



Honors College Theses

5-3-2022

The potential role of phenotypic plasticity in the ability of *Hydrocotyle bonariensis* to occupy two different habitats

Harold Ralph Parsons III
Georgia Southern University

Follow this and additional works at: <https://digitalcommons.georgiasouthern.edu/honors-theses>



Part of the [Biology Commons](#), [Plant Biology Commons](#), [Plant Breeding and Genetics Commons](#), [Population Biology Commons](#), and the [Terrestrial and Aquatic Ecology Commons](#)

Recommended Citation

Parsons, Harold Ralph III, "The potential role of phenotypic plasticity in the ability of *Hydrocotyle bonariensis* to occupy two different habitats" (2022). *Honors College Theses*. 758.
<https://digitalcommons.georgiasouthern.edu/honors-theses/758>

This thesis (open access) is brought to you for free and open access by Digital Commons@Georgia Southern. It has been accepted for inclusion in Honors College Theses by an authorized administrator of Digital Commons@Georgia Southern. For more information, please contact digitalcommons@georgiasouthern.edu.

The potential role of phenotypic plasticity in the ability of *Hydrocotyle bonariensis* to occupy two different habitats

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Biology.

By Harold Parsons

Under the mentorship of Dr. Heather Joesting

Abstract

Phenotypic plasticity is the ability of an organism to change morphology and/or physiology in response to changes in the environment. *Hydrocotyle bonariensis* is a coastal perennial herb found in both coastal sand dunes and inland coastal plain habitats in Georgia. The purpose of this study was to determine if there were differences in leaf morphology for populations of *H. bonariensis* in coastal sand dune and inland coastal plain habitats. Leaf morphology and microenvironmental variables were compared between *H. bonariensis* populations at Tybee Island (TI), GA, representing the coastal sand dune habitat, and Georgia Southern University Armstrong Campus (GSU) in Savannah, GA, representing the inland coastal plain habitat. According to results, populations at TI experienced significantly greater incident sunlight, air temperatures, and soil temperatures compared to GSU populations, and TI soil contained a greater percentage of sand particles, indicating lower water-holding capacity, and lower organic matter and nutrients. Furthermore, *H. bonariensis* leaves from TI had significantly greater leaf fresh weight, dry weight, water weight, thickness, and chlorophyll content than leaves from GSU. Leaf morphology results suggest that leaves from TI generally have a structure that facilitates relatively greater water-retention and chlorophyll content, likely influenced by the low water-holding capacity of the sandy soil and high incident sunlight characteristic of the sand dune habitat. These results will provide greater insight into the role of phenotypic plasticity in the ability of plants to occupy environmentally different habitats.

Thesis Mentor: _____

Dr. Heather Joesting

Honors Director: _____

Dr. Steven Engel

April 2022

Department of Biology

Honors College

Georgia Southern University

Acknowledgements

I would like to thank Dr. Heather Joesting for guidance and insight during the research process and the writing of this thesis. I would also like to thank the Georgia Southern University Honors College for providing funding for soil analysis and Dr. Marylou Machingura for the use of the MC-100 Chlorophyll Concentration Meter. I would also like to thank Georgia Southern Facilities Services for protecting our study species until the project was completed and the City of Tybee Island and Georgia Department of Natural Resources for permission to collect leaves from sand dunes at the north end of Tybee Island.

Introduction

Plants can respond to environmental factors through either phenotypic plasticity or local adaptation (Knight and Miller, 2004). Phenotypic plasticity is the ability of an individual to change its morphology and/or physiology in response to changes in the environment (Gratani, 2014; Chiarello and Joesting, 2018). Phenotypic plasticity may be influenced by biotic (e.g., herbivory, neighbor absence, presence, size, or density, or parasitism) or abiotic (e.g., sunlight exposure, pH, nutrient and water availability, soil type, elevation, or rapid climate change) factors, or a combination (Sultan, 1995; Callaway et al., 2003; Gratani, 2014; Chiarello and Joesting, 2018; Bakhtiari et al., 2019). On the other hand, local adaptation is the ability of a population to genetically alter its morphology and/or physiology in response to changes in the environment and often occurs when environmental selective pressure is greater than gene flow (Knight and Miller, 2004; Gratani, 2014; Moriuchi et al., 2016). Local adaptation has been shown to be influenced by herbivory, interspecific competition, climate, salinity, soil, or flooding (Lessen et al., 2004; Macel et al., 2007; Bischoff and Hurault, 2013; Busoms et al., 2015). Differences in microenvironmental factors can lead to phenotypic plasticity in plants, which has been demonstrated in the distinct leaf morphology of sun and shade leaves in response to sunlight quality and quantity (Gratani, 2014). Plants in coastal sand dune habitats are often exposed to high incident sunlight with little to no canopy cover, whereas plants in inland coastal plain habitats are exposed to variable incident sunlight with canopy cover (Joesting et al., 2012; Joesting et al., 2016; Chiarello and Joesting, 2018). Too little incident sunlight can lead to reduced photosynthesis, while too much incident sunlight may result in photoinhibition and damage to the photosynthetic apparatus (Joesting et al., 2012). Leaf chlorophyll content is often used as a proxy for photosynthesis, with higher leaf chlorophyll content correlated with greater photosynthetic gain and greater sunlight exposure

(Gratani, 2014; Croft et al., 2017). Furthermore, soils of coastal sand dune habitats are composed of approximately 100% sand while inland coastal plain habitat soils are often composed of a mixture of clay, sand, and silt (Chiarello and Joesting, 2018). The relatively smaller soil grain size and higher organic matter content of inland coastal plain soil compared to coastal sand dune habitats make this soil more suitable for nutrient and water retention, and thus plant growth (Chiarello and Joesting, 2018).

The purpose of this study was to determine whether differences exist in leaf morphology between native wild-type populations of *H. bonariensis* found in coastal sand dune and inland coastal plain habitats. It was predicted that *H. bonariensis* from a coastal sand dune habitat would have greater leaf chlorophyll content to manage the high incident sunlight characteristic of the sand dune habitat. Furthermore, it was predicted that *H. bonariensis* leaves occupying the coastal sand dune habitat would have overall thicker leaves with greater water content in response to the low water-holding capacity and organic matter content of the soil and increased evaporative demand due to high incident sunlight (Maun, 2009; Chiarello and Joesting, 2018).

