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ORIGINAL RESEARCH

Impact of herbivory by *Zerenopsis lepida* (Lepidoptera: Geometridae) on the endangered *Encephalartos eugene-maraisii* under field conditions

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Abstract

Encephalartos eugene-maraisii is an endangered, endemic plant in the Waterberg Mountain range of South Africa. This is the first report of herbivory by *Zerenopsis lepida* (Lepidoptera: Geometridae) on natural populations of *E. eugene-maraisii*. The impact of herbivory by folivorous arthropods on this cycad species has not been studied before. This study aimed to determine the incidence and intensity of *Z. lepida* herbivory on *E. eugene-maraisii* under field conditions. Local plant dynamics were studied by recording the plant location, sex, size and phenological stage for each plant. Damage surveys were carried out over two consecutive seasons (2020–2021 and 2021–2022). Leaf damage was assessed on 227 plants comprising 692 stems. Approximately 40% of the stems had signs of leaf herbivory by *Z. lepida* and 10% of the total leaf area was removed by *Z. lepida* larvae. Less herbivory was recorded in areas where plants synchronously produced new leaf flushes compared to areas with asynchronous leaf flushes. Greater apparency of plants led to a greater incidence of herbivory and level of damage. Repeated damage to plants may delay future leaf and cone flushing events and contribute to the threat against this cycad species.

Keywords Cycad · *Encephalartos* · Zamiaceae · *Zerenopsis* · Herbivory · Conservation biology

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Introduction

The consumption of plants by herbivores is among the most important species interactions in nature. Insects are one of the most abundant groups of herbivores and have significant impacts on plant population dynamics (Ancheta and Heard 2011; Agrawal et al. 2012; Myers and Sarfraz 2017). Most herbivores remain at low densities and impose minor but chronic damage on their host plant, a phenomenon referred to as background insect herbivory (Kozlov et al. 2015; Barrio et al. 2017; Shao et al. 2020). However, numerous studies have demonstrated that even minor herbivory can reduce plant health and reduce the rates of population growth (Baron and Bros 2005; Kettenring et al. 2009; Zvereva et al. 2012; Stephens and Westoby 2014; Zvereva and Kozlov 2014).

It is important to consider how insect herbivores impact populations of rare plant species as part of conservation programs (Myers and Sarfraz 2017). Specialist herbivores on rare plants raise interesting conservation questions because they may be rare and at risk of extinction themselves (Ancheta and Heard 2011; Moir et al. 2011, 2016; Ramírez-Restrepo et al. 2016; Koi 2017). Since rare plant species characteristically have small and isolated populations, with a specific habitat, specialist herbivores will likely have lower rates of colonization and higher probabilities of extinction during the decline of their host population (Moir et al. 2011; Taylor and Moir 2014). Indeed, reduced herbivore incidence and levels of herbivory, and in some cases even the complete absence of herbivores, have been reported in smaller host plant populations (Colling and Matthies 2004; Baron and Bros 2005). Increased herbivory has only rarely been reported in small host populations (Elzinga et al. 2005).

Globally, several groups of Lepidoptera feed on cycads although many cycadivorous Lepidoptera remains understudied (Whitaker and Salzman 2020). In Africa, an entire tribe of Geometridae moths, the Diptychini, consume leaves of the cycad genera *Encephalartos* and *Stangeria* (Sihvonen et al. 2015). The majority of Diptychini moths have only been recorded from a few cycad hosts and some are of conservation concern, e.g., *Callioratis millari* (Lepidoptera: Geometridae) in South Africa (Staude and Sihvonen 2014; Louw and Armstrong 2018; Terblanche 2018). Sufficient host plant populations that can sustain herbivore populations are becoming increasingly scarce and fragmented (Staude and Sihvonen 2014). *Zerenopsis lepida* (Lepidoptera: Geometridae), however, is widely distributed and occurs on wild populations of several species of *Encephalartos* and *Stangeria eriopus. Zerenopsis lepida* also made the transition to cultivated cycads outside their native range, including cycad genera from other world regions such as *Cycas* and *Dioon* (Donaldson and Bösenberg 1995; Staude 2008; Donaldson 2015). However, the unique genetic diversity of the various wild *Z. lepida* populations is still under threat from the large-scale removal of cycads from the wild and some wild populations of *Z. lepida* have been reported to have disappeared (Staude and Sihvonen 2014).

Zerenopsis lepida and other Diptychini have several characteristics that set them apart from other Geometridae i.e., gregarious larvae, aposematic colouration, diurnal adults, and lek mating behaviour whereby males choose specific lek sites from where they attract females (Staude et al. 2011; Bayliss et al. 2009). Early-instar larvae of *Z. lepida* always feed on new cycad leaves that are still soft while larger instars (≥third instar) can switch to a variety of secondary angiosperm host plants growing in the vicinity of their primary cycad host (Donaldson and Bösenberg 1995). *Carissa bispinosa*, *C. macrocarpa*, *Diospyros* *lycioides*, *D. whyteana, Apodytes dimidiata, Maesa alnifolia* and *M. lanceolata* have been recorded as secondary host plants for *Z. lepida* (Donaldson and Bösenberg 1995; Staude and Sihvonen 2014). Cycad-host preference amongst ovipositing females and early-instar larvae may primarily be influenced by the presence of methylazoxymethanol (MAM) glycosides in cycad tissue and secondary host plants are likely included in the larval diet when new and soft cycad leaves are depleted (Donaldson and Bösenberg 1995).

