Sodium Mediates Developmentally Plastic Responses in Plants and Herbivores

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SODIUM MEDIATES DEVELOPMENTALLY PLASTIC RESPONSES IN PLANTS AND HERBIVORES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
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Doctor in Philosophy

in

The Department of Biological Sciences

by

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My journey across my doctoral studies has been a dream made come true. Never in a million years would I have predicted that a person like me, coming from the humble and beautiful coffee farm in Ciales, Puerto Rico, would have the opportunity to develop so much fantastic science. Exploring life and its wonders has been a fundamental driver of my life and this dissertation is the result of it and a great start to, hopefully, a great academic career.

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ABSTRACT

Sodium plays a crucial role in organismal performance, trophic-level interactions, and eco-evolutionary dynamics. For plants, sodium impacts osmoregulation, growth, and water uptake. For animals, sodium is essential influencing osmoregulatory processes, muscle and neural development, and blood regulation. My dissertation aims to disentangle why sodium mismatch affects resource-consumer interactions and its influence on morphological and behavioral plasticity. First, I identified how sodium impacts plant performance and sodium accumulation strategies. I initially focused my research on understanding how increasing substrate sodium affects plant growth and tissue sodium accumulation strategies in controlled settings using a systematic review approach. I found that saltier plants reflected saltier substrates, even across crop and non-crop species, regardless of species identity. However, responses in plant growth showed phylogenetic conservatism across the taxa studied.

I aimed to describe how the phytochemical landscape of sodium forms across a heterogeneous landscape. I performed field collections of widespread plant genera, along with adjacent soil, across the southern United States. Based on the results, environmental sodium correlated with plant sodium concentrations, contrasting with other cations which are at strict homeostatic levels across plant tissues. These findings suggest that plant sodium concentrations are highly dependent on environmental conditions and that plants lack the ability to homeostatically control sodium in their tissues. We can deduce that plant sodium concentrations result from complex interactions with the environment and that herbivores experience substantial variation in dietary sodium across their range
To then link the influence of plant tissue sodium variation to herbivore performance and behavior, I exposed larvae of the bordered patch to common sunflower plants grown hydroponically across increasing treatments of substrate sodium. The findings from this experiment demonstrate how plants with high sodium concentrations reduce herbivore survivorship and extend developmental time, potentially due to imposed physiological stress. In plants grown in no-addition conditions, 25% of larvae were eaten by their siblings, demonstrating the first instance where variation in dietary sodium influences cannibalistic behavior. These findings show the importance of sodium for animal development and performance and how two extremes in the plant sodium concentration negatively affect herbivore fitness.
Plants, as well as most primary producers, form the basis of the movement of key elements in biogeochemical cycles, thus influencing trophic level interactions, food webs, and eco-evolutionary dynamics across ecosystems. The elemental composition of plants can vary across taxa with primary elements and secondary compounds being distributed unevenly across the species ranges. Understanding this phytochemical landscape, as envisioned by Hunter 2016, represents an important step in understanding bottom-up and top-down trophic interactions, especially in the context of the movement of elements across ecosystems. We can think about this phytochemical landscape as a set of contour maps of complex chemical structures which can focus on essential (i.e., Ca, K, Zn, Cu) and nonessential elements (i.e., Na, Cd, Al, Pb), plant-related toxins (i.e., cardenolides, terpenes, among others), and structural molecules (i.e., lignin, cellulose), that form the basis of plant and primary producers which exists across a heterogeneous landscape (Figure 1.1). Phytochemistry results from plants’ particular interactions with the environment and can result in direct and indirect feedback loops that can impact biogeochemistry as well as primary and secondary consumer activities given local nutrient availability and chemical phenotypes presented by autotrophs (Hunter 2016). Yet, most of our understanding of how the phytochemical landscape is formed and maintained across a heterogeneous landscape and how it directly influences herbivore activity remains poorly understood.

By using the phytochemical landscape as a framework to study plant-herbivore interactions, we can better understand general ecological and evolutionary patterns that shape
organismal performance, community assembly, and ecosystem dynamics, whilst taking into consideration the role the heterogeneous landscape of nutrients plays in such dynamics. We can also test patterns of ecological stoichiometry that might arise from the interaction of various trophic levels and the movement of nutrients across the landscape. Changes in ecological stoichiometry and can have substantial effects on organismal behavior and performance across their range. For instance, koalas (*Phascolarctos cinereus*) have been found to selectively forage for *Eucalyptus* leaves that have lowered toxic secondary compound concentration, a pattern that can be readily observed using mapping techniques and appropriate sampling (Moore et al. 2010). Yet, on average, the ways the phytochemical landscape influences trophic level interactions and organismal performance are poorly described. To achieve this, we must characterize and understand how the phytochemical landscape is formed and maintained, whether for primary elements, and/or secondary compounds across multiple plant taxa and across the landscape.

![Figure 1.1. A conceptual diagram of the phytochemical landscape framework and its interaction with other trophic levels. Contour maps indicate geographical variation in chemistry and herbivore activity. Blue arrows indicate direct interactions and orange arrows indicate indirect (Redrawn from Hunter 2016).](image-url)
SODIUM AND ITS INTERACTION WITH ORGANISMS

As the seventh most abundant element on the Earth’s crust, sodium is found across all ecosystems in highly variable degrees (Smith 2013). Sodium, in its purest form, is a silvery-white metal element, which can form many ionic associations with non-metals, and represents about 3% of the elemental composition of the Earth’s crust, yet not all of it is bioavailable (Anderson and Anderson 2010). Most of the sodium ions are locked in feldspar and other insoluble silicates formed after magma cooling. We mostly encounter sodium in its association with chloride (NaCl). Also, many other sodium-containing salts can be found in nature such as soda ash (NaCO$_3$), baking soda (NaHCO$_3$), and caustic soda (NaOH), among others, which are of economic and biological importance. However, the availability of sodium across the landscape is highly heterogeneous, especially in terrestrial environments showing at least a coefficient of variation of 88.1% across soils of the United States with a range of <0.01 to 6.41 percent weight (Smith 2013). Seawater is approximately 1.08% of sodium, the highest cation to make up seawater salinity (Culkin and Cox 1966).

As a biologically important element, sodium plays a crucial role in all biological organizations, from the cellular level to ecosystems. For instance, the presence and variation of sodium control and limit how plant communities structure on coastlines with special effects on mangroves and salt marshes (Clarke and Hannon 1970). It is also considered to be the catalyst for the evolution of nervous systems in animals as it is related to a calcium channel gene duplication coopted to use sodium, the sodium channels, a deep in evolutionary time trait that arose in the ancestor of all animals, except poriferans (Natochin 2007, Liebeskind et al. 2011). However, not all organisms use sodium, and its presence can become toxic even at low levels as is the case with most plant species.
For most plants, sodium tends to be toxic, having consequences on osmoregulatory processes, water movement, growth inhibition, and yield reductions (Flowers and Colmer, 2008, Santiago-Rosario et al. 2021). Plants are unable to prevent the intake of sodium as sodium increases in the substrate which is a physiological effect related to the uptake of potassium, a similar cation essential for plant metabolism and function (Cheeseman and Wickens 1986a, Flowers and Colmer 2008, Maathuis 2014, Santiago-Rosario et al. 2021). In most plants, sodium enters through the root system and rapidly is allocated to vacuoles which decreases water potential towards aboveground tissues, resulting in dehydration and drought-induced stress (Cheeseman and Wickens 1986a, 1986b, Pantha and Dassanayake 2020). Yet, some plants have evolved a certain mechanism to deal with pervasive sodium substrate presence. For instance, some plants have evolved salt glands and bladders to extrude sodium salts from their tissues aiding in ionic regulation of aboveground tissues (Grigore et al. 2014, Sanadhya et al. 2015, Dassanayake and Larkin 2017). Salt bladders in Amaranthaceae have been observed to reduce insect herbivory due to the exposure of insects to highly concentrated sodium crystals on leaf tissues (LoPresti 2014). Additionally, some plants have evolved a mechanism to prevent sodium intake from the roots which are mostly found across mangroves and other coastal plants (Parida et al. 2004, Kanai and Sakai 2021). Moreover, in some cases, plants have also evolved ways to use sodium as an osmoticum to increase water intake in xeric environments, a trait that is mostly related to halophytes, especially those found in the order Caryophyllales (Subbarao et al. 2003, Nieves-Cordones et al. 2016, Mancarella et al. 2016, White et al. 2017). Even though plants have evolved these mechanisms, sodium is still toxic at high levels with all plants unable to overcome salinity-induced stress and other physiological effects (Santiago-Rosario et al. 2021).
For animals, sodium is an essential element. Sodium maintains osmoregulatory processes, ensures cell stimuli via sodium channels, promotes muscle and neuron function, and maintains blood pH, pressure, and volume (NRC 2005). Sodium is particularly limiting for terrestrial animals, where herbivores are mostly impacted by sodium-deficient diets. Plants across terrestrial environments differ greatly in sodium concentrations (see Chapter 2) which has effects on herbivore performance, development, and behavior.

Physiologically, the abundance of sodium in animal tissues is controlled by the hormone aldosterone which helps maintain animal bodies from dehydrating. This process is tightly regulated by maintaining appropriate levels of sodium ions along with water across the extracellular matrix. Animals encountering sodium-deficient diets can experience hyponatremia, high levels of pica (abnormal appetite), inhibition of growth and lactation, agitation, and eventually death by dehydration. Most farmed animals require between 0.1 to 0.4 percent of sodium in their diets for optimal growth and productivity (NRC 2005). On the other hand, a high intake of sodium can result in multiple effects depending on the frequency of exposure. Single high dose toxicity results in ataxia, muscle spasms, vomiting, emesis, convulsions, hypernatremia (high blood sodium), and even death when given in high amounts (Blaxland 1946, Boyd and Shanas 1963, Todd et al. 1964, Hardy 1989, Senturk and Huseyin 2004). Acute and chronic exposure to sodium can result in ataxia, diarrhea, cerebral necrosis, convulsions, hypernatremia, increased water consumption, and death (Meneely et al. 1953, Krista et al. 1961, Hardy 1989, NRC 2005). Therefore, these effects sodium have across the spectrum of its availability and intake can impact animal performance and behavior greatly, from increased seeking behavior to total avoidance when possible.
Across most terrestrial animals, the ability to taste sodium is highly conserved. Sodium is
the most sought-after salt-inducing unique foraging behavior and evolved physiological
mechanisms by which animals forage for the element (Geerling and Loewy 2008, Puri and Lee
2021). For instance, in natural settings, elephants, parrots, and bats frequent salt licks and salt-
rich puddles (collpa water) engaging in geophagy (Holdø et al. 2002, Burger and Gochfeld 2003,
Bravo et al. 2010a, 2010b). Some insects have also been observed to increase visitations to salt-
rich sources. For example, male lepidopterans drink from sodium-rich sources including puddles,
feces, fruits, carrion, seawater, and animal tears, supplementing their dietary needs and
enhancing mating success by increasing spermatophore sodium concentrations (Pivnick and
Watanabe and Kamikubo 2005, Mitra et al. 2016). Additionally, the availability of sodium in the
animals’ diets has been observed to influence trophic level interactions. In some cases,
omnivorous animals increase carnivory when sodium is limiting thus changing intra- and
interspecific dynamics of populations. For example, omnivorous ants increase carnivory as
sodium becomes more limiting, a pattern that is mostly observed in inland rather than coastal
populations (Clay et al. 2017). Also, dietary sodium limitations can affect intraspecific
interactions. For example, dietary sodium-limitation influences a shift to increased cannibalism
and migratory behaviors in Mormon crickets, *Anabrus simplex* (Orthoptera: Tettigoniidae),
impacting inclusive fitness (Simpson et al. 2006). Nevertheless, understanding the effects of
variation in plant dietary concentration of animal activity across the landscape remains poorly
known, especially in the context of sodium.

As an important economical element, the movement of sodium by anthropogenic means
has seen a dramatic shift after industrialization and agricultural practice have become more
mechanized. Even though salts have been moved by humans for millennia, humans have been moving sodium in larger quantities in the past century (Cirillo et al. 1994, Ivushkin et al. 2019, Hintz et al. 2021). For example, road salts are used in high quantities in freeze-prone areas across North America and Europe which affects plant and animal communities found in otherwise sodium-deprived habitats. These recent inputs have been observed to have consequences on plants development and community structure as well as impact animal development and behaviors across large geographical regions (Bryson and Barker 2002, Marosz 2011, Snell-Rood et al. 2014, Mitchell et al. 2020, Hintz et al. 2021, Shephard et al. 2021). Also, poor irrigation practice has increased soil salinization across important agricultural areas globally which has had a substantial reduction in agricultural yields given its toxicity for plant development (Oster 1994, Shahid et al. 2018, Mukhopadhyay et al. 2021). Studying the effects of environmental and anthropogenically-induced sodium variation across the landscape on plant and animal performance and behavior represent an important avenue of research.

**DISSERTATION SYNOPSIS**

In this dissertation, I considered the influences sodium has on the phytochemical landscape taking into consideration how plants deal with sodium in laboratory and field settings. I also have considered how variation in sodium phytochemical landscape influences herbivore activity (development and behavior) using an empirical approach. This is a trophic story, and I will summarize each chapter herein.

In Chapter 2, by using a systematic review approach, I assessed how plants dealt with increases in substrate sodium in controlled studies taking into consideration how increases of sodium in the substrate affect plant growth and sodium accumulation strategies. This was done by collecting already published data on 107 plant populations across 67 species in 20 plant
families and compiling plant responses in growth (biomass) and sodium accumulation across increasing substrate sodium treatments. Additionally, I performed a phylogenetic signal analysis to quantify whether species responses to increases in substrate sodium reflected phylogenetic conservatism, a pattern that was observed for growth phenotypes but not for sodium accumulation. One of the main results of Chapter 2 was the origination of the No-Escape-From-Sodium hypothesis which posits that, on average, plants’ tissue sodium concentrations reflect that of the substrate they are found in, at least, in controlled settings.

In Chapter 3, I follow the finding of Chapter 2 into field settings. I wanted to test whether plants across a heterogeneous gradient of sodium followed the No-Escape-From-Sodium hypothesis and if this pattern holds for other essential cations like calcium, magnesium, and potassium. I also tested how abiotic conditions influence plant cation accumulation and whether plant cation concentrations show spatial autocorrelation. Briefly, the results from this study show that plants do follow the No-Escape-From-Sodium hypothesis across field settings meaning that as environmental sodium increases plant sodium increases accordingly. Yet, plants appear to be maintaining essential cations (Ca, Mg, and K) tightly homeostatically regulated. In terms of what herbivores experience, sodium varies greatly across the landscape at least up to 30-fold suggesting a marked influence of the cation on animal performance across their ranges. For essential cations, this pattern might not hold as animals might be experiencing similar concentrations of calcium, magnesium, and potassium across their range, at least when it comes to the plant tissues they consume.

In Chapters 4 and 5, I move to understand how the variation in plant sodium concentration impacts the development and behavior of herbivores. For these two chapters, I used the common sunflower, *Helianthus annuus* (Asteraceae,) and the sunflower specialist
bordered patch butterfly, *Chlosyne lacinia* (Lepidoptera: Nymphalidae). To test whether sunflower sodium variation influences the development and behavior of *C. lacinia*, I created a hydroponic setup that was used to carefully control sodium accumulation by plant tissues. I wanted to know if accumulated sodium directly affected the development and behavior of the herbivore. Previous studies have looked at the addition of sodium to artificial diets or sprayed sodium-rich solution on leaves surfaces, but none have tested the effects of internalized sodium on herbivore performance. I exposed sunflowers plants to four treatments of sodium: no addition (0 % NaCl), low (0.1 % NaCl), medium (0.5 % NaCl), and high (1 % NaCl). These values are within the ranges found in field conditions as described in Chapter 2. Subsequently, larvae were exposed to plants reared on each treatment in groups (gregarious) or individually (solitary). In Chapter 4, I describe the effect plant sodium variation has on the developmental time, growth, and sodium accumulation strategies of female and male *C. lacinia* individuals. In chapter 5, I explored the effects plant sodium variation has on the onset of cannibalistic behaviors and mortality rate based on the plant sodium concentration. These results highlight the disproportionate effect variation in plant sodium has on herbivore performance and behavior across a spectrum of sodium availability.
CHAPTER 2
NO ESCAPE: THE INFLUENCE OF SUBSTRATE SODIUM ON PLANT GROWTH AND TISSUE SODIUM RESPONSES

INTRODUCTION

Plants are key conduits in many, especially terrestrial, biogeochemical cycles (Elser & Bennett, 2011; Farago, 1994; Neubauer, Givler, Valentine, & Megonigal, 2005; Yuan & Chen, 2015). As intermediaries between soils and higher trophic levels, they often control, limit, or enhance the availability of elements to consumers. Plant phytochemistry varies substantially in elemental composition, stoichiometry, and concentration of essential micronutrients for animals and decomposers (Farago, 1995; Sterner & Elser, 2002). Hunter (2016) envisioned the geographic patterning of phytochemistry as the phytochemical landscape. The phytochemical landscape of micronutrients has considerable effects on plant-herbivore interactions, as well as community and ecosystem dynamics across landscapes that vary in soils, climate, etc. (Clay, Yanoviak, & Kaspari, 2014; Kaspari, Yanoviak, & Dudley, 2008; Moore, Lawler, Wallis, Beale, & Foley, 2010). Nonetheless, the composition, formation, and intermediary function of the phytochemical landscape remains poorly characterized and understood (Hunter, 2016), especially for certain elements like sodium (Kaspari, 2020).

Sodium is the seventh most abundant element in the Earth’s crust (Kaspari, 2020). However, its presence in terrestrial ecosystems is highly heterogeneous but spatially correlated with xeric conditions, certain geological formations and proximity to a marine coast or source of...
marine aerosols (Kaspari, 2020; Martin, Coombes, & Dunstan, 2010; Smith, 2013; Stallard & Edmond, 1981). Sodium is unusual as a nutrient for life because although it is a non-essential element for most plants, it is a key and essential element for animals and decomposers (Kaspari, 2020). Although sodium requirements vary among organisms, the availability and intake of sodium are tightly linked to organismal performance across ecosystems and form fundamental components of ecological and evolutionary dynamics (Baxter & Dilkes, 2012; Kaspari, Yanoviak, Dudley, Yuan, & Clay, 2009; Sterner & Elser, 2002).

