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Article

Osmotic characteristics of water retention structures of *Bursera microphylla* in relation to soil salinity

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SUMMARY

With a worldwide increase in soil salinity, as well as the climate crisis raising global temperatures, it is important to understand the effects of soil salinity on plant survival. One way to understand how plants may evolve to survive in these xeric desert environments is to gain a further understanding of existing halophytic (live in saline environments) and/or xerophytic (live in dry, arid environments) plants. *Bursera microphylla* in particular is quite interesting because it is both a halophyte and a xerophyte. One reason it is able to survive in dry environments is due to its bulbs at the base of the trunk and major limbs of the tree, which have been shown to store water during droughts. To facilitate mineral uptake, plants use ATP to transport NaCl ions into the plant, which facilitates water uptake. Because observed field conditions in the Baja California Peninsula were extremely xeric, most plants there have distinct evolutionary traits to facilitate water storage or retention. The Elephant Tree (*Bursera microphylla*) gets its name from the abnormal proportions of the base of its stem, which is much wider than much of the tree, and studies show that this disproportionately wide stem can store water for use during times of drought, which are common in the Baja California Peninsula. Our hypothesis was that sodium chloride was taken up through the plant's root structure to facilitate this water transportation, and that this sodium chloride accumulation was directly proportional to the soil salinity. We tested this hypothesis via light microscopy of plant tissues to observe cell tonicity under different concentrations of saline solution. soil salinity was measured using saturated extraction. The results of this experiment show that most cells within the "bulb" structures were isotonic at a concentration approximately twice as high as that of root tissue and ambient soil salinity, therefore supporting the presented hypothesis.

INTRODUCTION

B. microphylla is native to the extreme southern regions of the southwest United States and several states in Mexico, with its range contained mostly in the states of Sonora, Baja California, and Baja California Sur (3). *B. microphylla* is unique in that it is both a halophyte and a xerophyte, meaning that it can survive in very dry conditions and in soil with abnormal saline concentrations. *B. microphylla* is well suited for the Baja California desert, and in environments where most other vascular plant life cannot be found, *B. microphylla* naturally occurs. Soil salinity also plays a role in plant mineral uptake characteristics. Field site emulation refers to the methodology used to simulate field conditions in which a subject of experimentation lives to better understand how the subject interacts with its environment in a controlled and regulated laboratory environment. This ensures that emulated field sites are representative of organisms in their natural habitat. We performed field site emulation to emulate the environments observed in Baja California and Baja California Sur, by growing emulated field sites in a low-humidity, high temperature environment within a controlled lab space. Observed field sites in the Baja California Peninsula receive approximately 40 mm of rain per year and experienced an average temperature of 31.5°C (4). High soil salinity in certain regions of Baja California shows that plants that can survive in this region have specific adaptations that permit them to do so (5).

Mineral uptake is the process by which plants, generally in the wet season when water is relatively plentiful, transport ions across the cell membrane via active transport to use these ions for a variety of applications (1,2). One of these applications is to uptake water via osmosis, a process by which water moves across a semipermeable membrane towards a solution of a higher concentration on the other side of the membrane to establish equilibrium. Osmosis is performed through passive transport, which does not require ATP, meaning, in the context of this study, that it can be performed in plants during times of drought (1).

The "bulb" structures at the base of the tree's trunks and limbs have been shown in previous studies to store reserves of water (2). The hypothesis presented in this study is that these bulbs not only store water, but also the sodium chloride necessary to retain water from its environment during times of drought. This allows plants to survive during times of drought in the xeric Sonoran Desert of the Baja California Peninsula. In this study, we used field site emulation to simulate conditions observed in the natural habitat of *B. microphylla*, which was observed during June of 2023 by the first junior author, dissection and microscopic analysis of cell tonicity of plant root tissue and bulbous tissue under different concentrations of saline solution, which were tested on individual resected tissue samples, and saturated extraction to quantify soil salinity. We found that the isotonic concentrations of the bulbous tissue were approximately twice that of both the root tissue isotonic concentration and the soil salinity of the

soil of each respective "SB" ("store-bought, as they were purchased from a nursery) emulated field site, indicating that *B.microphylla* stores NaCl ions within the bulb structure, supporting our hypothesis.

