**Sampling *Bomarea* tubers in Antioquia: a preliminary evaluation of chemical defense in underground storage organs**

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Walk through an Andean cloud forest and you are likely to see at least one of the over one hundred species in the genus *Bomarea* growing among the moss-covered trees that hang heavy with epiphytic bromeliads and orchids. *Bomarea* is closely related to plants sold as a cut flower in florists and supermarkets as the Peruvian Lily. Unlike those short flowers, many species of *Bomarea* encircle the branches and trunks of small trees, snaking upwards to extend their brilliantly colored flowers and fruits more than 5 meters above the forest floor. But my interests lie below, in the swollen underground organs where these plants store energy-rich starch, similar to the way camels store fatty tissue in their humps.

My research in evolutionary biology focuses on how plants have adapted to survive in their unique environments and climates. I am interested in how plants like *Bomarea* defend their juicy tubers from the hungry insects, bacteria, fungi, and nematodes that live below the forest floor. Plants have evolved many defenses against herbivory, including specialized chemicals that repel would-be predators, and often these chemicals are uniquely suited to defend against the specific suite of herbivores that species most often encounters. For my PhD dissertation, I hope to study the evolution of these chemicals in *Bomarea* tubers over the distinct altitudes and climates the various species inhabit. While most species thrive in cloud forests, some grow closer to sea level in the dense humid forests typical of tropical lowlands, while others inhabit the high-altitude bogs found at the top of Andean mountains (the páramo ecosystem), up to 5,000 meters above sea level. I hypothesize that *Bomarea* species have evolved unique chemical defenses that have allowed them to diversify across this altitudinal gradient. Insight into this process will allow scientists to better understand how organisms adapt to novel climatic conditions.

I spent almost two months living in Medellín, Colombia working in collaboration with Dr. Fernando Alzate, a professor in the Universidad de Antioquia (U de A). I collected samples of *Bomarea* to evaluate what chemicals may be defending the tubers from underground herbivory, and if or how those chemicals differ from those produced in leaves of the same species. I also reviewed collections in the Herbario de la Universidad de Antioquia (HUA), a botanical library housing over 200,000 preserved plant specimens that have been carefully collected and curated over the past 50 years. Herbarium specimens consist of carefully pressed and dried plant material in addition to information on when and where the plant was collected, observations about the plant's condition or appearance that may not be preserved in the drying process (such as color, smell, etc.), and the general ecosystem in which the plant was growing. For a biologist interested in how plants adapt to their environments, the herbarium is an exhilarating source of information and inspiration.

The HUA is a particularly active herbarium. Botanical expeditions of students and researchers frequently embark to survey and document species in remote parts of Antioquia, a province in the northwest Colombia. Violence caused by internal conflict between guerilla groups, paramilitary brigades, and law enforcement has decreased significantly over the past decade, allowing researchers to record the biodiversity of regions that had previously been inaccessible. Despite this, I observed specimens of *Bomarea* that had been collected in particularly dangerous regions during the peak of the conflict in the late 1980s[1]. Researchers who work for the herbarium tell stories of fleeing the forest when a guerilla group blew up electricity towers nearby[2]. Despite the brave efforts of these biologists, many regions of Colombia remain a mystery, botanical black boxes to the scientific community. Colombia has the second highest number of plant species of any country on Earth[3], and herbaria like the HUA are at the forefront of documenting that biodiversity and advocating for its protection.

Herber Sarrazola is a Masters student at the U de A. On my first trip into the field, we rode his motorcycle out of the valley of Medellín up to a hilly community at close to 2,500 meters above sea level. I packed my supplies in my backpack and held my plant press tightly against my stomach, trying keep my balance on the winding mountain roads. We found *Bomarea bredemeyerana* growing at the edge of a clearing in a small secondary forest close to the town of Santa Elena. Several stems climbed up the tree trunks, easily spotted by the deep garnet color of the tightly clumped flowers. I took out the gardening kit I had purchased the day before at a local hardware store and began to dig at the base of the stem, searching for the tubers that should be growing from the roots below. After carefully digging for ten minutes, the plastic handle of the small spade bent in half. I began to realize why underground herbivory is understudied. I needed a better shovel. Undeterred, I used my hands to clear the dirt carefully away from each root I found, gently pulling to avoid breaking the only link to possible tubers below. From that first individual I found 5 tubers the size of fingerling potatoes dangling from the tips of the roots.