Plant populations and communities are exposed to a wide range of saline substrates across terrestrial landscapes. Many plants actively avoid or limit sodium intake and most plants tolerate sodium in soils to remarkably high levels (at milli-molar levels) before they show signs of growth defects compared to many other non-essential or toxic cations such as lithium or many heavy metals that induce toxicity symptoms at micro-molar levels (Nawaz, Iqbal, Bliek, & Schat, 2017; Pantha & Dassanayake, 2020; Shahzad et al., 2016; van Zelm, Zhang, & Testerink, 2020; Vithanage et al., 2019). Most plants can tolerate or can be acclimated to survive up to 200 mM NaCl in their growth media, but those plants that can complete their life cycles at salinity levels higher than 200 mM NaCl are generally identified as halophytes (Cheeseman, 2015; Flowers, Galal, & Bromham, 2010; Flowers, Hajibagheri, & Clipson, 1986). Unlike most plants, many halophytes need sodium to thrive and suffer growth defects under limited sodium (Bose et al., 2017; D. Wang et al., 2012). However, only about 1% of the global flora are considered halophytes; they are distributed in multiple plant clades that reflect their convergent evolution to saline environments (Flowers & Colmer, 2008).

Even though most plants do not need sodium, they cannot necessarily avoid it, nor escape having to cope with it. As sodium concentration increases in the substrate, its concentration in
plant tissue also generally increases, and in turn affects plant fitness, especially in plants highly sensitive to salt stress (Greenway & Munns, 1980; Pantha & Dassanayake, 2020; Yang & Guo, 2018; Zhu, 2001). With increasing sodium, plants have been shown to: decrease biomass accumulation; increase osmotic, oxidative, and ionic stress responses; and arrest growth due to changes in cellular biochemistry (Maathuis, 2014; Zhao, Zhang, Song, Zhu, & Shabala, 2020). Furthermore, variation in soil concentration of sodium salts has direct links to variation in foliar sodium, which in turn influences plant-herbivore interactions and higher trophic-level performance (Bravo, Harms, & Emmons, 2010, 2012; Cheeseman, 2015; Kaspari, 2020; Kaspari, Clay, Donoso, & Yanoviak, 2014; Snell-Rood et al., 2014).

Decades of physiological, biochemical, and genetic studies have contributed to our current understanding of how plants respond to salt stress. Even so, these studies have primarily targeted salt stress-sensitive model plants like Arabidopsis, salt-sensitive crops, or extremely tolerant halophytes. For example, most crops or Arabidopsis ecotypes will show signs of salt-stress at 100 mM NaCl (0.58 %) treatments, whereas some halophytes can survive salinities exceeding seawater strengths (3.5 %) (Debez, Saadaoui, Slama, Huchzermeier, & Abdelly, 2010; Flowers, 2004; Kazachkova et al., 2018; Zhu, 2000). However, these two extremes in the plant salt-tolerance spectrum represent less than 2% of all angiosperm diversity. Therefore, it is unclear how plants with varying degrees of salt-stress responses growing in diverse salinity conditions conform to general expectations of how sodium accumulates in plants and how this accumulation affects their growth.

We conducted a systematic review of 49 published studies that included 67 species and 107 cultivars or populations, to identify broad-scale patterns of salt accumulation and growth responses across terrestrial angiosperms. Employing a priori response models that we could test
against experimental data, we surveyed the relationships between plant biomass growth and substrate NaCl concentration from controlled experiments across taxa. We also characterized relationships between plant tissue sodium accumulation and substrate NaCl concentration across taxa and examined how biomass growth responses associate with sodium accumulation. Finally, we assessed phylogenetic patterning of growth and sodium accumulation responses to reveal the role that evolutionary history has played in the distribution of these traits.

MATERIALS AND METHODS

Article search and selection protocol

To determine the effects of experimentally controlled, laboratory- or greenhouse-based substrate sodium chloride (NaCl) treatments on plant biomass and sodium accumulation in their tissues, we searched for peer-reviewed studies using Web of Science in December 2017 and May 2019 following the PRISMA protocol (Moher et al., 2009). We performed an initial search in December 2017 using the search criteria: “sodium AND biomass AND plant AND growth;” a timespan of “All years;” and indexes “Sci Expanded.” These criteria yielded 6,503 articles. For a second search in May 2019, we used the keywords: “sodium AND biomass AND plant OR sodium AND growth AND plant OR sodium accumulation AND shoot AND root AND plant OR sodium AND plant AND halophytes AND biomass;” a timespan of “All years;” and indexes “Sci Expanded.” This search yielded 6,654 articles. Subsequently, 6,387 duplicates were removed from the dataset, which produced a total of 6,770 non-duplicate articles from the two searches.

The articles grouped into five unique categories: effects of sodium on growth, biomass, and tissue sodium accumulation in plants (1,305); salt related responses involving other taxa (animals, fungi, bacteria, protists, etc.) (906); transcriptomics, genomics, proteomics, or other
molecular responses (627); influences of other elements and/or compounds (1,750); and other miscellaneous articles (2,183). We used the 1,305 articles that provided data for growth (biomass accumulation) and sodium accumulation in plant tissues.

In plants, biomass or biomass growth are often used as proxies for fitness, because they are often highly correlated with plant fecundity and survivorship. In addition, these fitness metrics can be easily applied across taxa to answer comparable questions across multiple species (Younginger, Sirová, Cruzan, & Ballhorn, 2017). To investigate the relationship between substrate sodium and biomass changes, we retained 49 studies that reported above- and belowground dry biomass as well as above- and belowground sodium tissue concentration for a total of 107 cultivars, strains, or varieties (herein populations) of plants, in 67 species, 43 genera, and 20 families, across 16 orders (Supplementary Information: Table S1). Although these controlled experiments were conducted by different groups, in different controlled environments, and at different time scales, each used specific NaCl treatments between control and salt-treated plants for a uniform duration specific to each study, keeping all other macro- and micronutrients constant. The plant material subjected to NaCl treatments was mostly seedlings (80.37%), with the remaining studies conducted on cuttings (13.08%), rootstocks/grafts (3.74%), and bulbs (2.80%). Prior to analysis, we updated nomenclatural changes for all species considered in this study using Tropicos (www.tropicos.org) and NCBI taxonomical databases (Supplementary Information: Table S2).

Data extraction and compilation

Articles differed substantially in their data representation, ranging from tables to graphical illustrations. We directly extracted data from tables, whereas measurements in figures were extracted using WebPlotDigitizer (Rohatgi, 2019). Treatments of NaCl were converted
when necessary to mM. We focused on the mean responses of plants across treatments compared to their relevant control group as defined in each published study.

For biomass growth of above- (B_A), belowground (B_B) or total dry mass (B_T), we extracted and converted when necessary all measurements in grams. Above- and belowground biomass summed together equaled total plant biomass. We calculated relative biomass difference (RBD) for above-, belowground or total biomass as:

$$RBD = \frac{Treatment\ biomass}{Control\ biomass} - 1$$

Values of RBD greater than zero mean that growth under the treatment condition exceeded the growth observed for control plants. A negative RBD indicates that growth slowed in the salt-treated plants compared to the control plants. While we note that growth itself cannot be negative, negative RBD values may represent salt-induced shedding of leaves or similar plant responses that may directly affect the total biomass of experimental plants. RBD values corresponding to their raw experimental values for each study are given in Supplementary Information: Table S3.

Using the same methods described above, we extracted sodium concentrations per dry mass of above-, belowground or total tissues. It is important to note that some plants may have expelled sodium, by means of salt glands or other adaptations. Tissue sodium concentration was considered as reported by each study. Acceptable sodium concentration measurements included weight by weight basis (i.e., mg/g, mg/kg), molarity (i.e., µM, mM or M (mol/L)), molality (i.e., mol/g), percentage (%), or parts per million (ppm). We converted all measurements, when necessary, to percentage (%) values. Measurements of electrical conductivity (S/m or psu) were excluded because, unless stated, they do not necessarily reflect sodium concentrations accurately.
since conductivity results from multiple elemental ions (Carter & Gregorich, 2007). Above-ground (Na_A) and belowground (Na_B) tissue sodium concentrations (%) were used to calculate total plant sodium concentration (Na_T, %) using the formula:

$$Na_T = \left( \frac{B_A}{B_T} * Na_A \right) + \left( \frac{B_B}{B_T} * Na_B \right)$$

All extracted raw data for sodium accumulation have been organized in Supplementary Information: Table S4.

**Model design, selection, and population classification**

We postulated a set of *a priori* potential response models for both RBD (Table 1) and sodium accumulation (Table 2) as functions of substrate NaCl treatments. Each *a priori* model prediction was described by a mathematical function for the shape of the response curve. Three pairs of responses shared an underlying mathematical function. For growth (Table 1), the function for a straight line accounted for both linear increase and linear decrease models; the slope of the line was used to classify the respective response - positive slope indicated linear increase and negative slope indicated linear decrease. Also, the quadratic function accounted for both hump-shaped and non-linear decrease models. For sodium accumulation (Table 2), the quadratic function accounted for hump-shaped and non-linear increase. In these quadratic-function cases, we used the vertex value (a) to classify cases as hump-shaped (when a was negative) or non-linear decrease and non-linear increase (when a was positive).

We used an Information Criterion (IC) approach to select the model that best fit the data extracted for each population, using three different ICs: Akaike Information Criterion (AIC), the AIC small-sample corrected version (AICc), and Bayesian Information Criterion (BIC). We used
the R package ‘AICcmodavg’ to calculate AIC, AICc and BIC values (Mazerolle, 2020). Although we examined results from all three metrics, we based our conclusions on BIC, since this metric gave consistent results across the data sampled, it is more specific (reduced Type-I error or lower false-positive rate), and is considered a more conservative test, as advocated by Dziak, Coffman, Lanza, Li, and Jermiin (2020). AIC is mainly recommended for larger datasets and does not account for sample size. Furthermore, for AICc, the penalization that is given to the AIC formula increases the chances of overfitting the data due to the extremely small sample sizes for the data analyzed (Bolker, 2018; Dziak et al. 2020). The models from Tables 1 and 2 that best fit each response (i.e., the smallest BIC value) were used to designate a response shape for each population’s above-, belowground and total plant biomass growth and sodium accumulation, respectively. Since we based our conclusions on BIC, we provide the corresponding likelihood values, ΔBIC and BIC weights for each model chosen; we also share results from the other two IC metrics for comparison (Supplementary Information: Tables S5, S6, S7 and S8).

Fisher’s Exact Test contingency analysis with simulated p-values in R-Studio following recommendations from Broman and Caffo (2003) was used to test for significant differences between growth and Na accumulation. This test assumes that each population can be treated independently. This assumption may not be valid if the responses in certain groups are dependent on phylogenetic relationships (see next section for our analyses to test for such a bias).

To determine whether sodium accumulation differed by growth responses between above- and belowground tissues, for each growth response category we performed a Wilcoxon Test for paired values of above- vs. belowground tissue sodium concentrations. For this test we divided treatments into non-saline (0 mM treatment of NaCl) and saline treatments (30-300 mM...
treatment of NaCl). For the saline group, the highest treatment concentration for each population was selected within the treatment range of 30-300 mM of NaCl.
Table 2.1. *A priori* response predictions for relative biomass growth and models used to classify populations in plants exposed to increasing concentrations of NaCl in the substrate.

<table>
<thead>
<tr>
<th>Model ID</th>
<th>Equation</th>
<th>Classification</th>
<th>A priori representation</th>
<th>Criterion of classification</th>
<th>Total plant responses</th>
<th>Aboveground responses</th>
<th>Belowground responses</th>
<th>Biological significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$y = mx + b$</td>
<td>Linear increase</td>
<td><img src="image" alt="Graph" /></td>
<td>$m$</td>
<td>3 (2.8%)</td>
<td>3 (2.8%)</td>
<td>5 (4.7%)</td>
<td>Salt induced linear growth response.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Linear decrease</td>
<td><img src="image" alt="Graph" /></td>
<td>$-m$</td>
<td>40 (37.4%)</td>
<td>34 (31.8%)</td>
<td>31 (29%)</td>
<td>Salt sensitive linear decrease in relative growth.</td>
</tr>
<tr>
<td>II</td>
<td>$y = -e^x$</td>
<td>Threshold decline</td>
<td><img src="image" alt="Graph" /></td>
<td>$-e$</td>
<td>11 (10.3%)</td>
<td>11 (10.3)</td>
<td>15 (14%)</td>
<td>Salt insensitive growth at lower Na concentrations changed to rapid growth inhibition as external Na increases.</td>
</tr>
<tr>
<td>III</td>
<td>$y = ax^2 + bx + c$</td>
<td>Hump-shaped</td>
<td><img src="image" alt="Graph" /></td>
<td>$-a$</td>
<td>18 (16.8%)</td>
<td>18 (16.8%)</td>
<td>17 (15.9%)</td>
<td>Salt induced growth enhancement switches to growth inhibition as external Na increases.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-linear decrease</td>
<td><img src="image" alt="Graph" /></td>
<td>$a$</td>
<td>32 (29.9%)</td>
<td>33 (30.8%)</td>
<td>29 (27.1%)</td>
<td>Decelerating growth inhibition in response to increasing substrate salt.</td>
</tr>
<tr>
<td>IV</td>
<td>$y = b$</td>
<td>Zero slope</td>
<td><img src="image" alt="Graph" /></td>
<td></td>
<td>3 (2.8%)</td>
<td>3 (2.8%)</td>
<td>5 (4.7%)</td>
<td>Salt-insensitive growth.</td>
</tr>
</tbody>
</table>
Table 2.2. *A priori* response predictions for sodium accumulation responses and models used to classify populations in plants exposed to increasing concentrations of NaCl in the substrate.

<table>
<thead>
<tr>
<th>Model ID</th>
<th>Equation</th>
<th>Classification</th>
<th>A priori representation</th>
<th>Criterion of classification</th>
<th>Total plant responses</th>
<th>Aboveground responses</th>
<th>Belowground responses</th>
<th>Biological significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$y = mx + b$</td>
<td>Linear increase</td>
<td><img src="image" alt="Linear increase" /></td>
<td>$m$</td>
<td>35 (32.7%)</td>
<td>39 (36.4%)</td>
<td>35 (32.7%)</td>
<td>Plants steadily and monotonically increase accumulation of sodium as sodium in the substrate increases.</td>
</tr>
<tr>
<td>II</td>
<td>$y = e^x$</td>
<td>Exponential increase</td>
<td><img src="image" alt="Exponential increase" /></td>
<td>$e$</td>
<td>13 (12.1%)</td>
<td>12 (11.2%)</td>
<td>11 (10.3%)</td>
<td>Monotonic exponential increase in accumulation of sodium as sodium in the substrate increases.</td>
</tr>
<tr>
<td>III</td>
<td>$y = ax^2 + bx + c$</td>
<td>Hump-shaped</td>
<td><img src="image" alt="Hump-shaped" /></td>
<td>$-a$</td>
<td>12 (11.2%)</td>
<td>5 (4.7%)</td>
<td>14 (13.1%)</td>
<td>Accumulation of sodium increases to a maximum and then decreases as sodium in the substrate increases; this is a non-monotonic change, since the directionality of change reverses.</td>
</tr>
<tr>
<td>IV</td>
<td>$y = a - be^{-cx}$</td>
<td>Asymptotic increase</td>
<td><img src="image" alt="Asymptotic increase" /></td>
<td>$a$</td>
<td>7 (6.5%)</td>
<td>5 (4.7%)</td>
<td>6 (5.6%)</td>
<td>Monotonic increase in accumulation of sodium is non-linear as sodium in the substrate increases.</td>
</tr>
<tr>
<td>V</td>
<td>$y = \frac{1}{1 + e^{-x}}$</td>
<td>Sigmoidal increase</td>
<td><img src="image" alt="Sigmoidal increase" /></td>
<td>$1$</td>
<td>17 (15.9%)</td>
<td>16 (15%)</td>
<td>14 (13.1%)</td>
<td>Monotonic increase in accumulation of sodium is sigmoidal as sodium in the substrate increases.</td>
</tr>
<tr>
<td>VI</td>
<td>$y = b$</td>
<td>Zero slope</td>
<td><img src="image" alt="Zero slope" /></td>
<td>$b$</td>
<td>1 (0.9%)</td>
<td>1 (0.9%)</td>
<td>2 (1.9%)</td>
<td>Accumulation of sodium is unaffected by sodium in the substrate.</td>
</tr>
</tbody>
</table>
**Phylogenetic patterns among responses**

We performed a phylogenetic signal analysis to assess whether phylogenetic relationships may have influenced growth and Na accumulation responses in the diverse set of taxa used in our systematic review. Phylogenetic signal is the tendency of closely related species to resemble each other more in trait values than species drawn at random (Blomberg, Garland, & Ives, 2003; Münkemüller et al., 2012). We used a subset of the rooted and dated ALLMB phylogeny from Smith and Brown (2018) for our phylogenetic signal analyses; this phylogeny consists of a backbone from Magallón, Gómez-Acevedo, Sánchez-Reyes, and Hernández-Hernández (2015) and data from both GenBank and the Open Tree of Life (Smith & Brown, 2018; available from [https://github.com/FePhyFoFum/big_seed_plant_trees;](https://github.com/FePhyFoFum/big_seed_plant_trees; Supplementary Information: Table S2)). The phylogenetic tree of angiosperms was pruned using the ‘drop.tip’ function from the *ape* package (Paradis and Schliep 2019; v.5.3) to represent the species relevant to this study. In four cases (*Citrus sinensis*, *Solanum nigrum*, *Triglochin bulbosa*, and *Tripleurospermum maritimum*), subspecies were used as proxies in the phylogeny. For the genus *Narcissus*, we used the species *N. tazetta* for tree pruning (LoPresti, Pan, Goidell, Weber, & Karban, 2019). Additionally, for species that had multiple populations represented in our response dataset, we averaged population responses and selected the best models that fit the extracted data to assign overall responses for growth and sodium accumulation for each species (*Aeloropus lagopoides*, *Beta vulgaris*, *Brassica rapa*, *Cajan cajan*, *Eucalyptus camaldulensis*, *Gossypium hirsutum*, *Helianthus annuus*, *Lotus ceticus*, *Narcissus*, *Olea europaea*, *Oryza sativa*, *Phaseolus vulgaris*, *Solanum lycopersicum*, and *Solanum melongena*). A polytomy at the node for *Citrus* was resolved using the *phytools* package (Revell 2012) function ‘resolveNode’ and ‘multi2di’ function from the *ape* package (Paradis and Schliep 2019).
We tested for phylogenetic signals for the discrete characters of above-, belowground and total plant growth and sodium accumulation responses using the Maddison and Slatkin (1991) method in the ‘phylo.signal.disc’ function from Bush et al. (2016). This method estimates the minimum trait transitions at each node and compares this to a distribution sampled from a null model (Head et al., 2018; Paleo-López et al., 2016). We used 1000 randomizations to infer a significant result if the number of observed trait changes was significantly (alpha=0.05) less than the median of the null model distribution. All data were analyzed using R software version 3.6.3 (R Core Team 2020).

