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Environmental Stress Shifts Growth Strategies of Oak and Heath Shrubs in the Florida Scrub

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ABSTRACT

The ability of plant species to mitigate environmental stresses is crucial for maintaining their populations and communities. One potential mitigation approach is a growth strategy shift mediated by intraspecific trait variation. We evaluated potential stress-induced shifts in two growth strategies across six common Florida scrub shrub species in two major groups, oaks (*Quercus* spp., Fagaceae) and heaths (Ericaceae), along an elevational stress gradient. The acquisitive-conservative gradient explains how plants invest in aboveground tissues to photosynthesize more, while the collaboration gradient explains how plants attain belowground resources using a 'do-it-yourself' strategy or collaboratively 'outsourcing' with mutualistic mycorrhizal fungi. We expected high-stress environments to shift species towards conservative growth with increased symbiont collaboration compared to low-stress environments. For each species, we measured a suite of aboveground (all species) and belowground (oaks only) plant functional traits in two or more different habitats along an elevational stress gradient. Aboveground traits (leaf circularity and stomatal density) significantly decreased with stress across all species analyzed, suggesting a shift to a more conservative strategy. Belowground oak traits showed significant support for a shift to more collaboration with stress, with decreases in specific root length and increases in ectomycorrhizal tip density with stress. Overall, we found that oak and heath shrubs showed limited support for shifting to a conservative strategy with stress and relatively strong support for the oaks to shift to a more collaborative strategy. These findings advance our broader knowledge of how oak and heath species cope with environmental stress via shifting growth strategies.

Key words: Ectomycorrhiza, Ericaceae, intraspecific trait variation, plant traits, *Quercus*

INTRODUCTION

Plants face many environmental stresses and disturbances that are predicted to worsen with climate change, including more severe and prolonged drought (Trenberth 2005), higher fire frequency (Jones et al. 2022), and increasingly variable temperatures (Holle et al. 2010). Understanding how plant species mitigate these stresses is therefore crucial for maintaining their populations and communities. One approach by which plant species mitigate stress is via variation in their growth strategies (Nicotra et al. 2010; Henn et al. 2018; Laughlin et al. 2020). For example, shifts in growth strategy in response to water availability can allow species to establish populations across a broad precipitation gradient (Couso and Fernandez 2012). Importantly, the ability to shift growth strategies in the face of environmental stress likely relies on underlying intraspecific trait variation (e.g., Weigelt et al. 2021; Welles and Funk 2021; Westerband et al. 2021). Identifying environmental stress responses such as shifts in growth strategy and underlying trait variation is necessary toward explaining plant species persistence in a changing world.

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In particular, two growth strategies may shift with environmental stress. First, the acquisitive-conservative gradient (also referred to as the “fast-slow conservation gradient”) explains how plants invest in aboveground tissues (e.g., Wright et al. 2004; Reich 2014; Weigelt et al. 2021). An acquisitive strategy allows plants to produce inexpensive plant structures that grow quickly, such as thin leaves with large surface areas and low tissue density (i.e., high specific leaf area, low leaf dry matter content) to attain high photosynthetic rates. On the other hand, a conservative strategy allows plants to build sturdy, relatively expensive plant structures, such as thick leaves with low surface area and high tissue density (i.e. low specific leaf area, high leaf dry matter content) that may last years. While traits such as leaf size and leaf shape are best characterized as operating outside the acquisitive-conservative gradient at a cross-species scale (Reich 2014), these traits may still be informative of growth response within taxa, particularly with regard to environmental stress (Nicotra et al. 2011). For example, stressful, dry conditions can lead to narrow, less circular leaves that can shed heat and avoid water loss (Yates et al. 2010).

Second, the collaboration gradient describes how plants gather belowground resources and ranges from a ‘do-it-yourself’ strategy in which plants obtain most of their nutrients by investing in relatively cheap, fine roots to a collaborative ‘outsourcing’ strategy in which the plant invests in mutualists such as mycorrhizal fungi to attain resources (Bergmann et al. 2020; Weigelt et al. 2021). Collaboration allows the plant to expend resources on building sturdy roots with relatively low turnover rates and long lifespans (Bergmann et al. 2020). Under this collaboration gradient, plants under stress might be expected to invest more in collaborative interactions with mutualists to buffer water and nutrient stress (Pena and Polle 2014), while non-stressed plants may lack this selective pressure and by default invest more in fine roots for their nutrient acquisition.