Methods

Study Species

Hydrocotyle bonariensis Comm. ex Lam. is a large-leaf clonal perennial herb found in both the coastal sand dune and inland coastal plain habitats in coastal Georgia. Individual plants (i.e., clones) have underground, horizontally-connected rhizomes and nodes where leaves and roots are attached (Chiarello and Joesting, 2018). Individual members of a clone, called ramets, are composed of a single leaf and root system at each node, and ramets have been shown to share resources (e.g., nutrients, water, and photosynthates) with each other via the underground rhizome

(Evans and Whitney, 1992; Evans and Cain, 1995; Chiarello and Joesting, 2018). Sexual reproduction success rate has been observed to be low in the sand dune habitat (1-3%), and thus reproduction is primarily asexual through clonal growth (Evans, 1992; Chiarello and Joesting, 2018).

Field Data Collection

H. bonariensis leaves were collected from both the north end of Tybee Island, GA (TI; represented the coastal sand dune habitat) and Georgia Southern University Armstrong Campus (GSU; represented the inland coastal plain habitat) in August 2021. Two populations were selected at both GSU and TI, and fifteen healthy, mature leaves were randomly selected from each population. For each leaf, measurements of leaf chlorophyll content (i.e., SPAD) were taken in the field using the MC-100 Chlorophyll Concentration Meter (Apogee Instruments: Logan, UT), after which leaves were collected and stored in cool, wet conditions until laboratory measurements were conducted.

To characterize microclimatic factors of each habitat (i.e., GSU and TI), weather stations were erected at ~1 m off the ground within each population to record sunlight intensity [measured as photosynthetically active radiation (PAR)], air temperature, and soil temperature. Weather stations consisted of a HOBO Micro Station Data Logger (H21-002; Onset Computer Corporation, Bourne, MA) attached to a PVC pipe with three attached environmental sensors: a Photosynthetic Light (PAR) Smart Sensor (S-LIA-M003; Onset Computer Corporation, Bourne, MA) held level above the PVC Pipe with a bracket and two 12-Bit Temperature Smart Sensors (S-TMB-M006; Onset Computer Corporation, Bourne, MA). Diurnal PAR and air and soil temperature were measured every hour for one week at each population in August 2021. Daily PAR and air and soil

temperatures were then averaged to calculate a mean diurnal PAR and air and soil temperature for each population.

Soil was collected from TI and both populations at GSU in March 2022 and analyzed for soil type, pH, ammonium, nitrate, and organic matter content at the University of Georgia Extension's Agricultural and Environmental Services Laboratory (<https://aesl.ces.uga.edu/>).

Lab Data Collection

For each collected leaf, leaf fresh weight was recorded and images were taken of the labeled leaves next to a ruler for digital assessment of leaf area. Impressions of both leaf surfaces (i.e., top and bottom) were obtained using clear nail polish, which was peeled from the leaves and placed on a labeled microscope slide. After leaf impressions were made, each leaf was dried at 65°C for at least 24 hours. Dry leaves were then weighed, and leaf water weight (indicative of leaf water-holding capacity) was estimated using the following equation:

$$\text{Leaf water weight (g)} = \text{Leaf fresh weight (g)} - \text{Leaf dry weight (g)}$$

Images were taken of the leaf impression slides using an Olympus BX 60 microscope and a Q Color 5 camera (Olympus Corporation, Waltham, MA) at 10X magnification. The glue used to attach the cover slips onto the microscope slides created a white substance on some slides that interfered with microscope imaging, and thus slides with >10% area either indiscernible for stomata or lacking stomata altogether were not analyzed. To measure stomata density, leaf impression images were uploaded to Adobe Photoshop (12.1 x64, Adobe Systems Incorporated), a layer was added to the image, and dots were placed on each stomata identified. This layer was saved as an additional file and uploaded to ImageJ (National Institutes of Health: Bethesda, MD),

where dots (indicating stomata) were counted using the Analyze Particles tool. To estimate leaf area, leaf images described above were opened in ImageJ, and a scale was created using the ruler and Set Scale tool. The color threshold was changed to Black and White, and leaves were selected using the ROI Manager tool, after which leaf area (cm^2) was measured using the Measure tool.

Stomata density was calculated using the following equation:

$$\text{Stomata Density (\#/cm}^2\text{)} = \frac{\text{Number of stomata}}{\text{Leaf area (cm}^2\text{)}}$$

Specific leaf area (SLA) was calculated using the following equation:

$$\text{Specific Leaf Area (cm}^2\text{/g)} = \frac{\text{Leaf area (cm}^2\text{)}}{\text{Leaf dry mass (g)}}$$

Statistical Analyses

Diurnal curves for PAR, air temperature, and soil temperature were compared between populations within each habitat (Population effect) and between habitats (Habitat effect) using a repeated-measures ANOVA, with Time as the repeated effect and significance at $P \leq 0.05$. Prior to analysis, all leaf morphological variables were analyzed for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. Leaf fresh weight, dry weight, water weight, specific leaf area (SLA), and chlorophyll content could not be transformed to meet assumptions. Therefore, differences in these variables between populations within each habitat and between habitats were analyzed using a Wilcoxon Signed-Rank Test with significance at $P \leq 0.025$. Leaf area and stomata density on top and bottom leaf surfaces were compared between populations within each habitat and between habitats using a one-way ANOVA with significance at $P \leq 0.025$.