RESULTS

*Increasing substrate NaCl has varied effects on total plant growth responses*

Using model selection for each of our chosen 107 populations, we classified relative total plant growth responses as shown in Table 1 (Supplementary Information: Table S1). Growth was negatively affected as sodium increased in the substrate for most taxa. However, 12 taxa grew better in at least one treatment ≥200 mM NaCl. Growth was severely reduced in all populations that were exposed to NaCl concentrations >500 mM as compared to 0 mM of NaCl (Figure 1). None of the populations that we classified as having linear increase or zero slope biomass responses were exposed to treatments >360 mM NaCl.

Plant growth based on relative biomass difference showed similar trends in response to increased salinity regardless of the tissue sampled from above- or belowground (Supporting Information S1: Figure S1a, b). The overall growth patterns of above- or belowground tissue mirrored the patterns observed at the total plant level, as visualized by similarity in the alluvial plot (Figure 2a).
Figure 2.1. Populations’ responses to increasing substrate NaCl concentrations. Total relative biomass growth responses (a) across NaCl treatments for each population sampled in the study. Negative and positive values represent a growth inhibition or an increase, respectively, in growth relative to control NaCl substrate concentrations. Also, the effect of NaCl treatments on total plant sodium accumulation (b) across increasing NaCl substrate concentrations for each population. The main data shown cover the range from 0 to 600 mM treatments of NaCl. An inset with the complete dataset and treatments is included with each panel. Colors represent the responses that describe biomass growth and sodium accumulation responses, as in Tables 1 and 2.
Figure 2.2. Alluvial plot describing the association between above- and belowground phenotype responses to total plant biomass (a) and sodium accumulation (b). Thickness of each connector indicates the proportion of populations in each response group.
**Total plant sodium increases as substrate sodium increases**

Using model selection for each of the 107 populations, we classified total plant sodium accumulation responses into 6 groups shown in Table 2 (Supplementary Information: Table S1). The total sodium concentration within a plant generally increased as the substrate concentration of sodium increased (Figure 1b). However, the level of sodium accumulation was highly variable among populations and between above and belowground tissues (Supporting Information S1: Figure S1c, d). Notably, the aboveground sodium concentrations were generally higher than in belowground tissues for most populations in saline treatments (Supporting Information S1: Figure S1c, d). Additionally, regardless of the variation observed, both relative above- and belowground responses were similar to relative total sodium accumulation responses (Figure 2b).

**Crop species do not adequately represent general plant responses**

In our study, crop species represented 43.3% (29) with only 7 of them including populations surpassing 200 mM experimental exposure to substrate NaCl (Figure 3). Growth responses were generally more variable in non-crop populations, with hump-shaped growth responses being more prominent in non-crop (26.7 %) as compared to crop (4.8%) populations (Figure 3a). Moreover, percent differences in tissue sodium concentration varied more in non-crop as compared to crop populations (*i.e.*, variability in tissue sodium concentration was higher in non-crop taxa) (Figure 4).
Figure 2.3. Growth responses to increasing substrate NaCl for (a) crop and (b) non-crop populations.
Figure 2.4. Sodium accumulation responses to increasing substrate NaCl for (a) crop and (b) non-crop populations.

**Plant growth responses do not predict sodium accumulation responses**

Total plant biomass growth responses were largely independent of the type of sodium accumulation response, which we illustrate using an alluvial plot ($p = 0.43$; Figure 2.5).

Furthermore, irrespective of the growth response, tissue sodium concentrations increased monotonically in the majority of populations, *i.e.*, increase in plant sodium continued at a steady positive rate as sodium in the substrate increased or increased to a plateau for 77% of the populations (Figure 2.1 and 2.5).
Only those populations with hump-shaped growth responses differed significantly in sodium accumulation between above- and belowground tissues across saline treatments (Wilcoxon Test: n= 17, Z=1.9, p>0.046). There were no statistically significant differences for any other biomass growth responses between sodium accumulation of above- versus belowground tissues across saline treatments. Additionally, for non-saline treatments, there was no statistically significant difference for any biomass growth response groups when above-and belowground sodium accumulation was compared (Figure 2.6).

Figure 2.5. Alluvial plot describing the associations between biomass growth and sodium accumulation responses. Sodium accumulation responses were either monotonically increasing (grey) or not (maroon). Thickness of each connector indicates the proportion of populations in each response group. Responses for growth where abbreviated as follows: Hump- shape (HS), linear decrease (LD), linear increase (LI), nonlinear decrease (NLD), threshold decline (TD) and zero slope (ZS). For sodium accumulation responses were abbreviated as follows: Asymptotic increase (AS), exponential increase (EI), hump- shaped (HS), linear increase (LI), non- linear increase (NLI), sigmoidal increase (SI) and zero slope (ZS).
Figure 2.6. Mean log-transformed tissue sodium concentration (%) (and SE) for above- and belowground tissues across biomass growth responses for non-saline (0 mM NaCl) and saline treatments (30–300 mM NaCl). Significant differences (p < .001, Wilcoxon Test) for above- and belowground mean response comparisons are indicated by asterisks (***). Sample sizes for each growth response for above- and belowground tissues were the same for non-saline and saline treatments: hump-shaped = 17; linear decrease = 36; linear increase = 3; non-linear decrease = 32; threshold decline = 11; and zero slope = 3.

**Phylogenetic relationships predict biomass growth but not sodium accumulation responses**

Biomass growth, both above- and belowground, showed significant phylogenetic signal (i.e., phylogenetic relationships help explain the distribution of the trait across the phylogenetic tree in our dataset; p = 0.031 and p = 0.046, respectively; Figure 2.7). We recovered 28 observed evolutionary transitions (i.e., the change from one discrete trait to another) with a randomization
median of 35 for aboveground biomass growth response. Belowground biomass growth response showed 33 observed evolutionary transitions and a randomization median of 37 transitions. We found significant phylogenetic signal for total biomass response ($p = 0.012$) with 29 observed evolutionary transitions and 34 median randomization transitions. Most of the species in the order Caryophyllales, especially in the family Amaranthaceae, expressed a hump-shaped biomass growth response as sodium increased in the substrate. However, hump-shaped responses were also found in other plant orders, reflecting potential independent evolutionary origins.

Sodium accumulation responses (both above- and belowground) were not significantly phylogenetically organized, i.e., did not show significant phylogenetic signal ($p = 0.37$ and $p = 0.184$, respectively; Figure 2.8). For aboveground sodium accumulation response, there were 36 observed evolutionary transitions while the randomization median was 37. We found 35 observed evolutionary transitions and 37 randomized median transitions for belowground sodium accumulation response. No phylogenetic signal was found for total plant sodium accumulation response ($p = 0.161$) and we recovered 38 observed transitions with a randomized median of 40 transitions. For the orders most sampled, Caryophyllales and Poales, responses for sodium accumulation differed substantially across and within genera, with no apparent pattern observed. Plants appeared to accumulate sodium in different ways and patterns regardless of their biomass growth responses.
Figure 2.7. Total (T), above- (A) and belowground (B) plant biomass growth responses mapped onto a phylogeny. Tips represent species pruned from rooted and dated ALLMB phylogeny from Smith and Brown (2018). Plant orders are indicated to the right of the phylogeny.
Figure 2.8. Total (T), above- (A) and belowground (B) plant sodium accumulation responses mapped onto a phylogeny. Tips represent species pruned from rooted and dated ALLMB phylogeny from Smith and Brown (2018). Plant orders are indicated to the right of the phylogeny.

**DISCUSSION**

Understanding the influence of sodium in the substrate on plant performance (growth, fitness) and tissue sodium accumulation is essential to comprehend ecological and evolutionary dynamics of plants across terrestrial environments. Our study emphasizes that plant adaptations
to substrate sodium vary substantially across taxa in terms of growth and sodium accumulation with a degree of phylogenetic conservatism. However, regardless of growth responses, sodium accumulation mostly followed an increasing trend and did not have any apparent association to growth responses as substrate sodium levels increased. Additionally, we note that for the taxa for which we had data, domesticated plant species present a narrower range of variation among salt stress responses as compared to non-crop species. In order to understand how substrate sodium influences plant growth, we must consider non-crop species in our studies. Moreover, we advocate the importance of characterizing responses using a systematic approach and we provide recommendations on experimental designs to reach a broader understanding of plant-salt stress.

*Increasing substrate sodium influences plant growth and sodium accumulation in variable ways*

Saline soils are known to hinder plant growth and crop losses are reported when soil salinity is above a crop-specific threshold (Bernstein, 1975; Zhao et al., 2020; Zörb, Geilfus, & Dietz, 2019). Whereas our analysis is aligned with this general consensus on the negative impact of soil salinity on plant growth, it sheds light on how plant growth varied in response to substrate NaCl levels across plant taxa that ranged from highly studied crops to scarcely examined wild species (Table 2.1 and Figure 2.2 a, b). Despite the overall trend of decreased biomass concurrent to increasing substrate NaCl levels, several taxa in the order Caryophyllales (*e.g.*, families Amaranthaceae, Plumbaginaceae and Portulacaceae) showed a hump-shaped or linear increase in biomass growth to increasing substrate NaCl (Figure 2.1a and 2.7). Most halophytes are non-randomly distributed and the order Caryophyllales holds the greatest number of recorded halophytes among angiosperms (Flowers et al., 2010). Halophytes not only are tolerant of high NaCl, but also use Na$^+$ and Cl$^-$ ions for osmotic adjustment in an energetically favorable manner
and are equipped with structural and physiological traits that aid the compartmentalization of salts to promote growth while avoiding ionic or osmotic stress until threshold NaCl levels are reached (Munns, Passioura, Colmer, & Byrt, 2020; Slama, Abdelly, Bouchereau, Flowers, & Savouré, 2015). This set of characteristics would account for the positive growth in saline substrates that we observed within the Caryophyllales taxa (Figure 2.1a and 2.7). Furthermore, plants that follow these hump-shaped or linear increase growth responses to increasing substrate sodium follow a subsidy-stress gradient, i.e., at low substrate sodium levels overall plant growth is subsidized, reaching a threshold leading to growth inhibition due to salt stress as sodium in the substrate becomes toxic (Odum, Finn, & Franz, 1979). All plants that followed these trajectories in our analyses (Figure 2.7) are considered salt tolerant, as classified in the eHALOPH database (Santos, Al-Azzami, Aronson, & Flowers, 2016) and by the respective authors in each study (Supplementary Information: Table S1). Regardless, even among those salt-tolerant taxa, plant biomass eventually decreased at the highest NaCl concentrations (Figure 2.1a). The use of sodium as an inexpensive osmolyte has convergently evolved in many halophytes as well as other plants adapted to water deficit stress and is found in multiple orders of plants. For example, even at low sodium levels in the soil, the xeric adapted plant, *Zygophyllum xanthoxylum* (Zygophyllaceae), accumulates high concentrations of sodium in shoots, resulting in large mesophyll cells leading to leaf succulence (Xi et al., 2018).

The taxa that showed linear or non-linear decreases (Figures 2.1a and 2.7) as NaCl increased in the substrate are non-halophytes highly sensitive to salt stress where growth is inhibited by excess salts (Munns et al., 2020; van Zelm et al., 2020). Moreover, we found that closely related lineages resembled each other with respect to biomass growth responses (i.e., significant phylogenetic signal indicating shared physiological responses within clades); thus, the
patterns observed in this trait are at least somewhat explained by shared evolutionary history (Figure 2.7). However, phylogenetic patterns do not account for sodium accumulation responses (Figure 2.8).

In plants, tissue sodium concentrations are generally linked with increasing substrate sodium concentrations (Figure 2.1b). However, plant sodium accumulation seemed to be uncoupled from biomass growth responses and any discernible phylogenetic signal among taxa (Figures 2.5 and 2.8). Similar patterns were observed when aboveground sodium accumulation was compared in the species *Plantago maritima* and *P. media* as NaCl in the substrate was increased (Maathuis, 2014; note that these populations – among others in the literature – were not included in the current study since they did not meet the criteria for our selection). The variation in responses by each species was mainly due to differential and discrete tolerance thresholds and external sodium concentrations (Maathuis, 2014), which might explain the idiosyncratic variation that was observed among taxa used in this study in terms of sodium accumulation responses (Figures 2.1b and 2.8).

Additionally, the accumulation of higher amounts of sodium in aboveground (Supporting Information S1: Figure S1c) than belowground (Supporting Information S1: Figure S1d) tissues is apparent when comparing sodium accumulation responses for each population across increasing treatments of substrate NaCl (Figure 2.1b). This observation agrees with the current understanding that sodium, once in the transpiration stream, is retained in the shoots as phloem re-circulation to roots and is considerably less then xylem loading from roots to shoots (Munns, 2002; Munns & Tester, 2008). Sodium accumulation in the shoots is dependent on the local tissue and species-specific tolerance capacity. Plants are known to store excess sodium in older leaves to protect younger growing tissue from salt toxicity and sustain growth until species-
specific tolerance levels are reached (Munns & Tester, 2008). Alternatively, a few halophytes have developed salt glands to remove sodium from shoots against a concentration gradient – an adaptation that is found in several plant orders (Dassanayake & Larkin, 2017).

Once sodium enters the roots, plants have transporters that preferentially export sodium back to the soil at an energy cost. However, this capacity to export sodium at the soil-root interphase is easily exceeded even among halophytes and accumulation of sodium inside the plant is unavoidable when external sodium concentrations increase (Zhao et al., 2020). Therefore, other sodium transporters that facilitate ionic balance throughout the plant organs play critical roles in sustaining growth or survival during salt stress (Apse & Blumwald, 2007; Yamaguchi, Hamamoto, & Uozumi, 2013). Our systematic review agrees with previous studies investigating single or small groups of taxa subjected to salt stress to highlight that almost all plants accumulated sodium monotonically (or nearly monotonically) as sodium increased in the substrate (Figures 2.1b and 2.5). Plants that expressed the biomass growth hump-shaped response accumulated significantly higher concentrations of sodium in above- than belowground tissues. Alternatively, populations characterized by the other growth responses did not differ significantly in above- vs. belowground sodium accumulation in saline treatments but not in non-saline treatments (Figure 2.6). We discussed earlier that the hump-shaped response was preferentially represented by taxa in the order Caryophyllales, and that this clade is an evolutionary hotspot for halophytes, but this response is not confined to the order (Figure 2.7). Furthermore, Caryophyllales species often are shoot sodium hyperaccumulators; they are enriched in plants that develop salt glands and have a higher tolerance to higher tissue sodium levels compared to predominantly salt-sensitive orders (Dassanayake & Larkin, 2017; Flowers et al., 2010; White et al., 2017).
Domesticated plant species tend to occupy a narrow range of variation among salt stress responses

Our systematic review demonstrated a clear dichotomy between salt tolerance (deduced from growth responses) during increased external sodium in crops compared to wild species or plants that have not been subjected to domestication. Most wild species in our study tend to have a higher capacity to tolerate higher tissue sodium than crop or domesticated species (Figure 2.2a, b). The exception to this is seen with crops in Caryophyllales, such as *Beta vulgaris*, *Salicornia bigelovii*, and *Spinacia oleracea* (Choo, Song, & Albert, 2001; Wu, Liang, Feng, & Zhang, 2013; Yamada, Kuroda, & Fujiyama, 2016). Recent studies have illustrated how crop species have lost traits related to salt tolerance their ancestral wild relatives had before domestication (Quan et al., 2018; Rozema et al., 2015; Z. Wang et al., 2021, 2020).

The individual studies used for our systematic review are limited to small and variable sample sizes among populations, differing treatment concentrations of NaCl, and include a mixture of crop (43.3%) and non-crop (56.7%) species. Salt stress responses in plants are known to vary in how the salt treatment is given (acclimated treatment vs salt shock), duration of the treatment, the age of the plants, plant growth conditions (e.g., light levels, presence of other stresses, and grown hydroponically or in soil, tidal systems, submerged systems), plant habit (e.g., herb vs tree, creeper vs upright), life history traits (e.g., annual vs perennial, frequency of flowering), morphological traits of the plants (e.g., presence or absence of salt glands, ability to produce succulent leaves, structural adaptations in roots), among many other genetic and environmental factors (Polle & Chen, 2015; Zhao et al., 2020). Plant survival compared to growth may use different adaptive traits among plants and biomass may not be the only indicator nor the optimal indicator to measure salt responses among different groups of plants. Therefore,
systematic, and rigorous studies need to be performed to understand overall mechanisms underlying salt stress responses across taxa, as discussed in the next sections.

**Characterizing responses promotes our understanding of plant-salt stress**

The modeling approach that we used in this study provides a useful way to quantify and categorize individual plant population responses to variation in NaCl in the substrate. These models describe the response trajectories of biomass growth and sodium accumulation responses and could be used extensively across taxa of interest. By using an Information Criterion approach, one can select the best-fit model for each population, given that our formulated models (e.g., linear decrease, hump-shaped, etc.) effectively describe natural patterns (Brewer, Butler, & Cooksley, 2016), within and among species (Table 2.1 and 2.2). For many purposes, it may be more useful to categorize plants by their responses across a range of sodium conditions, as opposed to performance above and below strict thresholds, as is often done with halophytic or salt tolerant plants (see Grigore, Ivanescu, and Toma, (2014) for a review on definitions and descriptions related to halophytes).