This study reports for the first-time evidence of *Z. lepida* feeding on a wild population of *Encephalartos eugene-maraisii*, an endangered endemic plant in the Waterberg Mountain range of South Africa (IUCN Red List Criteria A2ad+4ad; B1ab(v)) with between 400 and 620 plants remaining in the wild (Bösenberg 2022). *Encephalartos eugene-maraisii* is a large cycad with aerial stems (up to 4 m long) that often becomes procumbent with age. Individual plants are often multi-stemmed through the production of basal suckers (Fig. 1). Vegetative production of suckers and the longevity of stems imply that individual plants can persist over long periods. Like all cycads, *E. eugene-maraisii* is dioecious although cones are produced infrequently. Leaves are produced from the stem apex with new leaf flushes first appearing in October and peaking in January-February. Leaves are initially soft and harden with maturity and can remain alive for many years. *Encephalartos eugene-maraisii* has a very slow growth rate, producing only one set of new leaves annually with intervals between leaf flushes spanning several years. No seedlings have been recorded in recent history. Seeds produced naturally do not germinate, whereas cones pollinated by hand produce viable seeds, indicating that pollinators may be absent (Bezuidenhout 2019). The slow-growing nature of *E. eugene-maraisii* and its lack of natural recruitment has made it especially sensitive to poaching.

Zerenopsis lepida is abundant and widespread in habitats that are generally frost-free and moist in the eastern parts of southern Africa while previous reports indicate that it is absent

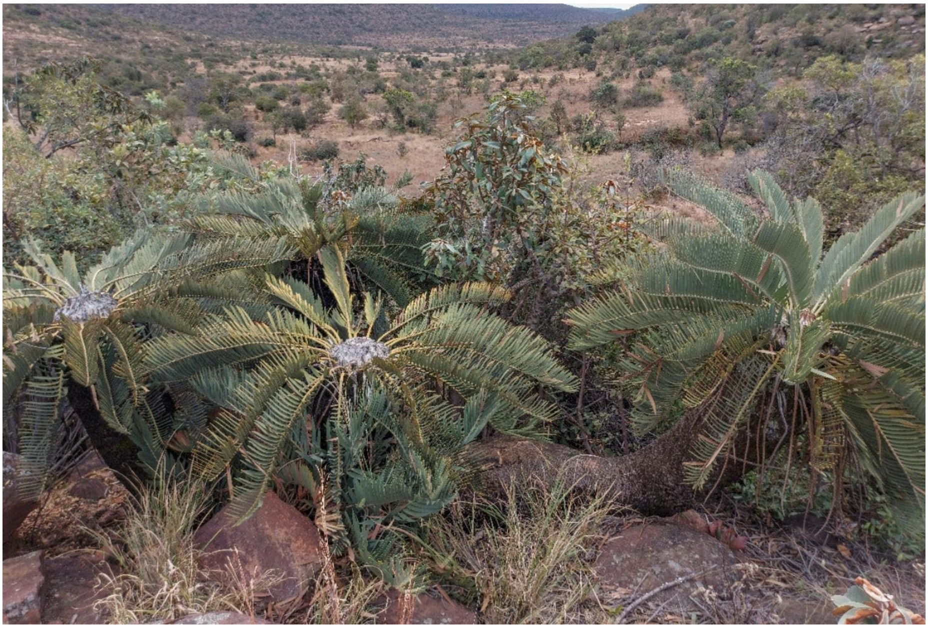

Fig. 1 Characteristic structure of an *Encephalartos eugene-maraisii* plant (P.D. Janse van Rensburg)

from drier and colder habitats above 1500 m a.s.l., including the Waterberg (Staude and Sihvonen 2014). The increasing availability of cultivated cycads (native and non-native) as host plants in transformed habitats may have potentially facilitated the expansion of its range (Donaldson and Bösenberg 1995; Staude and Sihvonen 2014). Host range expansion by *Z. lepida* is potentially problematic because herbivory is often severe. In the absence of secondary host plants, *Z. lepida* larvae complete their entire life cycle on cycads, often causing extensive damage. However, even though high levels of damage from *Z. lepida* have been observed on cultivated cycads, no published references addressed *Z. lepida* damage under field conditions. In this paper, we present quantitative estimates of *Z. lepida* damage on *E. eugene-maraisii* under field conditions. Field observations indicate that not all individuals in the population are equally damaged. We aim to investigate whether differences in herbivory are either random, dependent on plant features or related to environmental variables.

Materials and methods

Study sites

Encephalartos eugene-maraisii is distributed in isolated sub-populations in the Waterberg Mountain range in the Limpopo province, South Africa (Bezuidenhout et al. 2020). Two main conservation areas (Marakele National Park and Entabeni Safari Conservancy) remain in the wild situated at either extreme of the species' geographical range. Detailed locality information is not revealed to protect sensitive information on the distribution of remaining *E. eugene-maraisii* plants. Both areas form part of the Waterberg Biosphere Reserve.