The scrub of the Lake Wales Ridge in peninsular Florida, USA, is an ideal ecosystem to study the effects of stress on the intraspecific variation of plant traits. The Florida scrub ecosystem is a heterogeneous landscape across which small differences in elevation result in dramatic changes in water and nutrient availability, and ultimately plant productivity (Abrahamson et al. 1984; Weekley et al. 2007). Across this mosaic landscape, three major habitat types occur in close proximity along an elevational stress gradient—low elevation flatwoods, followed by medium elevation scrubby flatwoods, and high elevation rosemary scrub (Abrahamson et al. 1984; Hernandez et al. 2021); this landscape pattern results in replicated stress gradients that allow opportunities to study how changes in stress affect intraspecific plant variation (Menges and Gallo 1991; Abrahamson 2007; Ellsworth and Sternberg 2019). Flatwoods are savannah-like habitats dominated by shrubby and herbaceous species and may experience occasional standing water depending on rainfall; scrubby flatwoods are dominated by evergreen shrubs only and experience no standing water regardless of rainfall conditions; rosemary scrub habitats contain large patches of open sand inhabited by herbaceous species that are surrounded by and interspersed with shrubs (Abrahamson et al. 1984; Hunter and Menges 2002). Such stress gradients over short spatial scales (i.e., <0.25 km) generate populations of plants that are unlikely to be locally adapted to the specific habitat in which they occur, and, importantly, any trait differences among habitats are likely attributable to phenotypic plasticity.

Here, we evaluated potential stress-induced shifts in life-history strategies across six species of shrubs commonly found in Florida scrub habitat belonging to two major groups, oaks (*Quercus* spp., Fagaceae) and heaths (Ericaceae). To do so, we quantified intraspecific trait variation for a suite of aboveground and belowground functional traits of plants found in two or more habitat types along the elevational stress gradient that increasingly limit nutrient and water availability (Abrahamson et al. 1984; Weekley et al. 2007). We hypothesized that (1) the elevational stress gradient from lower elevations (flatwoods) to higher elevations (rosemary scrub) should induce a shift to a relatively conservative growth strategy with lower specific leaf area, stomatal density, and circularity, as well as higher leaf dry matter content, twig dry matter content, and root dry matter content. We further hypothesized that (2) the elevational stress gradient should induce a shift to a relatively collaborative growth strategy in which individual plants engage in more ectomycorrhizal (EcM) colonization and have lower specific root length (SRL).

MATERIALS AND METHODS

Study site

We conducted this study at Archbold Biological Station (Venus, Florida), located at the southern tip of the Lake Wales Ridge (27.18°N, 81.35°W) in south central Florida (Swain and Martin 2014). The white sand soils where our study took place (e.g., Duette, Satellite, and Basinger soil series) are highly acidic (pH range 3.5–5.2) with extremely low nutrient levels (Abrahamson et al. 1984). The subtropical climate experiences seasonally wet summers and relatively mild, dry winters. The landscape is actively managed using prescribed fire (Main and Menges 1997). Across this landscape are three major habitat types found in close proximity: lower elevation flatwoods (e.g., 38–46 m elevation; flatland with sand substrate; less xeric with poorly drained soils; open pine canopy with a layer of low shrubs and herbs), intermediate elevation scrubby flatwoods (e.g., similar 38–46 m elevation range though always higher elevation than flatwoods sites when in the same area; flatland with sand substrate; xeric with a widely scattered pine canopy and shrubby understory dominated by saw palmetto and scrub oaks), and high elevation rosemary scrub (e.g., 40–50 m elevation; upland with deep sand substrate; more xeric; dominated by the Florida Rosemary, *Ceratiola ericoides* Michx.; Abrahamson et al. 1984).

Study design

Three heath species (*Lyonia lucida* (Lam.) K.Koch, *L. fruticosa* (Michx.) G.S.Torr., *Bejaria racemosa* Vent.) and three oak species (*Quercus geminata* Small, *Q. inopina* Ashe, *Q. chapmanii* Sarg.) were sampled across sites that varied in time-since-fire (range 4–36 yr) and elevation (37–47 m). Each site was divided into three subsites in flatwoods, scrubby flatwoods, and rosemary scrub habitats. Habitats within sites generally had the same fire history. Oaks were sampled in all three habitats, while heaths were sampled in only flatwoods and scrubby flatwoods where they were more frequently encountered. For aboveground traits, we sampled 184 individuals across the nine sites (Table 1). For belowground traits, we limited our study to the three ectomycorrhizal-associating *Quercus* species across three sites and two subsite habitat categories: scrubby flatwoods and rosemary scrub resulting in 90 individuals being sampled.