RESULTS

Measurement of soil salinity allowed us to measure how much available NaCl was in the soil, which would allow us to predict the isotonic concentration of each "SB" site. Saturated extraction of the soil in which the emulated field sites were growing was performed to extract ions from the soil. This allowed us to calculate the expected isotonic molar concentration, in which the root cells of each respective field site would be isotonic under, meaning that the amount of salt in the soil and in the roots would be the same and no osmosis-influenced water uptake was occurring. Saturated extraction data was initially collected in micro-Siemens per centimeter (µS/cm). Siemens (S) are the SI unit for electrical conductivity, which is the number of total ions dissolved in a centimeter of solution. Because we were measuring saline conductivity, our electrical conductivity (EC) measurements refer to the total amount of NaCl ions dissolved in solution in µS/cm. It was determined that the average amount of ions added to the solutions by the filters used to filter the digested soil solution from the soil itself (see Methods) was equivalent to 10.33µS/cm ± 0.12 µS/cm (**Figure 1**). This amount was appropriately accounted for as a blank effect, a source of deviation which is represented here as an error bar, for the saturated extraction experiments. Microscopy allowed us to validate or refute our predicted isotonic values for each plant structure (roots, bulb, etc.) and to compare the isotonic concentrations of each plant structure to one another, this was done by observing thin sections of plant tissue under whichever saline concentration was being tested and observing if the majority of plant cells in the sample were hypertonic, hypotonic, or isotonic.

All emulated field sites were denoted with "SB" followed by their replicate number. These emulated field sites were grown under the same ambient conditions (temperature, watering, humidity, light exposure), with an exception being soil salinity. Three replicates were produced and experimented upon; they have been denoted as SB-1, SB-2, and SB-3.

The saturated extraction results for each emulated field site showed that SB-1 soil had an average soil salinity of 26.8 mg/g , SB-2 soil had an average soil salinity of 5.92 mg/g, and SB-3 soil had an average soil salinity of 7.42 mg/g (**Figure 2**). These values were then used to calculate the approximated isotonic solution of SB-1, SB-2, and SB-3 root tissues, which were grown in their respective soil (see Methods). The expected root isotonicity values, which were the concentrations in which no water uptake occurred, were 0.07M, 0.02M, and 0.02M, respectively.

Root tissue microscopy was then performed to produce mineral uptake and cell tonicity data that was used as an analog for comparison of bulbs tissue tonicity. We determined that root tissues of SB-1 were isotonic under 0.07M NaCl conditions, with a margin of error of ±0.01M. This was the predicted isotonic concentration (**Figure 3**). Root tissues of SB-2 were isotonic at the predicted concentration of 0.02M NaCl solution. Root tissues of SB-3 were isotonic at the predicted concentration of 0.02M NaCl solution. There was no standard deviation for SB-2 and SB-3 root values. Results of the root microscopy experiment show that the root tissue of each respective field site was isotonic at the predicted isotonic value, meaning that the root tissue of each SB site was isotonic in the soil it was in, meaning that there was no osmosis-influenced water uptake from the soil to the root tissues.

Light microscopy of cross-sections of plant bulb tissue provided the following tonicity data: SB-1 bulbs cross-section tissue was isotonic under 0.07M solution (±0.01M). SB-2

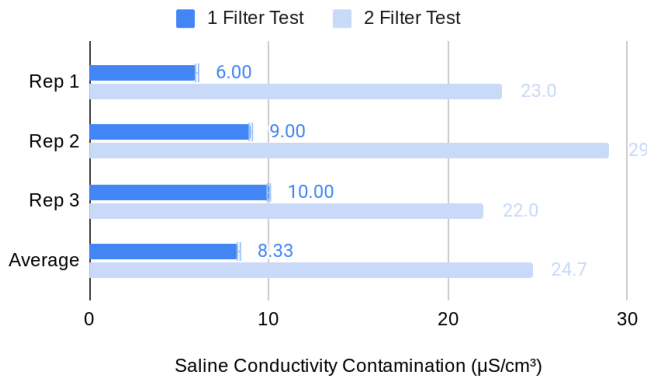

Figure 1. Amount of sodium chloride ions, in µS/cm added to the solution by the filters used. Tested by filtering 30 mL of distilled water through one or two coffee filters (One filter tests are shown in dark blue, while 2 filter tests are shown in light blue) and measuring the EC of this solution. 3 replicates were tested and an average was taken, the standard deviation was ±0.9 µS/cm per filter, this is reflected on the graph with error bars.

