

ORIGINAL ARTICLE

Phenological relationships of *Eunica bechina* (Lepidoptera: Nymphalidae) and its host plant, *Caryocar brasiliense* (Caryocaraceae), in a Neotropical savanna

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Herbivorous insects frequently fluctuate numerically throughout the year in response to variation in the availability and quality of plant resources. Here, we investigate the phenological relationship between the butterfly *E. bechina* and its host plant *C. brasiliense* in the Brazilian cerrado savanna. Abundance of *E. bechina* immature stages followed the temporal pattern of leaf production by the host plant, with increased numbers in the beginning of the rainy season (September–November). Ant visitation to extrafloral nectaries of *C. brasiliense* also increased in the humid period, following the production of new leaves and flower buds. Ant abundance, however, apparently had no major effect on the temporal occurrence pattern of *Eunica* immatures on host plants. Nevertheless, recent data on antagonistic and mutualistic ant–butterfly interactions have revealed that ants do affect host plant choice by butterflies in the ant-rich cerrado savanna. Future investigation in insect seasonality in cerrados should take into account not only climate and plant phenology, but also direct and indirect effects at the ant–plant–herbivore interface.

Keywords: ant–butterfly interaction; ant–plant interactions; Brazil; cerrado vegetation; extrafloral nectary; seasonality

Introduction

Populations of herbivorous insects frequently fluctuate numerically throughout the year in response to variation in the availability and quality of the plant resources on which they feed (Price 1997). In tropical environments, such fluctuations are normally associated with rainy and dry periods (Marquis et al. 2002). Because young nutritious leaves are produced mainly in the rainy season, this is usually the period more favorable for the growth and reproduction of most herbivorous insects (Janzen & Schoener 1968; Wolda 1978, 1988).

In the Brazilian cerrado savanna, the butterfly *Eunica bechina* (Hewitson 1852) (Nymphalidae: Biblidinae) uses the common shrub *Caryocar brasiliense* Camb. (Caryocaraceae) as host plant (Oliveira & Freitas 1991). Females oviposit on shoot tips and young leaves of *C. brasiliense* on which the larvae feed, whereas adults feed on rotting fruits and fermenting plant sap of many plant species (Freitas & Oliveira 1992).

Caryocar brasiliense sheds leaves in the dry season; new leaves and flowers are produced in the beginning of the rainy season (Araujo 1995; Leite et al. 2006; Silva & Oliveira 2010). Young leaves and floral buds bear extrafloral nectaries (EFNs) that are visited day

and night by numerous ant species (Figure 1A, B) that patrol the plant and decrease infestation levels by several insect herbivores including *E. bechina* (Oliveira & Brandão 1991; Oliveira 1997). Although *E. bechina* eggs are ignored by foraging ants, the larvae can be attacked and killed on host plants (Figure 1C–F; Freitas & Oliveira 1992, 1996). Females preferentially oviposit on plants without ants, and were recently shown to distinguish between aggressive and innocuous ant species when these co-occur on a plant (Sendoya et al. 2009).

The close relationship between specialist herbivores and their host plants makes insect populations strongly affected by plant phenology (Janzen 1993; Morais et al. 1999; Silva & Oliveira 2010). Myrmecophilous plants may produce extrafloral nectar that promotes visitation by aggressive ants in periods of high vulnerability to herbivores, such as during leaf flushing and reproduction (Rico-Gray & Oliveira 2007 and included references). Accordingly, climate may influence plant phenology, which in turn affects both herbivore and ant activity (Díaz-Castelazo et al. 2004). Although it is widely accepted that seasonal cycles of tropical herbivores are associated with plant phenological patterns, few studies have linked these parameters with the activity of

