ARIDUS

Bulletin of The Desert Legume Program of The Boyce Thompson Southwestern Arboretum and The University of Arizona

Volume 22, Number 1 April 2010

Legumes and Their Exudates

Jorge A. Santiago-Blay
Dept. of Paloebiology, MRC-121
Nat. Museum of Natural History
Smithsonian Institution
PO Box 37012
Washington, D.C. 20013-7012
blayj@si.edu

Joseph B. Lambert
Department of Chemistry
Northwestern University
2145 Sheridan Road
Evanston, IL 60208-3113
jlambert@northwestern.edu

Members of the Fabaceae (or Leguminosae) are ubiquitous in many arid regions of the world. Traditionally, legumes have been classified in three groups: caesalpinioids, such as *Senna*; mimosoids, like *Mimosa* and *Acacia*; and faboids (or papilionoids), such as *Pisum*. The overall importance of legumes to human civilization, particularly as sources of food is rivaled only by grasses (Poaceae). In addition, a number of legumes are sources of (or inspiration for) medicines, used in industrial processes, food for non-human animals, and coveted for their ornamental/forestry value (Figure 1).

Legumes have been a part of author JASB's life. When he was a boy in Puerto Rico, his first experience with legumes consisted of collecting bean fruits from the vines and removing the delicious green peas, *Pisum sativum* L. (subfamily Faboideae), with his grandparents, Pina and Julio, and his mother, Ángeles. Later on, still during his childhood, he encountered two leguminous trees: the Golden Shower Tree, *Cassia fistula* L. (Figure 2, see also another congeneric, Figures 3-4), and the algarrobo, *Hymenaea courbaril* L. (Figure 5), both in the Caesalpinioideae. At that time, children used the broken legumes of the Golden Shower tree and the seeds of the algarrobo trees to mimic mortal life events: war and cock fights (see End Note). Little did JASB know that years later, through the endless generosity of a wonderful friend, Patrick Craig (then living in Berkeley, California), he would have a life-changing reencounter with one of his childhood tree friends, *Hymenaea*, this time as

Figure 1. *Erythrina caffra* Thunb. (subfamily Faboideae) tree and flowers showing shapes and spectacular colors that make many legumes popular.

Figure 2. *Cassia fistula* L. (Mimosoideae). Species of *Cassia* tend to produce gums. Specimen 1933194 United States National Herbarium.

the fossilized plant exudate, known as amber (Santiago-Blay and Lambert, 2007). In the late 1980s, as JASB began studying biological inclusions in amber, he became intrigued by the botanical provenance of the fossilized materials he was studying as well as their relation to modern counterparts, such as the exudates of species *Cassia* (gums), *Hymenaea* (resins), and other plants worldwide.

When authors JASB and JBL met in the summer of 1998, a productive research collaboration (see papers by Lambert et al. in Literature Cited) was initiated to address those questions. Plant exudates are also commonly called "sap" although the word "sap" is used to describe any fluid that travels inside plants. In contrast, the word "exudate" refers to any such material when it is oozed out of the plant. Interestingly, it seems that different kinds of exudates (e.g. resins vs. gums) travel inside the plant using different types of tissues. Herein, we describe our research on plant exudates, or the gooey stuff that many plants ooze, emphasizing the materials produced by leguminous plants. Our ultimate research goal is to generate a chemical library of amber and plant exudates of the world.

Methods

An efficient way to get samples of exudates is to collect them from trees in botanical gardens or arboreta. Exudates are complex mixtures of organic compounds oozed by plants often, but not always, released as a result of injury. These products are rich in carbon and hydrogen atoms. We often garner only 1 gram or less of the exudates and do not purposely harm the plants. An advantage of visiting these venerable institutions is that there is a large diversity of identified plants, often from many parts of the world (Santiago-Blay, 2006). Also, many botanical gardens or arboreta carefully assign each plant an accession code and/or house herbaria, making it easier for other researchers to access the same plant (or the same species) we have sampled. Identified exudates are also obtained through generous donations from colleagues worldwide.

Once the solid exudates samples are at hand, they are pulverized and analyzed. We always use solid state nuclear magnetic resonance spectroscopy (SSNMR) for detecting the pattern of abundance of the carbon-13 isotope in the samples of all harvested materials. As much as possible, we analyze more than one sample per species. If the exudates samples are soluble, we also use a complementary technique, called proton or hydrogen-1 isotope NMR. These tools have the distinct advantage of analyzing samples in bulk and, while we cannot identify specific molecules in the samples, NMR does not discriminate against specific molecular components.