Results

There was overall significantly greater diurnal PAR ($F_{1,26} = 66.77$, $P < 0.001$), air temperature ($F_{1,26} = 17.75$, $P < 0.001$), and soil temperature ($F_{1,26} = 16.50$, $P < 0.001$) at TI compared to GSU (Figure 1). Furthermore, PAR, air temperature, and soil temperature achieved higher levels for a longer period of time at TI than at GSU (Figure 1). There was no significant difference in diurnal photosynthetically active radiation (PAR; $F_{1,12} = 0.042$, $P = 0.841$), air temperature ($F_{1,12} = 0.03$, $P = 0.867$), and soil temperature ($F_{1,12} = 2.789$, $P = 0.121$) between populations at TI, so the mean values were combined to represent single diurnal curves for TI. There was a significant difference between populations at GSU for diurnal PAR ($F_{1,12} = 64.83$, $P < 0.001$) and soil temperature ($F_{1,12} = 86.41$, $P < 0.001$). Thus, the mean diurnal values were kept separate for these populations, and the population with the greater PAR and soil temperature was designated as the sun population and the lower PAR and soil temperature the shade population. Finally, soil from the GSU sun population was neutral ($\text{pH} = 6.64$) loamy sand (76.1% sand, 17.8% silt, and 6.1% clay) with 1.15% organic matter and ammonium and nitrate content of 0.69 and 4.75 mg/kg, respectively. Acidic ($\text{pH} = 5.90$) loamy sand (80.3% sand, 11.48% silt, and 8.24% clay) with 1.39% organic matter and ammonium and nitrate content of 1.07 and 2.71 mg/kg, respectively, was found for GSU soil from the shade location. Alkaline ($\text{pH} = 7.99$) sand (93.2% sand, 4.66% silt, and 2.12% clay) with <1% organic matter and ammonium and nitrate content of <0.18 and 1.71 mg/kg, respectively, was found for TI soil.

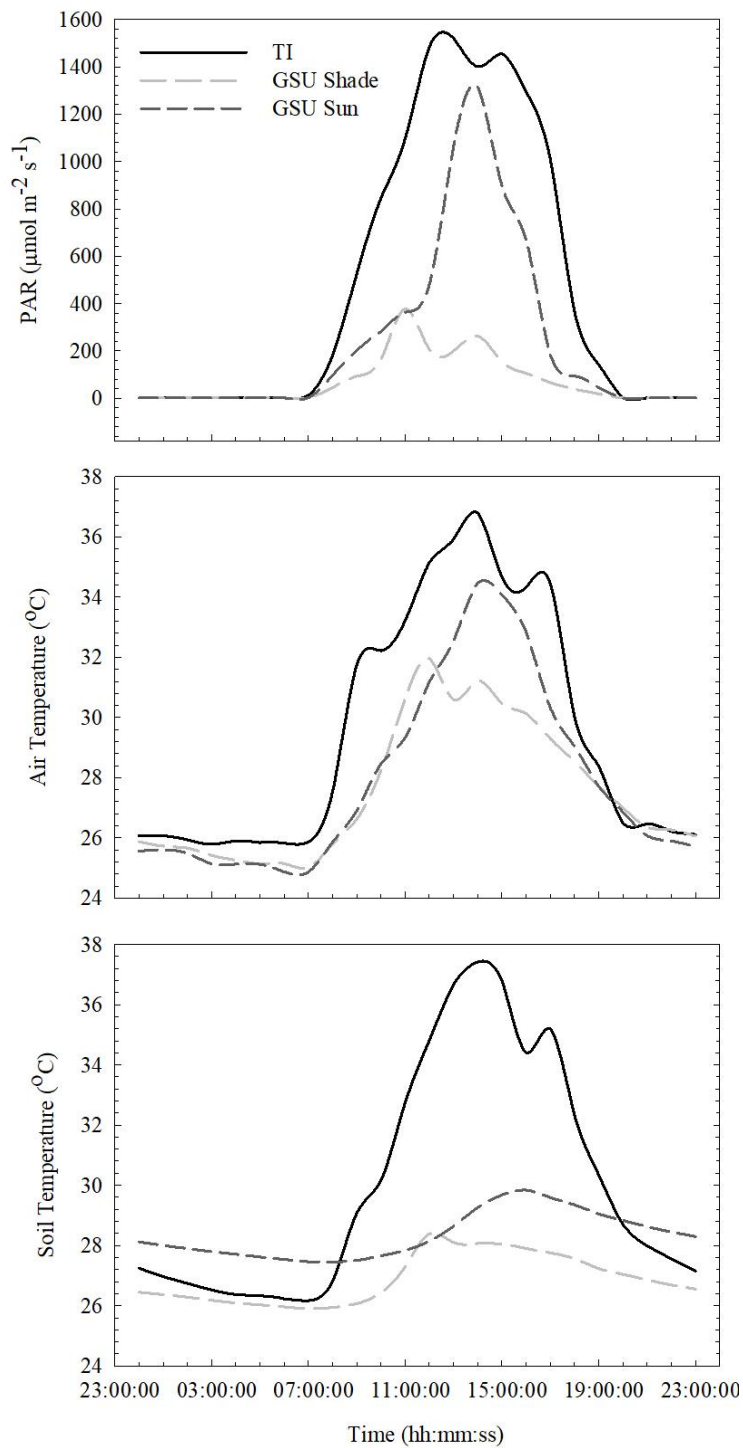


Figure 1. Mean photosynthetically active radiation (PAR), air temperature, and soil temperature over a 24-hour period in August 2021 at Tybee Island (TI) and sun and shade populations at Georgia Southern University (GSU).

There was no significant difference between habitats for leaf area and top or bottom stomata density (Figures 2-3). However, there was significantly greater leaf fresh weight ($\chi^2 = 11.42$, $P < 0.001$), leaf dry weight ($\chi^2 = 25.35$, $P < 0.001$), leaf water weight ($\chi^2 = 7.73$, $P = 0.005$), and leaf chlorophyll content ($\chi^2 = 23.66$, $P < 0.001$) at TI than at GSU and significantly greater specific leaf area ($\chi^2 = 37.10$, $P < 0.001$) at GSU than at TI (Figures 2, 4-5).

There was no significant difference between populations for leaf fresh weight, leaf dry weight, leaf water weight, leaf area, top or bottom stomata density, or leaf chlorophyll content at GSU (Table 1). There was, however, significantly greater specific leaf area at the sun population compared to the shade population at GSU (Table 1). While there was a significant difference in leaf water weight and leaf area between populations at TI, there was no significant difference in leaf fresh weight, leaf dry weight, specific leaf area, top or bottom stomata density, or leaf chlorophyll content at TI (Table 1).