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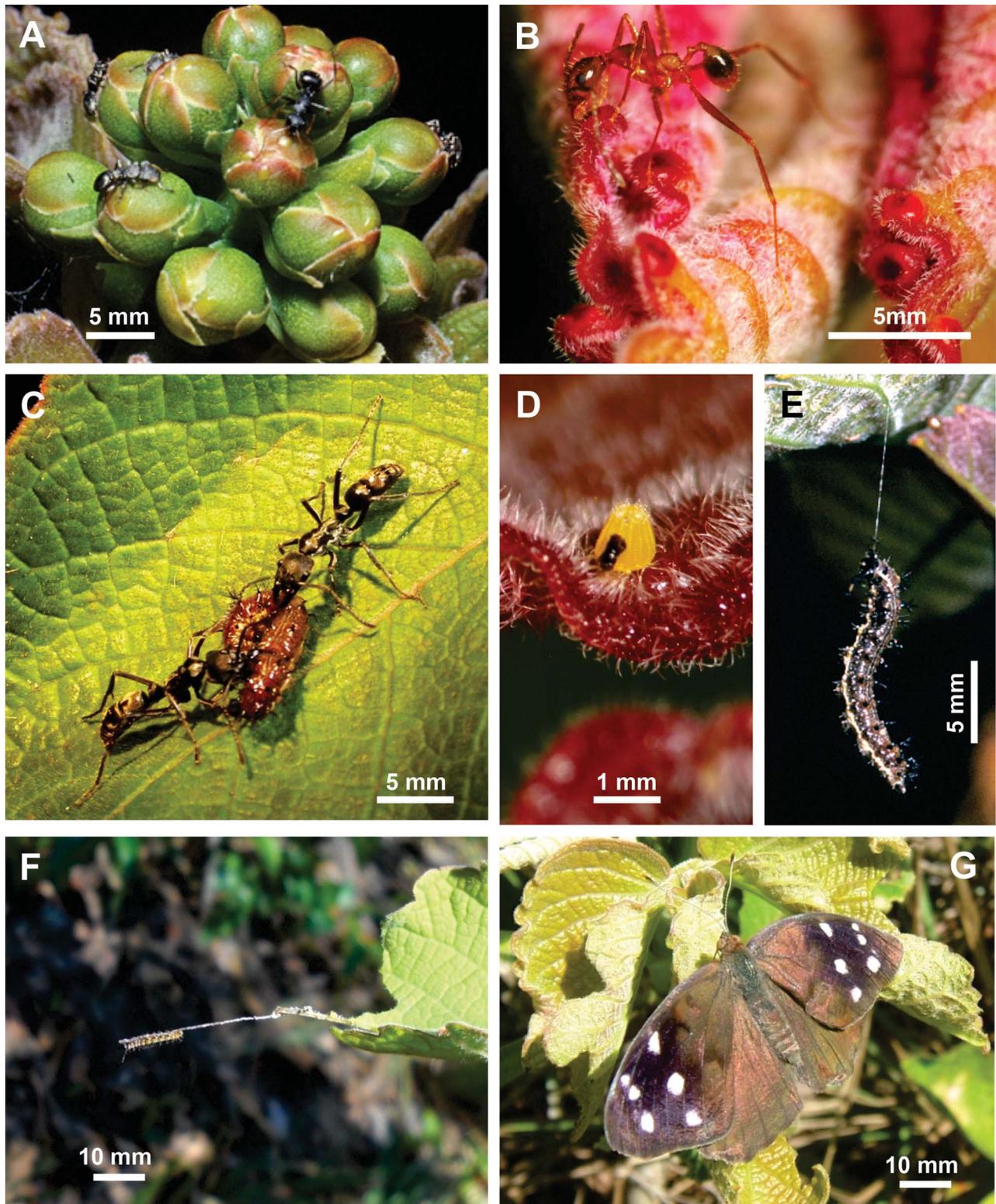


Figure 1. (Color online) A, *Cephalotes* and *Camponotus* ants visiting extrafloral nectaries (EFNs) on buds of *Caryocar brasiliense*; B, *Pheidole* sp. ant visiting EFNs on young leaves of *C. brasiliense*; C, workers of *Pachycondyla villosa* attacking fourth-instar of *Eunica bechina*; D, *E. bechina* egg; E, *E. bechina* larva hanging by a silk thread after attack by a large ant on a leaf; F, *E. bechina* larva resting on a frass chain; G, *E. bechina* adult male. Photographs (B, D) courtesy of H. Soares Jr., (F) courtesy of S. F. Sendoya.

their natural enemies (see Bentley 1977; Janzen 1993; Marquis et al. 2002).

In the current study we investigate the phenological relationship between *E. bechina* and its host plant *C. brasiliense* in cerrado savanna, taking into account the presence of predaceous ants attracted to the plant's EFNs and their possible effect on the pattern of host plant use by the butterfly.