Functional traits measurements

Acquisitive-conservative gradient. We measured six aboveground traits–specific leaf area (SLA, $mm^2\ mg^{-1}$), leaf dry matter content (LDMC, $mg\ g^{-1}$), twig dry matter content (TDMC, $mg\ g^{-1}$), and leaf size (cm^2), leaf shape (circularity, no dimension), stomatal density (number of stomata mm^{-2}), using standardized protocols (Pérez-Harguindeguy et al. 2013). All aboveground traits were measured for each species, except for stomatal density (data available only for *L. lucida*, *Q. chapmanii*, and *Q. inopina*) (Table 1).

Sampling was conducted May–July 2022. Whole twigs including fully-expanded leaves were collected from the south-facing canopy and subcanopy of full-grown shrubs, stored in bags with wet paper towels, and stored at 4°C for up to 36 hours until measurement. Two to three leaves including the petiole were then removed from the twigs, photographed, and weighed to obtain fresh mass (g). Images were analyzed using ImageJ (Schneider et al. 2012) to measure leaf area and circularity, and the leaves were oven-dried for at least 72 hours at 60°C to 80°C and weighed to determine dry mass (g). Specific Leaf Area was calculated as the ratio of leaf area divided by leaf dry mass ($cm^2\ g^{-1}$) and LDMC as the ratio of leaf dry mass divided by leaf fresh mass.

For both stomatal density and TDMC, the leaves were then washed under tap water, initially dried with paper towels, and air-dried for up to half an hour. For stomatal density, a thin layer layer of nail polish was applied, air-dried for up to 15 minutes, then peeled off with clear tape. Five images of each imprint were captured using a compound microscope (BH2-RFL-T3, Olympus Corp., Tokyo, Japan) with a camera attachment (MU300, AmScope, Irvine, CA, USA) at 100x magnification.

Table 1. Number of Individuals Sampled for each Functional Trait for each Species

Species	Acquisitive-Conservative Gradient	Collaboration Gradient
	•Specific Leaf Area (SLA) •Leaf Dry Matter Content (LDMC) •Twig Dry Matter Content (TDMC) •Leaf Size •Circularity •Stomatal Density (indicated with *)	•Specific Root Length (SRL) •Ectomycorrhizal (EcM) Tip Density
Bejaria racemosa	31	–
Lyonia fruticosa	31	–
Lyonia lucida	29*	–
Quercus chapmanii	39*	30
Quercus geminata	32	30
Quercus inopina	40*	30

Stomatal density was measured by dividing the number of stomata by the focal area (0.0263 mm^2). For TDMC, the leaves were removed, and the twigs were weighed for fresh mass, dried as above, and weighed for dry mass. Twig Dry Matter Content was calculated as twig fresh mass divided by twig dry mass.

Collaboration gradient. For the three species of oaks, fine roots were collected in May 2022 separately from the aboveground leaf sampling. Using shovels, we uprooted 90 saplings in three sites with two subsites from scrubby flatwood and rosemary scrub habitat, five individuals per species per habitat. Belowground tissues were collected and stored at 4°C. Fine roots were removed from the main root for analysis.

We measured two traits – ectomycorrhizal (EcM) tip density (number of colonized tips cm^{-1}) and specific root length of fine roots (SRL, m g^{-1}). Ectomycorrhizal tips were inspected under a dissecting microscope (Model SZX12, Olympus Corp., Tokyo, Japan). Samples were then stored in 60% ethanol for up to one month before SRL measurement (Freschet et al. 2021). For SRL, the five individual samples of each subsite's species were combined into a representative sample for that subsite due to the small mass of fine roots. SRL was measured as root length using the program RhizoVision (Seethepalli and York 2020) and then divided by dry mass.

To confirm that tips were colonized by ectomycorrhizal fungi, we sequenced DNA from a subset of EcM tips. During root processing, a total of 30 EcM tips were excised from each of the three oak species (four from *Q. chapmanni*, 20 from *Q. geminata*, and eight from *Q. inopina*). DNA was extracted using a rapid extraction procedure following the methods of Vandepol et al. (2020). The ITS ribosomal DNA was amplified using primers ITS1F and ITS4 (Gardes and Bruns 1993) using the methods for single EcM root tips outlined in Karlsen-Ayala et al. (2023). In cases where DNA failed to amplify, the ITS2 reverse primer (White et al. 1990) was used. PCR products were visualized on a 1.5% agarose gel using SYBR Green (Invitrogen, Carlsbad, CA). Amplicons were sequenced at Eurofins Genomics (Louisville, KY). Sequence quality was assessed and trimmed using Geneious v. 2020.1.2 (Biomatters Ltd., Auckland, New Zealand; Kearse et al. 2012). Identities of EcM fungi were assessed using BLAST searches against GenBank. All EcM fungi ITS sequences >200 bp were deposited into GenBank.

