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How Chemical Compounds Affect Fruit Bats' Plant Interactions

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Fruit bats are known to be able to discriminate, select, and track the essential oils of their preferred fruits. A few years ago, our research group hypothesized, experimented, and confirmed that these bats can be attracted with essential oils only – concentrated volatile aromas – of their preferred fruits both in forested and open areas. These findings led to the proposal of a restoration tool that uses essential oils of chiropterochoric fruits (fruits eaten by bats) to attract seed-dispersing bats to degraded areas with the objective to increase seed arrival and germination.

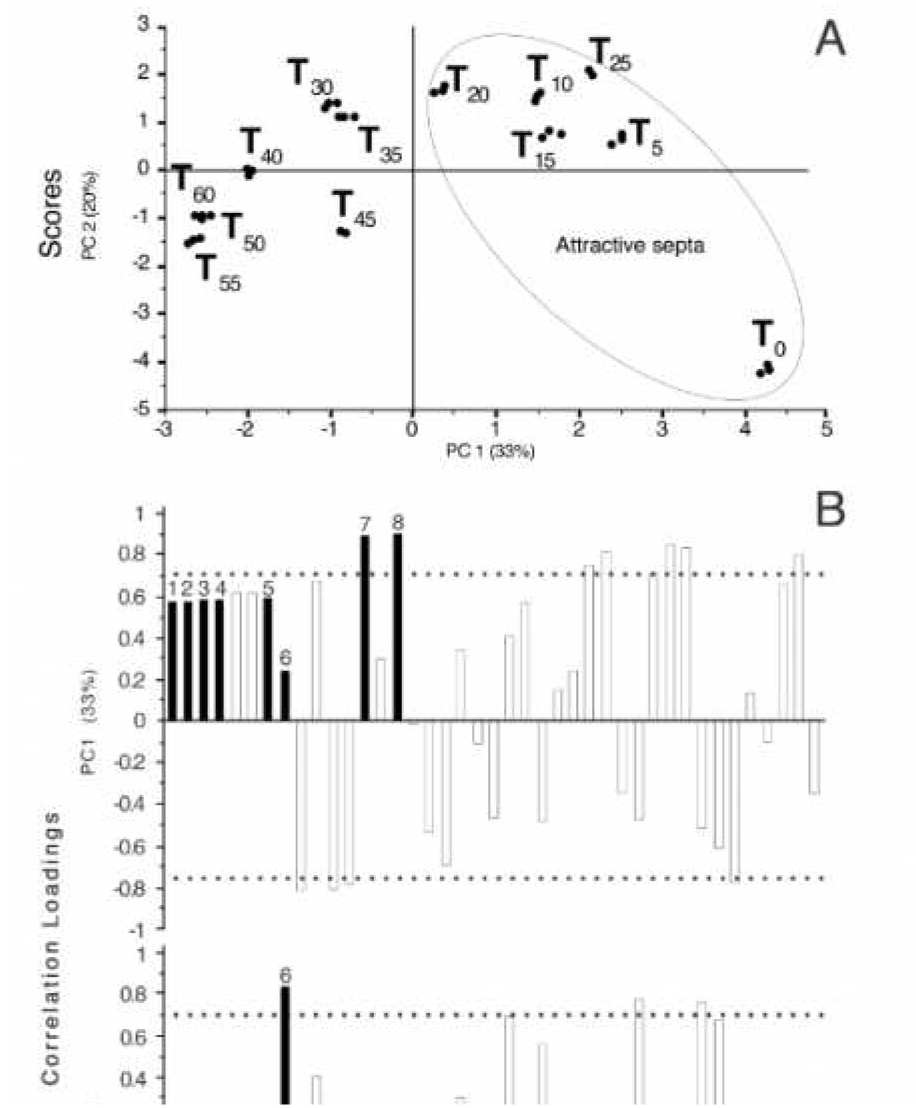
Nevertheless, the role played by different chemical compounds in attracting the bats was largely unknown. Now, our recent study, "Chemical compounds in Neotropical fruit bat-plant interactions," unveils how fruit bats can find fruiting trees and ripe fruits in the dark within dense canopies. To do so, we have combined gas chromatographic (GC) analysis and attraction trials with captive bats to investigate the molecular basis of fruit foraging in two common neotropical species: *Artibeus lituratus* and *Carollia perspicillata* (Figure 1).

Figure 1. Bat species used in captivity tests (L. C. Parolin).

We began by impregnating a series of rubber septa (Figure 2) with the raw essential oil of *Piper gaudichaudianum*, a chiropterochoric fruit highly appreciated by these animals. The septa were kept on a laminar flow cabinet until GC analysis or attraction trials, both conducted on the same day, and then we tested and analyzed the septa every 5 days for up to 60 days. The tests indicated whether the septa (with the remaining components of the essential oil) were still attractive to the bats after the oil had aged. The CG analysis allowed us to identify which volatile oil compounds were lost and which ones remained on the rubber septum throughout the trial and, consequently, could be responsible for bat attraction.

Figure 2. Rubber septum that was impregnated with essential oils of fruit eaten by bats (L. C. Parolin).

The tests with captive bats revealed that the bats' response toward the septum with essential oils decreased significantly beginning on the 25th day (Figure 3). The chemical analysis then suggested that eight oil compounds had a prominent role in bat-oil interaction – five monoterpenes: α-pinene, β-pinene, cymene, limonene, and dihydro carveol, and three sesquiterpenes: α-copaene, 9-epi-caryophyliene, and cis-eudesma-6, 11-diene (Figure 3B).