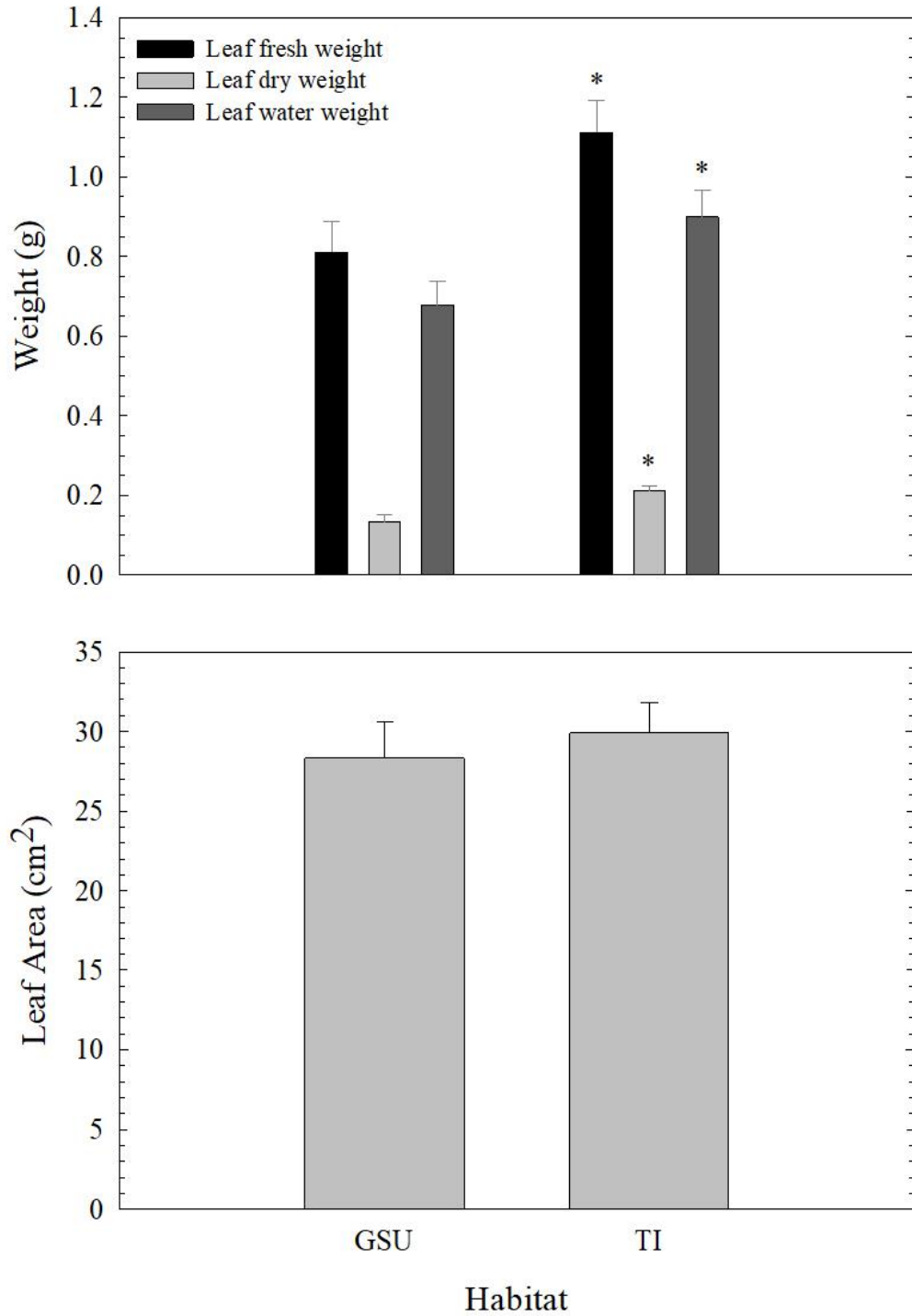


Figure 2. Mean leaf weights (wet, dry, and water weight) and leaf area for *H. bonariensis* leaves from Georgia Southern University (GSU) and Tybee Island (TI). Asterisks indicate a significant difference between habitats and error bars represent standard error.

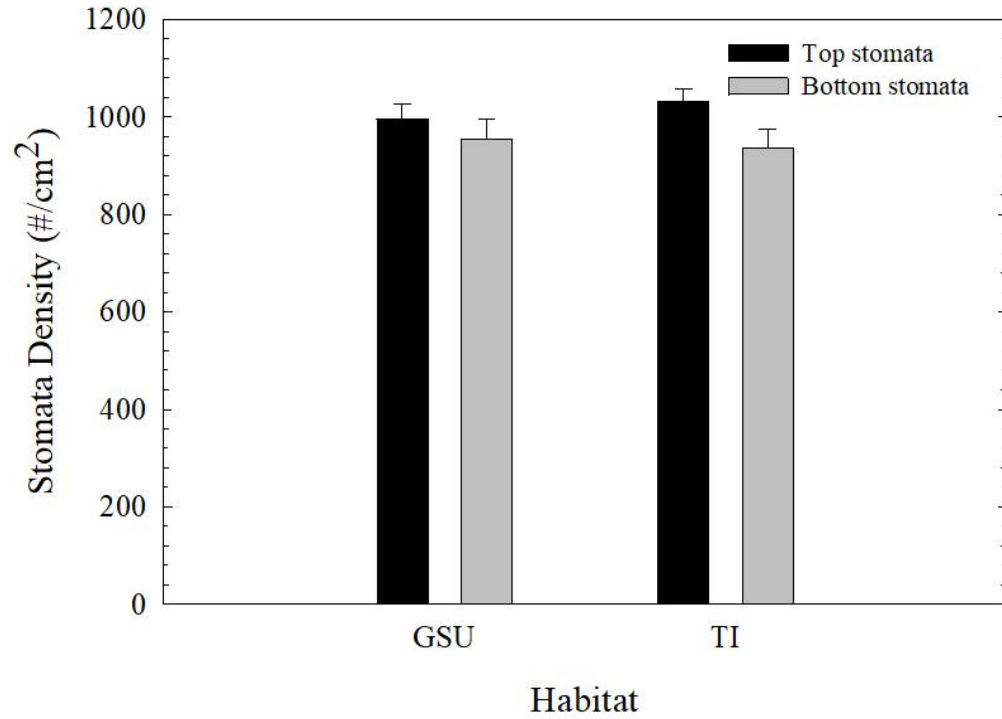


Figure 3. Mean stomata density for *H. bonariensis* leaves from Georgia Southern University (GSU) and Tybee Island (TI). Error bars represent standard error.

