Revista Brasileira de Entomologia 64(3):e20200021, 2020

SOCIEDADE BRASILEIRA DE ENTOMOLOGIA FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution

Effect of patch size of the exotic host plant *Calotropis procera* (Apocynaceae) on herbivory

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ARTICLE INFO

Article history:
Received 13 March 2020
Accepted 27 June 2020
Available online 24 August 2020
Associate Editor: Lucas Kaminski

Keywords:
Caatinga
Danaus
Insect-plant interactions
Invasive species
Resource concentration hypothesis

ABSTRACT

Calotropis procera is a xerophytic perennial milkweed shrub native to Asia and Africa, which currently is also widely distributed throughout the Brazilian Caatinga. *C. procera* is well defended against herbivores, especially with latex and toxic steroidal cardenolides. The goal of this study was to evaluate how patch size of this exotic host plant affect insect herbivory. Eight patches were selected containing a varied number of *C. procera* individuals (5, 8, 29, 31, 55, 79, 116, and 172 individuals/patch) in the Caatinga. Patches were divided into four categories according to the number of *C. procera* individuals: very small (5 and 8), small (29 and 31), intermediate (55 and 79), and large (116 and 172). The percentage of herbivory was greater in patch categories with more individuals. Among the herbivorous insects associated with *C. procera*, larvae of *Danaus* spp. (Lepidoptera: Nymphalidae) were commonly observed feeding on all patches. These herbivores present specialized behaviors to circumvent the presence of latex on the host leaves. Adult *Danaus* spp. females probably had higher probability to find large patches of *C. procera*, leaving a greater abundance of eggs and, consequently, increasing the herbivory by their larvae in these patches. In contrast to immigration tendency, emigration from large patches may be lower, because there is a higher probability of finding resources (e. g. suitable *C. procera* leaves) inside large patches compared with small patches. We conclude that patch size of the host plant *C. procera* is a major factor affecting herbivory.

The distribution of insect herbivores is primarily limited by the occurrence of their host plants. At a local scale, individual plants can vary in architectural complexity and herbivorous insects are more likely to be found on the most vigorous plants within a plant population and/or on the most vigorous modules within a plant (Price, 1991; Faria and Fernandes, 2001; Cornelissen et al., 2008). At the landscape scale, plant density (the number of individuals of a given species that occurs within a given sample unit or study area) and patch size (the number of host plants or the geographical extent of the stand) are important factors that can affect the abundance and diversity of insect herbivores (Root, 1973; Raupp and Denno, 1979; Kéry et al., 2001; Cassel-Lundhagen et al., 2008).

The resource concentration hypothesis (Root, 1973) states that larger host plant patches should have large insect densities because the probability of insects, particularly specialists, finding a large path is higher and the probability of an insect leaving a large patch is lower. However, several studies addressing the effects of host plant patch size on herbivore abundance have shown variable effects (e.g., Bach, 1988; Kareiva, 1983; Bukovinszky et al., 2010). Part of the inconsistency of the results in patch size experiments can be attributed to species-specific differences in the dispersal abilities of herbivorous insects and to the wide variety of systems studied. For example, the largest patches between studies ranged from a few square meters to dozens of hectares (e.g. Bach, 1988; Raupp and Denno, 1979; McCauley, 1991; Bukovinszky et al., 2010).

Calotropis procera (Aiton) WT Aiton (Apocynaceae) is a xerophytic perennial milkweed shrub native to Asia and Africa, which has been widely introduced as an ornamental plant into many other parts of the world (Hassan et al., 2015; Fig. 1A). In Brazil, its tolerance to drought and to acidic and nutrient-poor soils allowed the expansion into the Cerrado and Caatinga biome domains (Ulhôa et al., 2007; Rangel and Nascimento, 2011). In addition, this invasive exotic species presents rapid growth, dispersion by wind and easy propagation, which have allowed the formation of large populations mainly in disturbed areas, such as in pastures and roadsides (Ulhôa et al., 2007; Fabricante et al., 2013).

