How organisms reach and colonize bromeliads: a field experimental test of two of Picado’s hypotheses, and the effect of tree age and cardinal distribution on bromeliads in Cartago, Costa Rica

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ABSTRACT
Numerous studies have been conducted on the biodiversity of organisms that inhabit bromeliad water tanks. However, little is known about how organisms reach these tanks (also called “phytotelmata”). Two other aspects of bromeliad ecology, the effect of tree age and the cardinal distribution of bromeliads in canopies are slightly better known, but still little research has been done on these subjects for Central American bromeliads. To improve understanding of these subjects, we studied bromeliad ecology in Pejibaye de Jiménez, Cartago, Costa Rica. To avoid destroying natural phytotelmata, we built 150 artificial bromeliads with plastic cups to test Picado’s hypotheses that phytotelmata are colonized via rain and debris. We set them in the field in February 2012 and collected macroinvertebrates from them after seven weeks. We also measured bromeliad leaf length and trunk diameters in 100 Gliricidia sepium trees and counted bromeliads in the four cardinal directions of 60 trees. In agreement with Picado’s hypotheses, the bromeliads that did not receive rainwater had 2.9 times less invertebrates than the control, and the bromeliads that did not receive debris had 3.4 times less invertebrates than the control. Larger trees had more and larger bromeliads growing on them, possibly because they were older and had more structural complexity. Mean number of bromeliads was lower in the west side of canopies, the side that receives less sunlight. These findings not only address Picado’s hypotheses in the centenary of their publication, but also are potentially useful for conservation of bromeliads and the complex microecosystems that they house.

KEY WORDS
Bromeliaceae, colonization, bromeliad ecology, Gliricidia sepium, invertebrate, microecosystem, phytotelmata, living fence trees, conservation of bromeliads.

RESUMEN
Existen numerosos estudios sobre la biodiversidad dentro de los tanques de agua de las bromelias. Sin embargo, poco se sabe sobre cómo llegan los organismos hasta los tanques de agua ubicados en las partes altas del bosque. El efecto de la edad de los árboles y la distribución cardinal de las bromelias en el dosel son aspectos mejor conocidos, pero casi no hay datos para las especies centroamericanas, por lo tanto, estudiamos la ecología de las bromelias en Pejibaye de Jiménez, Costa Rica. Para no destruir bromelias naturales, fabricamos 150 bromelias artificiales con vasos de plástico para poner a prueba las hipótesis de Picado sobre la colonización de los tanques bromelícolas mediante lluvia y residuos que caen de las ramas. Colocamos las bromelias artificiales en el bosque en febrero 2012 y siete semanas después recolectamos los macroinvertebrados que las habitan. También medimos bromelias y diámetros de los árboles en 100 individuos de Gliricidia sepium y contamos las bromelias en los cuatro puntos cardinales del dosel en 60 árboles. En concordancia con las hipótesis de Picado, las bromelias que no recibieron lluvia tuvieron 2.9 veces menos invertebrados que el tratamiento control, y las bromelias que no recibieron residuos registraron 3.4 veces menos invertebrados que el control. Los árboles más grandes tienen más bromelias y éstas son más grandes, posiblemente debido a que son árboles más viejos, con mayor complejidad estructural. El número medio de bromelias fue menor en el lado oeste de las copas, justo la parte que recibe menos luz solar. Estos resultados no sólo evalúan las hipótesis de Picado en el centenario de su publicación, sino que también son potencialmente útiles para la conservación de las bromelias y de los complejos microecosistemas que hospedan.

PALABRAS CLAVE
Bromeliaceae, colonización, ecología de bromelias, Gliricidia sepium, invertebrados, microecosistemas, fitotelmas, cerca viva, conservación de bromelias.
The availability of year-round sunlight and above-freezing temperatures in the tropics allows plants to photosynthesize more freely than in other latitudes. In addition, neither plants nor animals need to enter a period of dormancy for lack of food or temperature. However, because of the constant access to solar radiation, there is also strong competition for light and in the case of Bromeliads (family Bromeliaceae), most species are epiphytic. Epiphytes are plants that use roots as holdfasts, but not to acquire nutrients. Bromeliads use this adaptation to attach themselves to large trees and access light high in the canopy (Ingram et al., 1995).