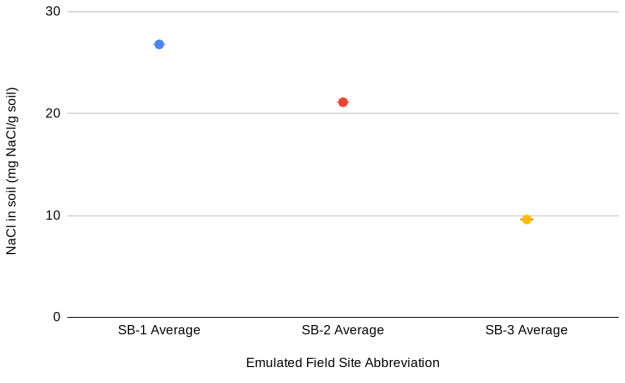

Figure 2. Saturated extraction data of each emulated field site (SB site). Soil salinity data for each SB site in milligrams of salt per gram of soil (mg/g) Saturated soil solution was filtered through coffee filters and measured with a saline EC meter. EC is adjusted to account for blank value contamination from filters and standard deviation is indicated.

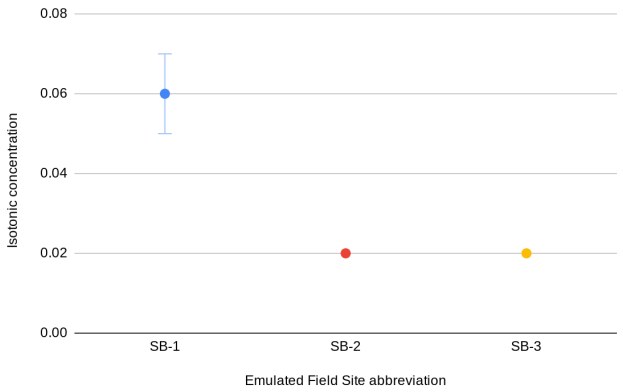

Figure 3. Root tissue tonicity chart. Average isotonic concentration of root tissue for all replicates of each SB site as observed during light microscopy under different molar concentrations of solution with standard deviation indicated.

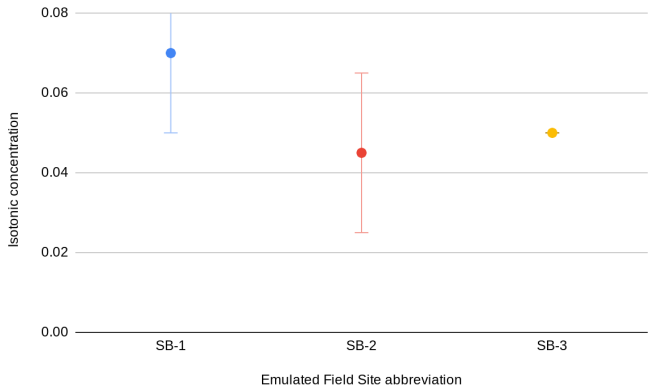

Figure 4. Bulb tissue tonicity chart. Average isotonic concentration of bulb tissue for all replicates of each SB site as observed during light microscopy under different molar concentrations of solution with standard deviation indicated.

bulbs tissue was isotonic under 0.045M solution (±0.007M) (**Figure 4**). All replicates of SB-3 bulbs tissue were isotonic under a saline concentration of 0.05M, there was no variance and thus no standard deviation. The bulb tissue microscopy displayed that the isotonic concentration of the space within this structure was notably higher than both the root and soil salinity. This shows that the bulbs' structure does accumulate and/or store sodium chloride.

DISCUSSION

Due to the isotonic concentration within the bulbs' structures of each respective field site being higher than that of their roots, and higher than the soil salinity of each field site, it can be concluded that an osmotic gradient is formed that permits an accumulation of sodium chloride within the bulb structure.

In summary, the results show that the "bulb" tissue of *B.microphylla*, located at the base of the trunk of the tree, uptakes and retains water by sequestering saline of a higher molar concentration than the root tissue and ambient soil. This study also shows that *B. microphylla* plants can survive in environments with ambient soil salinity between 9.61 and 26 mg/g of NaCl, further exhibiting their high drought and saline tolerance. This study also sheds light on general water retention by focusing on quantification of saline within plant vascular structure in relation to that of soil salinity.