Materials and methods

Study site

Fieldwork was carried out during 2009 and 2010 in a reserve of cerrado savanna in Itirapina, São Paulo, southeastern Brazil (22°15' S, 47°49' W). The vegetation consists of a scrub of shrubs and trees, which corresponds to the cerrado *sensu stricto* physiognomy (Oliveira-Filho & Ratter 2002), and the density of *Caryocar brasiliense* shrubs reaches up to 300 individuals/ha (Oliveira 1997). A hot and rainy season occurs from October to March (spring-summer), and a cool and dry season from April to September (fall-winter). Temperature and rainfall

data during the study period were obtained from the reserve's climatological station (see Figure 2).

Phenological patterns of *Eunica bechina* immature stages and *Caryocar brasiliense*

Comparative data on the abundance of *E. bechina* immature stages and the host plant *C. brasiliense* were collected between November 2009 and October 2010 along a trail in the cerrado savanna (600 m long). This dataset was further complemented with previous phenological data recorded between August 1986 and July 1987 (Oliveira 1988).

Fifty shrubs of *C. brasiliense* (0.5–1.0 m tall) were tagged and monitored weekly. The number of young and mature leaves and the presence of floral buds, flowers, and fruits were recorded in each visit. The number of *E. bechina* eggs and larvae on plants were recorded from January to October 2010. From August 1986 to July 1987, the presence of *E. bechina* immatures and the phenology of the host plant were recorded every 2 weeks for 80 tagged shrubs (0.5–1.0 m tall; Oliveira 1988).

