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Conditional outcomes in ant–plant–herbivore interactions influenced by sequential flowering

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Abstract

Mechanisms that affect a host plant’s ability to face herbivory are subjects of ongoing interest. Plant reproductive phenology plays a key role in the dynamics of communities in many ways. In ant–plant–herbivore interactions, host-plant phenology affects traits of its herbivores which in turn determine what traits ants must have to benefit the host-plant. Diversity of plant phenological traits could influence the ecological diversity of coevolved ant–plant mutualisms.

In the Brazilian savanna, members of several plant families resprout and bloom simultaneously. However, some shrubs of the family Malpighiaceae exhibit sequential flowering, and four of these species also present extraloral nectaries that attract ants. Here we determine whether their phenological patterns result in a shared herbivore guild and if this may be harmful to these plant species, making the association with ants critical to the plant optimal development and reproductive success. Plant phenology, herbivory, and the richness and abundance of ants and herbivores were recorded on control (with unrestricted ant access) and treatment (ant-excluded) shrubs. Floral-bud production and fruit set were also quantified. The plants flowered sequentially with brief periods of overlap, benefitting the guild of generalist herbivores. A cluster analysis indicated that 32 herbivore species were associated with these Malpighiaceae, with substantial species overlap. In all species, ants reduced leaf-area loss but not the damage to reproductive structures. Some floral herbivores presented adaptations to avoid or appease ants. We suggest that plant phenology directly influences the outcomes of these ant–plant–herbivore interactions and the related ecological networks.

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Introduction

As the base of terrestrial trophic chains, plants are consumed by a wide range of organisms, including microbes, invertebrates, and vertebrates. The pressure that herbivores exert over plant development and fitness has led plants to develop numerous defensive strategies (Marquis, 2012; Mortensen, 2013). While some defenses are constitutive, others are induced only upon the perception of an attack to allow optimal resource allocation (Campbell and Kessler, 2013; Karban and Baldwin, 1997). One interesting plant trait to escape from herbivory is to resprout or bloom during a season when the main herbivores are less common in the field (Coley and Barone, 1996). Some plant families produce new leaves and flowers during the same season; others produce leaves and flowers at distinct times, such as during the dry and wet seasons (Raven et al., 2012). According to Staggemeier and Morellato (2011), most tropical plant species rely upon animal vectors for pollination and seed dispersal, and temporal and spatial variations in flowering and fruiting phenology strongly affect animals that rely upon flowers or fruits as a food resource.

Plants present different patterns of flowering, such as aggregated, segregated (e.g., Gentry, 1974; Gotelli and Graves, 1996; Mural and Sukumar, 1994) or indistinguishable from what would be expected by chance (e.g., Rathcke, 1984). Several evolutionary hypotheses have been proposed to explain the distinct patterns observed in flowering times among species. Initially, Robertson (1895) suggested that plants with the same pollinators will avoid competition through staggered flowering and thus will increase their reproductive success. Another hypothesis states that staggered flowering could be a result of interaction between early-flowering and later-flowering species, wherein the first-flowering species facilitates the pollinators of the next species to flower (Waser and Real, 1979). The analysis of flowering strategies is a complex issue because they are the result of an interacting set of abiotic factors, plant traits, and plant–animal interactions (Armbruster, 1995). In a consumer–resource
perspective, sequential flowering may represent a plant defensive strategy against floral herbivores (Coley and Barone, 1996; Marquis and Lill, 2010).

Biotic defenses, like the association with predators, most commonly ants, may also represent an important defensive strategy against action of herbivores in terrestrial ecosystems. Ant–plant relationships have made enormous contributions to our understanding of tropical communities (Rico-Gray and Oliveira, 2007; Rousek et al., 2009), and these interactions are particularly pervasive in the Cerrado (the Brazilian tropical savanna) due to the high incidence of insect- and plant-derived exudates on foliage, which promotes intense ant activity on the vegetation (Rico-Gray and Oliveira, 2007, and references therein). The Cerrado is the most diverse tropical savanna in fauna and flora, and one remarkable characteristic of this ecosystem is its great seasonal variation (Oliveira-Filho and Ratter, 2002). The reproductive success of individual plant populations often depends on flowering phenology allowing plants to take advantage of temporally periods of favorable conditions (Mahoro, 2002). Studies comparing conditional outcomes in ant–plant–herbivore interactions mediated by temporal variation in host-plant phenology are of great relevance to the ecology of interactions and the conservation of natural communities. However, such studies are rare, especially in tropical America (Rousek et al., 2009).