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https://doi.org/10.1590/1806-9665-RBENT-2020-0021

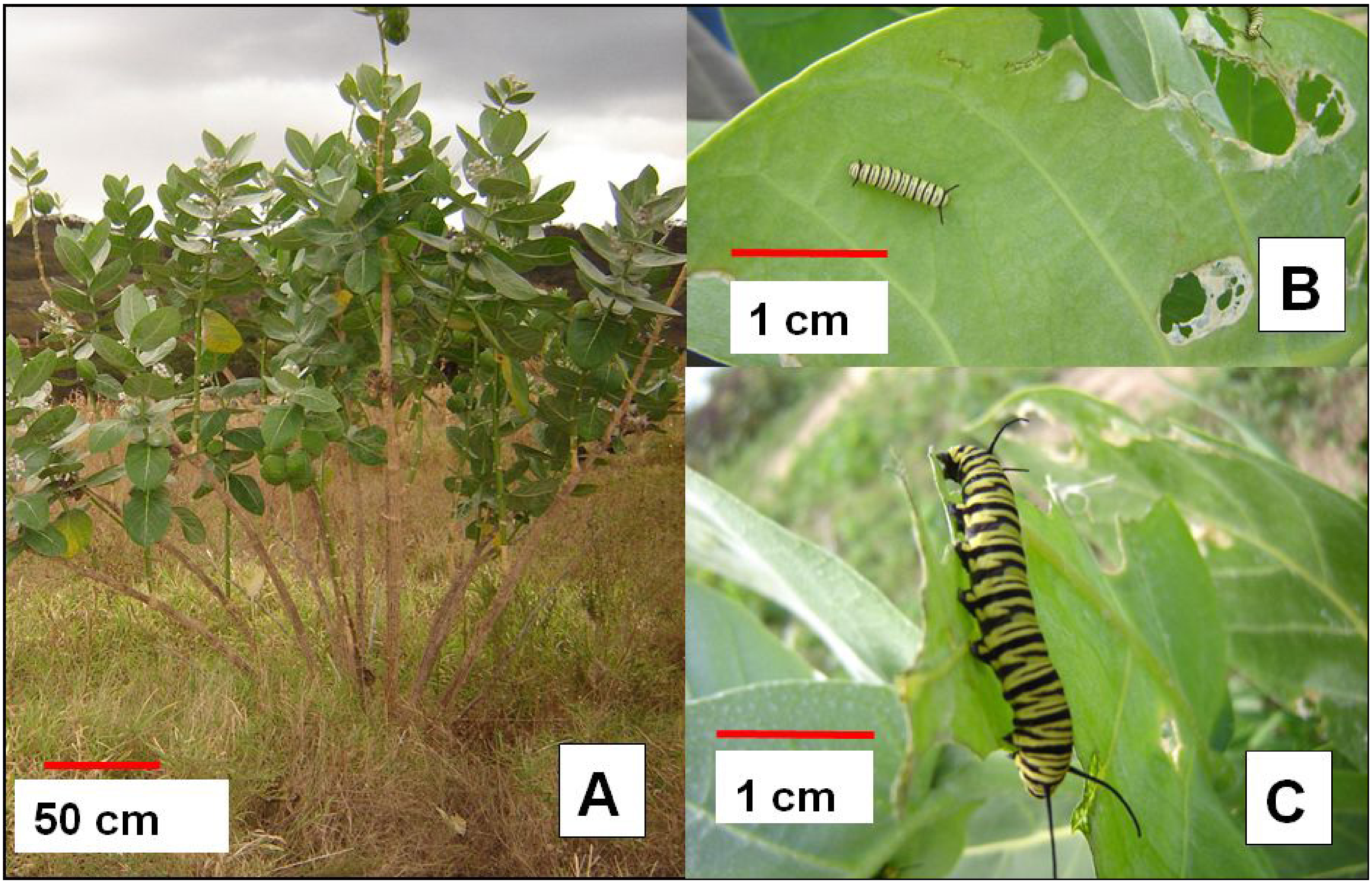

Figure 1 (A) Adult individual of *Calotropis procera* in a pasture area in the Caatinga, Pernambuco, Brazil; (B) early and (C) late instars of *Danaus erippus*.

The presence of patches of *C. procera* with vigorous and green leaves throughout the year may represent an important resource for herbivorous insects, especially in strongly seasonal environments such as the Brazilian Caatinga. Despite the fact that this species is very well defended against herbivores, especially with latex and toxic steroidal cardenolides (Malcolm, 1991; Agrawal et al., 2012), many insects from Coleoptera, Hemiptera and Lepidoptera orders have been reported consuming *C. procera* leaves (Chandra et al., 2011; Al Dhafer et al., 2011).