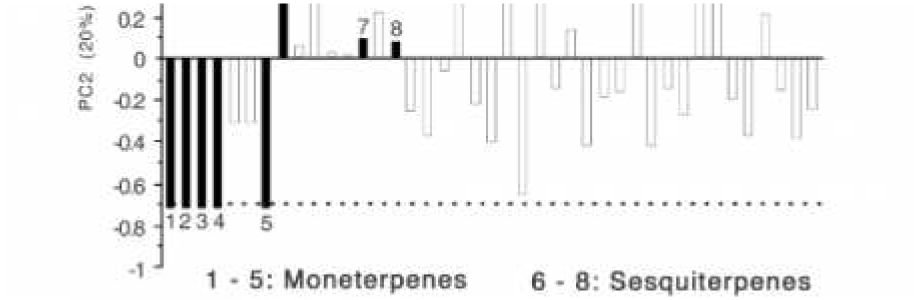


Figure 3. (A) PCA analysis of GC-MS profiles of the essential oil of *Piper gaudichaudianum* remaining in rubber septa – the first two principal components clearly separated attractive septa for *Carollia perspicillata*, (B) correlation loadings of the peak areas in terms of contribution to component 1 and 2 of Figure A, in which (1) α-pinene, (2) β-pinene, (3) cymene, (4) limonene, (5) dihydro carveol, (6) α-copaene, (7) 9-epi-caryophyliene and (8) cis-eudesma-6, 11-diene (Parolin et al., 2019). Republished with permission from Elsevier from https://doi.org/10.1016/j.mambio.2018.06.009

Out of these, α-copaene appears to have a key role in fruit-bat interactions, since its concentration decreased strongly until the turning point in bat response (Figure 4). Based on these results, additional trials (two for each compound and bat species) were conducted with two essential oil compounds: α-pinene and α-copaene against a blank septum (control).

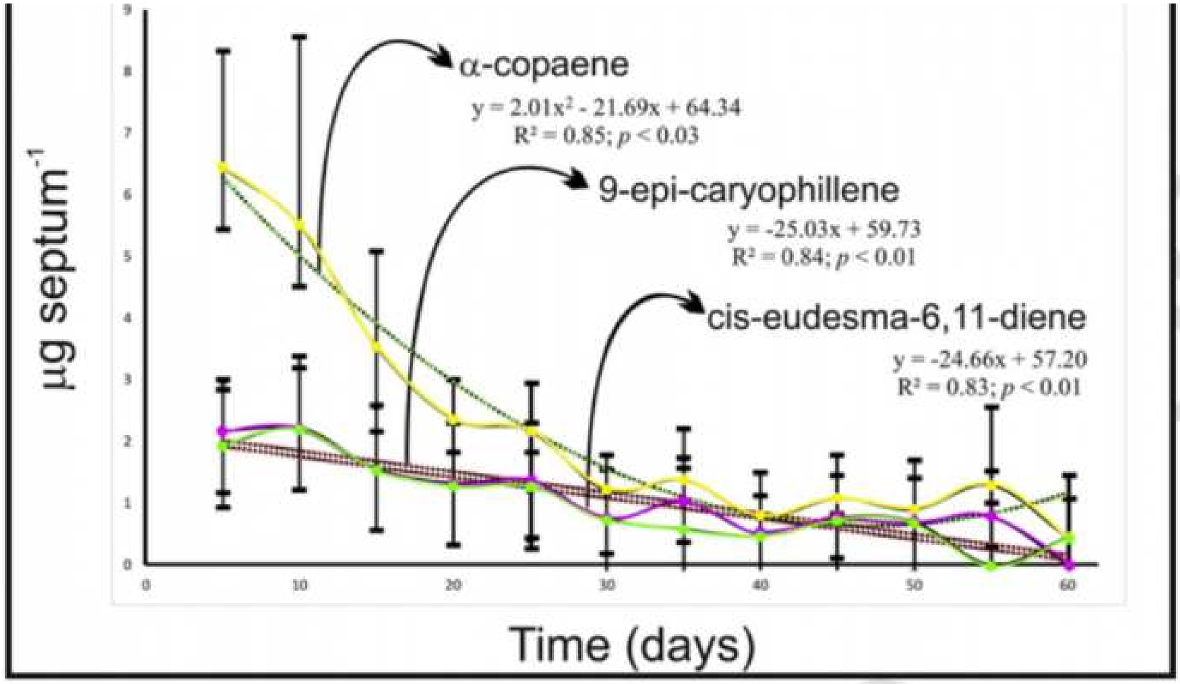


Figure 4. The behavior of α-copaene, 9-epi-caryophyllene, and cis-eudesma-6-11-diene along the duration of the experiment (60 days) (Parolin et al., 2019). Republished with permission from Elsevier from https://doi.org/10.1016/j.mambio.2018.06.009

In the end, we were able to demonstrate that fruit bats can discriminate between two classes of essential oil compounds: monoterpenes and sesquiterpenes. Monoterpenes seem to provide an initial short-living signal, allowing bats to locate fruiting trees while moving through the dark forest. Sesquiterpenes, on the other hand, seem to provide longer-lived information on fruit ripeness, thus allowing bats to make optimal foraging choices.

Such findings give a unique perspective on the molecular mechanism of bat-plant communication and have important implications in forest restoration. Specifically, simple mixtures of commercially-available mono and sesquiterpenes (e.g. α-pinene and α-copaene) could be used as attractants to seed-dispersing bats into degraded landscapes improving the density and diversity of plants.

These findings are described in the article entitled Chemical compounds in Neotropical fruit bat-plant interactions, recently published in the journal *Mammalian Biology*.