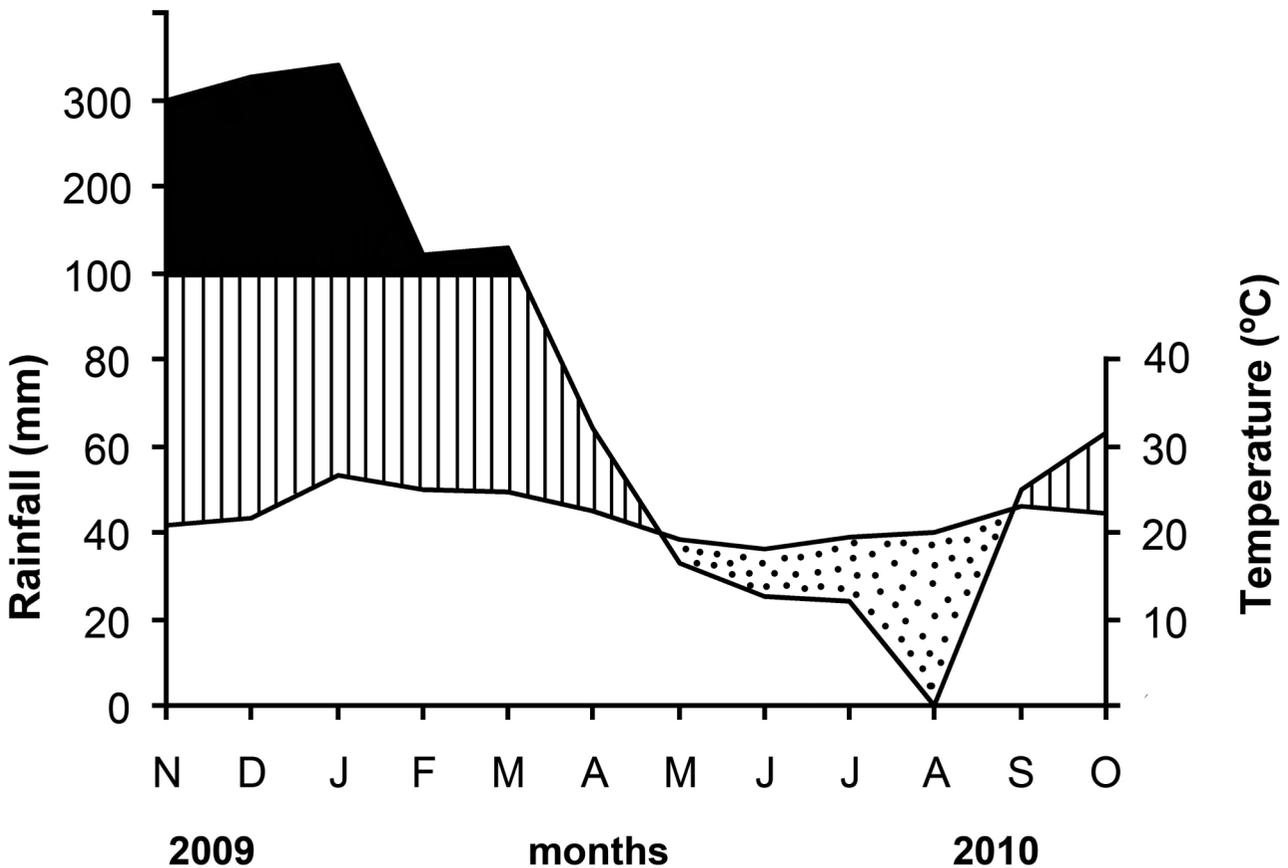


Figure 2. Climatic diagram at the Itirapina region in southeastern Brazil, from November 2009 to October 2010. Average temperature: 21.7°C; total rainfall: 1566 mm. Dotted = dry periods, hatched = humid periods, black = superhumid periods. Format following Walter (1985).

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Butterfly sampling

Adult *E. bechina* (Figure 1G) were sampled weekly from November 2009 to October 2010 using 20 fruit-feeding butterfly traps suspended 1.0–1.5 m above the ground, and baited with a standard mixture of mashed banana with sugar cane juice, fermented for at least 48 h (see DeVries 1987; Uehara-Prado et al. 2005). Traps were placed along five transects 30 m apart from each other; four traps were placed at a distance of 30 m from each other within each transect. Adults of *E. bechina* were individually numbered on the underside of both forewings with a black permanent felt-tipped pen and released. This procedure prevented us from recounting the same butterfly within a sampling period.

Ant censuses

Ant censuses were performed on 40 tagged *C. brasiliense* shrubs from September to November 1986. At each plant 20 seconds were spent recording the number of ant workers from different species; plants were monitored once per month and ants visiting EFNs were recorded at four different times: 08:00, 14:00, 20:00, and 02:00 h (Oliveira 1988, Oliveira & Brandão 1991).

Results

Occurrence of adults and immatures of *E. bechina* are illustrated in Figure 3. In the 2009–2010 sampling period, no clear temporal pattern was observed for eggs, but larvae increased consistently from August to October; adults were captured only from February to April, and later in September and October (Figure 3A). In the 1986–1987 sampling period, larvae and eggs were not present in August but were abundant in September, decreasing steadily towards January (Figure 3B).

Leaf phenology was similar in the 1986–1987 and 2009–2010 study periods, with a maximum number of leaves from November to January and a minimum from July to September (Figure 4A, B). In 2009–2010 leaves were produced all year round; young leaves were rare most of the year and increased markedly in September and October (Figure 4A).

Both in the 1986–1987 and 2009–2010 study periods, occurrence of EFNs on plants was more pronounced from August to November (Figures 3, 4B). Ant visitation to plants increased markedly in October and November, following the production of new leaves and flower buds (Figures 3B, 4B).

Discussion

Phenological relationships

In tropical habitats insects are usually more limited by biotic than by abiotic factors (Denlinger 1986), and populations of herbivorous insects may be regulated by the availability of the plant tissues on which they feed (Wolda 1978, 1988). Indeed, in both observation periods the abundance of *E. bechina* immatures followed the temporal pattern of leaf production by the host plant. A similar pattern has been recorded for the pentatomid *Edessa rufomarginata*, which also feeds on *C. brasiliense* phloem in the same study area (Silva & Oliveira 2010), and for other insect groups in the cerrado (Marquis et al. 2002) and elsewhere in the tropical region (Wolda 1978; Frith & Frith 1985; Braby 1995a).

Although abundance of lepidopteran larvae has been recorded to peak in the mid dry season in cerrado (Morais et al. 1999; Rodovalho et al. 2007), this is not the case for *E. bechina*, which feeds only on young leaves that are abundant in the early rain season (see also Oliveira & Freitas 1991).