Experimental ant-exclusion studies have shown that the absence of ants can increase leaf-area loss in plants possessing extrafloral nectaries (Oliveira, 1997; Korndörfer and Del-Claro, 2006). The absence of ants can also reduce fruit set (Nascimento and Del-Claro, 2010; Oliveira et al., 1999), seed production (Vesprini et al., 2003) and viability (Sobrinho et al., 2002). Moreover, data concerning the effectiveness of ant–plant mutualisms are sometimes controversial (see e.g., Holland et al., 2011; Nahas et al., 2012). Nevertheless, despite their importance to the study of mutualisms and to the structure and maintenance of natural communities (e.g., Bronstein, 2012; Rico-Gray and Oliveira, 2007), ant–plant interactions are most often studied in single plant species over a limited time period (Rousek et al., 2009), ignoring sequential events. Recently, some researchers have shown the impact of ant–plant–plants interactions on communities using diverse ecological network approaches (e.g., Blüthgen et al., 2007; Ings et al., 2009; Lange et al., 2013, and references therein) and have focused on the ecological factors that may cause a mutualism to vary in space and time, resulting in “conditional mutualism” (Alves-Silva and Del-Claro, 2013; Del-Claro and Oliveira, 2000; Herre et al., 1999).

In the Cerrado of central Brazil, shrubs of the family Malpighiaceae are diverse and abundant (Anderson, 1990; Gates, 1982). Several species exhibit sequential phenological development, in which individuals of different species resprout, bloom, and set fruit sequentially over time (Barros, 1992; Costa et al., 2006; Mendes et al., 2011; Torezan-Silingardi, 2007). These species also possess extrafloral nectaries (EFNs) and have close relationships with protective ants (Alves-Silva et al., 2013; Torezan-Silingardi, 2011). In Cerrado, the ant–plant–herbivore interactions occur within multitrophic systems whose outcomes are strongly influenced by plant phenology (Lange et al., 2013). Here we used a set of Malpighiaceae–ant–herbivore associations as model to determine the following: (a) whether herbivores shift among related host-plant species over time in response to variation in plant phenology; and (b) whether the outcomes of ant–plant interactions vary depending upon the associated herbivores and ants. Our major hypotheses are that the sequential flowering of related host plants results in a shared herbivore guild over time that may be quite harmful to these plant species, making the association with ants critical to the plants reproductive success.

Materials and methods

Study site and plant species

Fieldwork was carried out from May 2008 to June 2009 at the Reserva Ecológica do Clube Caça e Pesca Itororó de Uberlândia (CCPUI; 18° 59’S, 48° 18’W), Uberlândia, Minas Gerais State, Brazil. We used a 400 ha Cerrado site consisting of a dense scrub of shrubs and trees, known as Cerrado sensu stricto (Oliveira-Filho and Ratter, 2002). The climate is markedly seasonal, with a dry winter (April–September) and a rainy summer (October–March) – for additional details, see Rêu and Del-Claro, 2005).

Four Malpighiaceae species were selected for the study: Peixotoa tomentosa A. Juss., Banisteriopsis laevifolia (A. Juss.) B. Gates, Banisteriopsis campestris (A. Juss.) Little, and Banisteriopsis malifolia (Ness and Mart.). B. Gates. These species were chosen because they are small shrubs with paired EFNs on the leaf base and are common in the Cerrado and abundant at the study site (Torezan-Silingardi, 2007).

Experiments

During the month in which each species sprouted (when the shrubs bore vegetative buds but not leaves), we tagged 30 individuals of similar size and architecture (1–2 m tall, with 5–7 stems) that were at least 3 m apart. Thus, the ant-exclusion experiments began in May 2008 for P. tomentosa, July 2008 for B. laevifolia, November 2008 for B. campestris and February 2009 for B. malifolia. For each species, by the flip of a coin, we designated tagged shrubs as control plants (with unrestricted ant access; N = 15) or treatment plants (with ants excluded by applying Tanglefoot® resin on the main plant stem; N = 15). In treatment plants, ants were manually removed and the trunk was covered with a 5 cm broad adhesive paper strap to which a layer of sticky resin was applied. We removed surrounding vegetation that ants could use as bridges to gain access to these plants. To be sure that the sticky resin did not interfere in the results, in control plants we also covered the trunk with a paper strap, but we applied the resin only on one side of the trunk.