Data analysis

All data were analysed using R version 4.3.2 (R Core Team 2023).

Acquisitive-conservative gradient. We tested whether each intraspecific trait value varied across the habitat gradient. We constructed linear mixed-effects models (LMMs) which tested the fixed effects of habitat, species, and habitat × species using the random effect of site using the lme4 package (Bates et al. 2015). The response variables were each of the six aboveground traits (SLA, TDMC, LDMC, stomatal density, circularity, and leaf area). Habitat was modeled as a numeric predictor variable in which the flatwoods, scrubby flatwoods, and rosemary scrub habitat types were assigned values of −1, 0, and 1, respectively. Model terms were evaluated for significance using analysis of deviance in the car package (Fox and Weisberg 2019). For each trait, within-species LMMs were constructed to test the effect of habitat using the random effect of site, and similarly evaluated using analysis of deviance.

Collaboration gradient. Using the three oak species, we tested whether intraspecific SRL and EcM density varied across the habitat gradient. Because nearly all tips were colonized, we report the EcM results as the number of colonized tips per centimeter of fine root. LMMs were constructed with the fixed effect of habitat, species, and habitat x species, and random effects of site, and the response variables were the SRL and EcM density (natural-log transformed) and evaluated using analysis of deviance. For both traits, within-species LMMs were constructed and evaluated as above.

RESULTS

Acquisitive-conservative gradient

Analyses of the aboveground traits provided limited support that species shift to a more conservative strategy with stress (Figure 1E-F, Tables 2–3). Two traits (leaf circularity and stomatal density) significantly decreased with stress across all species analyzed. Within species analyses showed that this across-species finding was driven by *Quercus inopina* (leaf circularity $p<0.001$) and *Q. chapmanii* (stomatal density $p=0.080$), for these two traits, respectively. The only other within-species significant finding was an increase in TDMC for *Lyonia lucida* ($p<0.001$), which supports the shift to a more conservative strategy. None of the other traits differed across stress overall.

Collaboration gradient

Analyses of the belowground traits of oaks showed significant support for a shift to more collaboration with stress (Figure 2, Tables 4-5). Across all species, SRL significantly decreased with stress, and within species analyses showed that only *Quercus inopina* significantly decreased in SRL with stress ($p=0.017$) (Figure 2A, Table 5). EcM density significantly increased overall with stress, and all three species significantly increased (Figure 2B).

Sanger sequencing of EcM root tips revealed several taxa that associated with each of the oak species across rosemary scrub and scrubby flatwoods (Table 6). Eight EcM tips each were successfully sequenced from *Quercus inopina* and *Q. geminata*, and two tips from *Q. chapmanii*. Of the sequences from EcM root tips, all but 15 represented EcM fungi whereas the other 3 were contaminating soil fungi or mycoparasites. *Russula* Pers. species were observed on all three oak species, with *Russula arenicola* (S.L. Mill. & D. Mitch.) Trappe & T.F. Elliott identified on three tips of *Q. inopina* and one tip of *Q. geminata*. *Quercus inopina* also associated with a species of *Delastria* Tul. & C. Tul. and an unidentified Cantharellales fungus that likely belongs in the genus *Sistotrema* Pers. *Quercus geminata* also associated with *Alessioporus rubriflavus* J.L. Frank, A.R. Bessette & Bessette and *Aureoboletus pseudoauriporus* J.A. Bolin, A.R. Bessette, A.E. Bessette, L.V. Kudzma, A. Farid & J.L. Frank. *Quercus chapmanii* associated with *Lactarius chrysorrheus* Fr.

DISCUSSION

Intraspecific trait variation and growth strategies

Understanding how plants grow under a wide variety of environmental conditions is essential for understanding their current and future distributions, especially in the context of climate and land use changes. Studying how growth strategies and intraspecific trait variation shift across gradients is key to this effort (Welles and Funk 2021; Westerband et al. 2021). Noteworthy, in our study, all six species exhibited traits associated with a conservative growth strategy consisting of very low SLA (all mean values <10 mm^2 mg^{-1}) and high LDMC, an unsurprising finding given the general xeric and nutrient-poor, stressful environment across all three habitats of the Florida scrub studied.