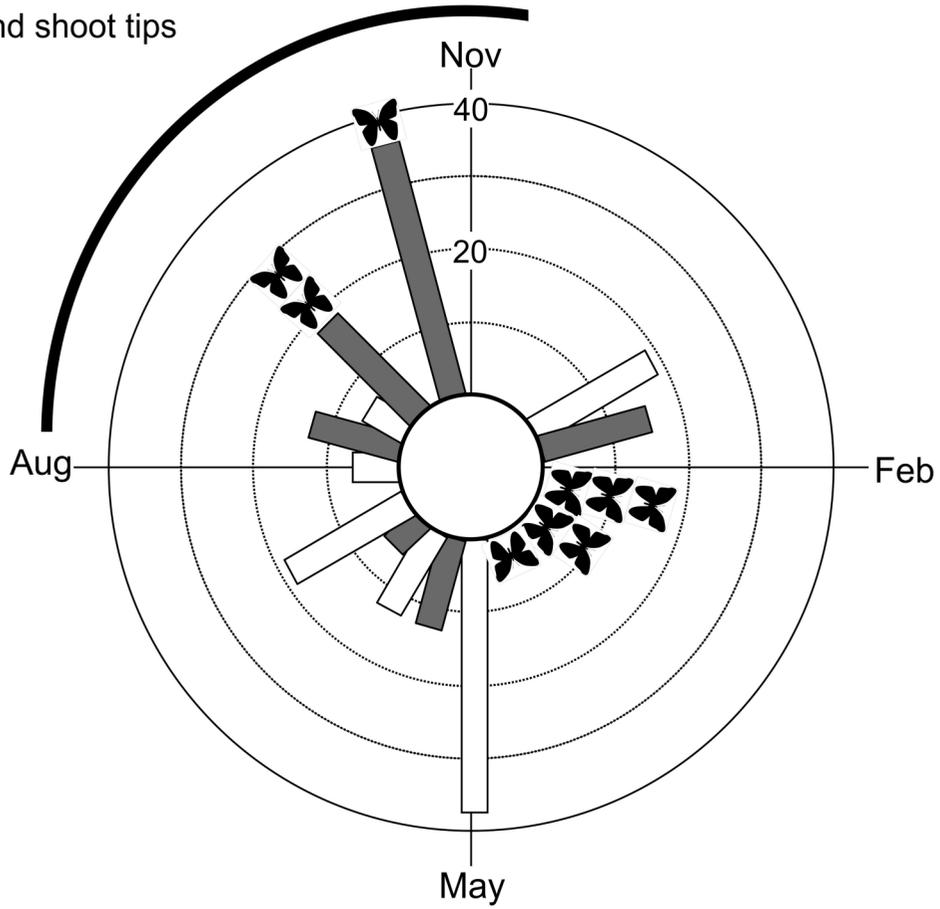
The role of parasitoids in regulating lepidopteran populations has been documented in several tropical habitats (Price et al. 1980; Brown 1992). Indeed, Campos et al. (2006) observed a lepidopteran population in southeastern Brazil to oscillate even with abundant larval resources present year round, due to migration and parasitoid attack. Although parasitoids could play some role in the abundance patterns of *E. bechina*, our data suggest that resource availability is a key factor explaining the temporal variation in the abundance of *E. bechina* immatures.

Many tropical insects undergo a “reproductive diapause” and remain relatively inactive during the dry season (Janzen 1973; Salomão & Vasconcellos-Neto 2010), including butterflies of other savannas (Braby 1995b). Even though a reproductive diapause is the most likely explanation for the temporal pattern of

Figure 3. A, Occurrence of *Eunica bechina* from November 2009 to October 2010 in an area of cerrado savanna at Itirapina, southeastern Brazil. Bars indicate the percentage of egg (white) and larva (gray) observations on 50 *Caryocar brasiliense* shrubs. Butterfly icons indicate number of trapped adults. The external thick line highlights months of high presence of extrafloral nectaries on shoot tips and flower buds. Eggs and larvae were not counted in November and December. B, Temporal occurrence of *E. bechina* immatures and ants on *C. brasiliense* at Itirapina, from August 1986 to January 1987. Bars show the percentage of observations of butterfly eggs and larvae on 80 tagged shrubs. Ant data are mean number of workers on 40 plants. The upper horizontal thick line highlights months of high presence of extrafloral nectaries on shoot tips and flower buds.