Data collection

Each plant was monitored every two weeks during its four-month reproductive period. The plants were always inspected by the same observer, and the richness and abundance of herbivores on the reproductive and non-reproductive plant parts were recorded. We quantified herbivory (leaf area loss) monthly in the first week of each month. To determine the mean monthly herbivory, we recorded data from nine leaves per plant, three from the most apical stem, three from a middle stem and three from the most basal stem. This procedure was done without leaf removal. Measurements of herbivory rates on leaves were assessed by placing leaves on a transparent grid (divided into millimeters). An index of herbivory from each leaf was calculated as the proportion of points in the grid falling within damaged and undamaged areas of the leaf blade (Moreira and Del-Claro, 2005). Additionally, data about behavior of the animals at the time of observation were noted, including their topical position (on leaves, stems, buds, or flowers feeding on EFNs). An animal was considered an herbivore if it was observed sucking, chewing, perforating or grasping the plant tissue. Voucher specimens were collected from non-experimental plants for identification. Plant phenology (developmental stage of leaves, buds, flowers, and fruits) was recorded by A. Vilela, following the practice of Torezan-Silingardi and Oliveira (2004) during May 2008–June 2009.
**Statistical analysis**

Circular statistical analyses were made using the following phenological variables: date of first bud, first flowering and first fruiting, date of peak of bud, flowering, and fruiting. To calculate the circular statistic parameters, months were converted to angles from 0 = January to 345° = December at intervals of 15° because the measurements were made every two weeks (Cardoso et al., 2012). The frequency of individuals at each phenological phase for all species (n = 4) was considered for calculating the parameters: the mean vector (μ̂), length of mean vector (r̂), median, circular standard deviation, Rayleigh test z and Rayleigh test p. The mean date for each phenophase is determined by converting the mean angular directions to corresponding mean dates (see Morellato et al., 2000, 2010; Staggebmer et al., 2010). The phenological data were analyzed with the statistical software Oriana 4.0.

A null model calculation was performed to indicate if the temporal overlap among species should be less than expected by chance, which represent a segregated or sequential phenology. Overlap was quantified via Pianka (Pianka, 1973) and Czechanowski (Feinsinger et al., 1981) indices and the values were generated using a randomization algorithm ('Rosario'). This algorithm was designed specifically for use with important interval data (Castro-Arellano et al., 2010). ‘Rosario’ maintains the shape of the empirical activity distributions for each species in the randomly generated matrices by shifting entire activity patterns over a random number of intervals. For each analysis, overlap indices were calculated for 10,000 randomly generated matrices of temporal flowering phenology, creating a null distribution of overlap values. Then a one-tailed test was conducted and the p-value was calculated as the proportion of randomizations that resulted in overlap that is equal to or less than the empirical overlap value (observed) – see, e.g., Brito et al. (2012). Simulations were conducted with the Time Overlap program (Castro-Arellano et al., 2010).

The similarity of herbivore composition among plant species was evaluated using a cluster analysis (Freitas et al., 2002). An incidence matrix was constructed with the presence or absence of each herbivore species on each plant species. A dendrogram was then constructed using the Jaccard index and single linkage with the software Systat 12 (Jurzenki et al., 2012). Differences in leaf-area loss were compared using repeated-measures ANOVA after arcsin transformation of the percentage values. The fruit set and herbivore abundance were compared using the Mann–Whitney U-test because the data were not normally distributed even after transformation.

**Results**

In the circular diagrams the mean angles of onset and peak for all phenophases were not significantly seasonal (Rayleigh test P > 0.05) and the lengths of mean vectors (r̂) were nearly 0 (Table 1).

The vector r varies from 0 (when phenological activity is distributed uniformly throughout the year) to 1 (when phenological activity is concentrated around one single date or mean angle), cf. Zar (1996). Thus, the results showed that phenological activity of the Malpighiaceae species studied is distributed uniformly throughout the year featuring a segregated phenology.

The null model simulations indicated that the distributions overlap was significantly less than expected by chance, which means that species exhibited sequential flowering with little overlap in their reproductive periods (Pianka = 0.202, P < 0.001; Czechanowski = 0.968, P < 0.001). The first to blossom was P. tomentosa in June 2008, followed by B. laevifolia, B. campestris and B. malifolia (Fig. 1).