The tightly spaced leaf axils of bromeliads capture water falling as rain. These water tanks (“phytotelmata”) are similar to the tanks of pitcher plants (i.e. Sarracenia; Fish, 1983) and allow bromeliads to absorb nutrients through their trichomes. A variety of invertebrates and vertebrates live in these phytotelmata (Picado, 1913; Frank, 2009), where species richness and diversity correlate with bromeliad size (Aráujo, 2007). There is also correlation between insect communities and both precipitation in the habitat and water volume in the tanks (Sodré, 2010). For over a century, botanists and ecologists have been studying these unique microecosystems (Gómez, 1972; Hernández-Baz, 2011), where for example, predatory spiders not only affect the species richness of the microecosystem itself, but also the surrounding ecosystems (Romero, 2010).

Bromeliads are of key importance to maintaining insect diversity (Gonçalves-Souza, 2010) but they are under pressure from deforestation, urbanization and destructive agriculture practices in Mexico and Central America. A study in Mexico found that slash and burn agriculture and selective logging put stress on arboreal frog populations that depend on bromeliad phytotelmata (Galindo-Leal, 2003); additionally, seedling mortality—more than germination failure—has been described as a limiting factor in the successful establishment of bromeliad species (Winkler, 2005).

Research regarding the distribution of bromeliads on a given tree is also poorly represented in the literature, yet crucial for understanding bromeliad ecology. By pinpointing the conditions that favor bromeliad growth and dispersal, conservation biologists can increase the abundance of possibly endangered species (Mondragon, 2006).

Another important and neglected aspect of bromeliad ecology is how aquatic organisms can reach phytotelmata high in the forest canopy. The colonization of other forms of phytotelmata, like hollows in tree bases and pitcher plant containers, is affected, among many other factors, by the color of the cup (Yanoviak, 2001). Bromeliad phytotelmata vary in their color and this factor could be important in colonization, but research in the field of colonization is close to non-existent, despite the fact that the question was published a century ago. Picado (1913) asked the question and proposed several hypothetical mechanisms by which small invertebrates and other organisms could reach bromeliad phytotelmata; most are passive, while the fifth is active: (a) rainwater transports organisms to the leaf axils; (b) spores in the air fall on the water; (c) rainwater washes along the bark of trees and other higher plants and subsequently falls into bromeliad phytotelmata; (d) fallen debris from higher plants transport the organisms; and (e) organisms colonize the phytotelmata by their own means of locomotion (Picado, 1913).

Our research objectives were to (1) experimentally test two of Picado’s hypotheses about how macroinvertebrates reach bromeliad phytotelmata, (2) see if there is a relationship between the size of trees and the number and size of bromeliads on them, and (3) compare the number of bromeliads among cardinal directions to evaluate the effect of physical factors such as light and wind.

**METHODOLOGY**

**Study Area**

We conducted our research in Pejibaye de Jiménez, Cartago, Costa Rica (9°49’N - 83°42’W). The climate is tropical with a mean annual precipitation of 4 572 mm and mean temperatures between 20-30°C. Elevations range between 750-1 950 meters above sea level. All statistical tests were run with Statgraphics Centurion XVI.

**Colonization**

To test passive colonization hypotheses, we used artificial bromeliads made with plastic cups. This way we avoided destroying natural phytotelmata and their complex communities, and we could also control for one colonization factor at a time. We made 150 plastic bromeliads using 0.47 l and 0.35 l red plastic cups, clear plastic string, metal wire, green plastic mesh and translucent plastic domes. Besides the 50 control bromeliads, 50 had a plastic dome that blocked rainwater and 50 had a mesh that blocked debris; in all cases light and air reached the insides of the cups (Fig. 1). We used red cups because other colors were not available and because our experiments were for passive colonization, not for active selection of bromeliads by the invertebrates. We used two sizes of cups to imitate real bromeliads: they have smaller circles of leaves inside the external leaf circle.