Nuclear magnetic resonance (NMR) spectroscopy exploits the behavior of atomic nuclei in a magnetic

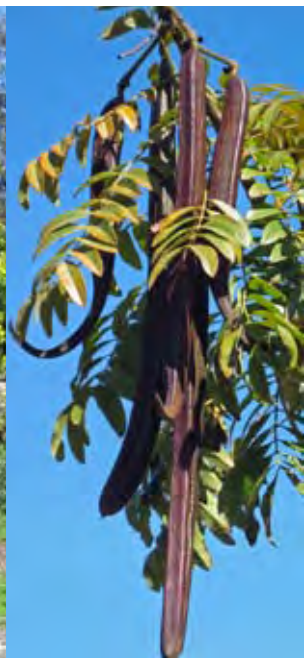


Figure 3. (left) *Cassia leptophylla* Vogel and *C. bicapsularis* L. (background, with yellow flowers). Many species of *Cassia* are exudates producers. **Figure 4. (right)** *Cassia leptophylla* seed pods which can grow 30 cm

field. When an electromagnetic pulse is applied to spinning nuclei in atoms, they absorb energy from the pulse and the nuclei radiate this energy back out. The energy radiated back out is at a specific resonance frequency that depends on the strength of the magnetic field and other factors. The results of an NMR experiment are represented in a graph where the horizontal axis represents the specific resonance frequency at which the energy has been released by the isotopes in the collected material. The height of the peak represents the relative abundance of that particular type of isotope.

Figure 5. Seeds ("gallitos") of *Hymenaea courbaril* L. (subfamily Caesalpinioideae), a resin producer. Specimen 409297 United States National Herbarium. Collected in Barrio Descalabrado, Coamo in November 23, 1889 by George P. Goll, accompanying O. F. Cook and G. N. Collins of the United States Department of Agriculture.

Results and Discussion

After 12 years of research, we have seen obvious patterns within the legumes and we summarize them herein. First, there are distinct classes of plant exudates: resins (basic unit is a five carbon molecule, called isoprene; Langenheim, 2003), gums (Figure 6), basic unit is a five or six carbon molecule, a monosaccharide; Nussinovitch, 2010), gum resins (mixtures of gums and resins), and kinos (Figure 1, basic units are six carbon ring molecules, such as phenol. There are a few other exudates that we have thus far been unable to classify into specific categories and we call them "others". Interestingly, all of those exudates are present in legumes.

Second, resins predominate in caesalpinioids and gums dominate the mimosoids as well as the faboids.

Our future plans include the analyses of as many legume exudates as possible. Although nobody knows exactly how many species currently placed in the 818 genera of legumes (Lewis et al., 2005) produce exudates, we know that the following additional genera, which we have not studied, produce exudates: the caesalpinioids *Afzelia*, *Berlinia*, *Brachystegia*, *Burkea*, *Ceratonia*, *Cercidium*, *Colophospermum*, *Dicorynia*, *Eperua*, *Erythrophleum*, *Gleditsia*, *Gossweilerodendron*, *Gymnocladus*, *Haematoxylum*, *Hardwickia*, *Isoberlinia*, *Julbernardia*, *Kingiodendron*, *Oxystigma*, and *Sindora*; the mimosoids *Chloroleucon*, *Dichrostachys*, *Entada*, *Faidherbia*, *Lysiloma*, *Piptadenia*, *Piptadeniastrum*, *Pithecellobium*, *Swartzia*, and *Xylia*, as well as the faboids *Abrus*, *Apoplanesia*, *Baptisia*, *Butea*, *Cajanus*, *Cordyla*, *Cyamopsis*, *Dalbergia*, *Deguelia*, *Dussia*, *Dipteryx*, *Erythrina*, *Inocarpus*, *Machaerium*, *Milletia* (also known as *Pongamia*), *Myrocarpus*, *Ougeinia*, *Oxytropis*, *Parkia*, *Sesbania*, *Sophora*, *Sphenostylis*, *Strophostyles*, *Tessmannia*, *Vicia*, *Vigna*, and *Virgilia*. We request our readers to contact us if they wish to make exudates of those and other legume genera available to us for study.

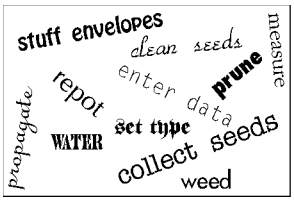
Acknowledgments

Mr. Matthew Johnson and Dr. Margaret Norem (both at the Desert Legume Program, University of Arizona, Tucson) invited us to write this paper. Mr. Johnson also provided guidance to collecting from the legumes in the Tucson and Yuma (Arizona) plots of the Desert Legume Program. Mr. Steve Carter (Boyce Thompson Arboretum, Superior, Arizona) received author JASB with hospitality during his December 2009 visit. Figures 1 was taken by author JASB at Balboa Park (San Diego, California) with the assistance of Crystal Ritchie and Kim Duclo (Department of Parks and Recreation, City of San Diego). Dr. Pedro Acevedo (Department of Botany, National Museum of Natural History, Smithsonian Institution) directed author JASB to *Cassia fistula*, with which Acevedo, another Puerto Rican scientist, also played during his boyhood.