Cluster analysis showed the greatest similarity in herbivore composition between P. tomentosa and B. laevifolia (70%). Between these two species and B. campestris the similarity was almost 65%. B. malifolia shared almost 50% of its herbivores with the other plant species (Fig. 2).

The presence of ants significantly reduced the leaf damage caused by herbivores in all plants (P. tomentosa: F = 616.6, P < 0.001, DF = 1; B. laevifolia: F = 604.8, P < 0.001, DF = 1; B. campestris: F = 603.6, P < 0.001, DF = 1; B. malifolia: F = 544, P < 0.001, DF = 1; Fig. 3) but did not affect fruit set (P. tomentosa: U = 49, P = 0.67, n = 30; B. laevifolia: U = 97, P = 0.52, n = 30; B. campestris: U = 101, P = 0.61, n = 30; B. malifolia: U = 89.5, P = 0.34; n = 30). In this plant–herbivore system, each plant species had a main group of more abundant herbivores (mostly floral herbivores) and a secondary group of less abundant herbivores (secondary herbivores). The main herbivores of each plant species caused considerable damage to their respective hosts, mainly injuring the reproductive structures and reducing the benefit of ants to the plants’ fitness (fruit set).

Thrips (Thysanoptera: Heterothrips peixotou) were the most numerous herbivores on P. tomentosa during the plant reproductive season (n = 587; Fig. 4), far outnumbering orthopterans (n = 12), hemipterans (n = 11), coleopterans (n = 10), and lepidopteran larvae (n = 8). Thysanopteran infested control plants and experimental plants equally (U = 85, P = 0.26, n = 30), while secondary herbivores were significantly more abundant on ant-excluded plants (U = 63, P = 0.04, n = 30; Fig. 5). Thus, ants effectively removed or chased away herbivores from P. tomentosa except thrips. Ectatomma tuberculatum was the most common visiting ant species on P. tomentosa above. Curculionid beetles (Curculionidae: Anthomusus sp.) were the most numerous herbivores on B. laevifolia (n = 3906; Fig. 6), followed by thysanopterans (n = 161), hemipterans (n = 76), other coleopterans (n = 15), and orthopterans (n = 9). Curculionid larvae are endophytic herbivores that infest the buds and flowers, eating the plant embryonic tissue (Torezan-Silangardi, 2011). These beetles infested control plants and ant-excluded plants equally (U = 102, P = 0.66, n = 30), while the other herbivores were significantly more abundant on ant-excluded plants (U = 59.5, 2009.

| Table 1 | Circular statistical analysis testing for seasonality of phenological patterns of four Malpighiaceae species in a Brazilian tropical savanna between June of 2008 and 2009. Rayleigh test were performed for significance of the mean angle, and the mean data were omitted because they were not significant. |
|---|---|---|---|---|---|---|---|
| Flower bud | Flower | Fruit |
| | Onset | Peak | Onset | Peak | Onset | Peak |
| Mean data | 16/07/2008 | 15/06/2008 | 02/07/2008 | 25/07/2008 | 17/07/2008 | 08/07/2008 |
| Number of observations | 110 | 109 | 97 | 99 | 87 | 89 |
| Mean vector (μ̂) | 197.3519\(°\) | 166.697\(°\) | 183.013\(°\) | 206.112\(°\) | 198.797\(°\) | 219.599\(°\) |
| Length of mean vector (r̂) | 0162 | 0010 | 0174 | 0169 | 0175 | 0177 |
| Median | 223 | 225 | 223 | 240 | 240 | 255 |
| Circular standard deviation | 109.408\(°\) | 122.594\(°\) | 107.109\(°\) | 108.082\(°\) | 106.992\(°\) | 106.622\(°\) |
| Rayleigh test (z) | 2.87 | 1.12 | 2945 | 2563 | 2661 | 2758 |
| Rayleigh test (p) | 0.06 | 0.32 | 0.06 | 0.08 | 0.07 | 0.07 |
P = 0.03, n = 30; Fig. 5). Thus, ants effectively removed exophytic herbivores but not endophytic larvae from these plants. *Cephalotes* sp. was the most common visiting ant species on *B. laevisola*.