The objective of this study was to evaluate how patch size of the exotic host plant *C. procera* affects leaf herbivory. We tested the resource concentration hypothesis (Root, 1973), and predicted that there will be a greater percentage of leaf herbivory on patches with more individuals of the host plant *C. procera*.

The study was carried out between March and April 2006 in an area belonging to the Agronomic Institute of Pernambuco, located in the municipality of Serra Talhada, Pernambuco, Brazil. The climate of the region is seasonal with an average annual precipitation of approximately 800 mm, concentrated from January to May, and annual average temperature of 26°C (Sampaio, 1995). The local biome is Caatinga, dominated by an arboreal-shrubby physiognomy (Ferraz et al., 1998). The herbaceous stratum is not very dense and is mainly composed of annual plants that grow only in the rainy season (Sampaio, 1995).

To evaluate the effect of patch size (number of individuals) on insect herbivory eight patches were selected containing a varied number of *C. procera* individuals (5, 8, 29, 31, 55, 79, 116 and 172 individuals / patch). In each patch all individuals were marked and had their heights estimated (co-variable). To estimate the percentage leaf herbivory in the patches, all plants higher than one meter and with more than ten branches were pre-selected. Of these, five were randomly selected in each patch. From each individual, three to six young stems were randomly selected, from which their leaves were collected, for a total of approximately 200 leaves per patch. Each *C. procera* individual was surveyed once. The leaf area removed was calculated by dividing the sum of all areas removed from a leaf by the total area of the respective leaf. The leaf images were analyzed using the Image Tool 1.1 program. The percentage of herbivory on each plant individual was determined by averaging the leaf area removed from each stem per individual.

First, a correlation analysis was performed between the independent variables (patch size and average patch height). The variables showed a strong correlation with each other ($r = 0.716$, $p < 0.001$), and therefore, average patch height was discarded from the model (see Clarke and Warwick, 2001). Patches were divided into four categories according to the number of *C. procera* individuals: very small (5 and 8), small (29 and 31), intermediate (55 and 79), and large (116 and 172). A Generalized Linear Model with a *"Quasi-binomial"* distribution of errors was used to test whether the percentage of herbivory would be higher in patch categories (very small, small, intermediate, and large) with a greater number of individuals. Analysis of variance (ANOVA) was used to test for differences in leaf herbivory between these categories. The package "*lsmeans*" was used for *a posteriori* comparisons (Lenth, 2017). The statistical analyses were conducted with the R software (R Core Team, 2020).

The percentage of branches with at least one leaf damaged was high in all patches (88-100%), while the percentage of herbivory varied from 2.78% to 11.74% among patches. The percentage of herbivory was greater in patch categories with more individuals (deviance = 0.974; $F = 10.442$;

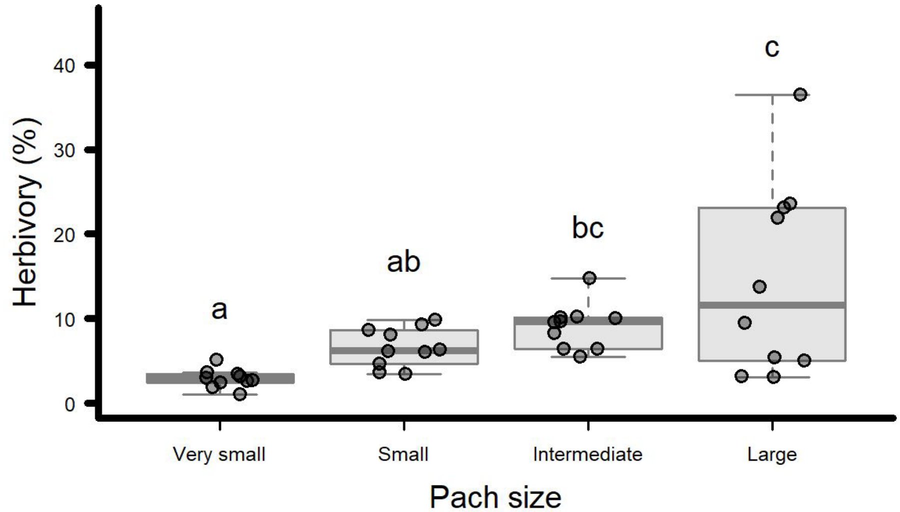

Figure 2 Boxplots of the percentage of herbivory between patches of *C. procera* of different sizes (number of individuals) in the Caatinga, Pernambuco, Brazil. Each circle represents the average percentage of herbivory of the branches of each individual sampled. The horizontal thick grey band represents the median value, the boxplot margins indicate first and third quartiles, the whiskers represent the maximum/minimum value within one and a half times the interquartile range.