**Study Design**

To test for active colonization hypotheses, we used 100 plastic bromeliads made with plastic cups. This way we could test Picado’s hypotheses about how macroinvertebrates colonize phytotelmata. We used red cups because other colors were not available and because our experiments were for active colonization, not for passive colonization due to the larger plastic cups we used. We used two sizes of cups to imitate real bromeliads: they have smaller circles of leaves inside the external leaf circle.

**Colonization Tests**

We conducted each test with three replicates. We used two sizes of cups to imitate real bromeliads: they have smaller circles of leaves inside the external leaf circle. We used red cups because other colors were not available and because our experiments were for passive colonization, not for active selection of bromeliads by the invertebrates. We used two sizes of cups to imitate real bromeliads: they have smaller circles of leaves inside the external leaf circle.

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**Statistical Analyses**

We conducted a chi-square test to determine if the number of invertebrates was significantly different among cardinal directions. We ran a one-way ANOVA to test for significant differences in the number of invertebrates among plastic bromeliads with different sizes of cups. We conducted a Student’s t-test to test for significant differences in the number of invertebrates between plastic bromeliads with and without plastic domes. We conducted a multivariate analysis of variance to test for significant differences in the number of invertebrates among plastic bromeliads with and without plastic domes.
We filled all of the cups with sterilized water and monitored water levels throughout the entire study so that the pools had sufficient water to allow invertebrate life. Sterilized water was added when needed to keep a proper level. We attached the cups to tree trunks with plastic string in clusters (control and two treatments). Each tree had five clusters and a total of ten trees were selected within the forest (Fig. 1). Trees were selected because they already had bromeliads on them. After seven weeks the organisms in the cups were preserved in 96% denatured alcohol for counting. We did not identify taxa because we did not have the resources for that and because this was not required to test Picado’s hypotheses, which dealt simply with means of arrival and colonization.

Relationship between trees and their bromeliads

We recorded DBH (diameter at breast height) of 100 *Gliricidia sepium* (Jacq.) Kunth ex Walp. (Fabaceae) trees planted along the roadside from downtown Pejibaye to the GUDIKAHO Farm and measured the longest whole leaf from base to tip of each bromeliad in those trees.

Cardinal distribution

Using a compass, we photographed each of the four cardinal parts of canopy in 60 trees of varying species near the town of Pejibaye. In order to keep results consistent, we stood one meter away from the trunk and set the camera facing straight upward to photograph the canopy. We counted the number of bromeliads in the photographs.

RESULTS

Colonization

Of 150 cups affixed to trees, 99 in total collected some form of macroinvertebrate: Control 82%, Rain 64% and Debris 56%. The mean number of invertebrates per bromeliad was: Control 5.49 (range 0-18), Rain 1.92 (0-18) and Debris 1.62 (0-22). The bromeliads that did not receive rainwater had 2.9 times less invertebrates than the control, and the bromeliads that did not receive debris had 3.4 times less invertebrates than the control. Using the mean number of macroinvertebrates collected in the control cups as a basis, we found that blocking rainwater decreased the mean number of insects by 8% and blocking debris decreased it by an even higher number: 26%. There was no difference in number of invertebrates found between trees (F=1.36, Df=98, p=0.2121), which indicates that the experimental design was correct and not affected by individual trees.

We decided to remove two cups from the analysis because they were full of drowned ants. Ants do not use bromeliad phytotelmata during the larval stage of development and most likely these fell into the cups and drowned.

Relationship between trees and their bromeliads

*Gliricidia sepium* trees had a mean diameter of 11.85cm (range 2, 19 to 33, 94cm; N=100) and a mean of 7.83 bromeliads per tree (Min. 0; Max. 54; N=729). Trees with thicker trunks had more bromeliads (F=34.40; Df=98; p<0.0001; R²=25.9) (Fig. 2).
in phytotelmata pools is a limiting factor for growth and colonization. Some reports claim that excessive organic matter actually decreases species richness in phytotelmata because the organic matter offsets vital water within the tanks (Jabiol, 2009). However, a different study assessed the effects of nutrient availability for phytelmata communities, and found a positive correlation between animal and debris content (Richardson, 2000).