Entabeni contains the weight of *E. eugene-maraisii* distribution as a species and is situated at the north-eastern extreme of its species distribution. Most of the remaining plants occur at high altitudes (1500–1750 m a.s.l.) where it grows on rocky outcrops in mainly the Waterberg-Magaliesberg Summit Sourveld (Gm 29) (Mucina et al. 2006). The vegetation is characterised by patches of open woodland of *Protea caffra* and open shrubland of *Englerophytum magalismontanum* and *Ancylobotrys capensis* (Steyn and Bezuidenhout 2020). The plants are growing in three main groups on individual rocky outcrops that are separated by large flood plains and were treated as three sub-populations (A1-A3).

Marakele is situated at the southwestern extreme of the distribution of *E. eugene-maraisii* in formally protected areas managed by SANParks. The site consists of small isolated sub-populations separated by mountains with rugged topography and steep cliffs in some areas. In a phytosociological classification, mapping and description of the Waterberg Mountain vegetation 12 plant communities were identified. One of these plant communities is the *Indigofera mollicoma-Encephalartos eugene-maraisii* shrubland, which is characterised by the presence of *E. eugene-maraisii* along with the trees *Heteropyxis natalensis* and *Protea caffra* and the grasses *Themeda triandra*, *Loudetia simplex* and *Trachypogon spicatus* (Van Staden et al. 2021).

The climate of both sites is warm in summer and cold and prone to frost in winter. Winter temperatures (coldest months June–July) are as low as −3.7 °C, whilst the summer temperatures (warmest months December to February) can reach a maximum temperature of 40.0 °C recorded at the Marakele weather stations. The rainfall, which is mainly during the spring and summer (September–March) seasons, can range from as high as 1196.9 to lower than 400 mm per year. Mean frost days per annum is 16 days with the earliest occurrence on 14 May and the latest occurrence on 23 July (Van Staden et al. 2021). Historically, fire has been a regular occurrence in the study areas due to a very high frequency of lightning strikes in this mountainous region. Fire likely plays an important role in the patterns and processes of cycad habitats, for example, stimulating the production of new leaves and cones (Cousins and Witkowski 2017) and fire scars were visible on cycads in both populations.

Herbivory scoring

Due to the low number of plants remaining in Marakele and difficulty in reaching plants, damage surveys were confined to Entabeni. Marakele was, however, visited and although damaged leaves were observed no new leaves and no eggs or larvae could be located to confirm the presence of *Z. lepida*. A total of 227 plants were selected at Entabeni after which the geographic position of each plant was recorded and a photo was taken for future identification of each of these plants. Herbivory scoring was conducted over two consecutive seasons (2020–2021 and 2021–2022). The scoring was done between March and April since, during this period, most of the new leaves were fully expanded and the majority of larvae have moved off of plants. Scoring of damage severity could be done up to 3 months after herbivory and was postponed this long to avoid underestimating damage on new leaves because feeding lesions may expand during leaf expansion.

Damage scoring during the 2020–2021 season was done for new and old leaves while only new leaves were assessed during the 2021–2022 season. New leaves refer to the leaves produced in the current year and are distinctively coloured for several months. Old leaves were those that developed during previous years. The feeding damage symptoms of *Z. lepida* herbivory are easy to observe and remain visible for several years, due to the toughness of leaves.

The level of herbivory was expressed as incidence (presence or absence of herbivory) and level of damage (percentage of leaf area removed). To determine the level of damage the percentage of leaf area removed for each leaf was estimated visually using different damage classes: 0%, 1–25%, 26–50%, 51–75%, and > 75%. These broad herbivory bins are effective for higher levels of herbivory and although potentially unsuited for estimating low levels of herbivory, we rarely observed leaves with low levels of herbivory (1–5%) due to the close association of *Z. lepida* with the new and expanding leaves of *E. eugene-maraisii* and the gregarious feeding behaviour of larvae. Visual damage estimation has been used in similar herbivory studies and was shown to be reliable (Johnson et al. 2016; Barrio et al. 2017; Brezzi et al. 2017; Castagneyrol et al. 2018). To avoid bias a single person performed all the herbivory assessments. We calculated the percentage of leaf area consumed by larvae by multiplying the number of leaves from each damage class with the midpoint of each damage class category e.g., 13% for the 1–25% class (Kozlov et al. 2015). The values of all classes were then summed and divided by the total number of leaves per stem.

Apparency of plants

We also tested whether the apparency of plants affected the level of herbivory. The apparency of plants is affected by several factors including the plant size, host-plant density and density of surrounding vegetation. Five plant size parameters were taken into account: (1)

the total number of leaves per plant, (2) the number of stems/suckers per plant, (3) basal circumference of the largest stem, (4), the length of the longest stem (distance between stem base and the base of the most distal petiole) and since stems were often procumbent the (5) vertical plant height (distance between the ground and the tip of the most distal leaflet of the tallest stem) were also measured. Based on heat maps of existing plants, we rated dense and sparse host-plant density areas, and areas in between as intermediate. The density of surrounding vegetation was scored as dense (host plant was completely covered with foliage from other plants), moderate (intermediate covering), and sparse (host was not covered by any other vegetation).