$p < 0.0001$; Fig. 2). Despite no quantitative data on the abundance of the insect herbivore species associated with *C. procera* in this study, larvae of *Danaus* spp. (Lepidoptera: Nymphalidae) were commonly observed feeding on all patches (M.F.V. Rodrigues-Menelau *personal communication*). Larvae of the three *Danaus* species that occur in the Brazilian Caatinga, *D. erippus*, *D. gilippus* and *D. eresimus*, were observed feeding on *C. procera*. In contrast, chewing insects from other orders, such as Coleoptera and Orthoptera, were observed feeding in few individuals of *C. procera* ($n < 10$; M.F.V. Rodrigues-Menelau *personal communication*).

The resource concentration hypothesis was supported in our study system, since we confirm our prediction that *Calotropis procera* plants within patches with a greater number of individuals would be more attacked by herbivorous insects than those within patches with few host plants. Previous studies have reported *Danaus* spp. as important herbivores feeding on milkweed species, presenting specialized behaviors to circumvent the presence of latex (Rodrigues et al., 2010; Ferreira and Rodrigues, 2015). Early instars of *Danaus* spp. have been observed performing both trenching and vein-cutting on milkweeds, while late instars, which have already acquired greater resistance to latex, are able to feed directly on the leaf margins (Rodrigues et al., 2010; Ferreira and Rodrigues, 2015; Fig. 1B and Fig. 1C). In addition, compared with the small number of South American herbaceous milkweed species (genus *Asclepias*), *C. procera* is a woody milkweed that is probably very apparent and extremely easy to find by specialized *Danaus* spp.

Positive relationships between host population size and the occurrence of associated specialized species have been reported for other lepidopterans (e.g., Förare and Solbreck, 1997; Kéry et al., 2001; Cassel-Lundhagen et al., 2008; but see Grez and Gonzalez, 1995). For example, population size of the butterfly *Cupido minimus* was positively related to the coverage of its larval food plant *Anthyllis vulneraria* (Krauss et al., 2004). Similarly, populations of the butterfly *Maculinea rebeli* were both more likely and larger on larger populations of its host plant *Gentiana cruciata* (Kéry et al., 2001).

Previous studies showed that specialized butterflies should have a higher probability of immigrating to large patches, which are a larger target (Kéry et al., 2001; Krauss et al., 2003). In our study, adult *Danaus* spp. females probably had higher probability to find large patches of *C. procera*, leaving a greater abundance of eggs and, consequently, increasing the herbivory by their larvae in these patches. In contrast to immigration tendency, emigration from large patches may be lower, because there is a higher probability of finding resources (e. g. suitable *C. procera* leaves) inside large patches compared with small patches (Root, 1973). Thomas and Jones (1993) demonstrated that the probability of colonization by the butterfly *Hesperia comma* increased with increase of the patch area of its host plant, while probability of local extinction declined with increasing patch area. Similarly, a study showed that the extinction rates of specialized butterfly species decreased with increasing fragment size (Krauss et al., 2003).

We conclude that patch size of the host plant *C. procera* is a major factor affecting herbivory. Despite no quantitative data on the abundance of the insect herbivores associated with *C. procera,* we highlight the presence of *Danaus* species commonly feeding on this exotic host plant. Much is yet to be learned on the colonization of *C. procera* by these *Danaus* species in the Brazilian Caatinga, and this might represents an interesting system in which one can test many important long standing hypotheses on insect plant relationships as well as to investigate how population dynamics of the different *Danaus* species may differ from each other.

Acknowledgments

We thank PROCAD/CAPES (grant number 0166057) for financing the project. This study was part of MFV Rodrigues-Menelau 'MSc at UFPE that was supported by CNPq. We also thank UFRPE and IPA of Serra Talhada for logistical support. SN thanks CAPES for the postdoctoral researcher scholarship (CNPq-CAPES-PELD - 88887.142635/2017–00). GWF and JSA thank CNPq for the productivity grant.

Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

GW Fernandes, MFV Rodrigues-Menelau and JS Almeida contributed to the study design. GW Fernandes and MFV Rodrigues-Menelau conducted the sampling. S Novais analyzed the data and wrote the paper. All authors have read and approved the manuscript.

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