With Herber's help, I was able to collect tubers and leaves from three individuals in that forest clearing. I sliced the tubers carefully with a mandolin and placed the slices and leaves in silica gel, a desiccant, to remove moisture quickly and prevent decomposition and degradation of the chemicals inside. The sliced tubers smelled like raw potatoes. I set one leaf per individual aside for DNA analysis. I cut the flowers from two stems and carefully arranged them in the plant press, creating a permanent record to which my chemical samples and DNA would be tied.

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[1] In 1989, Dr. Ricardo Callejas collected a specimen of *Bomarea diffracta* in one of the dangerous townships of Amalfi, a rural municipality in Antioquia. *Callejas 9051 HUA.*

[2] Liliana Londoño Ortiz, personal communication. July 2016.

[3] "Colombia - Country Profile." Main Details. Convention on Biological Diversity, n.d. Web. 02 Sept. 2016. <https://www.cbd.int/countries/profile/default.shtml?country=co#facts>.

In subsequent trips to the field, I brought a larger and more resistant shovel that allowed me to quickly loosen the dirt around the base of the plant before sifting through and looking for tubers. To reach the high-altitude páramo ecosystem where *Bomarea linifolia* thrives, Herber and I rented mules to take us up a steep trail that cut through canyon-like insets in mountain and passed through pits of mud so deep it went up passed the mules' knees. I gripped the saddle in front with one hand and behind with the other to steady myself as my mule lunged upward and downward over the rocky terrain. We passed through dense cloud forest, where the bark of the trees was completely obscured by thick layers of moss, and the light filtered dimly through the leaves of bromeliads clinging to the branches above. From the trail I observed two species of *Bomarea,* but we were short on time and the goal of this trip was *Bomarea linifolia,* which is restricted to the páramo ecosystem and is abundant particularly in this region, the Páramo del Sol.

After several hours, the trees began to clear and I noticed a small population of plants in the genus *Espeletia*, closely related to sunflowers and indicators of the unique flora found in páramo regions. Soon after, I spotted the distinctive red and yellow flowers of *Bomarea linifolia*. It was growing out of the side of a mound of peat, and after carefully peeling away thick, damp layers of moss I found the tubers – pea sized protrusions coming off of a central stem. That underground organ was just as long as the stem above.

After collecting four more individuals, we stopped for lunch. Our guide and the owner of the mules, Don José Herrera, told stories about various accidents that happened on the mountain. Two horses fell off the side of the trail a few years ago, broke their legs, and he killed them with a machete. A horse with broken legs cannot recover. Don José only takes mules up the mountain now. He talked about the violence and people that died from a decade ago, when the region was a hotbed of guerilla activity. In 2004, a group of students and a professor from the U de A were retained for three days by members of the FARC guerilla group in the Parque Nacional de las Orquídeas, around 20 km from the Páramo del Sol[4]. The violence, in addition to the difficult terrain, may have protected the Páramo del Sol from extensive human development. This páramo is the largest and most species-rich in Antioquia. After lunch, we went back down the mountain, choosing to walk for most of the trail. My arms ached from digging and I was worried I would not be able to hold onto the saddle.

In total, I collected specimens from five species of *Bomarea*. I learned that some individuals produce rhizomes, swollen underground stems like ginger, in addition to tubers. In one individual, the rhizome formed a complete spiral, mimicking the form

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[4]Eluniverso.com. "Grupo Insurgente Colombiano Liberó a Universitarios Y Docente Secuestrados - SEP. 13, 2004 - Internacionales - Historicos - EL UNIVERSO." Grupo Insurgente Colombiano Liberó a Universitarios Y Docente Secuestrados. El Universo, 13 Sept. 2004. Web. 02 Sept. 2016. <http://www.eluniverso.com/2004/09/13/0001/14/11A704F806454E2AB8E102098CA197A4.html>.

of juvenile stems above ground still looking for a tree or other support before extending upwards. For some individuals, I dug for almost an hour before deciding to move on, unable to find any tubers amongst the tangle of roots, rocks and dirt. I discovered that tubers of *Bomarea diffracta* have a unique deep yellow pigmentation of the outer skin, unlike the beige color of most other species. While I am still waiting on permits to ship my samples back to the Herbarium of the University of California, the differences in morphology and coloration give me hope that subsequent analyses will demonstrate variation in defensive chemicals. If so, I will return to Colombia to sample more extensively from regions beyond Antioquia, and to visit more herbaria such as the Herbario Nacional Colombiano.

Through this continued work, I hope to build more connections with local researchers and include local students in fieldwork as well as analytical stages of my project. Ultimately, I plan to reconstruct the evolutionary relationships of most species in the genus. This will allow me to understand the relationship between the altitude where the species grow and the evolution of their defensive chemicals.