Statistical analysis

Data were analyzed using non-parametric methods since the assumption of normality was not met for either data set. The level of damage was compared between plant sex, altitude, aspect, host-plant density and density of surrounding vegetation and tested for significant differences with the Kruskal-Wallis test. We analysed correlations between the incidence of herbivory and the level of damage with the different plant size parameters, using Spearman rank correlation analysis. Finally, we used Chi-square (χ^2) analysis to compare the frequency of new leaf flushes and the incidence of herbivory on new leaf flushes between subpopulations (site A1-A3). All analyses were done using SPSS version 27 (IBM Corp 2020).

Results

Descriptive data

A total of 227 plants were counted comprising 692 stems. No seedlings could be found. The total number of stems/suckers on an individual plant ranged from one to eight (3.01 ± 1.57 [mean ± SD]) and only 18.94% of the plants were single-stemmed. Stem length averaged 63.37 ± 75.13 cm and the longest stem recorded was 370 cm. By searching plants for the presence of live cones and old decaying cone material, the sex of 86 plants (37.89%) could be identified. Of these, 49 (56.98%) were male and 37 (43.02%) were female. The smallest stem with evidence of a previous coning event was 40 cm in height. This was taken as the minimum size of mature stems. In the 2020–2021 season, 335 stems flushed new leaves and 522 stems had old leaves while only 179 flushed leaves in the 2021–2022 season and 573 stems had old leaves (Table 1). Most new leaf flushes occurred in the summer with the first flushes appearing in October and the last flushes in March.

Field observations

Field observations were made of all development stages of *Z. lepida*. No adults could be observed during the winter months probably due to very low temperatures and frost that occur during some nights. Egg clusters were first observed in October when new leaf flushes first appear. Eggs were laid in clusters containing from 17 to 231 eggs (118.02±43.85) (Table 2). The majority (76.92%) of egg clusters were observed early in the season from

Table 1 Numbers of new and old leaves on stems of *Encephalartos eugene-maraisii* counted during 2020–2021 and 2021–2022

Leaf numbers	2020–2021			2021–2022		
	New	Old	Total	New	Old	Total
No. of stems with leaves	335 (48.41%)	522 (75.43%)	665 (96.10%)	179 (25.87%)	573 (82.80%)	653 (94.36%)
Total no. of leaves counted	4854	7772	12,626	2084	9150	11,234
Mean no. of leaves per stem (±SD)	14.49±9.63	14.89±12.71	18.46±15.12	11.64±8.79	15.75±11.18	16.18±12.75
Mean no. of leaves per mature stem (±SD)	21.80±5.58	22.83±12.51	29.88±12.51	21±4.91	23.27±8.52	25.76±9.80
Maximum no. of leaves per stem	40	62	74	35	52	58

Table 2 Number of eggs and egg clusters counted on *Encephalartos eugene-maraisii* plants during the 2021–2022 season

Date/location of egg clusters	Number of egg clusters	Mean number of eggs per cluster (±SD)	Total number of eggs
October-December	40 (76.92%)	119.93±48.57	4917
January-March	12 (23.08%)	110.91±17.35	1220
New leaves	34 (65.38%)	123.32±42.52	4193
Old leaves	18 (34.62%)	108.00±45.79	1944
Upper surface	10 (19.23%)	123.50±55.75	1235
Lower surface	42 (80.77%)	116.00±41.24	4902
Total	52	118.02±43.85	6137

October to December. The majority of egg clusters (80.77%) were located on the underside of leaves and several clusters were often found on a single leaf flush. The majority (65.38%) of egg clusters were recorded from new leaves (Table 2). Although egg clusters were often found on old and even dead leaves, they were always laid on stems with robust developed cataphylls that were about to either push leaves or cones (data not shown). Subsequent visits to these plants revealed no new damage to old leaves.

Four cases of early-instar larvae attacking the crown of the plant were observed. After a seven-day period, no larvae could be observed on any of these plants. Subsequent visits to these plants revealed the plants were still capable of flushing new leaves in the same season despite damage to the crown although leaf flushing was delayed. Four cases were recorded where egg clusters were laid on plants in the process of pushing male cones. Only a single female plant produced cones during the study period and no eggs or larvae could be located on this plant. Early instar larvae were attacking emerging male cones by scraping the surface of sporophylls but damage to the cones was only superficial. Larvae could also not be located on these plants after a seven-day period.

Early instar larvae fed gregariously on new leaves by scraping the surface beneath leaflets producing irregular patches (Fig. 2). The larvae spin a fine mat of silk on which they rest on the underside of leaves. Late instar larvae feed on the edge of leaflets often causing severe damage. As larvae became larger the number of larvae per group decreased. When larvae hatched on very young leaves (leaflets still folded tightly against each other) damage tended to be severe and the entire leaf flush was destroyed. Once the leaves have extended beyond ~50 cm in length and started to harden, larvae started dispersing either to neighbouring plants with new emerging leaves or secondary host plants. Of the known secondary host plants, *Apodytes dimidiata* and *Diospyros lycioides* were found in the study area. *Apodytes dimidiata* is abundant in the study area and *Z. lepida* larvae (≥ fourth instar) were often observed feeding on *A. dimidiata*. We observed no larvae feeding on *D. lycioides*. No other host plant species were recorded.