**Experimental design to achieve broader understanding**

Many studies have tested the effects of NaCl on plant growth and yield, especially in crop species (Cheeseman, 2015). However, because of differences in methodology, it is a challenge to make comparisons and contrasts of results across studies. We make several observations and recommendations for future studies:

a. Often, there is a lack of enough replication and/or treatments. For us, this prevented effective response pattern identification in some cases, especially in studies that presented only three treatments with few replicates.
b. The determination of treatments was often arbitrary. Limitations are imposed using independent categorical variables (ANOVA-based approach) instead of applying treatments as independent numeric discrete or continuous variables (regression-based approach). Experimental designs that cover a wide range of treatments may provide more accurate estimates. A regression-based approach allows one to better fit non-linear responses, which encompasses most of the responses we measured in our study (Inouye, 2001; Whitlock & Schluter, 2014). Additionally, when resources are limited, experimental design should prioritize increasing the number of treatments over increasing number of replicates per treatment. Furthermore, functional growth analysis (i.e., the assessment of absolute growth rate and relative growth rate) should be performed to better comprehend how plants manage resources at different life stages or across multiple environmental stresses, especially in the context of biomass growth and ionic accumulation (Cheeseman & Wickens, 1986; Tessmer, Jiao, Cruz, Kramer, & Chen, 2013).

c. Most of the plants in the studies selected were not exposed to the highest levels of sodium they could potentially encounter in nature. Lack of these data thwarts the complete description of responses associated with increasing substrate NaCl within and across taxa. Linear increase responses are highly unlikely across all NaCl concentrations observed in nature. This type of response in our study likely results from lack of high NaCl treatments. Under the full range of NaCl, these taxa would most likely have hump-shaped responses. Additionally, we observed that in non-saline treatments (0 mM substrate NaCl), substantially large amounts of sodium were found in some plant taxa. The reason for this could have been the lack of attention to the ionic salts used in the
Hoagland solution; some salts are combined with sodium (i.e., EDTA, Na$_2$MoO$_4$ 2H$_2$O, etc). Another reason could be the use of tap water instead of distilled or deionized water. Generally, a combination of copper, calcium, magnesium, and sodium is found in tap water on average at 1%, with some regional variation (Patterson, Pehrsson and Perry, 2013).

d. Many of the plants in the studies selected were grown under controlled conditions using watering regimes and nutrient mixes that do not closely reflect conditions in nature. Future research should focus on plant morphological, physiological, and adaptive responses to treatment solutions and/or substrates that truly match conditions (water availability, nutrient stoichiometry, etc.) potentially found in nature.

e. Studies generally focus on biomass to the exclusion of other fitness-related traits. Even though biomass is often an acceptable proxy for fitness measurements in plants (Younginger et al., 2017), observations on flower production, survivorship, seed set, and seed germination success should be quantified, to provide a more complete understanding of sodium’s influence on whole-plant performance and fitness (Primack & Kang, 1989).

f. Studies also should consider that salt stress is often combined with water deficit and heat stress, or other nutrient stresses in natural habitats. Additionally, biotic stresses such as herbivory and disease can compound the overall plant response to salt stress, with special consideration of wild taxa. The net outcome of plant performance under these natural conditions needs to be assessed and compared to responses observed under controlled environments to be able to model plant responses at community or ecosystem scales.
Moving toward an ecological-evolutionary perspective: from the lab to the field

We focused on plant performance and sodium accumulation strategies in controlled settings as reported in the literature, which emphasizes the physiological aspects of substrate sodium rather than the ecological and selective effects of sodium on plant performance, including fitness, under environmental conditions in nature. More importantly, this systematic review suggests support for the general No-Escape-From-Sodium Hypothesis, *i.e.*, that generally plants' tissue sodium levels reflect (at least in a ranked fashion) substrate/solution sodium levels irrespective of their growth responses to sodium (potentially with key and interesting exceptions). We still have a long way to go to be able to fully test this hypothesis, especially under the natural field conditions that truly matter for plant evolution, ecology, and farming.

Moreover, assessments of the phytochemical landscape of sodium across large geographical areas is increasing, with examples in *Ficus* in Central and South America (Bravo & Harms, 2017), *Asclepias* (milkweeds) in Minnesota (Mitchell et al., 2020), among roadside plant communities in Massachusetts (Bryson & Barker, 2002), and across global grasslands (Borer et al., 2019). These examples demonstrate that aboveground plant sodium accumulation co-varies closely with some abiotic factors, including but not limited to: effective distance from nearest coast/saline habitat; road salt pollution; and concentration of sodium in the soil. However, experimental designs that include comprehensive plant growth meta-data, phenotyping, and careful selection of target plants to allow rigorous, yet broad comparisons are needed. Following our recommendations would help advance our understanding of the complexity of the formation of the phytochemical landscape of sodium and its ecological and evolutionary consequences for plant performance, sodium accumulation and plant-herbivore interactions. In any case, saltier
plants in saltier soils is proving to be a broadly general pattern for sodium, which begs the future research question: how do plants respond to all of the other elements in their substrates?
CHAPTER 3
CONTRASTS AMONG CATIONIC PHYTOCHEMICAL LANDSCAPES IN THE SOUTHERN UNITED STATES

INTRODUCTION

As essential components of communities and contributors to ecosystem function, plants and other primary producers represent key conduits that link substrates to higher trophic levels in food webs and biogeochemical cycles (Austin & Zanne, 2015; Farago, 1994; Kaspari & Powers, 2016; Sterner & Elser, 2002; Wang, Houlton, & Field, 2007; Waring et al., 2015; Welti et al., 2017). Plants make soil-borne elements available to herbivores and other consumers, often while altering the relative proportions of those elements, i.e., the ecological stoichiometry of those elements (Sterner & Elser 2002). Thus, the formation and maintenance of the phytochemical landscape, as envisioned by Hunter (2016), strongly influences plant-herbivore interactions, community assembly, and ecosystem dynamics across landscapes that differ in climatic conditions, soil composition, and other properties (Filipiak & Weiner 2017, Mitchell et al. 2020, Moore, Lawler, Wallis, Beale, & Foley (2010), Stallard & Edmond (1981), Zhang, Zhang, Slik, & Cao 2012). Yet, our understanding of the formation, composition, and function of phytochemical landscapes of all biotically important elements remains relatively understudied, especially elements like sodium (Na) and other influential cations as noted by Hunter, (2016), Kaspari, (2021), and Kaspari & Powers, (2016).

As an often biologically critical element, Na is distinctive. It is generally considered nonessential for most plants, yet it is a key and essential nutrient for animals and decomposers (Clay, Yanoviak, & Kaspari, 2014; Kaspari, 2020, 2021; Kronzucker, Coskun, Schulze, Wong, & Britto, 2013). The distribution of Na across terrestrial habitats is exceptionally heterogeneous.
Dry or xeric habitats, certain geological formations (e.g., geological salt depositions, salt domes, sodium feldspar rocks), and coastal habitats often have the highest concentrations of environmentally available Na ions (Kapustina, 2001; Kaspari, 2020; Martin, Coombes, & Dunstan, 2010; National Atmospheric Deposition Program (NRSP-3), 2020; Smith, 2013; Stallard & Edmond, 1981). High variation and shortfalls in environmental Na have important consequences for organismal behavior, physiological performance, species interactions, and community assembly (Borer et al., 2019; Bradshaw & Bradshaw, 1999; Bravo, Harms, & Emmons, 2012; Brewer & Grace, 1990; Clay, Shepard, Garda, Mesquita, & Vasconcellos, 2022; Clay et al., 2014; Prather et al., 2018; Snell-Rood, Espeset, Boser, White, & Smykalski, 2014).

Sodium is considered nonessential for development of most plants (Grigore, Villanueva, Boscaiu, & Vicente, 2012; Kronzucker et al., 2013). Notable exceptions in which Na benefits development or performance include most halophytes (Cheeseman, 2015; Flowers & Colmer, 2008; Kanai & Sakai, 2021) in certain environmental conditions, including specific ranges of Na concentration in the substrate (Santiago-Rosario at al., 2021). Certain C₄ (photosynthesis via C₄ carbon fixation or the Hatch-Slack pathway) and crassulacean acid metabolism (CAM) plants benefit - at specific substrate concentrations - from slight increases in substrate Na (Furumoto et al., 2011; Subbarao, Ito, Berry, & Wheeler, 2003). Some C₄ plant species in the families Amaranthaceae, Asteraceae, Brassicaceae, Cyperaceae, Fabaceae, Poaceae, Portulacaceae, and Solanaceae, among others, found at relatively low concentrations of substrate Na, benefit from slight increases in Na by increasing biomass yield and reducing chlorosis (Brownell & Crossland, 1972; Johnston, Grof, & Brownell, 1988; Pessarakli & Marcum, 2000). For example, for species in the genus *Flaveria* (Asteraceae), Na is an essential nutrient as a transporter.
required for C₄ photosynthesis (Furumoto et al., 2011). Additionally, Na increases growth in the CAM species *Bryophyllum delagoense* (Crassulaceae) when substrate Na was increased to 0.1 meq/L NaCl as compared to individuals in basal culture solution (0.07 µeq/L NaCl) especially when grown under conditions of short-day length and high diurnal temperature variation (Brownell & Crossland, 1974). However, it is important to note that whether Na’s effect on these species relates to drought adaptations and whether Na represents a micronutrient to them remain unresolved topics among plant physiologists (Brownell, 1968; Subbarao et al., 2003).

The No-Escape-from-Sodium Hypothesis posits that plants' tissues broadly increase in Na concentration as the concentration of Na in the substrate or solution increases, irrespective of their growth responses, and there is empirical support for this pattern across plant taxa (Santiago-Rosario et al., 2021). However, our understanding of how plants respond to increasing substrate Na comes mostly from controlled laboratory and greenhouse settings, which may or may not align with patterns in nature. In the current study, I tested the No-Escape-from-Sodium Hypothesis in the field across the southern continental United States. I also included three additional cations: calcium (Ca), magnesium (Mg), and potassium (K) given their essentiality for plants, and their ubiquitous distribution across the soilscape (see Table 3.1 for a summary of their function in plants), and their affordable measurement. I aimed to characterize the phytochemical landscape for these four cations, in the southern United States, asking how they vary across plants, soils, and environments and whether there is evidence for homeostatic regulation and spatial autocorrelation. To achieve this aim, I collected plants from the widespread genera *Atriplex, Helianthus*, and *Opuntia*, along with adjacent soils.

<table>
<thead>
<tr>
<th>Cation</th>
<th>Function in plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (Ca)</td>
<td>- Cell wall stabilization&lt;br&gt;  o Structural component of the cell wall middle lamella&lt;br&gt; - Cell extension and secretory processes&lt;br&gt;  o Promotes cell elongation in roots, shoots, and pollen tubes&lt;br&gt;  o Aids in exocytosis of cell wall materials or secretion of mucilage.&lt;br&gt; - Membrane stabilization&lt;br&gt; - Maintaining homeostasis&lt;br&gt;  o Cation-anion balance&lt;br&gt;  o Osmoregulation&lt;br&gt; - As a second messenger of environmental stress&lt;br&gt;  o Variation in Ca concentration in the cytosol regulates cell division, cell elongation, among other cellular responses, in response to environmental influences and/or plant phenology</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>- Plays a major role in photosynthesis&lt;br&gt;  o Central atom of the chlorophyll molecule&lt;br&gt; - Maintaining homeostasis&lt;br&gt;  o Promoted enzymatic regulation&lt;br&gt;  o Regulates cellular pH&lt;br&gt;  o Regulates cation-anion balance&lt;br&gt; - Protein synthesis&lt;br&gt;  o Aggregation of ribosomal subunits</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>- Osmoregulation&lt;br&gt;  o Maintains osmotic potential of cells and tissues&lt;br&gt;  o Maintains turgor pressure&lt;br&gt;  o Promoted stomatal movement by controlling guard cell osmotic pressure&lt;br&gt;  o Influences photonastic and seismonastic movements&lt;br&gt; - Homeostasis maintenance&lt;br&gt;  o Facilitates stabilization of cytosol pH aiding enzymatic activation and reaction&lt;br&gt;  o Cation-anion balance&lt;br&gt;  o Promotes phloem transport of sucrose and other carbohydrates&lt;br&gt; - Stimulates protein synthesis by aiding protein translation processes&lt;br&gt;  o Promotes accumulation of soluble N compounds such as amino acids, amides, and nitrates&lt;br&gt; - Supports photosynthesis&lt;br&gt;  o Promotes stomatal regulation&lt;br&gt;  o Stimulates CO₂ fixation&lt;br&gt;  o Control respiration rates</td>
</tr>
</tbody>
</table>
MATERIALS AND METHODS

Plant taxa, field collections, and elemental analysis

I selected the plant genera *Atriplex* (saltbushes), *Helianthus* (sunflowers), and *Opuntia* (prickly pears) for our study, based on their photosynthetic pathways and their widespread distribution across the conterminous United States. *Atriplex* comprises 65 North American species using the C₄ photosynthetic pathway (Brown, 1956; Kadereit, Mavrodi ev, Zacharias, & Sukhorukov, 2010). *Helianthus* species use the C₃ photosynthetic pathway and include over 49 species across North America (Heiser, Smith, Clevenger, & Martin, 1969; Timme, Simpson, & Linder, 2007; Vanaja et al., 2011). Finally, *Opuntia* comprises ~110 species in North America, with all species using the CAM photosynthesis pathway (Cui & Nobel, 1994; Majure et al., 2012).

I collected *Atriplex*, *Helianthus*, and *Opuntia* plants in 51, 131, and 83 sites, respectively. Collection sites ranged from Florida (~86° W) to California (~123° W). Sampling was completed during the summers of 2018, 2019, and 2020 (Figure 1). I collected samples of aboveground tissues (leaves and stems), adjacent soil (top 10 cm), and one voucher specimen, for genus verification, from each site, along with GPS coordinates.

Plant and soil samples were oven-dried at 65° C for seven days. Aboveground tissues (*i.e.*, leaves and stems) were processed and ground into a powder for each site. Dried soil samples were passed through a 2 mm copper sieve to remove rocks and organic debris. Concentrations of Ca, Mg, K, Na, and P, on a dry mass basis, for soils and plant tissues were determined at the Soil Testing and Plant Analysis Laboratory at Louisiana State University (http://www.lsuagcenter.com), using inductively coupled plasma with atomic emission.
spectrometry (ICP-AES) following standard protocols (Munns, Wallace, Teakle, & Colmer, 2010). Soil pH (1:1 distilled water) was also measured.

**Climatic variables**

Bioclimatic data and elevation data were extracted for each site using the ‘raster’ package (Hijmans & van Etten, 2012) with a resolution of 2.5 arcminutes in R (Team, 2020). Climatic variables were annual mean temperature (°C*10), mean diurnal temperature range, temperature seasonality, annual precipitation (mm), precipitation in the wettest month (mm), precipitation in the driest month (mm), and precipitation seasonality (coefficient of variation). I also measured the distance from the nearest coast (km), as a proxy for the effective marine source of cations (as in Bravo & Harms, 2017) with Google Earth (http://www.google.com/earth/), using the circumference tool by expanding a circular area until the edge of the circle first contacted the coast. The radius of the circle was recorded as the effective distance to the nearest coast for each site. I considered potential marine cation sources using the Gulf of Mexico-Pacific Continental Divide since wind movement and precipitation on both sides of the divide are associated more closely with their respective oceanic sources (Adams & Comrie, 1997).

**Multivariate model selection and assessment of homeostasis**

To quantify the influence of abiotic conditions on plant cation concentrations, we performed a multivariate analysis with a model selection approach in R Studio using the packages ‘lmtest’ (Zeileis & Hothorn, 2002) and ‘MASS’ (Venables & Ripley, 2002) for each genus. Plant cation concentration data were initially normalized using a log$_{10}$ transformation for each site. We then examined linear relationships among the variables compared to plant cation concentration as the dependent variable. We assessed multicollinearity levels among variables
using a model selection approach. A model that included all variables was considered first using AIC. Using the function ‘vif’, the variance inflation factor (VIF) was quantified for each variable from the full model. Variables with a VIF >10 were removed to improve model accuracy and reduce the collinearity of the independent variables. Model accuracy was confirmed using the function ‘sigma’ by quantifying the associated error rate using the residual standard error with low values indicating high model accuracy.

I also performed a paired t-test to compare cation concentrations in aboveground plant tissues and adjacent soils along with the coefficient of variation for each cation and taxa. To calculate the standard error around the coefficient of variation, I used a bootstrapping method with replacement sampling. Additionally, to assess the potential for plants to regulate cations in the field I calculated the homeostasis coefficient $H$ (eta) for each cation and genus combination, as outlined in Sterner and Elser (2002). For this approach, I calculated a stoichiometric ratio based on phosphorus ($P$) concentration for plants and soils for each of the cations as follows:

$$\text{Equation 1: Stoichiometric ratio} = \frac{p}{\text{cation}}$$

Then, the homeostasis coefficient ($H$) was calculated using the modified formula:

$$\text{Equation 2: } \log y = \log c + \left(\frac{\log x}{H}\right)$$

where $y$ is the plant stoichiometry cationic ratio, $x$ is the cationic soil ratio, and $c$ is a constant. By plotting the logarithms of the plant versus soil stoichiometry, Sterner and Elser (2002) advise that slopes ($1/H$) between 1 and 0 indicate negative feedback between soil and plants, thus suggesting a homeostatic adjustment of cation concentration due to lower proportionality change in plant stoichiometry than in soil stoichiometry. An $H$ value of precisely 1 indicates a complete
lack of homeostasis, whereas as $H$ approaches infinity, the slope of consumer versus resource stoichiometry approaches zero, indicating strict homeostasis (Meunier, Malzahn, & Boersma, 2014; Sterner & Elser, 2002).

**Cation spatial autocorrelation analysis**

To quantify whether inter-site proximity influenced cation concentrations for aboveground plant tissues and adjacent soils, I performed a Mantel test using the package ‘ecodist’ (Goslee & Urban, 2007) for each genus separately. All cation concentrations were $\log_{10}$ transformed and compared from site to site in a pairwise manner. All values were compiled into a matrix of differences (*i.e.*, values closer to zero indicate more significant similarity in cation composition), and the absolute value was calculated to remove all negative values from the matrix. Additionally, I calculated the Haversine distance (km) (*i.e.*, the shortest distance between two points on the surface of a sphere) among sites using the package ‘geosphere’ (Hijmans, Williams, & Vennes, 2019).

I performed a Mantel test to assess whether: (1) plants nearby shared similar cation concentrations; (2) soils nearby shared similar cation concentrations; and (3) to test whether soil cation spatial patterns of similarity could explain plant cation spatial similarities. For a Mantel test, a significant result reveals that more minor differences in elemental composition are, on average, observed among pairs of sites that are close to each other than farther apart (Rossi, 1996). Correlations can be positive or negative, representing how the variables are associated. Correlation in the ‘ecodist’ package is calculated using a Spearman approach, and all Mantel test calculations were performed using 9,999 permutations. I also conducted a Mantel correlogram using the function ‘mgram’ of the package ‘ecodist’ by dividing the data into 20 distance classes.
A Mantel correlogram was performed for each cation and genus combination. All analyses were performed in R (Team, 2020).

RESULTS

*Cationic phytochemical landscapes differ between Na vs. Ca, Mg, and K*

Aboveground plant cation concentrations vary to differing degrees across the southern United States and among plant genera sampled. Ca (Figures 3.1a and 3.2a), Mg (Figures 3.1b and 3.2b), and K (Figures 3.1c and 3.2c) displayed overall low variation. In stark contrast, Na exhibited higher variation in plant tissue concentration across all genera sampled (Figures 3.1d and 3.2d).