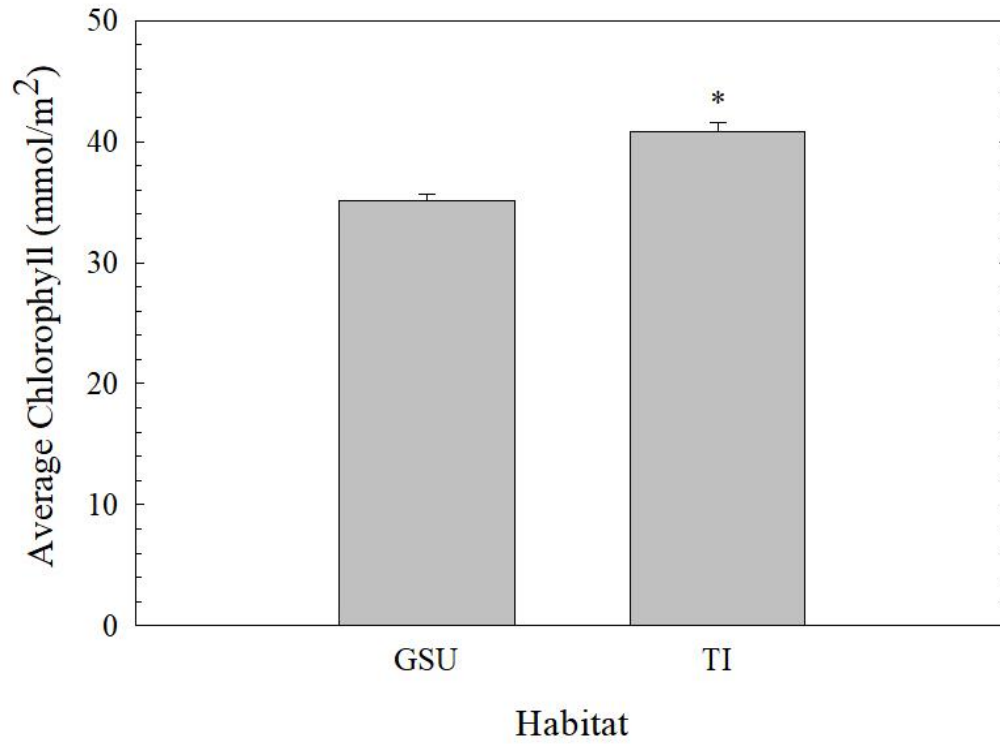


Figure 4. Leaf chlorophyll content for *H. bonariensis* leaves from Georgia Southern University (GSU) and Tybee Island (TI). Asterisk indicates a significant difference between habitats and error bars represent standard error.

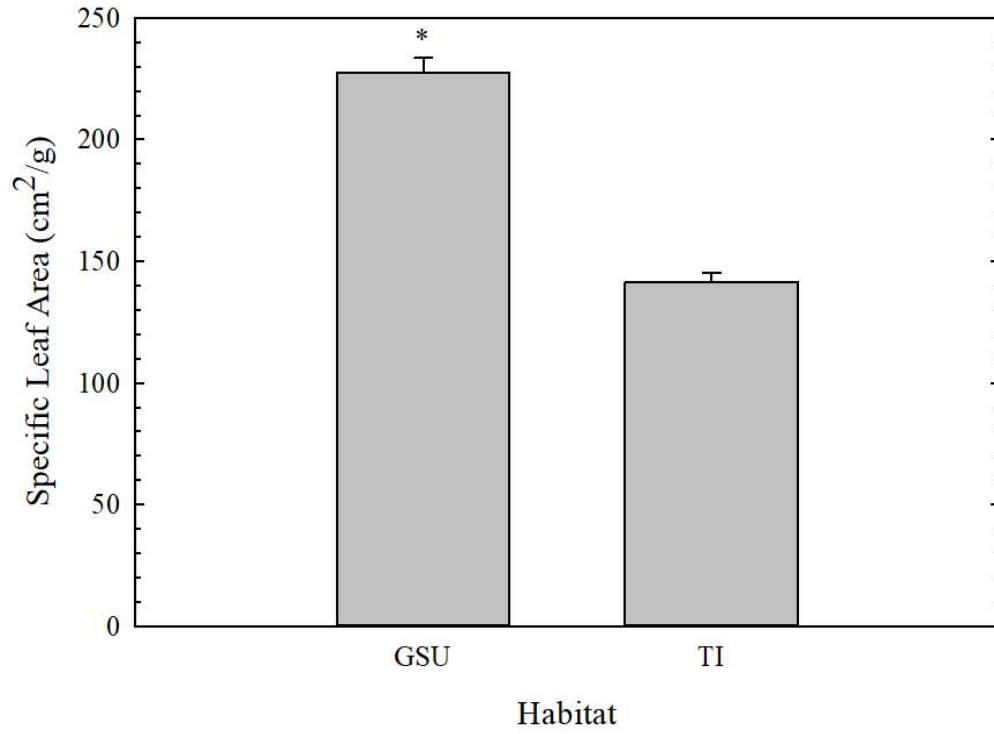


Figure 5. Mean specific leaf area (SLA) for *H. bonariensis* leaves from Georgia Southern University (GSU) and Tybee Island (TI). Asterisk indicates a significant difference between habitats and error bars represent standard error.

Table 1. Comparisons of leaf characteristics between *H. bonariensis* from Population 1 (shade) and Population 2 (sun) at Georgia Southern University (GSU) and *H. bonariensis* from Population 1 and Population 2 at Tybee Island (TI). χ^2 values are from Wilcoxon Signed Rank Test and F-values are from one-way ANOVAs, with significance at $P \leq 0.025$.