Levels of herbivory

The severity of damage to old leaves measured in the 2020–2021 season was lower than on new leaves for that season (Table 3). Estimates of old leaves likely under-estimated some aspects of damage because we recorded several instances where new leaf flushes were extensively damaged or destroyed that would otherwise not be visible on old leaf flushes. Incidence of herbivory on new leaf flushes ranged from 40.60% on new leaves from 2020 to 2021 to 40.78% on new leaves from 2021 to 2022 (Table 3). While the leaves on some

Fig. 2 Different development stages of *Zerenopsis lepida* observed in the Waterberg: **a** adult; **b** egg cluster; **c** newly hatched larvae; **d** mature larvae on cycad host; **e** mature larvae on secondary host plant (P.D. Janse van Rensburg)

cycad stems were heavily attacked, neighbouring stems or plants with newly flushed leaves often remained free of damage. The leaves from the majority of the stems did not have heavily damaged leaves (>25% leaf area removed). The mean amount of photosynthetic tissue removed ranged from 9.01% on new leaves from the 2020–2021 season to 10.61% on new leaves from the 2021–2022 season (Table 3).

There was no significant association between the level of damage and aspect, altitude and the sex of plants (data not shown). A Kruskal-Wallis test did, however, show significant differences in the level damage between different densities of surrounding vegetation for new leaves 2020–2021 (H = 7.132, P = 0.028), new leaves 2021–2022 (H = 11.529, P = 0.003), total leaves 2020–2021 (H = 9.059, P = 0.011) but not for old leaves 2020–2021 (H = 4.415,

Table 3 Incidence of herbivory and estimated percentage leaf area of *Encephalartos eugene-maraisii* consumed by *Zerenopsis lepida*

Level of herbivory	2020–2021			2021–2022
	New	Old	Total	New
No. of stems with signs of leaf herbivory (Incidence)	136 (40.60%)	200 (38.31%)	301 (44.01%)	73 (40.78%)
No. of stems heavily damaged (>25% leaf area removed)	38 (11.34%)	43 (8.24%)	63 (9.21%)	37 (20.67%)
No. of leaves with signs of herbivory	900 (18.54%)	1375 (17.67%)	2275 (18.01%)	543 (26.05%)
No. of heavily damaged leaves (>25%)	528 (10.87%)	765 (9.84%)	1293 (10.24%)	414 (19.86%)
Mean % leaf area lost per stem (stems with signs of leaf herbivory) (±SD)	22.27 ± 24.17	17.62 ± 18.75	16.81 ± 17.81	26.02 ± 15.91
Mean % leaf area lost per stem (all stems) (±SD)	9.01 ± 18.85	6.81 ± 14.46	7.62 ± 14.62	10.61 ± 16.34

P = 110). Pairwise comparisons indicated significantly less leaf area consumed on cycads that are surrounded by dense vegetation compared to sparse vegetation (Table 4). There were also significant differences in the level damage between different host-plant densities for old leaves 2020–2021 (H = 13.694, P = 0.001), new leaves 2020–2021 (H = 11.278, P = 0.004), total leaves 2020–2021 (H = 10.029, P = 0.007) but not for new leaves 2021–2022 (H = 1.008, P = 0.604). Pairwise comparisons indicated that significantly less herbivory occurred in low host-density areas compared to high host-density areas (Table 4).

Significant correlations were found between the incidence of *Z. lepida* herbivory and the level of damage with the number of leaves, the number of stems, stem circumference and stem height (Table 5). The strongest correlations were recorded between the number of leaves and the incidence of herbivory for new leaves 2020–2021 (r = 0.476, P = < 0.001) and total leaves 2020–2021 (r = 0.504, P = < 0.001) and between the number of stems with the incidence of herbivory for new leaves 2020–2021 (r = 0.451, P = < 0.001) and total leaves 2020–2021 (r = 0.452, P = < 0.001). The vertical plant height only had significant correlations with both the levels of herbivory (r = 0.255, P = 0.019) and incidence of herbivory (r = 0.216, P = 0.049) with new leaves from the 2021–2022 season.

The frequency of new leaf flushes varied dramatically between sub-populations during both the 2020–2021 (χ^2=77.092, df=2, P < 0.001) and 2021–2022 (χ^2=54.578, df=2, P < 0.001) seasons (Table 6). The proportion of stems with new leaf flushes that were damaged by *Z. lepida* decreased with an increasing number of stems flushing new leaves in 2020–2021 (χ^2=16.431, df=2, P < 0.001) but not significantly in 2021–2022 (χ^2=4.117, df=2, P = 0.128). (Table 6).

Discussion

Field observations

The biology of *Z. lepida* has been studied and described in previous studies (Donaldson and Bösenberg 1995; Staude and Sihvonen 2014). In this study, we report on a few idiosyncrasies regarding the behaviour of *Z. lepida* in the Waterberg region.