A potential source of error in the procedure occurred in which SB-1 was grown in slightly different soil than SB-2 and SB-3. This is because SB-1 was purchased from a different vendor than the other SB sites. This is why saturated extraction soil salinity was different for SB-1 than the other field sites. Other than that, no variables were changed for SB-1, and it was kept under the same conditions as the other SB sites for the entirety of experimentation. Other potential errors include the use of iodized salt to produce salt solutions, which may have affected measurements of molarity and introduced a gap between observed soil salinity and tested soil salinity, and potential cross-contamination between specimens on drop plates during storage. Cells within the tested structure were of several types of tissue cells (parenchyma, phloem, collenchyma, etc.), which may have resulted in cells of a certain type being isotonic under a certain concentration of sodium chloride, while others are of a different tonic type. We attempted to account for this by finding at least 10 different cells in different quadrants of the specimen and using the majority of these cells' tonic state to determine the total tonic state of the specimen under whichever test solution was being used. This experiment did not test any other vascular dicots which are known to contain salt for mineral uptake. A control was also not present in this study. Other potential errors could have been the presence of other ionic salts which contributed to water retention and/or mineral uptake; these were not tested in emulated field sites and would require separate experimentation and procedures.

Future experimentation may include use of actual field specimens and saplings taken from Baja California and Baja California Sur, rather than emulated field sites. The use of field specimens from a variety of habitats allows for a more accurate representation of the species, which permits a greater understanding of plants with similar water retention structures, including other species that are ecologically similar to *B. microphylla* (7).

Future experimentation could include further investigation of water retention and storage structures within halophytic and/or xerophytic species of *Burseraceae* and other genera. Other questions that remain pertain to the relation of soil salinity to vascular tissue dilation, the number of ions that a plant can store within a given interval, and the relation between soil salinity and plant adaptations, amongst other potential future scientific studies.

The implications of this study include further investigation into mineral uptake stress-testing to test the threshold under which xerophytes, such as *B. microphylla*, can effectively uptake water during times of drought and, therefore, survive.

Another potential study may include further investigation into the evolutionary roots of these water retention structures, especially in the *Burseraceae* family, as well as other vascular plants.

Climate change has profoundly affected world agriculture, with one of the primary effects being soil salinization, by which water, containing dissociated sodium and chloride ions, is evaporated by rising temperatures, leading to increasing concentrations of NaCl, which is toxic to most vascular plants. By studying plants that can survive in high-saline and arid environments, we can better understand the evolutionary characteristics of desert plants and their physiology.

MATERIALS AND METHODS

Field site emulation

Field sites in eastern Baja California and Baja California Sur were emulated by placing 3 young *Bursera microphylla* saplings (each of these had one "bulb" structure at the base of the tree) (Red Island Plants) under a heat lamp and near a window in a controlled laboratory environment with low humidity(between 0% and 20%), which provided the plants with sunlight and an increased temperature (30-50°C). This simulated sunlight setup was turned off for 8-9 hours to emulate a night cycle. Water was given to the plants on the 15th of each month. The amount of water given was dependent on the amount of rainfall in Loreto, Baja California Sur, México for that month given historical values for that given month (typically 15-20 mL per month). Field sites in eastern Baja California, at approximately 26 degrees north, -111 degrees west, were observed to be in dry, arid conditions, with dry, hardpan soil, with no fresh water nearby. Temperatures ranged from 26°C to 49°C at the time of observation. For the following experiments, three replicates per SB site were produced and tested for each experiment. No change of conditions occurred for any SB site throughout experimentation. The three SB sites were set up to better reflect the species and ensure procedural efficacy and replicability. Values for these parameters were chosen to best simulate conditions observed in the field sites. Plants were grown for 7-8 weeks before they were harvested for experimentation.

Production of test solution

The test solution was produced by calculating the grams of NaCl necessary to produce the desired molarity of solution. This was done with the molarity formula (Molarity = Mol_{Solute}/$L_{solvent}$). 200 mL of water was multiplied by the desired molarity, and this was multiplied by 58.44 g (the molar weight of NaCl). 200 mL of water was then measured with a graduated cylinder and a watch glass was placed over it to prevent evaporation. The salt was then weighed out with a scale (Thermo-Fisher Scientific) and added to a 250 mL beaker. The water was then added to this beaker and stirred thoroughly until the salt no longer precipitated out onto the bottom of the beaker.