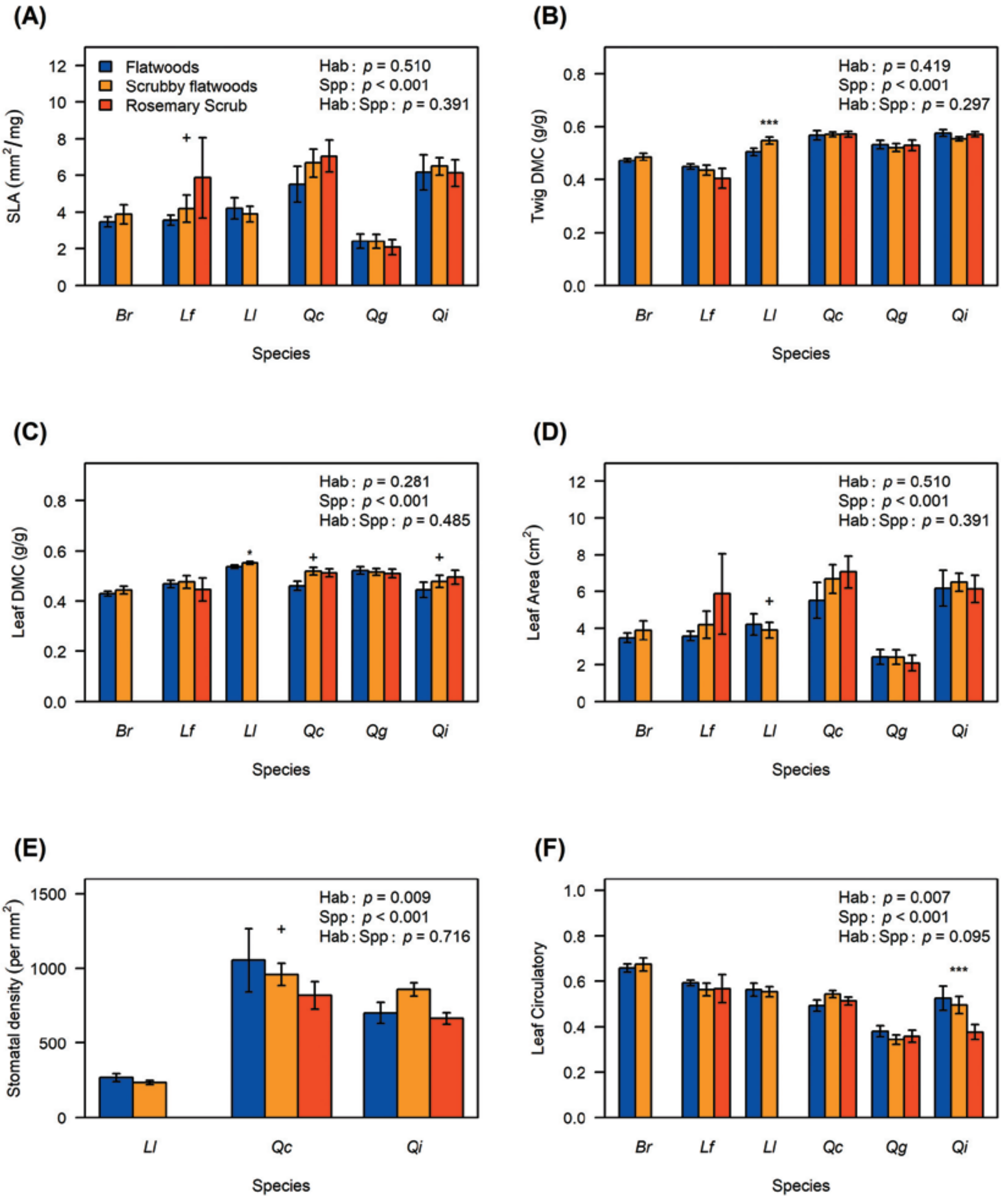

Figure 1. Aboveground Traits Across Six Shrub Species. **A.** Specific leaf area, **B.** Twig dry matter content, **C.** Leaf dry matter content, **D.** Leaf area, **E.** Stomatal density, and **F.** Leaf circularity. Statistical significance is shown for the global model across species. Asterisks denote within species differences across habitats +$0.05<p<0.1$, *$0.01<p<0.05$, **$0.001<p<0.001$, ***$p<0.001$. Species codes: *Br*=*Bejaria racemosa*, *Lf*=*L. fruticosa*, *Ll*=*Lyonia lucida*, *Qc*=*Q. chapmanii*, *Qg*=*Quercus geminata*, *Qi*=*Q. inopina*.