Zerenopsis lepida is widespread and has colonized a broad range of cycads from different clades and lineages in East and Southern Africa. This is the first record of *Z. lepida* in the Waterberg region of the Limpopo Province, which supports earlier indications that its host

Table 4 Pairwise comparisons of the effect of host-plant density and the density of surrounding vegetation on the percentage leaf area of *Encephalartos eugene-maraisii* consumed by *Zerenopsis lepida*

Density		Sparse	Intermediate	Dense	P
Host-plant density	Old 2020–2021	2.50 ± 6.15^{a}	7.29 ± 15.64^{b}	7.91 ± 14.93^{b}	0.001*
	New 2020–2021	5.18 ± 13.27^{a}	8.40 ± 16.54^{ab}	12.39 ± 24.47^{b}	0.004*
	Total 2020–2021	4.09 ± 6.45^{a}	6.84 ± 9.13^{ab}	9.72 ± 13.13^{b}	0.007*
	New 2021–2022	9.64 ± 14.65	11.98 ± 17.62	9.77 ± 15.83	0.604
Density of surrounding vegetation	Old 2020–2021	7.56 ± 11.71	7.77 ± 12.55	6.34 ± 16.38	0.110
	New 2020–2021	10.84 ± 18.98^{a}	6.90 ± 10.89^{ab}	0.59 ± 1.68^{b}	0.028*
	Total 2020–2021	8.29 ± 10.49^{a}	6.80 ± 7.08^{a}	5.84 ± 16.51^{b}	0.011*
	New 2021–2022	13.99 ± 15.21^{a}	5.69 ± 10.60^{b}	3.10 ± 8.59^{b}	0.003*

Means with different superscript symbols indicate significant differences

Table 5 Effect of plant apparency on the incidence and the level of damage from *Zerenopsis lepida* herbivory

Plant size		Incidence				Level of damage			
		2020–2021			2021–2022	2020–2021			2021–2022
		New	Old	Total	New	New	Old	Total	New
Number of leaves	r	0.476	0.334	0.504	0.319	0.389	0.215	0.320	0.295
	P	<0.001*	<0.001*	<0.001*	0.003*	<0.001*	0.002*	<0.001*	0.007*
Number of stems	r	0.451	0.229	0.452	0.201	0.379	0.203	0.339	0.170
	P	<0.001*	0.001*	<0.001*	0.067	<0.001*	0.004*	<0.001*	0.123
Stem circumference	r	0.207	0.239	0.231	0.292	0.163	0.176	0.184	0.303
	P	0.020*	0.001*	<0.001*	0.010*	0.068	0.014*	0.007*	0.007*
Stem height	r	0.194	0.196	0.257	0.302	0.098	0.090	0.132	0.315
	P	0.025*	0.005*	<0.001*	0.005*	0.261	0.203	0.047*	0.004*
Vertical plant height	r	0.105	0.116	0.126	0.216	0.064	0.087	0.075	0.255
	P	0.234	0.100	0.057	0.049*	0.467	0.216	0.259	0.019*

Asterisks represent significant relationships

Table 6 Percentage of *Encephalartos eugene-maraisii* stems with new leaf flushes and percentage of new leaf flushes with signs of *Zerenopsis lepida* herbivory

Leaf and herbivory phenology		2020–2021			2021–2022		
		Site A1	Site A2	Site A3	Site A1	Site A2	Site A3
Stems with new leaf flushes	Count	68	108	159	100	49	30
	%	28.9	48.9	69.7	42	21.90	12.90
New leaf flushes with signs of herbivory	Count	41	43	49	42	15	17
	%	60.3	39.8	30.8	42	30.6	53.3
Leaf area removed	%	15.86	5.92	4.92	14.10	7.82	15.78

and distribution range is expanding (Donaldson and Bösenberg 1995; Staude and Sihvonen 2014). This species probably arrived in the study area only during the last few years since it seems unlikely that its presence went unnoticed during previous surveys of *E. eugene-maraisii* in this region. Its ability to colonize new regions is probably aided by the increased use of its cycad hosts as ornamental plants in home gardens and the movement of plants between regions.

Wu et al. (2010) discussed a similar case of breakaway populations of *Chilades pandava* on cycads in Taiwan that were caused by range expansions of native populations of *C. pandava.* Host range expansion of native herbivores may have certain consequences for both the host and herbivore populations. Breakaway populations colonize host population previously unaffected by *Z. lepida*, including cultivated cycads in cities and will likely exhibit lower genetic diversity than wild populations (Olivier 2006; Wu et al. 2010). However, these breakaway population may also function as a bridge between natural populations of *Z. lepida* that have historically been isolated and have unique genetic features. This might increase interpopulation movement threatening the unique isolated wild populations of *Z. lepida* with genetic contamination (Olivier 2006; Staude and Sihvonen 2014). In addition to the threat of host range and distribution expansion, introduced cycad hosts in home gardens may increase the biomass of *Z. lepida* increasing its herbivory pressure on wild cycad populations.

Records generally indicate that *Z. lepida* is active throughout the year with two peak flight periods in mid-summer and autumn (Staude and Sihvonen 2014). However, the cold and frost-prone habitat of *E. eugene-maraisii* and the unavailability of food in the winter months means the area is likely unsuitable for *Z. lepida* to be active year-round and both immature and mature stages are only present during the spring and summer months of South Africa. Moths first appeared in the spring between October and November. The majority of egg clusters were found early in the season between October and December and new egg clusters were rarely observed later than January-February.