Universally applied methodology for microscopy

For microscopy, we aimed to measure what tonic stage (cellular hypotonicity, hypertonicity, or isotonicity) was present in the plant tissue samples. This was done by suspending tissue in a test solution (a solution used to simulate the extracellular fluid in structures) for 24 hours, and then observing it under a light microscope.

Tissue was collected with tweezers from an area of the plant that would best represent the average, uniform area of that specific structure (i.e., tissue was not from a section that appeared to be dying or abnormal). One leaf was then placed, with forceps, onto a drop plate containing the saline concentration it was to be tested in; It was then stored in that solution for 24 hours. The tissue sample was then placed onto a clean slide, and 1-2 drops of saline solution were then dropped with a clean pipette onto the sample until the sample was covered in the test solution. To efficiently test cell tonicity and to mitigate contamination of tissue specimens, DI water was used to wipe the coverslip three times, then a clean Kimwipe, before applying the new test solution to the coverslip. A coverslip was then gently lowered onto the leaf using tweezers. This slide was then stained at room temperature with approximately 2-3 mL of Carolina Biological Iodine solution (item no. 177020), added with a second clean pipette, which was washed with test saline solution until there was no visible discoloration underneath the coverslip. This slide was then observed under a light microscope until the tonic state of an individual layer, group, or cell is visible.

To observe cell tonicity, visual observations were made through the microscope at 100x-400x magnification. Hypertonic observations included: wrinkling or cytoplasm drawn farther back than usual from the cell wall, or a conjunction of both. Traits that indicate hypotonicity would be the cytoplasm "pressed" laterally against the cell wall and/or lateral curvature of the cell wall, making the cell look rounder. This cell was photographed with a phone or microscope camera and put into a shared document. The tonic state of the cell was then appropriately documented.

Dissection of vascular tissue

Vascular tissue was dissected via resection of tissue from plant growth of the trunk of the tree at a specific height determined by measuring with a ruler from the base of the plant to the superior bound of the resection. This was done by carving V-shaped areas approximately 2-3 mm deep into the plant's trunk. The sample was placed onto a drop container and transported to an area in which it could be dissected in a clean environment to mitigate contamination via biological factors that would hinder effective microscopy. The sample was then dissected into 3 parts, each with a width of about 0.5 mm, which would serve as replicates of the respective vascular tissue resection. Replicates of vascular tissue were then stored on a drop plate in the saline solution in which they would be tested. This drop plate was stored in a sealed plastic bag to prevent the test solution from increasing in

concentration due to evaporation of water. Root tissue was taken from the plants after all other vascular and leaf microscopy had been performed. This is because removal of root tissue could be detrimental to the plant's health. Root samples were resected from an area representative of average growth, and were taken, with a clean scalpel, from an area 3-4 mm from the tip of the root. This resection was then dissected, stored, and observed in an identical manner to that of vascular tissue. It is important to note that dissection has been performed with a dissection scope, scalpel, and forceps. Therefore, measurement estimates ranged between 0.3 mm and 0.7 mm in width.

Accounting for different cell types within a tissue specimen

Resected tissue tested in microscopy experiments contained many distinct types of cells. These cells may have behaved differently under a specific molar concentration of sodium chloride than cells of diverse types. This was accounted for by observing at least 10 cells from each specimen, with at least 2 cells from each quadrant (defined by the position of the cell relative to a vertical and horizontal midline, with quadrant I being on the right superior side of the specimen, and quadrant IV being on the inferior left side, and so on) being observed. The tonic state of the majority of these cells was recorded as the tonic state of the specimen at the specific saline concentration.