Generally, aboveground plant tissues contained higher cation concentrations than adjacent soils across all cations considered (Figure 3.2, Table 3.2). The only exception was for Na across sites where *Opuntia* was collected, in which there was no significant difference in aboveground (1.729 log(ppm) ± 0.08) and adjacent soil (1.674 log(ppm) ± 0.06) concentrations ($t_{(82)} = 0.750$, $p= 0.453$; Figure 3.2, Table 3.2). Additionally, Na was the most variable cation across sites in soils and plant tissue samples (Figure 3.2). For all the sites where *Atriplex* was collected, soils showed a higher variation of Na than aboveground tissues, which contrasts with the pattern observed in *Helianthus* and *Opuntia* (Figure 3.2d). Strikingly, variation in plant tissues of Ca, Mg, and K was minimal; more variation occurred in the soils found across the sampled landscape for these cations (Figure 3.3).
Table 3.2. Paired t-test results for plant and soil cation concentration [log_{10} (ppm)] with a summary of mean and standard errors for each genus studied. Significant results are in bold.

<table>
<thead>
<tr>
<th>Cation</th>
<th>Sample</th>
<th>Mean</th>
<th>SE</th>
<th>t-stat</th>
<th>p-value</th>
<th>Mean</th>
<th>SE</th>
<th>t-stat</th>
<th>p-value</th>
<th>Mean</th>
<th>SE</th>
<th>t-stat</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>Plant</td>
<td>4.13</td>
<td>0.0</td>
<td>3</td>
<td>6.799</td>
<td>4.47</td>
<td>0.0</td>
<td>1</td>
<td>20.89</td>
<td>4.73</td>
<td>0.0</td>
<td>2</td>
<td>19.86</td>
</tr>
<tr>
<td></td>
<td>Soil</td>
<td>3.83</td>
<td>0.0</td>
<td>5</td>
<td>3.60</td>
<td>0.0</td>
<td>1</td>
<td>6</td>
<td>3.77</td>
<td>0.0</td>
<td>5</td>
<td>3.83</td>
<td>0.0</td>
</tr>
<tr>
<td>Mg</td>
<td>Plant</td>
<td>3.86</td>
<td>0.0</td>
<td>3</td>
<td>17.78</td>
<td>3.69</td>
<td>0.0</td>
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Figure 3.1. Geographic locations and aboveground phytochemical landscape of (a) Ca, (b) K, (c) Mg, and (d) Na \((\log_{10} \text{ ppm})\) across the southern United States. Triangle, square, and circle shapes were used to depict sites where *Atriplex*, *Helianthus*, and *Opuntia* were sampled, respectively. A color gradient was used to demonstrate plant Ca, K, Mg, and Na concentrations, with darker shades indicating higher concentrations and lighter shades indicating lower concentrations.
Figure 3.2. Plant tissues (gold) and soil (grey) cation concentration in *Atriplex*, *Helianthus*, and *Opuntia* sampled sites. Response variables for (A) calcium, (B) magnesium, (C) potassium, and (D) sodium, shown in boxplots, are log-transformed. Asterisks (***+) indicate a significant difference (p<0.0001).
Coefficient of variation in concentration of cations for aboveground plant tissues (gold) and adjacent soils (gray) for (A) Atriplex, (B) Helianthus, and (C) Opuntia, across all sites sampled. Mean response and standard error for the coefficient of variation are shown in bar graphs for each genus considered. The standard error of the coefficient of variation was calculated by bootstrapping the data 10,000 times. All pairwise comparisons are significantly different (p<0.0001).

**Homeostasis coefficients vary across cations and taxa**

On average, the homeostasis coefficient across taxa differed substantially according to cation essentially for plants. For instance, Ca concentrations in Atriplex ($H=4.54$, slope=0.225, p<0.0001, Figure 3.4a) and Opuntia ($H=2.82$, slope=0.354, p<0.0001, Figure 3.4c) showed similar $H$ values. In contrast, Helianthus had a much more homeostatic pattern. ($H=38.46$, slope=0.022, p=0.401, Figure 3.4b). Magnesium showed the highest $H$ value in Helianthus ($H=21.79$, slope=0.049, p=0.099, Figure 3.4b), followed by Atriplex ($H=9.52$, slope=0.105, p=0.275, Figure 3.4a) and Opuntia ($H=5.12$, slope=0.235, p<0.0001, Figure 3.4c). The homeostasis coefficient for K varied less among genera than for Ca and Mg. For K, Atriplex had the highest $H$ value ($H=9.17$, slope=0.106, p=0.251, Figure 3.4a), followed by Helianthus ($H=5.46$, slope=0.183, p<0.0001, Figure 3.4b) and Opuntia ($H=4.26$, slope=0.235, p<0.0001, Figure 3.4c). The homeostasis coefficient for Na followed a different pattern than Ca, Mg, and
K, with consistently low values for all genera. *Helianthus* had the highest $H$ value ($H=1.68$, slope=0.593, p<0.0001, Figure 3.4b), followed by *Atriplex* ($H=1.51$, slope=0.659, p<0.0001, Figure 3.4a) and *Opuntia* ($H=1.47$, slope=0.678, p<0.0001, Figure 3.4c).

**Figure 3.4.** Homeostatic regulation of Ca, K, Mg, and Na in (a) *Atriplex*, (b) *Helianthus*, and (c) *Opuntia* as compared to soil stoichiometry (P: Cation ratio) The 1:1 dashed line indicates no homeostasis. A slope of 1 and an intercept of 0 indicates plant stoichiometry reflects that of the resources (soils). Additionally, departures from the 1:1 line indicate differential nutrient processing or other environmental influences (i.e., salt deposition from coastal environments). When slopes differ from 1 and intercepts are other than zero indicates that the plants’ cation concentrations are somewhat independent of the soil’s, a case that is mostly observed for Ca, Mg, and K (Sterner & Elser, 2002).

*The environment explains most of the variation in plant Na, but not for the other cations*

Sodium concentrations in aboveground tissues were highly influenced by the environment, with models selected representing 65.2% of the variation for *Atriplex*, 62.9% of the variation for *Helianthus*, and 71.1% of the variation for *Opuntia* (Table 3.4). All of the models for *Atriplex*, *Helianthus*, and *Opuntia* share soil pH (p < 0.0001) and soil Na concentration (p<0.0001) as the highest contributing variables influencing aboveground plant Na concentration across the heterogeneous landscape of the southern United States (Table 3.3).
Table 3.3. Model selection of environmental variable influences on plant cation concentration. Environmental variables considered are (AT) annual temperature (C*10), (MDR) mean diurnal range, (TS) temperature seasonality, (AP) annual precipitation (mm), (PWM) precipitation on the wettest month (mm), (PDM) precipitation on the driest month (mm), (PS) precipitation seasonality, (DNC) distance from the nearest effective coast (km), (E) elevation (m), (SpH) soil pH (1:1 water), and (SC) log₁₀[soil cation concentration (ppm)]. The environmental variables' influences on the models are displayed as coefficients. For each genus, models are given horizontally and are constructed using the following example: \( \log_{10}[\text{Plant Ca} \text{ (ppm)}] \sim \text{Precipitation on the driest month (mm)} + \text{Precipitation seasonality (\%)} + \log_{10}[\text{Soil Ca} \text{ (ppm)}] \). For each best model selected the intercept, Akaike Information Criterion (AIC), \( R^2 \), and error rates (\%) of the model are given.

<table>
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<th>Genera</th>
<th>Environmental variables</th>
<th>Intercept</th>
<th>AIC</th>
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<th>Error rate (%)</th>
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(Table cont’d)
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Table 3.4. Mantel test result summary for each genus by cations considered in the study. Significant results are highlighted in bold.
For Ca, Mg, and K, albeit significant (p<0.0001), environmental variables account for a small proportion of the variation in aboveground plant cation concentrations across all genera, especially as compared to the variation explained for Na (see Table 3.3).

*Spatial autocorrelation is strong for plant Na across all genera studied*

Aboveground plant Ca, Mg, and K exhibited low spatial autocorrelation across genera (Figure 3.5). *Atriplex* had weak spatial autocorrelation for plant Ca concentration (r=0.094, p=0.043; Figure 3.5a) and stronger spatial autocorrelation for K concentration (r=0.154, p=0.004; Figure 3.5a). *Helianthus* also had weak spatial autocorrelation in K concentration (r=0.054, p=0.050; Figure 3.5b, Table 3.4). In sharp contrast, there was strong significant spatial autocorrelation for plant Na concentration across all genera studied (p<0.0001, Figures 3.5a, b, and c). On average, the correlograms for Na were decreasing with positive significant spatial autocorrelation found for distances up to ~482 km for *Atriplex*, ~432 km for *Helianthus*, and ~162 km for *Opuntia*.

Soils sampled across sites, on average, exhibited varied spatial autocorrelation responses for each of the cations. Across *Atriplex* sites, Mg (r=0.201, p=0.001) and Na (r=0.119, p=0.009) revealed stronger spatial autocorrelation (Figure 3.6a). Only sites where *Helianthus* was sampled, Ca (r=0.052, p=0.026) and K (r=0.056, p=0.022) displayed weak spatial autocorrelation (Figure 3.6b). Sites where *Opuntia* was collected exhibited no spatial autocorrelation for any of the cations considered (Figure 3.6c).

Plants in soils with similar Na concentrations also shared similar plant Na concentrations (Figure 3.7). Accordingly, correlograms showed a decreasing pattern with significantly positive similarity distance classes of up to ~0.51 soil Na (ppm) for *Atriplex* and ~0.07 soil Na (ppm) for
Helianthus (Figure 7a, b). Opuntia did not show any significant similarity when aboveground plant Na similarity and soil Na similarity was compared in the correlogram (Figure 3.7c); however, there is a weak relationship in the overall Mantel test ($r = 0.105$, $p=0.041$).

Figure 3.5. Mantel correlograms for the plant cation similarity (in rows) spatial structure for Atriplex, Helianthus, and Opuntia (in columns). Mantel r values are plotted on the y-axis (note different value ranges for each plot). Closed circles indicate significant correlations ($p < 0.05$) after 9,999 permutations. Positive correlations in the smaller distance classes indicate positive spatial autocorrelation among sites sampled.

Figure 3.6. Mantel correlograms for the soil cation similarity (in rows) spatial structure (km) for Atriplex, Helianthus, and Opuntia (in columns). Mantel r values are plotted on the y-axis (note different value ranges for each plot). Closed circles indicate significant correlations ($p < 0.05$) after 9,999 permutations. Positive correlations in the smaller distance classes indicate positive spatial autocorrelation among sites sampled.
Figure 3.7. Mantel correlograms for the plant cation similarity (in rows) across soil cation similarity distance class with indices in parts per million (log_{10} ppm) for *Atriplex*, *Helianthus*, and *Opuntia* (in columns). Mantel r values are plotted on the y-axis (note different value ranges for each plot). Closed circles indicate significant correlations (p < 0.05) after 9,999 permutations. Positive correlations in the smaller distance classes indicate positive spatial autocorrelation among soil distance classes sampled.

**DISCUSSION**

Across natural settings, we found evidence that plants, on average, follow the No-Escape-from-Sodium Hypothesis when exposed to variation in environmental Na across their range, forming a complex phytocationic landscape structure for Na. This finding is congruent with patterns observed across laboratory studies (Santiago-Rosario et al., 2021). In contrast, the phytocationic landscapes of Ca, Mg, and K do not follow environmental concentrations of these elements, which are more likely to be influenced by homeostatic processes in all three genera of plants considered in this study.

**Plant Na concentration highly depends on the environment**

Sodium is an unusual biotically important cation for plants since there is no apparent metabolic or structural function for most plants (Benito, Haro, Amtmann, Cuin, & Dreyer, 2014; Kronzucker et al., 2013; Pardo & Quintero, 2002). Plants have multiple mechanisms to manage...
tissue Na’s presence, e.g., ionic vacuole accumulation (Apse, Aharon, Snedden, & Blumwald, 1999; Apse & Blumwald, 2007), salt gland extrusion (Dassanayake & Larkin, 2017), among other high-energy consuming mechanisms (Kazachkova et al., 2018; Pantha & Dassanayake, 2020), which help prevent overall tissue toxicity, thus promoting survival. Yet, plants generally cannot escape having to cope with Na, especially in environments where its availability is expected and persistent (Santiago-Rosario et al., 2021).

Plant Na in *Atriplex* and *Helianthus* appear to follow similar accumulation patterns, with plants having higher amounts of Na as compared to adjacent soils (Figure 3.2d). However, *Opuntia* aboveground tissues appeared to share similar amounts of Na with the soil, possibly indicating a unique and unusual strategy (Figure 3.2d). Moreover, variation in plant Na tissue concentration was approximately 40% for all genera, with *Helianthus* and *Opuntia* having higher variation in plant tissues than soil Na concentrations (Figure 3.2d). Variation in plant tissues of *Atriplex* was slightly less than soil Na concentrations, which might reflect the halophytic nature of the family Amaranthaceae and the use of Na as a possible osmoticum in this genus (Glenn, Olsen, Frye, Moore, & Miyamoto, 1994; White et al., 2017). Nevertheless, plant Na variation was substantially higher across all taxa than Ca, Mg, and K, suggesting a lack of homeostasis for Na across plant tissues in natural settings.

The results also indicate weak to no homeostasis of Na by plants in the field, which is consistent with the diversity of ways in which plants deal with Na. In most plants, physiological mechanisms that mediate internal Na concentration rely on complex pathways and energetically expensive mechanisms impacting growth, metabolism, and performance as substrate Na increases to toxic levels (Apse & Blumwald, 2007; Cheeseman & Wickens, 1986a, 1986b; Maathuis, 2014; Munns & Tester, 2008; Pantha & Dassanayake, 2020). Although the genera
sampled for this study are widespread across the southern United States, they only represent a small sample of the botanical phylogenetic diversity found in the area, suggesting that an expansion of taxa collected with the aim of understanding ecological stoichiometric patterns would be useful. This study represents the first instance where plants and soil cation concentrations have been assessed in a broad heterogeneous landscape with the aim to understand how the phytochemical landscape is formed and maintained for microelements. Additionally, to better understand the mechanisms giving rise to patterns observed in the field, laboratory experiments designed to manipulate substrate and plant Na stoichiometry across multiple plant taxa would be useful (Santiago-Rosario et al., 2021).

Concerning the phytochemical landscape of Na, its formation and maintenance are highly dependent on environmental factors such as the amount of Na in the soil, soil pH, and proximity to the coast, among other environmental conditions (Table 3.3). The lack of homeostasis and the amount by which abiotic variables explained plant tissue concentration variation in our study reflects the influence of the environment on plant Na concentrations across the landscape. Both lack of homeostasis and environmental influences on plant Na accumulation can be used to predict the phytochemical landscape of Na at different geographical scales. This is especially important in light of ongoing coastal salt intrusion, and natural deposition (Dasgupta, Hossain, Huq, & Wheeler, 2015; National Atmospheric Deposition Program (NRSP-3), 2020; Rahman et al., 2018), soil salinization due to poor irrigation practice and drought (Ivushkin et al., 2019; Mukhopadhyay, Sarkar, Jat, Sharma, & Bolan, 2021; Shahid, Zaman, & Heng, 2018), deicing road salt deposition across urban and rural areas (Bryson & Barker, 2002; Heintzman, Titus, & Zhu, 2015; Hintz, Fay, & Relyea, 2022; Mitchell et al., 2020; Snell-Rood et al., 2014), among other anthropogenic influences might have on plants in the future (Konkel, 2016).
The phytochemical landscapes of Ca, Mg, and K appear to be controlled by homeostasis

Homeostasis appears to play a prominent role in cations’ phytochemical landscapes, and responses differ substantially among genera (Wang et al., 2019). Calcium, Mg, and K were found at higher concentrations than adjacent soils across all genera sampled. In general, plants accumulate these cations because of their essential biochemical and physiological functions (see Table 3.1). However, the variation of these cations in plant tissues differs substantially from Na in that Ca, Mg, and K variation is extremely low, accounting for approximately 5% among genera (Figure 3.2). Although the ions displayed homeostasis, the concentrations did vary among genera (Gilroy, Bethke, & Jones, 1993; Leigh, 2001; Tang & Luan, 2017). In Helianthus, Ca and Mg displayed strict homeostasis (according to Sterner & Elser’s (2002) definition), and Mg and K also appeared to be kept in strict homeostasis in Atriplex (Figure 3.4). Strict homeostasis results from organisms maintaining similar levels of an element or resource regardless of external availability (Sterner & Elser, 2002; J. Wang et al., 2019). For the genera mentioned above, soil Ca, Mg, and K concentrations do not influence plant concentrations of the same ions, other than being the sole or primary source of the element (Farago, 1994). More importantly, these three cations are kept at homeostatic levels, even across highly heterogeneous substrates (Figure 3.4).

Homeostasis might explain why, on average, few of the climatic variables considered in this study weakly explained the variation of Ca, Mg, and K across plant tissues. The only exception was K in Atriplex, in which around 40% of the variation was explained by the environment, although this is not unexpected since plants differ in micronutrient concentration requirements (Zhang et al., 2012). The roles that Ca, Mg, and K play in plants vary greatly, from a fundamental structural component to metabolism and enzymatic reactions (Broadley, Brown,
Cakmak, Rengel, & Zhao, 2012). Therefore, the performance of plant tissues is tightly linked to the acquisition and maintenance of these elements, among others (Alemán, Nieves-Cordones, Martínez, & Rubio, 2009; Sardans & Peñuelas, 2021; Tang & Luan, 2017; White & Broadley, 2003; Xu et al., 2020)

Because of anthropogenic global warming and increases in atmospheric CO₂ concentrations, plant quality and stoichiometric coping mechanisms might be highly impacted, especially in the context of highly regulated elements such as Ca and Mg. Evidence suggests warming conditions and increasing CO₂ concentrations will affect the environmental availability of some essential elements and their regulation by plants (i.e., N and P) unevenly across their range (Dijkstra et al., 2012; Gu, Zamin, & Grogan, 2017). For instance, grassland in semiarid Wyoming, USA, has shown that elevated CO₂ can alter the relative availability of P and N but warming can reduce P uptake in plants affecting plant performance and other trophic level interactions (Dijkstra et al., 2012). Moreover, the nutrition dilution hypothesis posits that increases in atmospheric CO₂, water availability, and temperature promote increased carbohydrate production in primary producers resulting in increases in plant biomass accumulations with low foliar nutrient quality which, in turn, promotes a decline in herbivore abundance (Welti, Roeder, de Beurs, Joern, & Kaspari, 2020). Whether these stoichiometric patterns replicate across other micronutrients and nonessential elements across plant taxa and different habitats remains unquantified.