Donaldson and Bösenberg (1995) indicated that they could not locate any egg clusters on the leaves of *Encephalartos altensteinii* once its leaves had extended beyond 30 cm. However, we frequently recorded egg clusters on old leaves during the early months of summer. During this period new leaf flushes are still scarce and the ovipositing females likely have no choice in laying eggs on old leaves. Similarly, *Eumaeus atala* (Lepidoptera: Lycaenidae) were also observed laying eggs on older leaves and even stems and cones of *Zamia integrifolia* when the availability of new leaf flushes was low (Koi and Daniels 2015).

Although eggs were laid on old leaves, the stems of plants that they oviposited on had robust cataphyll complexes that were in the early stages of either flushing leaves or pushing cones. However, at such an early stage of an organ flush, the moths did not seem to be able to distinguish between a leaf flush or cone flush as several egg clusters were deposited on plants that were in the process of pushing cones. While several egg clusters were often recorded on single stems that were in the process of a new organ flush, no clusters were observed on adjacent stems or neighbouring plants that were not about to undergo an organ flush.

Various Lepidoptera species select young leaves for oviposition. Young leaves are thought to be selected because they provide a better food source for young larvae and they also lack mechanical protection. Cycads produce rich amounts of unique secondary compounds that may impact herbivore populations (Snyder and Marler 2011; Whitaker et al. 2022). It’s been suggested that the host preference of *Z. lepida* and other cycadivorous Lepidoptera may be based on a factor ubiquitous in most cycad lineages such as MAM glycosides (Donaldson and Bösenberg 1995; Koi 2017). Before a new leaf or cone is flushed, the cataphyll complex becomes mitotic and robust, mobilizing MAM-glycosides which may inadvertently function as a chemical cue to stimulate *Z. lepida* oviposition and feeding behaviour. The MAM glycoside concentrations tend to decrease as leaves expand and become older (Prado et al. 2014).

Levels of herbivory

The proportion of leaf area removed by larval feeding is a good measure of the loss of photosynthetic tissues. Herbivory by *Z. lepida* is easy to identify by searching leaves for typical feeding damage which persists for several years. Over two years we reported that a mean leaf area ranging from 9.01 to 10.61% was eaten away which seems fairly high compared to losses of foliage due to background herbivory that is generally reported in terrestrial ecosystems (Turcotte et al. 2014; Kozlov et al. 2015; Meyer et al. 2017; Zang et al. 2017; Gálman et al. 2018). Although very few studies have investigated the level of herbivory by folivorous cycad consumers, it falls into the same range as estimates in other reported cases for cycads (Clark and Clark 1991; Beltrán-Valdez and Torres-Hernández 1995; Prado et al.

2014; Santos Murgas and Abrego 2016). However, even minor, but chronic herbivory may have significant effects on the growth and reproduction of plants (Baron and Bros 2005; Kettenring et al. 2009; Zvereva et al. 2012; Myers and Sarfraz 2017). This may have far-reaching effects on rare plant populations such as the already endangered *E. eugene-maraisii.*

Cycads are slow-growing with long-lived leaves and make large investments in defence. Plant species with slow growth rates and low turnover rates of tissue in resource limited environments are expected to make large investments in anti-herbivore defences because the potential impact of herbivory could be very high (Endara and Coley 2011). Species such as this generally exhibit lower levels of herbivory (Cebrián and Duarte 1994; Fine et al. 2006). It is expected that fast-growing plant species are capable of tolerating higher levels of herbivory because they can more quickly replace lost leaf area while the health costs of a given level of herbivory will be higher for slow-growing species (Massad 2013; Gianoli and Salgado-Luarte 2017). Due to the slow turnover of plant tissue, slow-growing species might also take longer to recover should herbivory occur. Furthermore, even minor herbivory may reduce leaf life span (Zvereva and Kozlov 2014). Levels of damage may have been underestimated because measures of leaf area loss do not record leaves that were prematurely senesced due to herbivory.

Damage may further be underestimated if damage to the crown and potential damage to cones is considered. Flushing new leaves expose the vulnerable growth point for potential stem damage and death (Clark and Clark 1991). We reported several instances of larvae attacking the crowns of plants in the early stages of leaf flushes. These plants were monitored and although damage to the crown delayed the upcoming leaf flushes, plants were still able to flush new leaves. *Zerenopsis lepida* has previously been reported to opportunistically feed on female cones and burrow in the crown of *Encephalartos* species although damage is generally low (Donaldson and Bösenberg 1995; Zunckel 1995; Staude and Sihvonen 2014). Only one female plant coned during the study period but no *Z. lepida* larvae were present. However, we observed early instar *Z. lepida* larvae attacking emerging male cones, but that damage was only superficial.

Since cycads do not flush leaves continuously, plants are not able to regenerate damaged leaves until the following flush. During the 2020–2021 season, new leaf flushes from 38 stems were heavily damaged (>25% leaf area removed). Six of those re-flushed leaves during the 2021–2022 season. For most, however, it may take several years to recover and flush new leaves depending on the severity of the damage. Repeated severe damage will likely reduce growth and plant health. This may also delay coning and reduce the reproductive output of *E. eugene-maraisii.* Due to the absence of pollinators, there is no natural recruitment. Recovery programs are in place (Bezuidenhout 2019) but are dependent on wild plants coning in order to perform hand pollination. Cone production is already a rare event which may be exacerbated by chronic herbivory. However, the chances of experiencing severe herbivory in successive leaf flushes also appear to be low, e.g., of the 43 stems that had heavily damaged old leaves in 2020–2021, 27 flushed new leaves during the 2020–2021 and 2021–2022 seasons and only four of those were heavily damaged.

