress.

    Brazilian Journal of Plant Physiology, *21*, 187-195. Retrieved from

    http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1677-

    04202009000300003&nrm=iso

Heiser CBJ, Smith DM, Clevenger SB, Martin WCJ. (1969). The North American Sunflowers:

    Helianthus. Memoirs of the Torrey Botanical Club *22*(3), 1-218.

Hernández F, Poverene M, Presotto A. (2018). Heat stress effects on reproductive traits in

    cultivated and wild sunflower (Helianthus annuus L.): evidence for local adaptation

    within the wild germplasm. Euphytica *214*(8).

Hu, Y., & Schmidhalter, U. (1998). Spatial distributions and net deposition rates of mineral

    elements in the elongating wheat (Triticum aestivum L.) leaf under saline soil conditions.

    Planta, *204*(2), 212-219. doi:10.1007/s004250050249

Hu, Y., & Schmidhalter, U. (2005). Drought and salinity: A comparison of their effects on

    mineral nutrition of plants. Journal of Plant Nutrition and Soil Science, *168*(4), 541-549.

    doi:https://doi.org/10.1002/jpln.200420516

Hussain, S., Saleem, M.F., Cheema, M.A., Ashraf, M.Y., & Haq, M.A. (2010). Abscisic acid, a

    stress hormone helps in improving water relations and yield of sunflower (helianthus

    annuus l) hybrids under drought. Pakistan Journal of Botany, *42*(3), 2177-2189.

Jabeen, N., & Ahmad, R. (2012). Improvement in growth and leaf water relation parameters of sunflower and safflower plants with foliar application of nutrient solutions under salt stress. Pakistan Journal of Botany, *44*, 1341-1345.

Kantar MB, Sosa CC, Khoury CK, Castaneda-Alvarez NP, Achicanoy HA, Bernau V, Kane NC, Marek L, Seiler G, Rieseberg LH. (2015). Ecogeography and utility to plant breeding of the crop wild relatives of sunflower (Helianthus annuus L.). Front Plant Sci *6*, 841.

Kazan K & Lyons R. (2016). The link between flowering time and stress tolerance. Journal of Experimental Botany *67*(1): 47-60.

Läuchli A., Grattan S. (2007). Plant growth and development under salinity stress. In: Jenks M.A., Hasegawa P.M., Jain S.M. (eds) Advances in Molecular Breeding Toward Drought and Salt Tolerant Crops. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-5578-2_1

Martin AR, Rapidel B, Roupsard O, Van den Meersche K, de Melo Virginio Filho E, Barrios M, Isaac ME. (2017). Intraspecific trait variation across multiple scales: the leaf economics spectrum in coffee. Functional Ecology *31*(3): 604-612.

Mason, C. M., & Donovan, L. A. (2015). Evolution of the leaf economics spectrum in herbs: evidence from environmental divergences in leaf physiology across Helianthus (Asteraceae). *Evolution, 69*(10), 2705-2720. doi:10.1111/evo.12768

Moriondo, M., Orlandini, S., & Villalobos, F. J. (2003). Modelling compensatory effects of defoliation on leaf area growth and biomass of sunflower (Helianthus annuus L.). European Journal of Agronomy, *19*(2), 161-171. doi:https://doi.org/10.1016/S1161-0301(02)00022-9

Munns, R., Gardner, P., Tonnet, M., & Rawson, H. (1988). Growth and development in NaCl-treated plants. II. Do Na^+ or Cl^- concentrations in dividing or expanding tissues determine growth in barley? Functional Plant Biology, *15*(4), 529-540. doi:https://doi.org/10.1071/PP9880529

Nazar, R., Iqbal, N., Syeed, S., Khan, N. A. (2011). Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. *Journal of Plant Physiology*, *168*(8), 807-815. doi: https://doi.org/10.1016/j.jplph.2010.11.001

Pigliucci, M. (2007). Finding the way in phenotypic space: the origin and maintenance of constraints on organismal form. *Annals of Botany, 100*, 433-438.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytologist, *182*(3), 565-588. doi:https://doi.org/10.1111/j.1469-8137.2009.02830.x

Rawson, H., & Hindmarsh, J. (1982). Effects of temperature on leaf expansion in sunflower. Functional Plant Biology, *9*(2), 209-219. doi:https://doi.org/10.1071/PP9820209

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*, 671–675.

Shipley, B., M. J. Lechowicz, I. Wright, and P. B. Reich. (2006). Fundamental trade-offs generating the worldwide leaf economics spectrum. *Ecology*, *87*, 535–541.

Sultana, N., Ikeda, T., & Kashem, M. A. (2001). Effect of foliar spray of nutrient solutions on photosynthesis, dry matter accumulation and yield in seawater-stressed rice. Environmental and Experimental Botany, *46*(2), 129-140. doi:https://doi.org/10.1016/S0098-8472(01)00090-9

Szalai, G., Kellős, T., Galiba, G., Kocsy, G. (2009). Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. *Journal of Plant Growth Regulation*, *28*, 66-80. doi: 10.1007/s00344-008-9075-2

Takeno K. (2016). Stress-induced flowering: the third category of flowering response. Journal of Experimental Botany *67*(17): 4925-4934.

Temme, A. A., Kerr, K. L., Masalia, R. R., Burke, J. M., & Donovan, L. A. (2020). Key traits and genes associate with salinity tolerance independent from vigor in cultivated sunflower. Plant Physiology, *184*(2), 865-880. doi:10.1104/pp.20.00873

Tran VH, Temme AA, Donovan LA. (2020). Wild and cultivated sunflower (Helianthus annuus L.) do not differ in salinity tolerance when taking vigor into account. Agronomy *10*(7), 1013.

Vasseur F, Violle C, Enquist BJ, Granier C, Vile D. (2012). A common genetic basis to the origin of the leaf economics spectrum and metabolic scaling allometry. *Ecology Letters, 15*(10), 1149-1157.

Velikova, V., Arena, C., Izzo, L. G., Tsonev, T., Koleva, D., Tattini, M., . . . Loreto, F. (2020). Functional and structural leaf plasticity determine photosynthetic performances during drought stress and recovery in two Platanus orientalis populations from contrasting habitats. *International journal of molecular sciences, 21*(11). doi:10.3390/ijms21113912

Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu J-K. (2006). Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. The Plant Journal *45*(4): 523-539.

Wang Y, Donovan LA, Temme AA. (2020). Plasticity and the role of mass-scaling in allocation, morphology, and anatomical trait responses to above- and belowground resource limitation in cultivated sunflower (Helianthus annuus L.). Plant Direct *4*(10).

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., . . . Midgley, J. J. (2004). The worldwide leaf economics spectrum. *Nature*. doi:10.1038/nature02403

Wright, I. (2009). GLOPNET - Global Plant Trait Network. Retrieved March 15, 2021, from http://bio.mq.edu.au/~iwright/glopian.htm

Xiong, D. & Flexas, J. (2018). Leaf economics spectrum in rice: leaf anatomical, biochemical, and physiological trait trade-offs. *Journal of Experimental Botany, 69*(22), 5599-5609. doi:10.1093/jxb/ery322

Zhang, J. & Wagner, G. P. (2013). On the definition and measurement of pleiotropy. *Trends in Genetics, 29*(7), 383-384.