In this study, I highlight that among plants’ essential elements, such as Ca, Mg, and K, herbivores, and detritivores appear to encounter a stable and similar dietary distribution of these elements across their range, yet no such patterns were observed for Na, at least in the current climate. Na is an essential element for animal metabolism and development (National Research
Council (U.S.), 2005), and its highly variable distribution across the phytocationic landscape might be influencing animal communities disproportionately. For instance, low levels of plant Na concentrations promote salt-seeking behaviors in animals (i.e., *collpa* and salt lick visitation, increase in carnivory, among others) (Boggs & Dau, 2004; Bravo, Harms, & Emmons, 2010; Burger & Gochfeld, 2003; Clay, Lehrter, & Kaspari, 2017; Holdø, Dudley, & McDowell, 2002), whereas high levels of plant Na concentrations have generated unique herbivore adaptations to prevent salt-induced stress encountered in some halophytic plant taxa that accumulate Na in plant tissues to evade herbivory (Kenagy, 1973; Renault, Wolfe, Markham, & Avila-Sakar, 2016). Yet, the mechanism by which Na gradients influence animal behavior across natural settings remains poorly described, especially when considering herbivore species with extended heterogenous ranges.

**Plants share similar Na concentrations the closer they are to each other**

Sodium’s high variability differs geographically, especially across soil Na gradients and proximity to coastlines with persistent salt deposits from marine aerosols (Borer et al., 2019; Bravo & Harms, 2017; Doughty, Wolf, Baraloto, & Malhi, 2016). Not surprisingly, plant Na concentration exhibited strong spatial autocorrelation across all genera sampled, emphasizing the weak homeostatic regulation plants have for this cation along with the environmental influence on plant Na acquisition. Individuals closer to each other share similar levels of tissue Na across a heterogeneous landscape. Moreover, Ca, Mg, and K, patterns of spatial autocorrelation were complex, albeit mostly weak across genera. K showed strong spatial autocorrelation behavior only in *Atriplex*. A similar pattern was observed across several plant families sampled geographically broadly in China, where K and Na showed strong spatial autocorrelation across
leaf tissues but not Ca, thus suggesting that these patterns might be shared across plant taxa and habitats (Zhang et al., 2012).

CONCLUSION

This study illustrates the utility of focusing attention on the formation and maintenance of the phytochemical landscapes of essential (i.e., Ca, Mg, and K) and generally nonessential elements to plants, such as Na. The No-Escape-from-Sodium hypothesis was supported across field-collected plants, which suggests that plant tissues tend to reflect Na in the substrate, similar to patterns observed in controlled settings (Santiago-Rosario et al., 2021). The difference in cation variation and spatial autocorrelation observed in this study appear to be linked to homeostatic regulation, or lack thereof, depending on elemental essentiality to plants. Thus, identifying general phytochemical patterns of essential and nonessential elements to plants across the landscape represents a key step toward better understanding biogeochemical cycles and their effects on trophic-level interactions and ecosystem dynamics (Hunter, 2016; Sterner & Elser, 2002). Expanding this type of research to other essential and nonessential elements and other taxa would broaden our understanding of the evolutionary and biogeographic processes that give rise to phytocationic landscapes.
CHAPTER 4
EFFECTS OF VARIATION IN PLANT SODIUM ON CHLOSYNE LACINIA
DEVELOPMENT AND SODIUM ACCUMULATION

INTRODUCTION

For animals, sodium represents an essential element that promotes osmoregulation, participates in nerve stimuli and muscle movement, helps control blood pH, and carries out other important bodily functions (Soetan, Olaiya, & Oyewole, 2010). For example, sodium is the most abundant extracellular solute; it plays a regulatory role in water intake or excretion in animal tissues. On vertebrate animals, extracellular osmolarity is tightly maintained at around 290 mosmol\(^{-1}\) with the vast majority contributed by sodium ions at ~140mM (Geerling & Loewy, 2008; Soetan et al., 2010). Intra- and extracellular dehydration causes aldosterone hormone control of water or sodium uptake or excretion (Geerling & Loewy, 2008; Young, McCaa, Pan, & Guyton, 1976). For most animals, long-term sodium deficiencies will cause death from dehydration, yet sodium excess, in the short and long-term, can cause hypernatremia, convulsions, muscle spasms, and death (NRC, 2005). In insects, sodium has similar osmoregulatory influences and bodily functions as compared to vertebrate animals, which likely represents a conserved function of sodium across animals. For instance, sodium helps control water loss from insect cuticles, especially those found in xeric environments (Zachariassen, 1996). All terrestrial animals have developed a particular lust for sodium chloride, the only salt known to have such an influence on animals (Dudley, Kaspari, & Yanoviak, 2012; Puri & Lee, 2021).

As shown in Chapter 2, plants can vary greatly in their sodium concentrations across an herbivore’s geographic range. Therefore, the heterogeneous distribution of sodium across the landscape can have substantial consequences in terrestrial and aquatic ecosystems for both
animal behavior and performance (Kaspari, 2020). For instance, most ungulates, certain frugivorous bats, and some frugivorous birds have been observed visiting salt licks or resorting to geophagy to supplement sodium dietary needs, especially when the plant food source is sodium-deficient (Bravo, Harms, & Emmons, 2010a, 2010b, 2012; Burger & Gochfeld, 2003; Holdø, Dudley, & McDowell, 2002), or preferentially eating or avoiding plant tissues with higher sodium concentrations (Bradshaw & Bradshaw, 1999; Kenagy, 1973; Mares et al., 1997). Insects also resort to particular behaviors to forage for sodium. For example, most male lepidopterans forage for sodium in puddles, feces, animal tears and sweat, rotting fruits, carrion, seawater, and any other salt source available, a behavior called puddling (Arms, Feeny, & Lederhouse, 1974; de Lima Moraes, 2019; F. Molleman, Grunsven, Liefting, Zwaan, & Brakefield, 2005; Pola & García-París, 2005; Smedley & Eisner, 1995, 1996). Increased male mating success because of puddling has been shown in Battus philenor (Papilionidae), Byclicus anynana (Nymphalidae), Gulphisia septentrionis (Notodontidae) Papilio xunthus (Papilionidae), and Thymelicus lineola (Hesperiidae), where individuals that managed to accumulate more sodium increased in mating proficiency and fitness by supplementing spermatophores with sodium as a nuptial gift (Mitra, Reynoso, Davidowitz, & Papaj, 2016; Freerk Molleman, Zwaan, & Brakefield, 2004; Pivnick & McNeil, 1987; Smedley & Eisner, 1995; Watanabe & Kamikubo, 2005). Moreover, some insect herbivores shift their diets to become more omnivorous by resorting to cannibalism or opportunistically feeding on other animal taxa when they encounter sodium-deficient diets (Clay, Lehrer, & Kaspari, 2017; Kaspari, Welti, & Beurs, 2020; Peterson, Welti, & Kaspari, 2021; Simpson, Sword, Lorch, & Couzin, 2006). Yet, the effects of variation in sodium across the natural phytochemical landscape on herbivore development and performance is poorly understood.
The objectives of this study were to test whether variation in plant sodium concentrations affects the development and sodium accumulation in the bordered patch butterfly, *Chlosyne lacinia*. This butterfly species has a short generation time (~25 days), lays eggs in clusters, and feeds on *Helianthus annuus*, a host that can withstand high salinity levels in natural and control settings. For this study, I predicted that growth (measured as larval length and pupal and adult weight) for both sexes would vary as dietary sodium varied among feeding trials. Additionally, I predicted that males would accumulate a higher proportion of sodium in their tissues from dietary sodium sources, given the important role that sodium plays in nuptial gifts in Lepidoptera.

**MATERIALS AND METHODS**

*Hydroponics and plant sodium concentrations*

Domesticated common sunflower (*Helianthus annuus*), Sunspot cultivar (supplied by Urban Farmer [https://www.ufseeds.com](https://www.ufseeds.com)) were raised across increasing sodium concentrations using a hydroponic floating raft method (van Delden et al., 2021). Seeds were sown in groups of six in 9-oz Styrofoam cups filled with 1:2 parts sand and vermiculite and kept in 49-L trays with 16 L of hydroponic solution. Hoagland’s modified basal salt mixture was used as the hydroponic solution (1M Ca(NO₃)₂.4H₂O, 1M KNO₃, 1M NH₄H₂PO₄, 1M MgSO₄.7H₂O, 9.2mM MnCl₂.4H₂O, 0.77mM ZnSO₄.7H₂O, 0.32mM CuSO₄.5H₂O, 0.11mM MoO₃, 90mM FeSO₄.7H₂O, and 0.5M H₃BO₃) supplied weekly at half strength (Asher & Edwards, 1983; Hoagland & Arnon, 1950). Four sodium treatments were used, each supplemented as laboratory-grade sodium chloride (NaCl): Control (0 % NaCl); Low (0.1 % NaCl); Medium (0.5 % NaCl); and High (1.0 % NaCl). Once a week, trays were purged of the hydroponic solution and replenished with new solution and treatments. Water levels were kept constant with distilled
water. Three trays for each treatment were used, and within each tray there were 36 individual planted pots. Plants were raised using a full spectrum light regime of 16L/8D, at a temperature of 23 ± 3 Cº and relative humidity of 60 ± 10 % for a month. Five individuals per tray were selected for plant sodium concentration analysis using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (Munns, Wallace, Teakle, & Colmer, 2010). Plant sodium concentrations across hydroponic treatments were analyzed using linear regression in R. Additionally, I measured specific leaf area (mm/gr) and leaf water content (%) for the same five individuals sampled from each tray.

Establishment of Chlosyne lacinia colony

*Chlosyne lacinia* larvae and adult individuals were collected from Dilley, TX (28.660640, -99.220975), Sullivan City, TX (26.277365, - 98.564303), and Mission, TX (26.176123, -98.335364) in May 2021. Individuals were raised on sunflower plants (Mammoth Grey cultivar, supplied by Papaw’s Garden Supply [https://papawsgarden.com/](https://papawsgarden.com/)) grown in a greenhouse. Larvae were raised in mesh cages on sunflower plants in the following conditions: light regime of 16L/8D, at a temperature of 23 Cº± 3 Cº, and relative humidity of 60% ± 10%. After the first generation, adult butterflies were mixed across rearing cages to increase genetic diversity and reduce inbreeding depression for subsequent generations. First-instar larval clusters (each cluster constituted a group of siblings) of the F2 generation were randomly selected for feeding trials.

Larval exposure to dietary sodium and data analysis

Larval exposure to increasing dietary sodium treatments was performed in two ways: larvae in groups of six (herein Gregarious) and as individuals (herein Solitary). I exposed individuals to solitary feeding to assess whether the effects of increasing plant sodium concentrations influence development differently in a non-competitive environment, a behavior
observed among gregarious individuals, and to measure sodium concentrations in individuals that do not have the opportunity to cannibalize its siblings. The Gregarious trial was performed by using six individual sibling larvae selected from random 1st-instar clusters and added to 32-oz Styrofoam cups containing *ad libitum* sunflower plants grown on the randomly assigned treatment salt concentrations. Solitary larvae followed the same protocol as in the Gregarious trial, but each cup had a single larva. Larvae were kept on live plants until death or pupation, and then raised to adulthood in their original cups. Each plant sodium treatment had ten replicates. The study lasted 38 days. Every three days (for the first 15 days which encompassed the larval stages), larvae were photographed for measurements to be used to assess growth (measured as length (mm) from head to anal plate). This measurement was used to prevent destructive sampling and injury to tiny 1st-instar larvae. All length measurements were made from Image J using a digital caliper. For the Gregarious trial all larval lengths were averaged per cup, since following each individual through time was not practical. Pupal weight for each individual was also collected at the beginning of the pupal stages, but after cuticle hardening. After eclosion, adult individuals were preserved in a -20°C freezer and date of eclosion was recorded for each individual. Each individual for the Gregarious and Solitary trials was sexed in the adult stage based on the presence or absence of an ovipositor (female). All individuals were then dried at 60°C for two days. Each adult body was dissected into its abdomen and thorax, each of which was dried to constant mass and weighed.

**Sodium concentrations in butterfly tissues**

Dried dissected abdomen and thorax tissues from Solitary individuals were selected for sodium concentration analysis.
An Inductively Coupled Plasma with Mass Spectrometry (ICP-MS) approach was used to characterize sodium concentrations of the abdomens and thoraxes of 66 Solitary individuals that were reared in plants grown across the four sodium treatments. Briefly, tissues were digested in 1 mL trace metal free HNO₃ acid and 0.5mL of trace metal free H₂O₂ overnight. Using this approach completely digested the tissues. For sodium measurements, a dilution of 1/10 was performed with trace metal free water. This level of dilution was necessary because of the high values for sodium concentrations, which otherwise prevented accurate analysis by the ICP-MS. Diluted samples were then analyzed using the ICP-MS following standard protocols. All tissues were compared relative to a standard (Inorganic Ventures, IV-ICPMS-71A), which was used to generate a calibration curve. All measurement of concentrations of sodium are given in parts per million (ppm).

Data analysis

An analysis of interactions (two or three-way ANOVA) characterized how the variable’s experiment type (i.e., Gregarious or Solitary), sodium treatment, sex, and tissue type (i.e., abdomen and thorax in adults) influenced morphological and developmental phenotypes, as well as sodium concentrations. In the case of the adult body tissues, I removed the feeding trail variable given that it does not influence the results in any way after the model comparisons were performed. To assess whether variation across groups of treatments differed, a Bartlett test was performed, which is based on a comparison to the null hypothesis that all groups share the same variance. All ANOVA analyses were performed in R using the function ‘aov’ (Team, 2020). When a nonparametric test was needed, such as in the case of larval length comparisons, a Kruskal-Wallis test was used using the function ‘kruskal.test’ in R
RESULTS

Sodium treatment influences on hydroponically grown sunflowers

On average, plant sodium concentrations significantly increased by 1.145 log(ppm) as hydroponic sodium concentrations increased ($F_{1,10}=4.613$, $R^2=0.25$, $p=0.05$, Fig. 4.1A). Specific leaf area (i.e., leaf area / leaf dry mass) also significantly increased by 1,120 mm/gr as hydroponic sodium increased ($F_{1,10}=12.71$, $R^2=0.52$, $p=0.005$, Fig. 4.1B). Additionally, leaf water content significantly increased by 1.726 % as hydroponic sodium increased ($F_{1,10}=8.973$, $R^2=0.42$, $p=0.013$, Fig. 4.1C).
Figure 4.1. Plant sodium concentration [log(ppm)] (A), specific leaf area (mm/gr) (B), and leaf water content (%) (C) responses across hydroponically controlled sodium treatments (%).
**Time to eclosion**

The time to eclosion (measured in days) did not differ among treatments ($F_3=2.178$, $p=0.09$, Fig. 5.2, Table A4.1) nor between Gregarious and Solitary feeding trial ($F_1=1.414$, $p=0.24$, Fig. 5.2, Table A4.1). On average, mean responses were similar among treatments and between Gregarious and Solitary feeding trial (Table 4.1). However, the variance was on average 2-fold higher among treatments in the Solitary feeding trial, with the high-sodium treatment having a 61-fold increase in variance in days as compared to the no addition treatment (Bartlett test: $K$-squared=27.812, df=3, $p<0.0001$, Fig. 4.2). Eclosion time in Gregarious individuals did not show any differences in variance among treatments (Bartlett test: $K$-squared= 2.633, df=3, $p=0.45$, Fig. 4.2).

Figure 4.2. Days to adult eclosion from the pupal stage among sodium treatments, and between Gregarious (yellow) and Solitary (orange) feeding trials. The start date, not shown here was recorded as the date each larva emerged from the egg.
Table 4.1. Summary of eclosion mean responses (measured in days) between Gregarious and Solitary feeding trails and among sodium treatments.

<table>
<thead>
<tr>
<th>Feeding trial</th>
<th>Treatment</th>
<th>Mean (days)</th>
<th>Standard error</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gregarious</td>
<td>No addition</td>
<td>25.2</td>
<td>0.4</td>
<td>4.03</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>26.13</td>
<td>0.4</td>
<td>3.71</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>26.09</td>
<td>0.39</td>
<td>5.62</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>26.37</td>
<td>0.45</td>
<td>6.33</td>
</tr>
<tr>
<td>Solitary</td>
<td>No addition</td>
<td>24.57</td>
<td>1.35</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>25</td>
<td>1.19</td>
<td>6.00</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>24.5</td>
<td>1.26</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>27.33</td>
<td>1.19</td>
<td>38.25</td>
</tr>
</tbody>
</table>

**Larval growth**

On average, total live larval length (mm) did not significantly differ among treatments for Gregarious (Kruskal-Wallis test: $X^2_{(3)}=1.82$, $p=0.61$, Fig. 4.3A) or Solitary (Kruskal-Wallis test: $X^2_{(3)}=4.76$, $p=0.189$, Fig. 4.3B) feeding trails when measured at 12 days (the day the first individual started pupation). However, larval length varied significantly more among individuals exposed to the high-sodium treatment than individuals from other treatments in Solitary larvae (Bartlett test: K-squared= 13.673, df=3, $p=0.003$, Fig. 4.3B), as opposed to larvae in the Gregarious feeding trials (Bartlett test: K-squared= 3.657, df=3, $p=0.301$, Fig. 4.3A).

Table 4.2. Summary of larval length mean responses (measured in mm) at 12 days between Gregarious and Solitary feeding trails and among sodium treatments.

<table>
<thead>
<tr>
<th>Feeding trial</th>
<th>Treatment</th>
<th>Mean (mm)</th>
<th>Standard error</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gregarious</td>
<td>No addition</td>
<td>19.06</td>
<td>1.58</td>
<td>22.50</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>14.96</td>
<td>1.18</td>
<td>13.91</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>17.67</td>
<td>1.41</td>
<td>19.93</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>15.51</td>
<td>2.18</td>
<td>47.48</td>
</tr>
<tr>
<td>Solitary</td>
<td>No addition</td>
<td>22.04</td>
<td>1.47</td>
<td>17.34</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>21.03</td>
<td>1.66</td>
<td>27.52</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>21.121</td>
<td>0.42</td>
<td>1.59</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>18.36</td>
<td>1.87</td>
<td>31.33</td>
</tr>
</tbody>
</table>
Figure 4.3. Total live larval length means and standard error across time (days) for each sodium treatment (No addition: brown, Low: red, Medium: yellow, and High: gray) in (A) Gregarious and (B) Solitary larval feeding trials.

**Pupal mass**

There is a three-way interaction between treatment, sex, and feeding trial influencing pupal mass ($F_3=2.871$, $p=0.039$, Figure 4.4B, Table A4.2).
Figure 4.4. Pupal masses (mg) for each sex among all treatments (No addition: brown, Low: red, Medium: yellow, and High: gray) in (A) Gregarious and (B) Solitary larval feeding trials.

**Adult body-segment development**

Tissue type and sex showed a strong two-way interaction ($F_1 = 251.96$, $p < 0.0001$, Fig. 4.5, Table A4.3) in dry weight with abdomens and thoraxes. Females exhibited ~2.5-fold larger abdomens ($18.15 \pm 0.28$ mg) overall as compared to males ($7.41 \pm 0.25$ mg) (Fig. 4.5A and C). The same pattern was observed for thoraxes where females ($10.94 \pm 0.28$ mg) were 1.25-fold larger than males ($8.77 \pm 0.26$ mg) (Fig. 4.5B and D).