Figure 6. *Acacia velutina* DC (Mimosoideae). Members of this genus are typically gum producers.

Figure 7. *Tipuana tipu* (Benth.) Kuntze (Faboideae), a kino producer.

continued on page 6



Staff and Volunteers in Action

Twenty-two Years, and How Many Volunteers ?

Volunteers crowded back into our work space on January 13th, to begin the 23rd year with the Desert Legume Program. I choose the word "crowded" for perspective, because we now fit 18 to 30 or more volunteers into the space we used when a "big" session included ten to twelve people. During the 22 years of the program, 254 individuals have offered their time and talents working toward awareness, investigation and improved preservation of critical legume species. You have read about the changes in our volunteer group over the years; the spirit of the group has persisted in its dedication regardless of those changes. Volunteers continue to meet on the second Wednesday of each month, between September and May, with occasional special sessions throughout the year. This year's spring sessions have been well attended and each has included a slide presentation organized by one of the volunteers. These presentations have allowed us to take virtual trips to France, Brazil, the moon, and nearer destinations. Thanks to Ed Weber, Kay Fagan, Mike Fagan, Roy Williams, Jack Stroehlein, and Ramona Johnson for sharing these enjoyable "escapes" with us. Our next session is scheduled for May 12th. The tentative schedule for the autumn sessions is September 8, October 13, November 10 and December 8.

After the end of a mild and wet winter in Tucson I hit the road headed west for my annual excursion to our Yuma fields. Upon my arrival it was apparent that southwestern Arizona shared that winter climate. (The distance between Tucson and Yuma is ~ 235 miles and conditions can be dissimilar.) The desert between here and there was green, robust, and lush with wildflowers. Plants in our Yuma field looked healthy and happy. At that site for our frost sensitive specimens for the past 18 years, the maturing plants continue to yield more data for our program. Professional and student interest in the Yuma plantings has increased during recent years. In December, plant bark exudates for chemical evaluation were collected there by researchers from the eastern U.S. Recently, students at Arizona Western College asked for our assistance as they anticipate evaluating potential agronomic uses for *Faidherbia albida*. Two *F. albida* specimens in our Yuma fields are the tallest trees at that location. Initially, saplings of less than 4 foot height were planted in 1992 and are now 60^{+} feet in height, with truck diameters of approximately 3 feet, d.b.h. ("diameter breast high"). In March I met with Xavier Sanders, one of the students, to discuss those specimens. Sanders has unique ideas for incorporating that species into agricultural utility for the southwestern U.S. Before meeting with Sanders, my question was "Why *Faidherbia albida*?" After chatting and working with him in March, my question became "Why NOT *Faidherbia albida*?" We are glad to share information about my propagation work with that species and their observed growth habits over the past 18 years. Stay tuned for more about this!

A few faithful volunteers have joined our Yuma "crew" during the past 5 years. While Gail Culver and Pam Honaker have both been volunteering at our Yuma fields for 10 years, Terry Donovan and Joe Principe are the "new" Yuma guys. After my March trip, I think Xavier Sanders might be our newest volunteer. Thanks to each of them for their continued participation. Please contact me, kcoppola@ag.arizona.edu or call (520) 647-2460 to learn about volunteering with Desert Legume Program. **(KC)**

Literature Cited

Lambert, J. B., E. R. Heckenbach A. E. Hurtley, Y. Wu, and J. A. Santiago-Blay. 2009. Nuclear magnetic resonance spectroscopic characterization of legume exudates. Journal of Natural Products 72:1028-1035.

Lambert, J. B., M. A. Kozminski, C. A. Fahlstrom, and J. A. Santiago-Blay. 2007. Proton nuclear magnetic resonance characterization of resins from the family Pinaceae. Journal of Natural Products 70(2):188-195.

Lambert, J. B., M. A. Kozminski, and J. A. Santiago-Blay. 2007. Distinctions among conifer exudates by proton magnetic resonance spectroscopy. Journal of Natural Products 70(8):1283-1294.