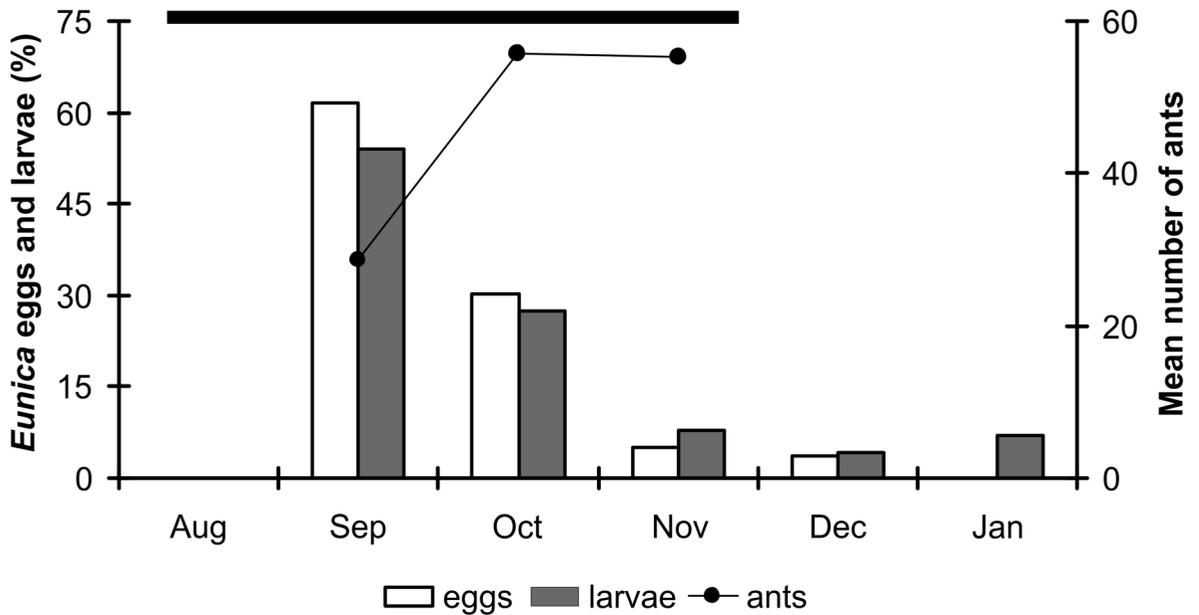
A

EFNs on flowerbuds and shoot tips



B

EFNs on flower buds and shoot tips



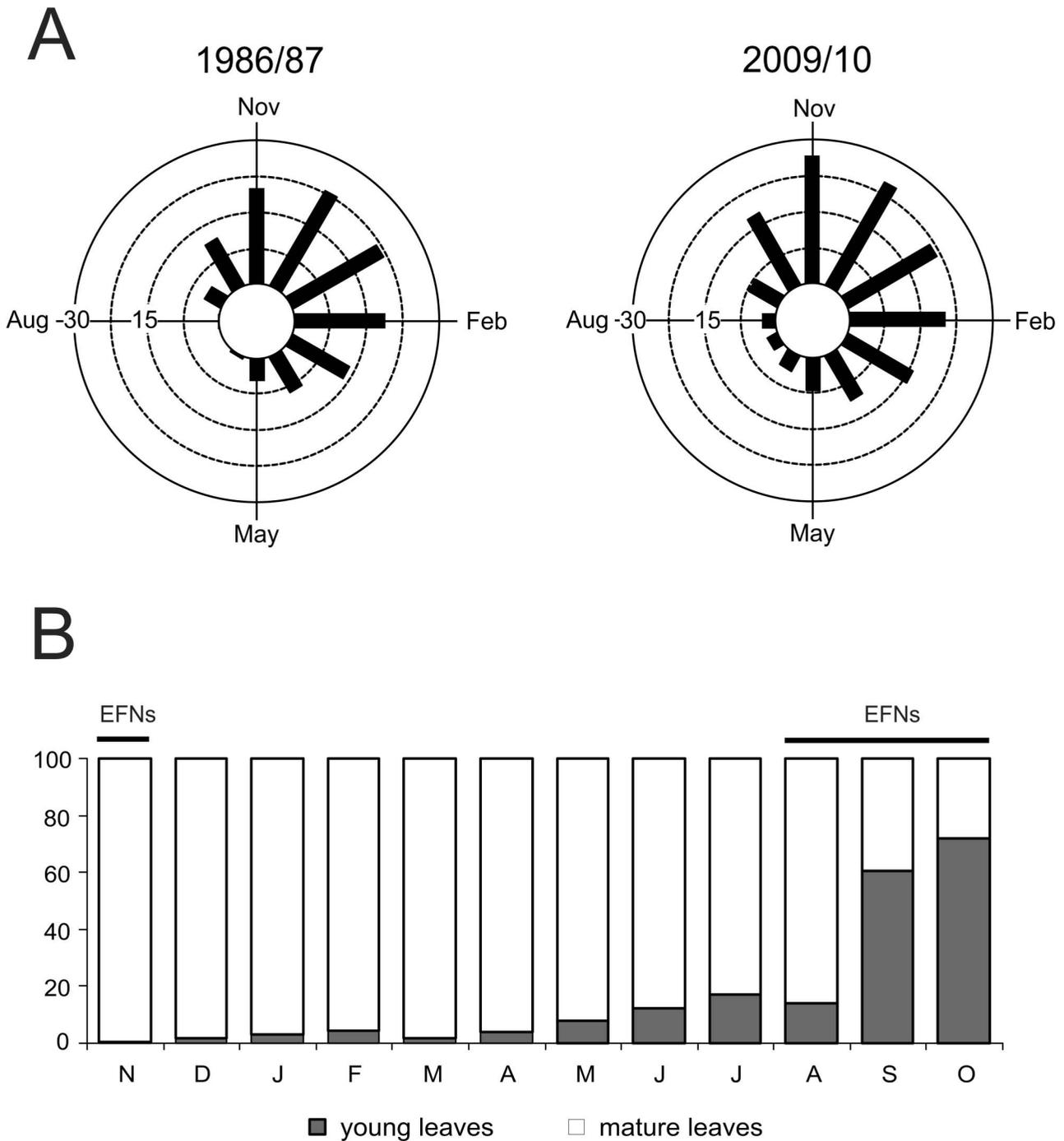


Figure 4. A, Mean number of leaves per *Caryocar brasiliense* shrub from November 2009 to October 2010 ($n = 50$), and from August 1986 to January 1987 ($n = 80$) in an area of cerrado at Itirapina, southeastern Brazil. B, Percentage of young and mature leaves of *C. brasiliense* shrubs ($n = 50$), from November 2009 to October 2010. The upper horizontal thick line highlights months of high presence of extrafloral nectaries on shoot tips and flower buds.

occurrence that we observed, we have no evidence that this process occurs in *E. bechina* based on present data. This strategy is only possible if adults are relatively long-lived, as they must survive for about three months during which time larval resources are low. Because

adult *E. bechina* were not recaptured, we cannot estimate their life-span, but fruit-feeding butterflies can be very long-lived (e. g. Molleman et al. 2007) and we did recapture other Biblidinae in the same area three months after marking individuals (Muniz 2010).

Relationship with ants

In periods of high vulnerability to insect herbivory, ant-visited plants frequently secrete food rewards that attract pugnacious ants whose aggressive behavior towards herbivores reduces plant damage (Bentley 1977; Koptur 2005; Rico-Gray & Oliveira 2007). *Caryocar brasiliense* is intensively visited day and night by over 30 ant species that actively search for extrafloral nectar on shoot tips and floral buds (see Oliveira & Brandão 1991), and rates of ant visitation are especially high in the beginning of the rainy season when *Eunica* immatures are most abundant.