Additionally, there is also a significant two-way interaction between sex and treatment ($F_3 = 3.655$, $p = 0.013$, Fig. 5.5, Table A4.3). Females from the high treatment were 1.16-times larger ($15.48 \pm 0.46$ mg) albeit only being to females in the low treatment ($13.29 \pm 0.37$ mg). No three-way interaction was observed across tissues, sex, and treatments ($F_3 = 1.189$, $p = 0.314$, Fig. 5.5, Table A4.3).
4.5, Table A4.3) and there were no differences among feeding trials ($F_1 = 0.05$, $p=0.824$, Fig. 4.5).

Figure 4.5. Abdomen and thorax dry mass (mg) for each sex and treatment (No addition: brown, Low: red, Medium: yellow, and High: gray) for (A and B) Gregarious and (C and D) Solitary larval feeding trials.
**Butterfly-tissue sodium concentrations**

On average males accumulated 8.4% more dietary sodium in their tissues than females ($F_1= 5.89, p=0.0189$, Fig. 4.6, Table A4.4). Also, males in the high treatments accumulated 26.29% higher sodium concentrations as compared to males from the no addition treatments ($F_3= 3.099, p=0.035$, Fig. 4.6, Table A4.4). Two-way interaction between sex and treatment was non-significant ($F_3=2.639, p= 0.06$, Fig. 4.6, Table A4.4). There is no evidence that suggests that there are differences in sodium accumulation across tissues ($F_1= 1.406, p=0.241$, Figure 4.6, Table A4.4) meaning that both abdomen and thorax show no difference within each category albeit that testis is present in the abdomen. Additionally, I found evidence that suggests that females tend to maintain similar levels of sodium across treatments ($F_3= 1.445, p=0.259$, Figure 4.6, Table A4.4) in both abdomen and thoraxes.

![Figure 4.6](image-url)  
**Figure 4.6.** Body-segment sodium concentration (ppm) for (A) abdomens and (B) thoraxes of Solitary individuals across all sodium treatments (No addition: brown, Low: red, Medium: yellow, and High: gray). Data were log-transformed.
DISCUSSION

The availability and variation of dietary sodium in plant tissues can influence development, behavior, and performance of herbivores across their ranges. For lepidopterans, sodium is important for muscle and neuron development and has been shown to be crucial during mating, in which it is used as a nuptial gift (Mitra et al., 2016; Smedley & Eisner, 1995, 1996; Snell-Rood, Espeset, Boser, White, & Smykalski, 2014). Males visit salt licks, feces, fruits, and carrion, among other sodium sources, to extract salts, by puddling, and accumulate salt in their tissues to pass to the female during mating. Yet, it is unclear how plant sodium variation would affect the development and sodium accumulation strategies of lepidopterans. In this study, we set out to characterize how variation in plant sodium influences developmental time, morphological expression, and tissue sodium accumulation in Chlosyne lacinia.

Plant responses to increasing substrate sodium are as expected

Hydroponically grown sunflowers accumulated sodium as a function of hydroponic sodium following the No-Escape-From-Sodium hypothesis (Chapter 1), which posits that plants accumulate sodium as sodium in the substrate (or hydroponic solution) increases (Santiago-Rosario et al., 2021; Manivannan et al., 2008; Wu, Jiao, & Shui, 2015). Additionally, responses of increased specific leaf area and leaf water content are correlated with increases in substrate sodium exposure. Increases in specific leaf area are related to drought responses in plants, which sodium induces under osmotic stress (Battie-Laclau et al., 2013; Melo, Yule, Barros, Rivas, & Santos, 2021). Moreover, leaf water content has been shown to increase in Suaeda fruticosa (Amaranthaceae), Tamarindus indica (Fabaceae), and Ziziphus spina-christi (Rhamnaceae) as sodium in the substrate increased and is related to an increase in salt-induced leaf succulence (Gebauer, El-Siddig, Salih, & Ebert, 2004; Khan, Ungar, & Showalter, 2000; Sohail, Saied,
Gebauer, & Buerkert, 2009). All these responses are directly related to increases in substrate sodium and, therefore, they might all function in concert to influence herbivore responses directly and indirectly.

**Increasing sodium minimally influenced morphology and developmental time**

Whether in the Gregarious or the Solitary feeding trials, individuals of *Chlosyne lacinia* did not experience a significantly extended developmental time (measured in days). This does not mean that all individuals reach the imago stage at the same time. In the high treatments of the solitary trial, two individuals extended their developmental time to adulthood to 38 days when the average was ~25 days. Whether this observation was due purely to high plant sodium concentrations or an effect imposed by solitary feeding remains to be evaluated. Studies on the cotton bollworm, *Helicoverpa armigera* (Noctuidae), showed that, on average, in sodium-deficient artificial diets individuals grew faster, a finding that was correlated with high food intake, high cannibalism rates, and lower survivorship (Xiao, Shen, Zhong, & Li, 2010). This pattern was not observed in *Chlosyne lacinia*.

Also, this study shows that, on average, larval stage development is not affected by plant sodium concentrations when individuals are reared in groups or solitarily. Perhaps what is striking is the degree of variation found in all treatments and groups. In terms of larval growth, solitary larvae appeared to show higher levels of variation, specifically at the high treatment. This might be explained by physiological stress experienced by the larvae as they encountered high concentrations of sodium in the plant tissues. This has been shown across increasing concentrations of sodium added to artificial diets, e.g., larvae of *H. armigera* experienced diminished growth based on stress due to a high sodium diet (Xiao et al., 2010). A similar pattern was observed for the weight accumulation of the grasshopper *Melanoplus differentialis*. 

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(Acrididae), in which individuals raised on high sodium artificial diets accrued lower biomass than individuals raised on lower-sodium diets (Peterson et al., 2021).

Additionally, we found a three-way interaction of sex, treatments, and feeding trails influencing pupal mass (Fig. 4.4). Female pupae were, on average, heavier than males across all treatments and feeding trials. Moreover, in adults, there was a two-way interaction between sex and treatment and between sex and tissue type. On average, female abdomens and thoraxes were larger than males (Fig. 4.5). Moreover, females appear to differ in the mass of their respective tissues across treatments to a higher degree than males. In Chlosyne lacinia, there is size dimorphism among the sexes with females being larger than males; this is a pattern found in other lepidopterans (Bissoondath & Wiklund, 1997; Kons, 2000; Singer, 1982).

**Males store sodium from dietary sources**

Males of C. lacinia tended to accumulate higher concentrations of sodium than females, especially when sodium in the plants was high (Fig. 4.6). Females tended to have similar sodium concentrations among treatments, suggesting regulation of sodium across environmental variation. This is the first study in which this pattern is described for lepidopterans.

As mentioned previously, many adult male lepidopterans puddle to extract sodium from a wide variety of sources (i.e., puddles, feces, fruits, vertebrate tears and sweat, seawater, among other sources) (Arms et al., 1974; F. Molleman et al., 2005; Pivnick & McNeil, 1987; Pola & García-París, 2005; Watanabe & Kamikubo, 2005). Sodium has been shown to be an integral component of mating in Lepidoptera because males supplement their spermatophore with sodium as a nuptial gift (Mitra et al., 2016; Freerk Molleman et al., 2004; Smedley & Eisner, 1996). This behavior is believed to have evolved to increase female performance and brood
success, yet this is currently not well understood, especially for *C. lacinia*. Given that males are storing sodium from larval dietary sources, this might have substantial consequences on adult behavior. Potentially, males that feed on plant tissues high in sodium concentrations might avoid the costly behavior of puddling and rapidly engage in courtship behaviors and mating as compared to males whose larval host plants had low sodium levels.

**Implications of the phytochemical landscape on herbivore activity and performance**

As shown in Chapter 3, plants vary greatly in sodium concentrations across their ranges (e.g., at least 30-fold for *Helianthus*), which suggests a varied landscape of herbivore activity, especially regarding sodium-driven behaviors. In the case of *C. lacinia* and their plant host (plants in the tribe Heliantheae) their ranges are extensive (Neck, 1973), covering coastal and inland areas which differ substantially in the pools and fluxes of environmental sodium, thus potentially, influencing phytochemistry and *C. lacinia* behavior and performance among populations. The population dynamics in terms of sodium-driven behaviors and performance might be changing due to anthropogenically-mediated sodium depositions (i.e., sea salt intrusion and drought due to climate change, irrigation practices, winter road salt additions, and cattle farming) across herbivore ranges (Bryson & Barker, 2002; Heintzman, Titus, & Zhu, 2015; Hintz, Fay, & Relyea, 2022; Kaspari, Chang, & Weaver, 2010; Oster, 1994), having large consequences on selection patterns, eco-evolutionary dynamics, and trophic level interactions.
CHAPTER 5
SIBLINGS AS SALTY SNACKS: LOW SODIUM AVAILABILITY IN HOST PLANTS INDUCES CANNIBALISM IN A LEPIDOPTERAN HERBIVORE

INTRODUCTION

Sodium plays a relatively unusual role within ecosystems, as a nonessential element for most plants, yet as a highly essential element for animal consumers and most decomposers (e.g., bacteria, fungi) (Adams, Early, & Bamford, 2008; Garrill, Clipson, & Jennings, 1992; Kaspari, 2021; Kaspari & Powers, 2016; Kaspari, Yanoviak, Dudley, Yuan, & Clay, 2009; Kronzucker, Coskun, Schulze, Wong, & Britto, 2013; National Research Council (U.S.), 2005; Soetan, Olaiya, & Oyewole, 2010). In most cases, plants are unable to entirely prevent the uptake of sodium from the substrate, which has important consequences for plant fitness and eco-evolutionary dynamics (Maathuis, 2014; Santiago-Rosario, Harms, Elderd, Hart, & Dassanayake, 2021). As an essential element for animals, sodium plays an important role in osmoregulatory processes, promotes muscle and neural tissue development and function, and controls blood movement, among other functions (Hodgkin, 1951; National Research Council (U.S.), 2005; Puri & Lee, 2021). Posing challenges for animal foragers, the availability of sodium across terrestrial environments (e.g., soils and plants) is highly heterogeneous (Bravo & Harms, 2017; Smith, 2013).

Overall environmental variation of available sodium and stochiometric mismatch (i.e., differences between what plants have and what animals need) influences animal behavior and performance, especially among herbivorous or non-carnivorous animals (Bravo & Harms, 2017; Bravo, Harms, & Emmons, 2012), since animal prey provides plenty of sodium. For instance, in sodium-deficient habitats, lack of dietary sodium promotes geophagy (e.g., salt lick visitation) (Bravo, Harms, & Emmons, 2010b, 2010a; Bravo et al., 2012; Burger & Gochfeld, 2003; Holdø,
and uptake of mineral-rich water (e.g., puddling) (Arms, Feeny, & Lederhouse, 1974; Mitra, Reynoso, Davidowitz, & Papaj, 2016; Molleman, Grunsven, Liefting, Zwaan, & Brakefield, 2005; Pola & García-París, 2005; Smedley & Eisner, 1995, 1996; Watanabe & Kamikubo, 2005); causes selective plant feeding of sodium-rich tissues (Bradshaw & Bradshaw, 1999; Fraser, Chavez, & Paloheimo, 1984; Kenagy, 1973; LoPresti, 2014; Mares et al., 1997; Weeks & Kirkpatrick, 1978); induces migratory behaviors and changes in animal population density (Aumann & Emlen, 1965; Simpson, Sword, Lorch, & Couzin, 2006); and can promote a shift in omnivores' diets toward higher trophic levels (Clay, Lehrter, & Kaspari, 2017; Kaspari, Welti, & Beurs, 2020; Zhang, Berg, Leeuwen, Blonk, & Bakker, 2018). The lust for sodium appears to be shared by many terrestrial and aquatic animals, representing a conserved condition, especially for strict primary consumers whose dietary needs rely solely on primary producers (strict herbivores and many detritivores) (Dudley, Kaspari, & Yanoviak, 2012; Filipiak et al., 2017; Puri & Lee, 2021; Yarmolinsky, Zuker, & Ryba, 2009).

Cannibalism, the consumption of conspecifics, has been observed extensively in otherwise non-carnivorous insects (Richardson, Mitchell, Reagel, & Hanks, 2010). For instance, sodium-deficient artificial diets have been shown to increase cannibalism incidence in the grasshopper *Melanopus differentialis* (Peterson, Welti, & Kaspari, 2021) and in the cotton bollworm *Helicoverpa armigera*, mainly when larger individuals encountered smaller ones (Xiao, Shen, Zhong, & Li, 2010), among other herbivorous taxa (Barros-Bellanda & Zucoloto, 2005; Chapman et al., 2000; Tea, Soong, Beaver, & Lohman, 2021). This behavior is not taxonomically limited (Fox, 1975; Richardson et al., 2010). Cannibalism has been found when there was a protein and salt deficiency (Simpson et al., 2006), in the acquisition of defensive chemicals (Tea et al., 2021), during parental care (Bishop, Onoufriou, Moss, Pomeroy, & Twiss, 2004).
2016; FitzGerald, 1992; Toyama, 1999), and during courtship and reproduction (Birkhead, Lee, & Young, 1988). Yet, theoretical models predict that cannibalism prevalence among taxa should result from a balance between cost (e.g., increasing risk of injury and loss of inclusive fitness when harming siblings) and benefits (e.g., reducing the prevalence of parasites, intraspecific competition, and nutritional scarcity) (Richardson et al., 2010; Rudolf, Kamo, & Boots, 2010; Van Allen et al., 2017).

Figure 5.1. Sibling larvae of *C. lacinia* feeding gregariously on a leaf of common wild sunflower (*Helianthus annuus*) in southern Texas. The photo was taken by Luis Santiago-Rosario.

Studies that have evaluated the influence of dietary sodium on insect herbivore performance have mostly applied sodium treatments in artificial diets or in solutions sprayed
onto the foliage (Peterson et al., 2021; Snell-Rood, Espeset, Boser, White, & Smykalski, 2014; Xiao et al., 2010). To our knowledge, no study of insect herbivore behavior or performance has manipulated plant-tissue sodium concentrations. We manipulated plant-tissue sodium to test whether sodium concentration would influence an herbivore's cannibalistic behavior and survival. We hypothesized that: 1) cannibalism would decrease as plant sodium increased from very low levels, owing to sodium deficiency at low levels; and 2) larval survival would suffer under plant sodium extremes, owing to physiological stress at high levels, following a subsidy-stress response (Odum, Finn, & Franz, 1979). We used the bordered patch butterfly, *Chlosyne lacinia* (Nymphalidae), a highly variable species with a geographic range from Oklahoma, USA, to northern Argentina (Kons, 2000; R.W. Neck, 1976; Santiago-Rosario, 2021). The larvae are oligophagous on members of the tribe Heliantheae (Asteraceae) which can be found from coastal areas high in sodium to inland sodium-deprived habitats (Brochero Bustamante, Colorado Martínez, & Sepúlveda-Cano, 2012; Gorodenski, 1969; Heiser, Smith, Clevenger, & Martin, 1969; Raymond W. Neck, 1977). Adult females oviposit large egg clusters (25-450 eggs) on the undersides of host leaves, and larvae feed gregariously, producing characteristic skeletonized leaf damage (Fig. 5.1) (Brochero Bustamante et al., 2012; Clark & Faeth, 1997; Drummond, Bush, & Emmel, 1970; Stamp, 1977).

**MATERIALS AND METHODS**

*Hydroponics and plant sodium concentrations*

We grew domesticated common sunflower (*Helianthus annuus*), Sunspot cultivar (supplied by Urban Farmer [https://www.ufseeds.com](https://www.ufseeds.com)), across increasing sodium concentrations using a hydroponic floating raft method (van Delden et al., 2021). Seeds were sown in groups of
six in 9-oz Styrofoam cups filled with 1:2 parts sand and vermiculite and kept in 49-L trays with
16 L of hydroponic solution. Hoagland’s modified basal salt mixture was used as the hydroponic
solution (1M Ca(NO$_3$)$_2$.4H$_2$O, 1M KNO$_3$, 1M NH$_4$H$_2$PO$_4$, 1M MgSO$_4$.7H$_2$O, 9.2mM
MnCl$_2$.4H$_2$O, 0.77mM ZnSO$_4$.7H$_2$O, 0.32mM CuSO$_4$.5H$_2$O, 0.11mM MoO$_3$, 90mM
FeSO$_4$.7H$_2$O, and 0.5M H$_3$BO$_3$) supplied weekly at half strength (Asher & Edwards, 1983;
Hoagland & Arnon, 1950). Four sodium treatments were used, each supplemented as sodium
chloride (NaCl): Control (0 % NaCl); Low (0.1 % NaCl); Medium (0.5 % NaCl); and High (1.0
% NaCl). Once a week, trays were purged of the hydroponic solution and replenished with new
solution and treatments. Water levels were kept constant with distilled water. We used three trays
for each treatment, and within each tray, there were 36 individual pots. Plants were reared using
a full spectrum light regime of 16L/8D, at a temperature of 23 ± 3 C°, and relative humidity of 60
± 10 % for a month. Five individuals per tray were selected for plant sodium concentration
analysis using Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES) (Munns,
Wallace, Teakle, & Colmer, 2010). Plant sodium concentrations across hydroponic treatments
were analyzed using linear regression.

**Butterfly colony establishment**

We collected larvae and adult individuals from Dilley, TX (28.660640, -99.220975),
Sullivan City, TX (26.277365, -98.564303), and Mission, TX (26.176123, -98.335364) in May
2021. Individuals were reared on sunflower plants (Mammoth Grey cultivar, supplied by
Papaw’s Garden Supply [https://papawsgarden.com/]). Larvae were reared in mesh cages on
sunflower plants in the following conditions: light regime of 16L/8D, at a temperature of 23 C°±
3 C°, and relative humidity of 60% ± 10%. After the first generation, adult butterflies were mixed
across rearing cages to increase genetic diversity and reduce inbreeding depression for
subsequent generations. We randomly selected 1st-instar larval clusters (each cluster constituted a group of siblings) of the F2 generation for the feeding trials.

