The ‘Herbivory Uncertainty Principle’: application in a cerrado site

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Abstract

Researchers may alter the ecology of their studied organisms, even carrying out apparently beneficial activities, as in herbivory studies, when they may alter herbivory damage. We tested whether visit frequency altered herbivory damage, as predicted by the ‘Herbivory Uncertainty Principle’. In a cerrado site, we established 80 quadrats, in which we sampled all woody individuals. We used four visit frequencies (high, medium, low, and control), quantifying, at the end of three months, herbivory damage for each species in each treatment. We did not corroborate the ‘Herbivory Uncertainty Principle’, since visiting frequency did not alter herbivory damage, at least when the whole plant community was taken into account. However, when we analysed each species separately, four out of 11 species presented significant differences in herbivory damage, suggesting that the researcher is not independent of its measurements. The principle could be tested in other ecological studies in which it may occur, such as those on animal behaviour, human ecology, population dynamics, and conservation.

Keywords: cerrado, herbivory, savanna, Uncertainty Principle.

O ‘Princípio da Incerteza da Herbivoria’: aplicação em uma área de cerrado

Resumo

Pesquisadores podem alterar a ecologia de seus organismos estudados, mesmo conduzindo atividades que são aparentemente benéficas, como em estudos sobre herbivoria, quando podem alterar a quantidade de dano foliar nas plantas. Testamos se a visita do pesquisador alterou o dano por herbivoria, como prevê o Princípio da Incerteza da Herbivoria. Em um fragmento de cerrado no Estado de São Paulo, estabelecemos 80 parcelas em que amostramos os indivíduos arbustivos ou arbóreos. Usamos quatro frequências de visitação (alta, média, baixa e controle), quantificando, ao fim de três meses, o dano por herbivoria por espécie em cada tratamento. Não corroboramos o Princípio da Incerteza da Herbivoria, uma vez que a frequência de visitação não alterou o dano por herbivoria, ao menos quando analisamos todas as espécies simultaneamente. Porém, quando analisamos as espécies separadamente, quatro das onze espécies amostradas apresentaram diferenças significativas, sugerindo que o pesquisador não é independente de suas medições. Esse princípio pode ser testado em outros estudos ecológicos em que possa ocorrer, como nos de comportamento animal, etnoecologia, dinâmica de populações e conservação.

Palavras-chave: cerrado, herbivoria, Princípio da Incerteza, savana.

1. Introduction

In 1927, the German physicist Werner Heisenberg introduced the “Uncertainty Principle”, according to which the act of measuring the physical properties of a particle alters its behaviour; that is, locating it in a small region of space makes the momentum of the particle uncertain, whereas measuring precisely its momentum makes the position uncertain (Heisenberg, 1927). If this in fact occurs, then the way that a physicist understands a problem and how conclusions are drawn from his or her study are affected (Heisenberg, 1927). Recently, this principle was extended to ecology, namely to the interactions between plants and herbivores (Cahill et al., 2001). In this sense, visiting a plant to measure herbivory may alter herbivory rates, biasing results (Cahill et al., 2001). If so, the implications could be similar to those of physicists studying particles: the act of measuring herbivory in target plants could change the herbivory rate, that is, the researcher would not be independent of the outcomes of the study (Cahill et al., 2001). Cahill et al. (2001) called this phenomenon the “Herbivory Uncertainty Principle” (HUP), to which little attention is paid in plant science (Boeck et al., 2008).

If repeated plant measurements, either destructive or non-destructive, affect plant functioning, implications could be far-reaching (Boeck et al., 2008). Since herbivory can alter plant growth (Louda, 1984), population demography (Louda and Potvin, 1995), and community dynamics (Fritz and Simms, 1992), researchers could change the behaviour and ecology of their studied organisms, even if carrying out apparently beneficial activities, such as non-destructive measurements and observations (Schnitzer et al., 2002). If there is indeed a HUP, and since a small change in the herbivory rate can reverse competitive order among species, the simple act of visiting and measuring plants could ultimately change community composition (Schnitzer et al., 2002). So, our aim was to test, in a cerrado site, the HUP, comparing plots with four different visitation intensities. We tried to answer whether the increase in visitation alters the amount of leaf damage by herbivores.

2. Material and Methods

The study site belongs to the Federal University of São Carlos and is located in São Carlos, SE Brazil (21° 58'-22° 00' S and 47° 51'-47° 52' W), 850 m asl, at least 4 km away from urban areas, and protected from visitation. We placed 80 5 m × 5 m quadrats, distributed in eight transects. Each transect was 10 m apart from the other. We collected data for 12 weeks, from May to June 2006. We used for visitation intensities: (1) high intensity (once in a week), (2) medium intensity (once every two weeks), (3) low intensity (once every four weeks), and (4) control (visited only at the beginning and at the end of the experiment). We assigned randomly the treatment in each quadrat, with 20 quadrats per treatment. In each quadrat, we tagged all individuals with stem diameter at soil level equal to or higher than 3 cm, identifying them to species level. Then, we selected all species with at least one individual in each treatment to allow comparisons. In each individual of the species included in the analysis, we randomly tagged leaves from the third or fourth nodes, as long as the leaves were fully expanded and without obvious symptoms of herbivore or pathogen damage (Cornelissen et al., 2003).