Table 2. Effects of Habitat and Species on Aboveground Traits. For each trait, the χ^2 and p-value are shown from an analysis of deviance of a mixed-effects model. Each analysis used habitat as a continuous predictor variable for 6 species, with the exception of stomatal density which considered 3 species. Significant p-values (≤ 0.05) are bolded and underlined.

Terms	d.f.	Specific leaf area		Twig DMC		Leaf DMC		Leaf area		Stomatal density		Leaf circularity	
		χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Habitat	1	0.43	0.510	0.65	0.419	1.16	0.281	0.43	0.510	6.88	**0.009**	7.15	**0.007**
Species	5	182.74	<**0.001**	240.22	<**0.001**	123.04	<0.001	182.75	<**0.001**	525.51	<**0.001**	238.72	<**0.001**
Habitat x Species	5	5.21	0.391	6.09	0.297	4.46	0.485	5.21	0.391	0.67	0.716	9.37	0.095

Table 3. Effects of Habitat on Aboveground Traits for each Species. For each trait, the estimate, standard error, χ^2, and p-value are shown from an analysis of deviance of a mixed-effects model. Each analysis used habitat as a continuous predictor variable. Significant p-values (≤ 0.05) are bolded and underlined.

Species	Specific leaf area				Twig Dry Matter Content				Leaf Dry Matter Content				Leaf area				Stomatal density				Circularity			
	Est.	SE	χ^2	P	Est.	SE	χ^2	P	Est.	SE	χ^2	P	Est.	SE	χ^2	P	Est.	SE	χ^2	P	Est.	SE	χ^2	P
Bejaria racemosa	0.11	0.13	0.69	0.405	0.03	0.03	0.93	0.334	0.03	0.04	0.77	0.381	0.11	0.13	0.69	0.405	–	–	–	–	0.03	0.04	0.36	0.551
Lyonia fruticosa	0.20	0.12	2.73	0.099	−0.04	0.03	1.87	0.172	0.00	0.03	0.00	0.996	0.20	0.12	2.73	0.099	–	–	–	–	−0.04	0.04	1.03	0.311
Lyonia lucida	−0.08	0.13	0.33	0.564	0.08	0.02	20.14	<**0.001**	0.03	0.01	3.92	**0.048**	−0.08	0.13	0.33	0.564	−0.13	0.09	1.97	0.160	−0.02	0.05	0.10	0.756
Quercus chapmanii	0.10	0.08	1.58	0.209	0.00	0.01	0.04	0.843	0.03	0.02	2.78	0.096	0.10	0.08	1.58	0.209	−0.14	0.08	3.07	0.080	0.00	0.03	0.02	0.882
Quercus geminata	−0.06	0.10	0.41	0.523	0.00	0.02	0.04	0.847	−0.01	0.02	0.52	0.472	−0.06	0.10	0.41	0.523	–	–	–	–	−0.04	0.03	1.35	0.245
Quercus inopina	−0.01	0.08	0.02	0.893	0.00	0.01	0.03	0.855	0.05	0.03	2.82	0.093	−0.01	0.08	0.02	0.893	−0.08	0.06	1.74	0.187	−0.18	0.05	15.90	<**0.001**

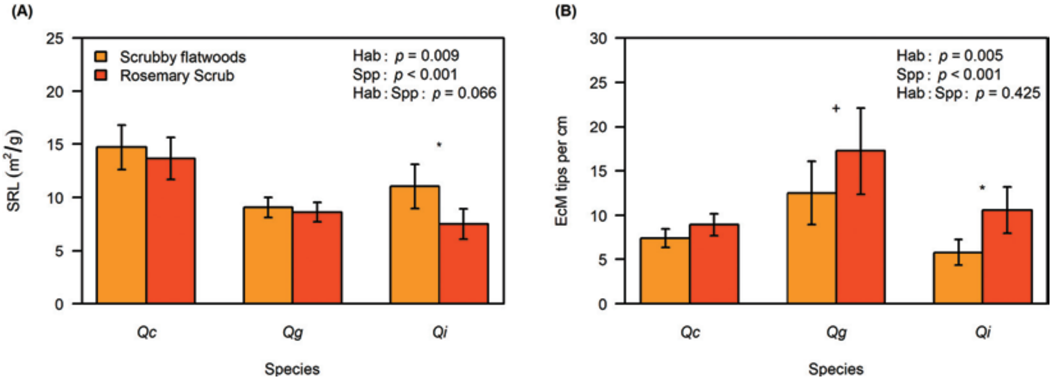

Figure 2. Belowground Traits across Three *Quercus* Species. **A.** Specific root length and **B.** Density of ectomycorrhizal colonized tips cm^{-1}. Statistical significance is shown for the global model across species. Asterisks denote within species differences across habitats +$0.05<p<0.1$, *$0.01<p<0.05$, **$0.001<p<0.001$, ***$p<0.001$. Species codes: *Qc*=*Q. chapmanii*, *Qg*=*Quercus geminata*, *Qi*=*Q. inopina*.