*Larval exposure to dietary sodium and data analysis*

Larval exposure to increasing dietary sodium treatments was performed with groups of six sibling larvae; for each group, individuals were selected at random from a single 1st-instar cluster. Each group was added to a 32-oz Styrofoam cup containing *ad libitum* sunflower plants grown on the randomly assigned treatment salt concentration. Larvae were kept on live plants until death or pupation and then reared to adults in each cup. Each plant sodium treatment had ten replicates. The study lasted 33 days. We observed each replicate every three days and determine the number of larvae alive at each stage, the number of cadavers (which died of causes other than cannibalism), and the number of individuals cannibalized (i.e., the absence of the molt or larval body was inferred to result from cannibalism, since with daily exhaustive checks on the containers, a dead body could not have sufficiently rapidly decayed to be missed). We also quantified pupal survival and the number of individuals reaching the adult stage at the end of the trial. To test plant sodium concentrations' influence on larval fate, we performed a 3x4 contingency table analysis using a Pearson Chi-squared test with treatments in columns and fate in rows. All analyses were performed in R (Team, 2020)

**RESULTS AND DISCUSSION**

*Sunflower tissue sodium increased as substrate sodium increased*

Sodium concentrations in sunflowers increased across hydroponic at a function 1.145 log(ppm) as of NaCl treatments increased (No addition= 568 ± 152 ppm; Low= 668 ± 245 ppm; Medium= 934 ± 427 ppm; and High= 2296 ± 1026 ppm) (F(1,10)=6.02, R²=0.38, p=0.034, Figure
5.2A). The values for plant sodium concentrations across treatments fall within the range of concentrations observed for sunflower foliar tissues in the wild across the geographic range of *C. lacinia* (minimum= 3 ppm, maximum= 29,791 ppm: LYSR unpublished data). Therefore, the patterns observed in this study support the No-Escape-from-Sodium Hypothesis, which posits that most plants’ tissues become saltier as the availability of Na in the environment increases (Santiago-Rosario et al., 2021).

*The fates of larvae depended on plant sodium concentrations*

Larvae of *C. lacinia* exhibited contrasting behavioral and mortality responses as plant host sodium concentrations increased. On average, larval survival was contingent on plant sodium concentrations ($X^2=12.59$, df=6, p<0.0001, Figure 5.2B). However, larval mortality resulted from two different mechanisms; cannibalism and death from other causes (observed as cadavers). Most cadavers were found in the High plant sodium treatment (58.3 %), suggesting a substantial mortality response to dietary sodium-induced stress. Cannibalism was only observed once in this treatment (1.7 %) (Figures 5.2C and D). In contrast, in the No addition and Low treatments, both cannibalism and cadavers were observed at high frequencies (Figures 5.2C and D). In both No addition and Low treatments, cannibalism killed 26.7%. Other causes killed 28.3% in the No addition and only 16.7% in the Low treatment (Figures 5.2B, C, and D).
Figure 5.2. (A) Linear regression of plant sodium concentration as a function of treatment of NaCl (%) in the plants’ hydroponic solutions. (B) Pearson Chi-square test association plot showing the influence of plant sodium treatments on larval fates. Each rectangle’s width is proportional to the square root of the expected frequency for each cell. The height of each rectangle is proportional to the standardized Pearson residual. Purple and gold rectangles depict significantly positive and negative residuals, respectively, highlighting which cells have more or fewer observations than expected within the Pearson Chi-Square test. (C) The cumulative number of cadavers through time and among treatments. Measurements were collected every three days from the start of the feeding trails. (D) The cumulative number of larvae cannibalized through time and among treatments. Measurements were collected every three days from the beginning of the feeding trails.
Most terrestrial animals have evolved in environments in which sodium is scarce. Thus, an intricate network of mechanisms has evolved for terrestrial and freshwater aquatic animals to conserve sodium within their bodies and tissues (Geerling & Loewy, 2008; Liebeskind, Hillis, & Zakon, 2011). Yet, when found in excess, animals must overcome sodium toxicity through excretion, which is an energetically taxing response (Boné, 1947; National Research Council (U.S.), 2005; Scheibener, Richardi, & Buchwalter, 2016). We found that high sodium levels resulted in higher mortality, a response most likely due to osmotic stress. Similar responses have been observed in other lepidopteran species. For instance, monarch (*Danaus plexippus*) larvae experienced high mortality when exposed to milkweed (*Asclepias*) collected near roads in Minnesota, where salts are used for deicing, with plants being 16 times richer in sodium concentration along roadsides, as compared to plants collected in natural habitats away from roads (Snell-Rood et al., 2014). However, we found that the lack of dietary sodium in plant tissues induced cannibalism in a species that feeds gregariously during the larval stages. In this case, larval clusters for each replicate consisted of siblings. Since cannibalism occurred among siblings, their lust for sodium must have been particularly powerful, i.e., to overcome the inclusive fitness losses, when the plant host proved deficient. 

CONCLUSION

Low plant sodium concentration promotes cannibalism in larvae of *C. lacinia*, an otherwise non-carnivorous, gregariously feeding herbivore. Mortality from other causes was also elevated at the lowest levels of plant tissue sodium, presumably owing to sodium deficiency. Non-cannibalistic mortality was elevated at the highest levels of plant tissue sodium, presumably owing to sodium toxicity. Therefore, our study highlights the disproportionate influences plant sodium concentrations have on animal behavior and performance, especially in the expression of
cannibalism. Sources of sodium for animal consumers are highly variable across terrestrial phytochemical landscapes (Hunter, 2016), which can have very consequential influences on micro- and macroevolutionary patterns, local adaptation, community assembly, and food web and eco-evolutionary dynamics.
CHAPTER 6
CONCLUSIONS

My dissertation investigated how sodium can influence plant and herbivore development, and their interactions align with how the phytochemical landscape of sodium is formed and maintained across heterogeneous gradients. Throughout a systematic review, field studies, and experimental approaches, I focused on how environmental and manipulated variation in sodium influences plant development and the formation of the phytochemical landscape and how the variation of sodium across the plant shapes animal development and behavior. My dissertation work highlights the importance of understanding the influence of plant nonessential elements (like sodium) on bottom-up effects across trophic levels. This interaction has been affected dramatically by anthropogenic influences in recent decades.

In Chapters 2 and 3, I assessed substrate sodium’s influence on plant growth and sodium accumulation strategies in controlled (Chapter 2) and field settings (Chapter 3). Both chapters are focused on how the phytochemical landscape of sodium is formed and maintained. In Chapters 4 and 5, I test how variation in plant sodium concentrations influences herbivore development and behavior using the sunflower- Chlosyne lacinia system.

Using a systematic review (Chapter 2), I summarized plant growth and sodium accumulation responses across increasing substrate sodium treatments in controlled settings, considering 107 populations in 66 species in 20 plant families. I found that the effects of increasing substrate sodium in plants growth related mostly to evolutionary history; some plants benefit while most experience increasing substrate sodium concentrations toxic at lower levels. At high substrate sodium concentrations, all plant species experienced growth inhibition. Sodium accumulation strategies mainly were similar across plants, with the highest sodium concentration
on their tissues as sodium in the substrate increases monotonically. These responses did not show a phylogenetic signal suggesting that plants species seem to be responding to increases in substrate sodium idiosyncratically. These findings let me generate the No-Escape-From-Sodium hypothesis, which posits that plants’ sodium accumulation responses reflect the increases in substrate sodium for most plant taxa, which also considers some unique exceptions. I also expose the problematic way plant responses to substrate sodium are assessed in controlled settings and give recommendations on improving the experimental design to achieve a general understanding of the physiological, ecological, and evolutionary consequences of substrate sodium inducing phenotypes across plants taxa regardless of their economic importance.

Considering the findings from Chapter 2, Chapter 3 focuses on assessing whether plants in their natural ranges follow the No-Escape-From-Sodium pattern based on intrinsic environmental sodium variation across the soilscape. For this experiment, I collected plants from the genus *Atriplex*, *Helianthus*, and *Opuntia* across 51, 131, and 83 sites, respectively, across the Southern United States and adjacent soils. After chemical analysis of all plant and soil samples, I found that, on average, plants from each taxon accumulated sodium positively across the varied amount of sodium in the substrate, with substrate sodium being the highest influencer of plant sodium concentrations. Strikingly, regardless of soil variation for calcium, magnesium, and potassium, plants appear to keep string levels of each element across the ranges they are found, suggesting that these elements are regulated. Since sodium is nonessential for plant metabolism and growth, a lack of homeostasis for the element is expected, a pattern contrary to the essential cation’s calcium, magnesium, and potassium. I also found that plant sodium showed a spatial autocorrelation pattern across the southern United States, demonstrating that closer plants respond similarly to environmental sodium concentrations contrary to calcium, magnesium, and
potassium. These findings suggest that herbivores might be experiencing varying amounts of sodium in plant tissues across the ranges they are found, but to a lesser degree for calcium, magnesium, and potassium. Given that sodium is an essential element for animals, this variation found across plant tissues might impact animal behavior and eco-evolutionary trajectories and possibly help shape plant-animal interactions and community assembly across a heterogeneous phytochemical landscape.

Chapter 4 tested whether variation in plant-sodium concentrations affected herbivore development and sodium accumulation strategies in males and females of *C. lacinia* by controlling sodium in plants hydroponically. I found that, on average, increasing treatments of sodium on plant tissues did not affect butterfly development. Still, in some cases, especially in the high treatments, developmental time was extended for some individuals. Surprisingly, in terms of sodium accumulation, I found that males accumulated more sodium than females as dietary sodium increased. Females tended to keep the same amount of sodium in their tissues across treatments. These findings show that males can accumulate sodium from their larval diets and carry it over to adulthood, with potential consequences on courtship behavior and mating success.

Finally, in chapter 5, I tested whether plant-sodium variation influences the behavior of the herbivore *C. lacinia* by using the same setup as in Chapter 4. In this study, I found that larvae developed on plants with high sodium concentration showed a higher risk of mortality (53% died). On the other hand, larvae that developed on plants deprived of sodium (i.e., control and low treatments) showed a higher risk of succumbing to cannibalism by their siblings. In the control treatments, there was also high mortality. These findings suggest that variation in sodium in plant tissues has a disproportionate effect on herbivore performance and behavior. These
patterns are also found in natural habitats, resulting in different strategies of survivorship, ecological interactions, and evolutionary consequences across the host and herbivore ranges.

This dissertation demonstrated sodium’s role in organismal performance across a varied phytochemical landscape. Therefore, I suggest that sodium should be regarded as a “keystone” element because of its disproportionate effect on ecological interactions, plant and animal performance, community assembly, and eco-evolutionary dynamics. Understanding the environmental role sodium and other microelements have on the phytochemical landscape and in trophic level interactions is a crucial question in ecological and evolutionary research.
APPENDIX A. SUPPLEMENTARY MATERIAL FOR CHAPTER 4

Table A.1. Two-way ANOVA result output for day of eclosion.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Df</th>
<th>Sum squares</th>
<th>Mean squares</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>42.4</td>
<td>14.133</td>
<td>2.178</td>
<td>0.093</td>
</tr>
<tr>
<td>Feeding trial</td>
<td>1</td>
<td>9.2</td>
<td>9.173</td>
<td>1.414</td>
<td>0.236</td>
</tr>
<tr>
<td>Treatment: Feeding trial</td>
<td>3</td>
<td>24.2</td>
<td>8.062</td>
<td>1.242</td>
<td>0.297</td>
</tr>
<tr>
<td>Residuals</td>
<td>140</td>
<td>908.3</td>
<td>6.488</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A.2. Three-way ANOVA result output for pupal mass.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Sum squares</th>
<th>Mean squares</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1</td>
<td>0.0926</td>
<td>0.0926</td>
<td>190.74</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>0.0005</td>
<td>0.0002</td>
<td>0.308</td>
<td>0.819</td>
</tr>
<tr>
<td>Feeding trial</td>
<td>1</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.126</td>
<td>0.724</td>
</tr>
<tr>
<td>Sex: Treatment</td>
<td>3</td>
<td>0.0036</td>
<td>0.0012</td>
<td>2.451</td>
<td>0.066</td>
</tr>
<tr>
<td>Sex: Feeding trial</td>
<td>1</td>
<td>0.0015</td>
<td>0.0015</td>
<td>3.057</td>
<td>0.083</td>
</tr>
<tr>
<td>Treatment: Feeding trial</td>
<td>3</td>
<td>0.0014</td>
<td>0.0005</td>
<td>0.983</td>
<td>0.403</td>
</tr>
<tr>
<td>Sex: Treatment: Feeding trial</td>
<td>3</td>
<td>0.0042</td>
<td>0.0014</td>
<td>2.871</td>
<td>0.039</td>
</tr>
<tr>
<td>Residuals</td>
<td>131</td>
<td>0.0636</td>
<td>0.0005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A.3. Three-way ANOVA result output for adult body-segment development.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Df</th>
<th>Sum squares</th>
<th>Mean squares</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1</td>
<td>2925.1</td>
<td>2925.1</td>
<td>563.81</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>54.4</td>
<td>18.1</td>
<td>3.496</td>
<td>0.016</td>
</tr>
<tr>
<td>Tissue</td>
<td>1</td>
<td>520.7</td>
<td>520.7</td>
<td>100.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex: Treatment</td>
<td>3</td>
<td>56.9</td>
<td>19</td>
<td>3.655</td>
<td>0.013</td>
</tr>
<tr>
<td>Sex: Tissue</td>
<td>1</td>
<td>1307.2</td>
<td>1307.2</td>
<td>251.96</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment: Tissue</td>
<td>3</td>
<td>4.1</td>
<td>1.4</td>
<td>0.263</td>
<td>0.852</td>
</tr>
<tr>
<td>Sex: Treatment: Tissue</td>
<td>3</td>
<td>18.5</td>
<td>6.2</td>
<td>1.189</td>
<td>0.314</td>
</tr>
<tr>
<td>Residuals</td>
<td>277</td>
<td>1437.1</td>
<td>5.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table A.4. Three-way ANOVA result output for body-segment sodium concentrations in Solitary individuals.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Df</th>
<th>Sum squares</th>
<th>Mean squares</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1</td>
<td>3.81</td>
<td>3.811</td>
<td>5.88</td>
<td>0.019</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>6.02</td>
<td>2.006</td>
<td>3.099</td>
<td>0.035</td>
</tr>
<tr>
<td>Tissue</td>
<td>1</td>
<td>0.91</td>
<td>0.910</td>
<td>1.406</td>
<td>0.241</td>
</tr>
<tr>
<td>Sex: Treatment</td>
<td>3</td>
<td>5.12</td>
<td>1.708</td>
<td>2.639</td>
<td>0.060</td>
</tr>
<tr>
<td>Sex: Tissue</td>
<td>1</td>
<td>0.52</td>
<td>0.519</td>
<td>0.802</td>
<td>0.375</td>
</tr>
<tr>
<td>Treatment: Tissue</td>
<td>3</td>
<td>0.98</td>
<td>0.326</td>
<td>0.504</td>
<td>0.681</td>
</tr>
<tr>
<td>Sex: Treatment: Tissue</td>
<td>3</td>
<td>0.45</td>
<td>0.151</td>
<td>0.243</td>
<td>0.872</td>
</tr>
<tr>
<td>Residuals</td>
<td>50</td>
<td>32.36</td>
<td>0.647</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX B. COPYRIGHT INFORMATION

No escape: The influence of substrate sodium on plant growth and tissue sodium responses

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Abstract
1. As an essential micronutrient for many organisms, sodium plays an important role in ecological and evolutionary dynamics. Although plants mediate trophic fluxes of sodium, from substrates to higher trophic levels, relatively little comparative research has been published about plant growth and sodium accumulation in response to variation in substrate sodium. Accordingly, we carried out a systematic review of plants' responses to variation in substrate sodium concentrations.

2. We compared biomass and tissue-sodium accumulation among 107 cultivars or populations (67 species in 20 plant families), broadly expanding beyond the agricultural and model taxa for which several generalizations previously had been made. We hypothesized a priori response models for each population's growth and sodium accumulation as a function of increasing substrate NaCl and used Bayesian Information Criterion to choose the best model. Additionally, using a phylogenetic signal analysis, we tested for phylogenetic patterning of responses across taxa.

3. The influence of substrate sodium on growth differed across taxa, with most populations experiencing detrimental effects at high concentrations, irrespective of growth responses, tissue sodium concentrations for most taxa increased as sodium concentration in the substrate increased. We found no strong associations between the type of growth response and the type of sodium accumulation response across taxa. Although experiments often fall to test plants across a sufficiently broad range of substrate salinities, non-crop species tend toward higher sodium tolerance than domesticated species. Moreover, some phylogenetic conservatism was apparent, in that evolutionary history helped predict the distribution of total-plant growth responses across the phylogeny, but not sodium accumulation responses.

4. Our study reveals that saltier plants in saltier soils prove to be a broadly general pattern for sodium across plant taxa. Regardless of growth responses, sodium accumulation mostly followed an increasing trend as substrate sodium levels increased.

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LIST OF REFERENCES


106


Hijmans, R. J., & van Etten, J. (2012). raster: Geographic analysis and modeling with raster data.


R Core Team (2020). R: A language and environment for statistical computing.


doi: 10.1086/692734


https://doi.org/10.1016/j.plaphy.2011.10.009

https://doi.org/10.1016/j.foreco.2019.117637


https://doi.org/10.1111/pbi.13443

https://doi.org/10.15252/embj.2019103256

https://doi.org/10.1098/rspb.2015.1001


of the National Academy of Sciences, 117(13), 7271–7275. doi: 10.1073/pnas.1920012117


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VITA

The main scholarly interest of Luis Y. Santiago-Rosario lies at the intersection of ecology, development, evolution, and behavior. In particular, Luis is interested in understanding how phenotypes arise given environmental influence. His interest in the biological sciences, ecology, and natural history began on a coffee farm in Ciales, Puerto Rico, where he grew up surrounded by tropical forests and beautiful Caribbean fauna. Luis started his academic career whilst doing a bachelor’s degree in biology from the University of Puerto Rico in Aguadilla, Puerto Rico. After earning his bachelor's degree, he worked for the Department of Agriculture of Puerto Rico (DAPR) and pursued a master’s degree in Biology from the Inter-American University of Puerto Rico, Bayamón, Puerto Rico. During his master’s degree and by working at DAPR, he developed a passion for ecology, a passion that brought him to pursue his doctoral degree at Louisiana State University (LSU), Baton Rouge, LA, under the guidance of Dr. Kyle E. Harms. Luis’s work at LSU has focused on understanding how sodium mediated plastic responses on plants and animals across heterogeneous gradients along with other projects. After finishing his Ph.D., Luis will be a postdoctoral researcher in Dr. Sarah Mathews’s lab at LSU where he will be working on elucidating whether Australian mistletoes in the family Loranthaceae mimic their host morphologically and chemically. Also, Luis has been awarded the NSF Postdoctoral Research Fellowship in Biology which he will start his appointment on March 2023 at the University of Minnesota (UMN), Twin City Campus. He will be training with Dr. Emilie Snell-Rood and Dr. Sara Hobbie from UMN, and with Dr. Dylan Craven from the Universidad Mayor in Santiago, Chile. His work will be focusing on elucidating how plants’ heavy metal and salt concentrations are influenced by urbanization and how plants of differing life histories deal with these elements and pollutants.