The mean bromeliad leaf length was 6.85 cm (Min. 0.60; Max. 50.68; N=729). Trees with thicker trunks had larger bromeliads (F=6.69; Df=727; p<0.0001; R² = 8.28) (Fig. 3).

Cardinal distribution

We found a mean of 12.67 bromeliads per photograph (Min. 0; Max. 56; N=240). Mean number of bromeliads was lower in the west side of canopies (F=2.54; Df=3, 177; p=0.058; Fig. 4).

DISCUSSION

Research concerning the colonization of bromeliad phytotelmata is sparse within the literature. The research that is available focuses on the nutrient-filled water of the phytotelmata. The availability of freshwater in phytotelmata pools is a limiting factor for growth and colonization. Some reports claim that excessive organic matter actually decreases species richness in phytotelmata because the organic matter offsets vital water within the tanks (Jabiol, 2009). However, a different study assessed the effects of nutrient availability for phytelmata communities, and found a positive correlation between animal and debris content (Richardson, 2000).

The data from our research indicate that more invertebrates colonized control cups than no rainwater and no debris cups. Control cups allowed rainwater and falling debris to enter the artificial phytotelmata unimpeded. Though the rainwater and debris cups had some traces of organic matter within their phytotelmata, the control cups had more leaves, pieces of bark, and decaying invertebrates within them. This input of organic matter provided a more suitable habitat than cups that only had clear water.
Since there were fewer insect colonizers in both the no-debris and the no-rainwater groups, our data are in agreement with the two Picado’s (1913) hypotheses that we tested: rainwater and debris are means for phytotelmata colonization.

Our results agree with previous findings. In a study conducted in Mexico on the tree epiphyte *Tillandsia*, Valverde (2005) concluded that small trees had less bromeliads. Wider trunks usually belong to older trees that have been exposed longer to colonization and there is a greater probability that bromeliad seeds from nearby plants will find a suitable substrate. Furthermore, many bromeliad seeds are airborne and settle onto crags in bark (Wester, 2011); thus, older, coarser bark is more likely to capture bromeliad seeds and lead to successful germination. Our finding that trees with thicker trunks also have larger bromeliads also agrees with the interpretation that these trees were older. Seed availability through time could also affect and we suggest it for future studies.

There were fewer bromeliads on the west sides of trees. We believe that the differences are based on a combination of local weather (including wind flow) and strong competition for light. During the study period it rained nearly every day, almost always in the afternoon. Rain falls equally on each side of the tree, but rain clouds affected the amount of sunlight accessible to the bromeliads. Cloud cover obscured sunlight during the afternoons and evenings, when the sun is in the west, and this reduction in light on the west side may explain the reduced occurrence of bromeliads on that side of the canopies.

We estimate that less than 3% of invertebrates escaped from cups when they were removed for preservation and this is not an important limitation in our study, but future research could consider this and also the possible effect of using artificial versus real bromeliads. More prolonged study periods can also be tried, but from the point of view of our goal, results show that the time we sampled was satisfactory to test the hypotheses. Regarding the other two studies, we believe that experimental error in the counting and measuring of bromeliads was insignificant. We did not have the resources needed to conduct the experiment for a longer period or to study the microscopic organisms inside the cups. Future studies could complement ours with that kind of information as well as other factors such as effect of cup color, height above ground and chemicals released by real bromeliads.

Bromeliad phytotelmata are at risk of desiccation based on slash and burn agriculture and climatic changes (Galindo-Leal, 2003), so our findings on how they are colonized by invertebrates, and on how tree size and cardinal direction affect bromeliad communities, are all potentially useful to conservation efforts, for example on how

![FIG. 4. Number of bromeliads per cardinal direction in Pejibaye de Jiménez, Cartago, Costa Rica (2012).](image-url)
to maintain phytotelmata and propagate bromeliads. In conclusion, our findings not only address two of Picado’s hypotheses (Picado, 1913) in the centenary of their publication (three hypotheses remain to be tested), but also are potentially useful for conservation of bromeliads and the complex microecosystems that they house.

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