	Population 1	Population 2	Test Statistic	P-value
<i>Georgia Southern University</i>				
Leaf fresh weight (g)	0.71 ± 0.067	0.91 ± 0.139	$\chi^2 = 0.99$	P = 0.319
Leaf dry weight (g)	0.11 ± 0.011	0.16 ± 0.036	$\chi^2 = 0.87$	P = 0.351
Leaf water weight (g)	0.60 ± 0.056	0.757 ± 0.104	$\chi^2 = 1.03$	P = 0.310
Leaf area (cm ²)	24.04 ± 2.123	32.64 ± 3.827	F _{1, 28} = 3.87	P = 0.059
Specific Leaf Area (cm ² /g)	215.56 ± 6.734	239.04 ± 9.729	$\chi^2 = 6.09$	P = 0.014
Top Stomata Density (#/cm ²)	976.23 ± 58.678	1006.50 ± 36.484	F _{1, 19} = 0.22	P = 0.648
Bottom Stomata Density (#/cm ²)	937.21 ± 59.790	974.39 ± 51.525	F _{1, 21} = 0.218	P = 0.645
Leaf Chlorophyll Content (μmol/m ²)	34.77 ± 0.548	35.50 ± 0.801	$\chi^2 = 0.72$	P = 0.395
<i>Tybee Island</i>				
Leaf fresh weight (g)	1.31 ± 0.124	0.92 ± 0.069	$\chi^2 = 4.84$	P = 0.028
Leaf dry weight (g)	0.24 ± 0.018	0.18 ± 0.01	$\chi^2 = 4.83$	P = 0.028
Leaf water weight (g)	1.07 ± 0.107	0.73 ± 0.056	$\chi^2 = 5.30$	P = 0.021
Leaf area (cm ²)	35.05 ± 2.913	24.77 ± 1.643	F _{1, 28} = 9.45	P = 0.0047
Specific Leaf Area (cm ² /g)	146.84 ± 6.664	135.68 ± 4.017	$\chi^2 = 0.8710$	P = 0.3507
Top Stomata Density (#/cm ²)	1046.05 ± 41.873	1016.10 ± 36.77	F _{1, 25} = 0.29	P = 0.594
Bottom Stomata Density (#/cm ²)	1001.63 ± 58.08	876.53 ± 50.089	F _{1, 26} = 2.69	P = 0.113
Leaf Chlorophyll Content (μmol/m ²)	41.43 ± 1.008	40.14 ± 1.11	$\chi^2 = 1.76$	P = 0.184

Discussion

The results suggest leaf morphological differences between *H. bonariensis* populations that were likely influenced by microenvironmental differences between habitats. Populations at TI experienced significantly greater PAR, air temperature, and soil temperature than populations at GSU. Soil from TI was alkaline ($\text{pH} > 7.5$) with less organic matter, ammonium, and nitrate, while soil from GSU was acidic ($\text{pH} < 6.5$) with relatively more organic matter and ammonium and nitrate content. Corresponding leaf measurements revealed significantly greater leaf weight (fresh, dry, and water) and chlorophyll content at TI and greater SLA in leaves from GSU (Figures 2-3, 5). Results also suggested differences between populations within habitats, especially at GSU. Although populations at TI did not experience significantly different levels of PAR, air temperature, or soil temperature, the sun population at GSU did experience significantly greater PAR and soil temperature than the shade population at GSU (Figure 1). Additionally, leaves from the sun population at GSU had significantly greater SLA than leaves from the shade population, likely in response to the higher sunlight incidence.

Environments

Results indicated that *H. bonariensis* populations at TI were exposed to significantly greater PAR, air temperature, and soil temperature compared to GSU. There is generally little to no canopy cover in coastal sand dune habitats, and individual plants are often located within bare patches of sand (Joesting et al., 2016). On the other hand, plants found in inland coastal plain habitats often grow in communities with a canopy of shrubs and/or small trees, which provide shade (Chiarello and Joesting, 2018; *personal observation*). High incident sunlight at TI may contribute to overall greater air temperature and soil temperature, which also persisted for longer periods of time at TI than at GSU (Figure 1). There is likely also increased evaporative demand in

coastal sand dune habitats due to higher incident sunlight, which may lead to increased transpiration and subsequent water loss for plants in the sand dune (Maun, 2009). Furthermore, the greater canopy cover at the GSU shade population compared to the GSU sun population may have contributed to the lower PAR levels and soil temperature at the shade population.

The soil at TI was coarse-grained (93.2% sand) and contained less organic matter content compared to GSU. Coarse-grained sand has a lower specific heat than soils with greater moisture content (i.e., fine-grained clay), and the greater sand content at TI compared to GSU likely contributed to the greater soil temperature (Abhu-Hamdeh, 2003). The coastal sand dune habitat likely experienced higher soil temperatures for a longer period of time than the inland coastal plain habitat because less heat was required to increase soil temperature by one degree Celsius. Additionally, the greater organic matter content at GSU (1.15% and 1.39% at sun and shade population, respectively) may have contributed to lower soil temperature compared to TI, which had an organic matter content of 0.11%. Organic matter helps soil retain water, which may decrease soil temperature (Bhadha et al., 2017). The higher soil temperature at the GSU sun population compared to the shade population may have also been due to differences in organic matter content. Finally, the higher sand content and lower organic matter content at TI compared to GSU indicates that the water-holding capacity of TI soil was substantially lower, which can lead to reduced water availability in coastal sand dune populations. Finally, soil at TI was alkaline (pH > 7.5) compared to GSU soils (pH 6.5 - 7.5 and pH < 6.5 at sun and shade population, respectively). Alkaline soil, such as TI, is outside of the optimal pH range for plants, which is between 5.5 and 6.5, and high sodium levels in alkaline soil can prevent stomatal closure and thus allow more water loss (Msimbira and Smith, 2020).