Allocation of extrafloral nectar to periods of production of young leaves and flowers reduces infestation by four different herbivores, including *E. bechina*, due to intense ant visitation (Oliveira 1997). *Eunica bechina* larvae, however, developed several morphological and behavioral mechanisms to diminish ant-induced injury and predation on *Caryocar* (Figure 1E, F; see Freitas & Oliveira 1992; Oliveira & Freitas 2004). In addition, the specialization on such a risky ant-defended plant has also resulted in discriminatory behavior by egg-laying females in the search for less vulnerable oviposition sites (Freitas & Oliveira 1996; Sendoya et al. 2009).

Recent research and the present study on *Caryocar* illustrate how the combination of biotic and abiotic factors may explain the seasonal pattern of occurrence of a tropical insect. In this case, plant phenology apparently has a strong influence in the year-round pattern of occurrence of *Eunica* immature stages on host plants. Behavioral data from antagonistic and mutualistic ant–butterfly interactions revealed that ants do affect host plant choice by butterflies in cerrado savanna (Freitas & Oliveira 1996; Sendoya et al. 2009; Kaminski et al. 2010). Thus, future investigation in insect seasonality in cerrados should take into account not only climate and vegetation variables, but also the range of direct and indirect effects at the ant–plant–herbivore interface.

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