Saturated extraction for soil salinity

Soil salinity was measured using the saturated extraction method. A saline electrical conductivity (EC) meter was used to measure electrical conductivity of NaCl ions dissolved in solution. Firstly, 5.0 g of soil from the pots of one field site were weighed. Once measured to be at 5.0 g, deionized (DI) water was added to the beaker until the soil solution was entirely saturated. The beaker was then labeled with "DSD" (Direct Soil Digestion), the abbreviation of the field site (SB-1, SB-2, etc.), the date, and the replicate number (rep 1, rep 2, etc.). A watch glass was placed over this beaker, and it was left to sit for 24 hours. A flask that had a funnel with a coffee filter inside the neck of the flask was then set up. After 24 hours, the DSD beaker was emptied into the filter, any remaining soil in the beaker was sprayed with a DI water squirt bottle until all soil was emptied into the filter. A watch glass was then placed over the filter, and the solution was left to filter through for 24 hours. Once this had occurred, the saline conductivity probe was rinsed with DI water 3 times, wiped dry with a Kimwipe, set to mS/cm, and the filtered solution in the flask was tested with the saline conductivity meter. These measurements were considered stable once the meter had stayed on one value for 3 seconds or longer. The solution in the flask was then poured into a graduated cylinder to calculate the volume of solution in the flask. Measurements for the respective field site and replicate were entered into a spreadsheet, along with the volume of solution in the flask, and the grams of soil. The soil salinity, in mg/g, was then calculated via TDS (total dissolved solids) $ppm_{550} = 550 \cdot \sigma$, where σ is the conductivity in mS/cm. In this case, ppm is defined as the mg of solute per liter of solvent. TDS was then multiplied by mL of water extracted to get the number of milligrams of NaCl extracted out of the soil sample. The number of mg was divided by the number of grams of soil to get the milligrams of salt per gram of soil.

Accounting for contamination from filters

To account for the contamination brought about by the filters used (in this case, coffee filters), 30 mL of high purity distilled water was filtered into a beaker. The average saline concentration of these solutions was 10.3 µS/cm ± 0.12 µS/cm per filter. This value was a blank effect and was subtracted from the average EC value for each field site during the saturated extraction experimentation.

Interpretation of saturated extraction data to yield predicted isotonic value

Data from the saturated extraction experimentation was entered into a Google sheet formula which would perform the following calculations described here. The mass of soil was divided by the volume of solution in the FSS (Filtered Soil Sample) flask to yield the ppm value. Total dissolved solids (TDS) were calculated by multiplying the electrical conductivity (EC) value by 550 and dividing this number by the ppm. This number was then divided by the liters of solution in the FSS flask to yield the milligrams of salt in the soil, and the milligrams of salt was then divided by the liters of solution to yield the milligrams of soil per grams of salt, which is the standard format for reporting saturated extraction soil salinity. The mathematical formulas used for saturated extraction interpretation are below:

$$\text{TDS} \times \frac{\text{grams of soil}}{\text{milliliters of solvent}} = 550 \times \text{EC}$$

$$\text{TDS} = 550 \times \sigma \times \frac{\text{milliliters of solvent}}{1000}$$

$$\text{milligrams of salt} = \text{TDS} \times \frac{\text{milliliters of solvent}}{1000}$$

$$\frac{\text{milligrams of salt}}{\text{grams of salt}} = \text{soil salinity}$$

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REFERENCES

1. Chabaux, Francois, *et al*. "Plant-soil-water Interactions: Implications From U-Th-Ra Isotope Analysis in Soils, Soil Solutions and Vegetation (Strengbach CZO, France)." *Geochimica Et Cosmochimica Acta*, vol. 259, Aug. 2019, pp. 188–210.
2. H, Lundegardh. "TRANSPORT OF WATER AND SALTS THROUGH PLANT TISSUES." *Nature*, May 1946.
3. Mooney, H. A., and W. A. Embodedn. "The Relationship of Terpene Composition, Morphology, and Distribution of Populations of Bursera Microphylla (Burseraceae)." *Brittonia*, vol. 20, no. 1, Jan. 1968, p. 44.
4. Turner, Raymond M., *et al*. *Sonoran Desert Plants: An Ecological Atlas*, 2005.
5. Ezcurra, Exequiel, and Eric Mellink. «Desert ecosystems.» (2013): 457-478.
6. Curry, Tierra R. "Mexico's Tropical Dry Forests." *Imperiled: The Encyclopedia of Conservation*, 2022, pp. 76–84.
7. Espinosa, David, *et al*. "Historical Biogeographical Patterns of the Species of Bursera (Burseraceae) and Their Taxonomic Implications." Journal of Biogeography, vol. 33, no. 11, Nov. 2006, pp. 1945–58.