Taken together, the microenvironment of TI likely imposed a greater risk of reduced photosynthesis and photoinhibition to *H. bonariensis* populations. Specifically, the relatively high incident sunlight may lead to excess light absorption and subsequent reduced photosynthesis through photoinhibition (Joesting et al., 2016). Photoinhibition occurs when excess photons cannot be processed by the light harvesting complexes of chloroplasts and often leads to reduced photosynthesis resulting from reduced efficiency in light processing. Furthermore, the increased evaporative demand due to the high sunlight combined with the low water-holding capacity and high pH of the soil may result in greater water stress in TI populations, further exacerbating photoinhibition risk.

Differences in Leaf Morphology between Habitats

Overall, *H. bonariensis* leaves at TI were thicker and contained greater water and chlorophyll content compared to leaves at GSU. Specifically, TI leaves had significantly greater leaf fresh, dry, and water weight, and the lower SLA found in TI leaves was likely due to an increase in volume, since no difference was found in leaf area between the two habitats. The overall thicker leaves at TI may function to hold more water to mitigate water loss and more chloroplasts (as suggested by the greater chlorophyll content) to process the greater sunlight characteristic of the coastal sand dune habitat.

There were observed differences between populations within habitats as well. SLA in GSU leaves was significantly higher in the sun population than the shade population, which is likely due to the difference in incident sunlight between the populations. SLA is a measurement of the leaf surface area to volume ratio (i.e., leaves with relatively high SLA have thinner leaves and greater surface area compared to leaves with low SLA). Leaves in sunnier habitats often have a thicker leaf due to the presence of thicker palisade parenchyma, spongy parenchyma, and

epidermal tissue than leaves in shadier habitats (Gratani, 2014). However, this was not observed between populations within GSU. There was also a significant difference in leaf water weight and leaf area between the two populations at TI. According to Knight and Miller (2004), higher elevations on sand dunes are often dry and open, whereas lower elevations are periodically flooded by high tides. Furthermore, lower elevations often have more vegetation than higher elevations, which can provide shade to the soil and reduce evaporative water loss at the soil surface (Knight and Miller, 2004). *H. bonariensis* leaves have also been observed to increase leaf area when there is surrounding vegetation (Evans, 1982). Differences in elevation, and hence potential vegetation cover, between the two populations at TI may explain the significant differences observed for leaf water weight and leaf area. Further research is required to determine if differences in elevation, and hence tidal flooding and vegetation, in sand dunes influences leaf morphology in *H. bonariensis* coastal sand dune populations.

Chiarello and Joesting (2018) observed phenotypic plasticity in leaves from the coastal sand dune planted in both sand dune and inland coastal plain soil. In this study, leaf area and petiole (i.e., leaf stem) fresh weight, thickness, and length were significantly greater in the leaves grown in inland coastal plain soil. According to Chiarello and Joesting (2018), the greater petiole fresh weight and thickness was likely due to more water content in the inland coastal plain soil (loamy sand) compared to sand dune soil (sand). Furthermore, the greater petiole length and leaf area in sand dune soil were suggested to be due to lower organic matter content and nutrients of coastal sand dune soil. While this study suggests that differences in leaf area are due to phenotypic plasticity influenced by soil characteristics, the role of phenotypic plasticity or local adaptation in petiole thickness and fresh weight in *H. bonariensis* is still to be elucidated.

Chiarello and Joesting (2018) found no significant differences in leaf thickness or weight (fresh, dry, and water) between soil types. This indicates that leaf thickness did not respond plastically to differences in soil characteristics. In the present study, leaf thickness and weight were significantly greater in leaves from TI than from GSU, and leaf thickness, fresh weight, and dry weight have also been seen to be significantly greater in leaves from the coastal sand dune regardless of the soil they are grown in (i.e., coastal sand dune or inland coastal plain; Kim and Joesting, *unpublished data*). Therefore, the observed differences in leaf thickness and leaf weight between *H. bonariensis* populations may be due to local adaptation.

In this study, there was not a significant difference in bottom stomata density between habitats. This is not consistent with data reported from Chiarello and Joesting (2018), which suggested that *H. bonariensis* leaves from the sand dune habitat grown in inland coastal plain soil had significantly greater bottom stomata density than individuals grown in sand dune soil. There was also not a significant difference in top stomata density between habitats in the present study, which is consistent with the findings of Chiarello and Joesting (2018). Furthermore, other data suggests that there is no significant difference in top or bottom stomata density between leaves from the coastal sand dune and inland coastal plain populations, regardless of the soil type (i.e., coastal sand dune and inland coastal plain) they are planted in (Kim and Joesting, *unpublished data*). Taken together, this suggests that stomata density does not respond plastically to environmental variables but rather is species-specific in *H. bonariensis*. However, previous studies indicate that sun leaves often have greater stomata density compared to shade leaves to maximize CO₂ absorption (Gratani, 2014). While top and bottom stomata density in *H. bonariensis* from both a coastal sand dune habitat and an inland coastal plain habitat were not significantly different, *H. bonariensis* from a coastal sand dune habitat employs leaf inclination to reduce midday incident

sunlight and prevent photoinhibition, which may not necessitate plastic responses in stomata density (Joesting et al., 2012; Joesting et al., 2016). However, it is not known if leaf inclination is utilized by *H. bonariensis* populations in the inland coastal plain habitat.

Both adaptive and inevitable phenotypic plasticity can simultaneously occur in a plant in response to the abiotic and biotic environment (Sultan, 1995). Macdonald and Lieffers (1993) found that *Calamagrostis canadensis* had fewer and shorter rhizomes in low-temperature soil with interspecific competition and expanded via clones into warmer soils without interspecific competition. In other words, the plant responded to unfavorable conditions through clonal expansion, but the inevitable result of unfavorable conditions was locally fewer and shorter rhizomes. Similarly, some traits observed in *H. bonariensis* may be due to adaptive phenotypic plasticity or be the result of inevitable morphological limitations. In a study by Eiguchi et al. (1993), it was discovered that stem elongation length of rice was not due to a genetic difference between a flood-intolerant strain and a flood-tolerant strain, but rather the timing of stem elongation. The flood-tolerant strain was prevented from surviving a flood because the shoot elongated too late to raise the plant above the flood water level. Therefore, it is possible that the response of *H. bonariensis* to high incident sunlight may not be mediated through greater stomata density but through other means (e.g., leaf thickness and leaf inclination). Likewise, such a response may be either through phenotypic plasticity or local adaptation, and whether phenotypic plasticity is adaptive or inevitable remains to be elucidated (Sultan, 1995; Knight and Miller, 2004).