We hypothesized that the elevation gradient from lower elevation (flatwoods) to higher elevation (rosemary scrub) would lead to shifts to a more conservative growth strategy, as nutrient and water limitations increase. However, support for this hypothesis was limited. This finding was surprising since studies have highlighted how change in elevation drives water availability, nutrient level, and community composition in the Florida scrub (Abrahamson et al. 1984; Boughton et al. 2006). It could be that these species are all at the extreme conservative end of the acquisitive-conservative gradient, and there is relatively little variation in these associated traits. One of the species that did show evidence of this shift to conservative growth, *Quercus inopina*, exhibited variable leaf morphology and LDMC, shifting to a less circular, more pointed leaf with higher LDMC when growing in more stressful environments. In addition to the traits measured, *Q. inopina* exhibits leaf rolling, a known conservative growth strategy for water-stressed plants to reduce transpiration rates (O'Toole et al. 1979).

In contrast, the oaks showed strong support for a stress-induced shift to a relatively collaborative strategy. Oak species were characterized by lower SRL and higher EcM colonized tip density in the rosemary scrub habitat than in the scrubby flatwood. The collaborative gradient was recently introduced as a cross-species phenomenon (Bergmann et al. 2020; Weigelt et al. 2021); here, we inferred that intraspecific trait variation with respect to SRL and ectomycorrhizal colonization was similarly indicative of shifts along the collaboration gradient and, ultimately, a plant's growth strategy. Our findings support the idea that, when species are confronted with environmental stress, they shift their investment from fine roots to their mycorrhizal fungal partners. Fungi, and microbial partners in general, are critical for mitigating the stresses that plant species face (e.g., Kivlin et al. 2013; Afkhami et al. 2014), including many herbaceous species of the Florida scrub (David et al. 2018; David et al. 2020; Revillini et al. 2023). Importantly, our findings demonstrate that ectomycorrhizal investment increases while SRL decreases, suggesting that stress induces a different root growth strategy for acquiring nutrients.

Of the study species, the distribution of *Quercus geminata* is arguably the most dynamic in its expansion into new scrub habitats. Though a common shrub species in many scrub habitats, it is also a weedy native species that encroaches into other habitat types, particularly sand hill and oak-hickory scrub (AS David, Archbold Biological Station, pers. observation). This expansion has largely been observed by land managers, and to date, has not been investigated scientifically. Though the causes of this recent expansion are not well understood, the species is believed to respond positively to less-than-ideal management with regard to the use of mechanical treatment or burn seasonality and quickly spread via runners. Our findings suggest that the ability to invade

Table 4. Effects of Habitat and Species on Belowground Traits. For both traits, the χ^2 and p-value are shown from an analysis of deviance of a mixed-effects model. Both analyses used habitat as a continuous predictor variable for 3 species. Significant p-values (≤0.05) are bolded and underlined.

Terms	d.f.	Specific Root Length		EcM density	
		χ^2	P	χ^2	P
Habitat	1	6.78	**0.009**	7.93	**0.005**
Species	2	43.69	<**0.001**	19.40	<**0.001**
Habitat x Species	2	5.43	0.066	1.71	0.425

Table 5. Effects of Habitat on Belowground Traits for each Species. For both traits, the estimate, standard error, χ^2, and p-value are shown from an analysis of deviance of a mixed-effects model. Each analysis used habitat as a continuous predictor variable. Significant p-values (≤0.05) are bolded and underlined.

Species	Specific Root Length				EcM density			
	Est.	SE	χ^2	P	Est.	SE	χ^2	P
Quercus chapmanii	–0.08	0.09	0.64	0.424	0.18	0.18	0.96	0.328
Quercus geminata	–0.05	0.10	0.27	0.601	0.31	0.17	3.49	0.062
Quercus inopina	–0.38	0.16	5.72	**0.017**	0.57	0.28	4.09	**0.043**

Table 6. Ectomycorrhizal Fungi Identified from Colonized Oak Root Tips using Sanger Sequencing