Mealybugs (Hemiptera: Coccoidea) were the main floral herbivores on *B. campestris* (n = 478) and *B. malifolia* (n = 386; Fig. 7). These mealybugs are trophobiont insects, attracting and feeding attending ants while sucking at the base of the floral pedicel. In *B. campestris*, the secondary herbivores were thysanopterans (n = 133), coleopterans (n = 51), hemipterans (n = 46), and orthopterans (n = 28). In *B. malifolia*, the secondary herbivores were thysanopterans (n = 112), orthopterans (n = 98), hemipterans (n = 86), coleopterans (n = 32) and lepidopteran larvae (n = 2). Mealybugs were significantly more abundant on control plants of both *B. campestris* (U = 47, P < 0.05, n = 30) and *B. malifolia* (U = 65, P < 0.05, n = 30), while secondary herbivores were more abundant on ant-excluded plants of *B. campestris* (U = 60, P < 0.05, n = 30; Fig. 8) and *B. malifolia* (U = 42, P < 0.05, n = 30; Fig. 8). *Crematogaster* sp. and *Camponotus* sp. were the most common visiting ant species on *B. campestris* and *B. malifolia*, respectively.

**Discussion**

The hypothesis was confirmed that the sequential flowering of related host plants results in a shared herbivore guild over time that may be quite harmful to these plant species. The sequential flowering of the studied species of Malpighiaceae favors the use of these plants by a similar herbivore guild over time. The interactions with ants were important to plants to reduce the abundance of sharing herbivores (Fig. 5 and Fig. 8) and foliar herbivory (Fig. 3). The strength of positive effects on reproductive structures was affected by the variation in the morphological and behavioral characteristics of certain herbivore groups and ants associated with particular host plants. Thus, the association with ants is critical to the optimal development of these plant species that present sequential flowering in the Brazilian tropical savanna.

Sequential flowering can be initiated by seasonal changes in rainfall, temperature and gain of solar radiation (Rathcke and Lacey, 1985). Plants with sequential flowering, such as the shrubs studied here, succeed each other in producing floral rewards throughout the year, generating the conditions needed to maintain pollinators and other floral visitors (Gentry, 1974; Newstron et al., 1994; for examples in Malpighiaceae see Costa et al., 2006; Sigrist and Sazima, 2004). Our results show that the sequential respouting and flowering of Malpighiaceae species in the Cerrado also provides an uninterrupted food supply to a diverse herbivore guild. In Malpighiaceae, immature structures (e.g., young leaves, buds, and flowers) generally have low structural resistance to physical damage, making the shoots, and inflorescences especially attractive to chewing and sucking insects (Del-Claro et al., 1997). This fact, associated with the close resemblance in floral structure among Malpighiaceae species (Anderson, 1979), may contribute to the observed similarity of the herbivore fauna.

**Fig. 1.** Linear graphic with the intensity of flowering of Malpighiaceae species showing the sequential flowering of *Peixotoa tomentosa*, *B. laevisola*, *B. campestris*, and *B. malifolia*, respectively, in a Brazilian tropical savanna between June of 2008 and 2009 (axis and mean intensity of flowering phenology being 1 = 25% and 2 = 75%, n = 30).

**Fig. 2.** Cluster analysis of the guild of herbivores associated to four Malpighiaceae species (*Peixotoa tomentosa*, *B. laevisola*, *B. campestris*, and *B. malifolia*) in a Brazilian tropical savanna between June of 2008; and 2009 (Jaccard index).
The shared herbivore guild may be quite harmful to these members of Malpighiaceae, making the association with ants decisive for optimal development of these plants. Our experimental manipulative study showed that the protection provided by ants is significantly biased against foliar herbivory, as observed in other Cerrado plants (Costa et al., 1992; Del-Claro et al., 1996; Oliveira, 1997). However, these benefits do not extend to the reproductive structures. The lack of effective ant protection for the plants’ reproductive structures can be explained by seasonal and temporal variation (Alves-Silva and Del-Claro, 2013). Temporal variations in the abundance of the main plant herbivores and behavioral characteristics of the herbivores and ants may reduce or eliminate the beneficial effects of ants on plant productivity. On the other hand, the significant reduction in leaf area loss due ant presence may improve plant survivorship and future reproductive success (Marquis, 2012). Thus, our results corroborate the hypothesis that the outcomes of ant–plant interactions depend upon the ecological and behavioral features of associated ant and herbivore species (Del-Claro and Oliveira, 2000).