Effect of plant apparency

The level of damage was significantly higher on more apparent plants. An individual plant may be more apparent if it is taller, multi-stemmed, occurs in high host-density areas and surrounded by sparse vegetation. This supports earlier observations that female *Z. lepida* moths avoid ovipositing on cycads under a closed canopy (Staude and Sihvonen 2014). This is also similar to findings by Bayliss et al. (2009) who found that the incidence of larval damage of *Callioratis grandis* (Lepidoptera: Geometridae) was associated with more mature individuals of its cycad host *Encephalartos gratus*.

While large plants may have certain advantages such as high competitiveness or increased fecundity, they may also have higher costs such as increased apparency to herbivores (Blanckenhorn 2000). Larger plants may be easier for herbivores to locate and colonize and larger plants may also be more attractive since they provide greater amounts of resources (Schlinkert et al. 2015). Therefore, a trade-off might exist between the benefits of plant size and the cost of herbivory. The effects of herbivory are likely counterbalanced by increased abilities for tolerance of herbivory damage due to their larger size and capacity for storage of energy (Boege and Marquis 2005) and higher ability to compete with surrounding vegetation for resources (Schlinkert et al. 2015).

Potential regulatory role of synchronized leaf flushes

In addition to the production of secondary metabolites, cycad leaves are also mechanically protected through leaf toughness and spines (Prado et al. 2014, 2016). Cycad leaves are initially soft and harden with maturity. Young leaves are vulnerable to herbivore attack during leaf expansion when the majority of cycad folivorous arthropods consume new leaves (Staude and Sihvonen 2014; Prado et al. 2014; Koi and Daniels 2015). Two phenological defence mechanisms are suggested to reduce damage to expanding leaves i.e., the production of leaves when herbivore abundance is low and satiating herbivores with a superabundance of edible tissue by synchronising leaf flushes (Clark and Clark 1991; Gong and Zhang 2014; Lamarre et al. 2014).

Our results support the idea that synchronised leaf flushes may satiate herbivore populations. The results were especially visible for site A3 during 2020–2021 when the majority of stems flushed new leaves and correspondingly the proportion of plants with damage and the severity of damage was the lowest in site A3. During mass leaf flushing events the sheer number of new leaves would satiate the *Z. lepida* population meaning the majority of plants escape damage. Unsynchronised leaf flushes would lead to a yearly steady supply of new leaves which should lead to a consistently high population of *Z. lepida*. Synchronised leaf flushes at long unpredictable intervals would ensure difficulty for adult moths to locate suitable sites for larval development (Clark and Clark 1991).

A possible explanation for the higher synchrony at site A3 is a severe hail storm that occurred at the start of the 2020–2021 season that severely damaged and defoliated several plants. Environmental disturbances (e.g., fire and strong winds) causing leaf damage and defoliation have been reported to result in a synchronised leaf flush afterwards (Hirsh and Marler 2002). The habitat of *E. eugene-maraisii* is fire-prone due to frequent lightning strikes, and fire likely plays an important role in the ecological processes in its habitat. Fire scars occur on the majority of plants in both populations and the plants are seemingly well adapted to fire. However, the majority of the Entabeni sites haven't burned in recent years. Fire could potentially synchronise leaf flushes (Swart et al. 2019). Manipulating the timing of fire may also be used to ensure the majority of new leaf flushes emerge outside peak herbivore densities (Zunckel 1995). We observed the majority of *Z. lepida* egg clusters between October and December. Stems that flush leaves later in the season may avoid severe damage.

Conclusion

The colonization by *Z. lepida* of this region where one of the last populations of *E. eugene-maraisii* occur adds to the threat against this species. The levels of damage recorded in this study are fairly high compared to what is often reported for terrestrial plants, and may be especially important in the context of *E. eugene-maraisii.* Slow growing species such as *E. eugene-maraisii* that make large investments in defence and have low turnover rates of tissue will take long to recover should herbivory occur. Damage to the crowns of plants delays upcoming leaf flushes, and plants are not able to regenerate damaged leaves until the following flush. Since some plants may take several years to recover and flush new leaves, repeated severe damage will likely reduce growth and plant health which may delay coning and reduce the reproductive output of *E. eugene-maraisii.* This study emphasises that young leaves are an ephemeral resource and that aspects of leaf phenology may serve as a defence mechanism. Plants may escape high levels of damage by producing leaves in synchrony to satiate herbivores or leaves may be produced during periods when herbivore abundances are low. Lastly, we recorded that *Z. lepida* feeding damage increases with plant size and canopy openness, as the apparency of large and open plants is likely more attractive to *Z. lepida* and larger plants likely provide greater amounts of resources.

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Data Availability The datasets generated during the study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and research permits Ethics approval (no: NWU-01552-20-A9) for this study was granted by the North-West University, Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC). A permit (no: ZA/LP/111179) to do scientific research on plants in the Limpopo Province of South Africa was granted by the Department of Economic Development, Environment and Tourism (LEDET).

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