Lambert, J. B., J. A. Santiago-Blay, and K. B. Anderson. 2008. Chemical signatures of fossilized resins and recent plant exudates. Mini Review. Angewandte Chemie (International Edition) 47:9608-9616. Also published in German, with the following bibliographic information: Chemischer Fingerabdruck von fossilen Harzen und rezenten Pflanzenexsudaten. Angewandte Chemie 120:9750 – 9760

Lambert, J. B., Y. Wu, and J. A. Santiago-Blay. 2002. Modern and ancient resins from Africa and the Americas. Chapter 6, pp. 64-83. *In*, Archaeological Chemistry. Materials, Methods, and Meaning. Symposium Series No. 831. K. A. Jakes (Editor). American Chemical Society. Washington, District of Columbia, U.S.A. 261 pp.

Lambert, J. B. Y. Wu, and J. A. Santiago-Blay. 2005. Taxonomic and chemical relationships revealed by nuclear magnetic resonance spectra of plant exudates. Journal of Natural Products 68(5):635-648.

Lambert, J. B., Y. Wu, and M. A. Kozminski, and J. A. Santiago-Blay. 2007. Characterization of *Eucalyptus* and chemically related exudates by nuclear magnetic resonance spectroscopy. Australian Journal of Chemistry - an International Journal for Chemical Science 60:862-870.

Langenheim, J. H. 2003. Plant Resins: chemistry, evolution, ecology and ethnobotany. Timber Press. Portland, Oregon, USA. 586 pp

Lewis, G., B. Schrire, B. Mackinder, and M. Lock (Editors). 2005. Legumes of the World. Royal Botanic Gardens. Kew, Richmond, Surrey, UK. 592 pp.

Nussinovitch, A. 2010. Plant gum exudates of the World. Sources, distribution, properties, and applications. CRC Press. Taylor and Francis Group. Boca Raton, Florida, USA. 401 pp.

Santiago-Blay, J. A. 2006. Marketing mostly intangible goods: the case of botanical gardens and arboreta. Also, Case Teaching Notes for "Marketing Mostly Intangible Goods: The Case of Botanical Gardens and Arboreta". Case Studies in Science. The National Center for Case Study Teaching in Science Case Collection. University of Buffalo. Buffalo, New York, U.S.A. http://www.sciencecases.org/garden/garden.asp, http://www.sciencecases.org/garden/garden_notes.asp . (Also at http://www.bgci.org/worldwide/news/0306/)

Santiago-Blay, J. A. and J. B. Lambert. 2007. Amber's botanical origins uncovered. American Scientist 95:150-157. Reprinted, with permission as, Aux sourcl'ambre. Pour la Science [(Paris, France) French version of Scientific American] 356 (June 2007):70-75.

End Note: The huge, conveniently "segmented" cylindrical seed pods of *C. fistula* (Figure 2) provide plenty of ammunition for hand-thrown "bullets" in children's war games. On the other hand, the legumes of *H. courbaril* (Figure 5) contain seeds covered by a foul-smelling material. The seeds are used to play a game resembling a cock fight. Once the offending smell was removed, the seeds were bored and attached to a string. These tethered seeds, were called "gallitos", or little roosters. When two "gallitos" were poised to fight, one child would place the "gallito" on the ground; the other child would aim it at the opponent's "gallito". A child turn lasted until missing the opponent's little rooster or until either "gallito" was broken, the end of the fighting life for the losing little rooster.

Terry Donovan, Gail Culver and Joe Principe take a break from their volunteer work in DELEP's Yuma field. (KC)

Tree Logo Here

Opportunities for Participation

DELEP's bulletin Aridus, is published three times annually to stimulate interest in desert legumes, to inform our readers of DELEP's activities, and to encourage support for DELEP's programs. Manuscripts related to legumes are welcome and should be mailed to the editor for review. Subscriptions are complimentary and are available by contacting the DELEP office. Aridus is published by The University of Arizona on behalf of The Desert Legume Program.

Financial support for DELEP is provided by private industries and individuals through contracts, grants and contributions. Dedicated volunteer work is an integral component of DELEP. Our volunteers have many different backgrounds and work on a variety of projects including wild seed collecting, seed processing organization of special events, and office work. DELEP volunteers meet once a month. To volunteer call (520) 647-2460 or email kcoppola@ag.arizona.edu

To Contribute:
Call to discuss a pledge, restricted gift or estate planning.
Make a check, payable DELEP/U of A Foundation

Mail to:
The Desert Legume Program
2120 E. Allen Road
Tucson, Arizona 85719

The University of Arizona
Desert Legume Program
2120 East Allen Road
Tucson, Arizona, 85719
U.S.A.

Return Service Requested

NON-PROFIT
ORGANIZATION
U.S. POSTAGE PAID
PERMIT NO. 190
TUCSON, ARIZONA

Faidherbia albida in Yuma field (KC)