The peak abundance of thysanopteran insects coincided with the reproductive period of *P. tomentosa*. This group of herbivores consists of small phytophagous and mycophagous insects (Mound and Kibby, 1998) that develop largely in floral chambers (Alves-Silva and Del-Claro, 2013; Del-Claro et al., 1997) and consume developing flowers, pollen and fruits (Del-Claro and Mound, 1996; Pinent et al., 2005). These insects are by far the main herbivores of *P. tomentosa*. In addition to the morphological characteristics of thrips, we considered certain morphological characteristics of the ants associated with the plants. Inside the floral chambers, thrips can be captured only by minute ants, such as *Crematogaster*/*Myrmicinae* and *Brachymyrmex*/*Formicinae* (Del-Claro et al., 1997). The ants observed during the flowering season of *P. tomentosa* were mainly *Ectatomma* (*Ectatomminae*) and *Camponotus* (*Formicinae*) species, which have morphological constraints (larger size) that prevent them from entering the floral chambers. Therefore, the ants that interact with *P. tomentosa* are ineffective in removing and reducing the abundance of thrips, allowing severe damage to the plants’ reproductive structures regardless of the presence of ants, as observed by Del-Claro et al. (1997). This relationship between the morphological limitations of certain ant genera and the inefficient removal of thysanopteran herbivores indicates that the identity of the ant species present in the field during the *P. tomentosa* reproductive season may be more critical to the outcome of the ant–plant interaction than the high abundance of thrips.

The *B. laevifolia* shrubs may have been strongly affected by the high abundance of their main herbivore. The peak abundance of beetles of the genus *Anthonomus* (*Anthonominae*: *Curculionidae*) coincided with the flowering period of *B. laevifolia*, and the ants were not significantly effective in removing these beetles. These herbivores feed and complete their development inside the flower buds. Larval development within plant structures benefits beetles by ensuring a steady food supply, protection against sun and drying winds, and shelter against predators and parasites (Clark and Martins, 1987; Torezan-Silingardi, 2011). Therefore, the high abundance of *curculionids* during the reproductive period of *B. laevifolia* most likely causes extensive damage to the buds of this species, which may not be intensely or fully protected by the associated ants. In a previous study by Torezan-Silingardi (2007),
beetles of the genus *Anthonomus* (Anthonominae: Curculionidae) had the greatest species richness (eight species), but their low abundance mitigated the damage to their plant hosts.

In this study, mealybugs were the major herbivores on *B. campestris* and *B. malifolia* and were present mainly on plants with ants. Del-Claro and Oliveira (2000) have shown that the benefits to the host plant in plant–ant–hemipteran interactions vary depending upon the behavior of the associated herbivores and ants. Here, the association of ants with coccoid herbivores most likely provides benefits for the plant leaves, as observed in similar systems (Moreira and Del-Claro, 2005). However, the high abundance of these animals during the reproductive periods of *B. campestris* and *B. malifolia* negatively affects these plants’ reproductive success.

The studied system shows that the interactions between ants, plants, and their herbivores occur within multitrophic networks whose dynamics, which determine the outcomes of the interactions, are strongly influenced by the sequential flowering of plants (e.g., Lange et al., 2013). In addition to various bottom-up effects, the phenological synchronization between herbivores and their host plants frequently determines the quantity and quality of food resources and abundance of herbivores, directly impacting populations and communities (Kerslake and Hartley, 1997; Yukawa, 2000). Unfortunately, few entomologists record detailed phenological data on host plants, and little information is available linking sequential resprouting and/or flowering with the associated herbivore fauna. We hope that these conditions will change in the near future because accumulation of phenological data is necessary also to assess the effects of global warming on the synchronization of herbivores with host-plant phenology (e.g., Yukawa, 2000).

**Fig. 5.** Comparative analysis of abundance of secondary herbivores (median ± 1 SE) between control (white bars – ant access) and treatment (gray bars – ant exclusion) shrubs of *Piptocha tomentosa* and *B. laevifolia* in a Brazilian tropical savanna. The asterisks (***) means *P* < 0.05 (non-parametric test, Mann–Whitney *U*-test).

**Fig. 6.** Circular scatterplots for the occurrence of: plants flowering; stems per plant with active extrafloral nectaries (NEF); plants visited by ants; Curculionidae abundance; on plants of *B. laevifolia* (*n* = 30), in a Brazilian tropical savanna between August and November of 2008. The straight line between bars indicates the circular median with standard deviation (95% CI). The four histograms show marked seasonality (*P* < 0.001, Rayleigh test *z*).
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