At the beginning of the experiment, in all treatments, we simulated measurements, by touching the leaves on both the upper and lower sides, taking care not to damage them. In the control, we did not touch the leaves; in the other treatments, we simulated measurements in the same way, with frequencies that ranged from one simulation in a week (high intensity), through one simulation every two weeks (medium intensity), to one simulation every four weeks (low intensity). We randomly picked ten tagged leaves for a given species to have the same number of replicates for each species. At the end of the experiment, we collected these leaves, placed them in paper bags, and in the laboratory, digitalised them.

To quantify the amount of herbivore damage, we used the ImageJ 1.36 software (Rasband, 2006), which measures the leaf area. First, we calculated the area of each leaf taking into account herbivore damage. Second, we calculated total leaf area, not considering herbivory damage; in the case of attacks from the leaf edge, we prolonged the margin, so we could assess this type of damage as well. Thus, for each leaf, we had two values: observed leaf area, taking into account herbivory damage, and potential leaf area, the area the leaf was supposed to have were it not attacked by herbivores. By dividing the former by the latter, we found the proportion of leaf area attacked by herbivores, which was transformed by its arcsine (Zar, 1999).

We compared these proportions with Kruskal-Wallis non-parametric analysis of variance (Zar, 1999). First, we tested the null hypothesis ($\alpha = 0.05$) that herbivore damage was equal in all four treatments. In this case, we used data from all leaves, according to the treatment, without distinguishing the species. Second, we tested the null hypotheses ($\alpha = 0.05$) that herbivore damage was equal in all four treatments in relation to the species. When we rejected the null hypothesis, we carried out Nemenyi's multiple comparison test (Zar, 1999) to test for differences among treatments.

3. Results

We identified 11 species present in all treatments, comprising 333 individuals (Table 1). When we analysed the treatments without distinguishing species identity, we did not find significant differences ($P = 0.27$; $H = 3.89$; $df = 3$), with herbivory proportion of 0.029 ± 0.073 (mean ± standard deviation) in the control, 0.030 ± 0.064 at low visitation intensity, 0.033 ± 0.074 at medium visitation intensity, and 0.029 ± 0.063 at high visitation intensity. When we analysed each species separately, our visits and measurements altered herbivory damage significantly in four species: for *Connarus suberosus*, an increase in herbivory in the control, for *Diospyros hispida*, an increase at low visitation intensity, for *Eriotheca gracilipes*, an increase at medium visitation intensity, and for *Stryphnodendron adstringens*, an increase at high visitation intensity (Table 2). For the remaining seven species, there were no significant differences in herbivory damage.

When we analysed all four treatments without taking species identity into account, we did not find the variation expected by the HUP. But, if the researcher visits influence herbivory rate, increasing in some species and decreasing in others (Schnitzer et al., 2002), there could have been a compensatory effect in the community, with the HUP implying an increase in some species and a decrease in others. Conversely, when we analysed each species separately, herbivory damage varied for four out of 11 species. Visitation did not affect all species similarly, with each one of these four species exhibiting an increase in herbivory in one of the treatments. Even if we used the same number of leaves for each species and each treatment, one bias in our analysis was the presence of just one individual for some species in some treatments, such as *C. suberosus*.

Table 1. Species and number of individuals found in the four treatments (high, intermediate and low visiting frequency, as well as a control) in a cerrado site inside the Federal University of São Carlos campus.

Species	Control	Low intensity	Medium intensity	High intensity
Aegiphila lhotzkiana Cham.	6	2	6	2
Connarus suberosus Planch.	1	1	3	2
Diospyros hispida A. DC.	9	7	9	8
Eriotheca gracilipes (K. Schum.) A. Robyns	13	5	5	6
Piptocarpha rotundifolia (Less.) Baker	2	4	19	3
Rourea induta Planch.	2	1	9	5
Stryphnodendron adstringens (Mart.) Coville	18	12	7	19
Stryphnodendron polyphyllum Benth.	30	26	24	18
Styrax ferrugineum Nees and Mart.	4	3	2	11
Tabebuia ochracea (Cham.) Standl.	5	7	1	2
Vernonia sp.	2	2	8	2
Total	92	70	93	78

Table 2. Proportion of herbivory (mean ± standard deviation) in woody species in the cerrado site inside the Federal University of São Carlos campus.