Sample ID	Species	Habitat	BLAST	ID (coverage)	GenBank Accession
16	*Q. chapmanii*	Rosemary scrub	*Lactarius chrysorrheus*	97% (99%)	PP660635
15	*Q. chapmanii*	Rosemary scrub	*Russula* sp.	96% (82%)	
27	*Q. geminata*	Rosemary scrub	*Alessioporus rubriflavus*	100 (100%)	
14	*Q. geminata*	Rosemary scrub	*Russula arenicola*	99% (93%)	
24	*Q. geminata*	Rosemary scrub	*Russula* sp.	95% (100%)	PP648228
25	*Q. geminata*	Rosemary scrub	*Russula* sp.	93% (98)	
9	*Q. geminata*	Scrubby flatwoods	*Aureoboletus pseudoauriporus*	99% (95%)	PP648225
10	*Q. geminata*	Scrubby flatwoods	*Aureoboletus pseudoauriporus*	99% (99%)	PP648226
28	*Q. inopina*	Rosemary scrub	*Sistotrema* sp.	90% (100%)	PP648229
29	*Q. inopina*	Rosemary scrub	*Sistotrema* sp.	90% (100%)	PP648230
30	*Q. inopina*	Rosemary scrub	*Delastria* sp.	97% (98%)	PP648231
12	*Q. inopina*	Rosemary scrub	*Russula arenicola*	91% (100%)	
1	*Q. inopina*	Scrubby flatwoods	*Russula arenicola*	98% (97%)	
2	*Q. inopina*	Scrubby flatwoods	*Russula arenicola*	100% (92%)	PP660634
11	*Q. inopina*	Scrubby flatwoods	*Russula* sp.	95% (95%)	PP648227

could further be connected to the species' ability to invest in its ectomycorrhizal partners in a stressful environment, and less likely attributable to intraspecific trait variation with respect to the acquisitive-conservative gradient. Yet, it is also worth noting that *Q. geminata* had the lowest SLA of any species, suggestive of a highly conservative, though less variable, growth strategy compared to the other shrubs.

Heterogeneous landscape of the Florida scrub

The Florida scrub consists of a mosaic of various upland scrub habitats, as well as wet habitats such as seasonal ponds, swales, and bayheads (Abrahamson et al. 1984). The heterogeneity of the aboveground vegetation across the landscape is paralleled belowground, where microbes, particularly ectomycorrhizal and arbuscular mycorrhizal fungi, reside (David et al. 2023). In particular, ectomycorrhizal abundance in the soil increases along the environmental stress gradient from flatwoods to rosemary scrub as oaks become relatively more common and heath and palmetto abundance decline (David et al. 2023). Our findings agree with this previous work, as ectomycorrhizal tip density increased with stress, likely because there was sufficient fungal inoculum in the soil to meet the demands of the relatively stressed plants. Furthermore, although less well studied in general and rarely detected in scrub soils (David et al. 2023), the ericoid mycorrhizae associated with many species of heaths could follow similar patterns with respect to their host plants.

Our findings also have implications for trait variation across species, particularly for oaks. Oak species of the southeastern USA are hypothesized to coexist primarily via resource partitioning as well as through facilitation among individuals and species (Cavendar-Bares 2019). Our work demonstrates that oak species differ substantially in their trait variation both above- and belowground, which could alter species coexistence at larger spatial scales.

Intraspecific trait variation in a pyrogenic ecosystem

While not explicitly studied here, intraspecific trait variation may respond to fire, particularly in the time immediately following fire. For example, fire can decrease SLA across plant communities (Mitchell et al. 2021). In our system, morphological changes to the leaves and stems of resprouting *Lyonia fruticosa* post-fire are typically observed within the first 4–6 yr after a fire and are particularly strong within the first two years (Dole et al. 2023). While fire is an important component of the ecosystem, scrub habitats are burned relatively infrequently; for example, the typical fire return interval of low elevation flatwoods is 6–9 yr, while that of high elevation rosemary scrub burns is 20–59 yr (Main and Menges 1997). Our dataset, of which the lowest time-since-fire of any sampling location was 4 years, did not show any effects from fire in preliminary analyses. Fire could affect intraspecific trait variation in years immediately following fire, though any fire-attributed intraspecific trait variation detected in the long-lived, resprouting shrubs would likely be limited to the brief period following infrequent fires.

CONCLUSIONS

Our study of shifting growth strategies of long-lived shrubs of the Florida scrub shows that when growing in relatively stressful conditions, oak and heath species show limited shifting to a conservative strategy and substantial evidence for shifting to a collaborative strategy. Our findings establish a baseline for intraspecific trait variation for two dominant plant families in the southeastern United States, especially for belowground studies.

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