Conclusion

In summary, results suggested that the overall greater PAR, air temperature, and soil temperature at TI played a role in the observed thicker leaves with more water and chlorophyll

content in TI *H. bonariensis* populations compared to GSU. Comparison of the results of this study with other studies comparing leaf morphology between coastal sand dune and inland coastal plain habitats (Chiarello and Joesting, 2018; Kim and Joesting, *unpublished data*) suggest that differences in leaf thickness and leaf weight may be due to local adaptation while phenotypic plasticity may control differences in leaf area and chlorophyll.

While it has been shown that morphological variables differ between *H. bonariensis* leaves from the coastal sand dune and inland coastal plain habitat, it was not possible at this time to determine whether such differences are the result of phenotypic plasticity or local adaptation. In order to determine whether differences in leaf morphology are due to adaptive phenotypic plasticity or local adaptation, a transplant study is required (Sultan, 1995; Knight and Miller, 2004).

It is important to understand the role of phenotypic plasticity and local adaptation in native populations of an inexpensive and abundant species to better understand how phenotypic plasticity and local adaptation occur in plants. Moreover, *H. bonariensis* ramets have different characteristics which may be important for coastal sand dune restoration. *H. bonariensis* leaves provide underground sediment stabilization to coastal sand dunes, which is important for successful dune restoration. Therefore, an understanding of how the source of a *H. bonariensis* plant may impact its efficiency in dune restoration is important in barrier island preservation. Furthermore, an understanding of the role of phenotypic plasticity and local adaptation in how plants respond to the environment may give clues about how plants such as rice (*Oryza sativa*) and wheat (*Triticum aestivum*) may be bred in various conditions to induce changes in traits through either phenotypic plasticity or local adaptation.

Literature Cited

- Abhu-Hamdeh NH. 2003. Thermal properties of soils as affected by density and water content. *Biosystems Engineering* 86(1):97-102.
- Bakhtiari M, Formenti L, Caggia V; Glauser G, Rasmann S. 2019. Variable effects on growth and defense traits for plant ecotypic differentiation and phenotypic plasticity along elevation gradients. *Ecology and Evolution* 9:3740-3755.
- Bhadha JH, Capasso JM, Khatiwada R, Swanson S, LaBorde C. 2017. Raising soil organic matter content to improve water holding capacity. University of Florida Institute of Food and Agricultural Sciences Extension.
- Bischoff A, Hurault B. 2013. Scales and drivers of local adaptation in *Brassica nigra* (Brassicaceae) populations. *American Journal of Botany* 100(6):1162-1170.
- Busoms S, Teres J, Huang X, Bomblies K, Danku J, Douglas A, Weigel D, Poschenrieder C, Salt DE. 2015. Salinity is an agent of divergent selection driving local adaptation of *Arabidopsis* to coastal habitats. *Plant physiology* 168:915-929.
- Callaway RM, Pennings SC, Richards CL. 2003. Phenotypic plasticity and interactions among plants. *Ecology* 84:1115-1128.
- Chiarello SD, Joesting HM. 2018. Examination of phenotypic plasticity in *Hydrocotyle bonariensis* in response to two soil types. *Bios*: 65-73.
- Croft H, Chen JM, Luo XZ, Bartlett, P, Chen B, Staebler, RM. 2017. Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. *Global Change Biology* 23(9):3513-3524.
- Eiguchi M, Sano R, Hirano H-Y, Sano Y. 1993. Genetic and developmental bases for phenotypic plasticity in deepwater rice. *Journal of Heredity* 84:201-205.

- Gratani L. 2014. Plant phenotypic plasticity in response to environmental factors. *Advances in Botany* 2014:1-17.
- Joesting HM, Counts J, Grier J, Reilly HZ. 2016. Leaf inclination in the coastal sand dune herb *Hydrocotyle bonariensis* Comm. ex Lam. *Flora* 224:159-166.
- Joesting HM, Sprague MO, Smith WK. 2012. Seasonal and diurnal leaf orientation, bifacial sunlight incidence, and leaf structure in the sand dune herb *Hydrocotyle bonariensis*. *Environmental and Experimental Botany* 75:195-203.
- Knight TM, Miller TE. 2004. Local adaptation within a population of *Hydrocotyle bonariensis*. *Evolutionary Ecology Research* 6:103-114.
- Lessen JPM, Van Kleunen M, Fischer M, De Kroon H. 2004. Local adaptation of the clonal plant *Ranunculus reptans* to flooding along a small-scale gradient. *Journal of Ecology* 92:696-706.
- Macdonald SE, Loeffers VJ. 1993. Rhizome plasticity and clonal foraging of *Calamagrostis canadensis* in response to habitat heterogeneity. *Journal of Ecology* 81:769-776.
- Macel M, Lawson CS, Mortimer SR, Šmilauerova M, Bischoff A, Crémieux L, Doležal, Edwards AR, Lanta V, Bezemer TM, Van Der Putten WH, Igual JM, Rodriguez-Barrueco C, Müller-Schärer H, Steinger T. 2007. Climate vs. soil factors in local adaptation of two common plant species. *Ecology* 88(2):424-433.
- Maun MA. 2009. The sand dune environment. *The Biology of Coastal Sand Dunes*. Oxford University Press: New York.
- Moriuchi KS, Friesen ML, Cordeiro MA, Badri M, Vu WT, Main BJ, Aouani ME, Nuzhdin SV, Strauss SY, von Wettberg EJB. 2016. Salinity adaptation and the contribution of parental environmental effects in *Medicago truncatula*. *PLoS ONE* 11(3):1-19.

Msimbira LA, Smith DL. 2020. The roles of plant growth promoting microbes in enhancing plant tolerance to acidity and alkalinity stresses. *Frontiers in Sustainable Food Systems* 4(106):1-14.

Sultan SE. 1995. Phenotypic plasticity and plant adaptation. *Acta Botanica Neerlandica* 44:363-383.