Species	Control	Low intensity	Medium intensity	High intensity
Aegiphila lhotzkiana	$0.000^{a} \pm 0.000$	$0.024^{a} \pm 0.034$	$0.005^{a} \pm 0.012$	$0.002^{a} \pm 0.017$
Connarus suberosus	$0.123^{c} \pm 0.128$	$0.065^{b} \pm 0.097$	$0.054^{ab} \pm 0.100$	$0.012^{a} \pm 0.029$
Diospyros hispida	$0.009^{a} \pm 0.041$	$0.071^{b} \pm 0.037$	$0.012^{a} \pm 0.058$	$0.007^{a} \pm 0.026$
Eriotheca gracilipes	$0.022^{b} \pm 0.041$	$0.022^{ab} \pm 0.037$	$0.039^{b} \pm 0.058$	$0.014^{a} \pm 0.026$
Piptocarpha rotundifolia	$0.029^{a} \pm 0.029$	$0.028^{a} \pm 0.050$	$0.025^{a} \pm 0.044$	$0.087^{a} \pm 0.068$
Rourea induta	$0.039^{a} \pm 0.064$	$0.036^{a} \pm 0.051$	$0.035^{a} \pm 0.064$	$0.041^{a} \pm 0.049$
Stryphnodendron adstringens	$0.018^{a} \pm 0.045$	$0.032^{b} \pm 0.068$	$0.038^{b} \pm 0.090$	$0.042^{b} \pm 0.081$
Stryphnodendron polyphyllum	$0.028^{a} \pm 0.088$	$0.025^{a} \pm 0.061$	$0.026^{a} \pm 0.072$	$0.026^{a} \pm 0.063$
Styrax ferrugineus	$0.018^{a} \pm 0.044$	$0.012^{a} \pm 0.110$	$0.008^{a} \pm 0.017$	$0.012^{a} \pm 0.024$
Tabebuia ochracea	$0.068^{a} \pm 0.087$	$0.046^{a} \pm 0.013$	$0.068^{a} \pm 0.100$	$0.022^{a} \pm 0.013$
Vernonia sp.	$0.026^{a} \pm 0.042$	$0.014^{a} \pm 0.018$	$0.031^{a} \pm 0.039$	$0.011^{a} \pm 0.020$

[a,b,c]Different letters above means indicate significant differences among treatments (n = 10 for each species and each treatment).

4. Discussion

Overall, our results were as inconclusive as in other studies. For instance, Cahill et al. (2001) carried out an experiment for eight weeks, with six species in SE USA, in which they visited and touched one half of the plants, whereas the other half was not visited. Visitation did not change herbivory rates in four of the six species, but increased in one and decreased in another (Cahill et al., 2001). Schnitzer et al. (2002) quantified the amount of herbivory and pathogen damage in 13 plant species at four different visitation intensities in N USA and found no evidence to support the HUP at any intensity of visitation. Similarly, Bradley et al. (2003) examined plant performance and herbivory on 14 plant species in three geographic regions, finding out that site differences affected herbivory rates more than visitation did. Boeck et al. (2008) compared stem diameter in three poplar species in plots that had been handled for six years and outside these plots. Biomass was reduced by up to 50% because of handling, and the observer effect differed among species (Boeck et al., 2008).

There is a debate about data analyses and interpretation that could lead to either support (Cahill et al., 2004; Boeck et al., 2008) or rejection (Louda et al., 2004) of HUB. Nevertheless, despite some failed test in supporting HUB (Schnitzer et al., 2002; Bradley et al., 2003), the concerns it raises about bias remain (Wolski et al., 2004). Quantitative analyses of such potential biases are critical to draw appropriate conclusions about biological significance (Bradley et al., 2003). Estimates may be biased if (1) trampling changes either the visibility of target plants or the competitive interactions, (2) human scent left on plants attracts or repels insects, (3) the release of volatile chemicals by plants are altered, or (4) herbivore composition in the community is disturbed (Cahill et al., 2001).

Occasionally, the HUP may occur, at community level, only in situations with more researchers manipulating the leaves and at higher frequency – for example, daily visits. Visitation by researchers to focal plants can alter herbivory rate and plant growth due to both effects of handling focal plants and trampling neighbours (Cahill et al., 2001, 2002). Methods other than repeated visitation may be available, such as remote censusing and satellite imaging, but there are many questions that can be answered only with the physical presence of the researcher (Cahill et al., 2004). In such cases, it would be prudent to carry out a study to assess the consequences of the researcher's impact on the community, establishing additional experimental units that are monitored infrequently (Cahill et al., 2004). If a design such as this is not possible for practical reasons, time, or money, researchers should minimise their visits and collect data only to answer their specific question (Cahill et al., 2004). On the other hand, if the HUP does not occur in cerrado plant communities, the outcomes of studies on plant-herbivore interactions would be reliable.

Within ecology, this "Uncertainty Principle" may not be restricted to herbivory. It is possible to imagine many situations in which the researcher may affect his or her results. This principle has already been studied in other ecological systems, including studies on bird nestling survival and predation (Marshall et al., 2002) and seed removal by vertebrates (Duncan et al., 2002), but can also influence other studies on animal behaviour or on herbaceous plants. Long-term experiences on community composition and functioning may also be affected by the researcher's presence (Tilman and Lehman, 2001). So, Heisenberg's main idea, that making scientific measurements can alter the outcome of the measurement, limiting one's ability to understand how systems operate when they are not being studied (Heisenberg, 1927), is valid and worth testing in many situations.

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