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Proceeding Paper

Effect of Altitudinal Variation on Phenology and Herbivory in *Trifolium repens* †

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Abstract: Phenology is an important ecological feature that can be influenced by many aspects. Mountainous regions are great sites to perform studies to help the understanding of the reproductive cycle of plants and herbivores. In this work, the phenological cycle and leaf damage rate caused by herbivores in *Trifolium repens* were observed, among three different altitudes in the Itatiaia National Park, from June to August of 2021 and statistical analysis were performed using linear mixed effects models. Preliminary results show that altitude affected vegetative phenophases and herbivory ($p < 0.01$). The highest altitude sampled stands out for having a less open and damaged leaves, and for being the only altitude without any flowering events. Nevertheless, the influence of growing season climate on phenology is often observed in transplanting experiments, in which at lower altitudes plants typically develop earlier than those in their higher altitude native sites. As for the damage caused by herbivores, it is known that environmental conditions of higher altitudes can reduce the aptitude of various insects.

Keywords: life cycle; white clove; altitude gradient

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1. Introduction

Montane environments have several biotic and abiotic variations related to elevation, which can affect plant phenology [1,2]. Climatic conditions related to increasing of elevation can influence the reproductive phenology of plants (e.g., affecting seed production and size [3–7]). Moreover, the temperature decreases is the most important environmental factor that affects the life cycle and activity of insects and herbivores [8–12].

Trifolium repens L. (Fabaceae) is one of the most important and widely used legumes throughout the world as a forage crop and nitrogen fixative [13]. Popularly known as white clover, it is a perennial and stoloniferous herb native to Eurasia [14,15]. Commonly sown with grasses in temperate pastures, naturalized populations of the species can also be found in highland grasslands [16]. It can reproduce vegetatively through stolons and is also capable of sexual reproduction through flowering and seed production [17].

The present work aims to evaluate the phenology of white clover (*T. repens*), and damage by herbivores at different altitudes (between 1.700 and 2.400 m) in the Itatiaia National Park (PNI), Brazil.

2. Materials and Methods

2.1. Study Site

The Itatiaia National Park (PNI) was created on 14 June 1937 (and expanded on 21 September 1982), being the first National Park established in Brazil. It is located between the states of Rio de Janeiro and Minas Gerais (22°15′ and 22°30′ S, 44°30′ and 44°45′ W) (Figure 1). It is entirely inserted in the Atlantic Forest Biome [18], with a Cwb—mesothermal climate [19], presenting an annual average temperature of 11.5 °C [20] and precipitation of 2600 mm [21]. The biological importance of this integral protection protected area is related to the extensive altitudinal gradient of more than 2.000 m in height. It allows the occurrence of different phytophysiognomies related to the Dense Ombrophilous Forest, in addition to the Campos de Altitude, which are a Brazilian tropical mountain grassland vegetation found above the limit. forest (1.800–2.000 m altitude) [22]. In the PNI, *Trifolium repens* is found in the elevated region, inhabiting an altitudinal gradient and is found on road sides (Figure 1 and Table 1).

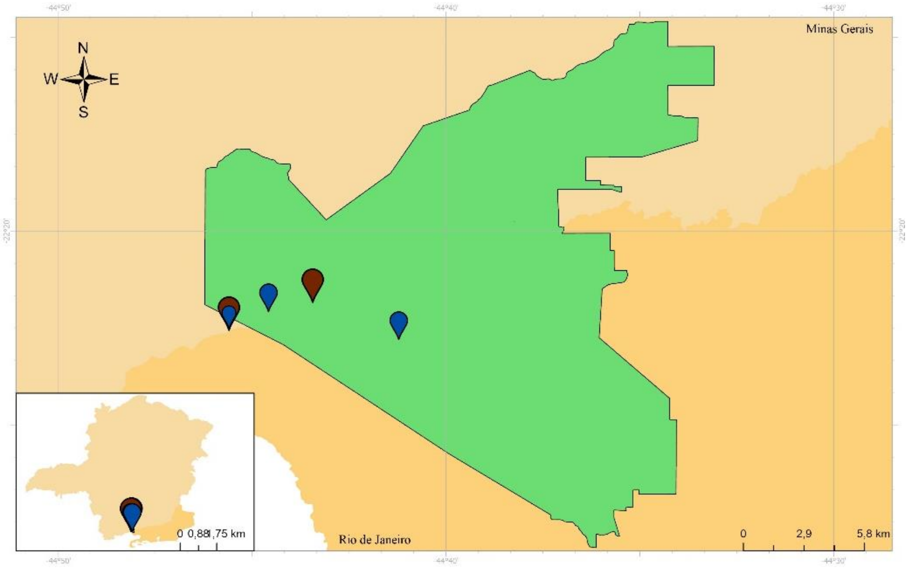

Figure 1. Illustrative map of Itatiaia National Park (in green), located between the limits of the states of Rio de Janeiro and Minas Gerais. The collection sites for the study of morphological data are in blue (1.700, 2.000 and 2.400 m).

Table 1. Location and number of samples of *Trifolium repens* at different altitudes in the Itatiaia National Park. ID = code of the collection area; L = low; M = medium and H = high.

ID	Latitude	Longitude	Samples	Altitude (m)
L	−22.375.710	−44.760.847	15	1.721
M	−22.368.405	−44.743.229	15	2.001
H	−22.380.871	−44.687.722	15	2.406

2.2. Fenology and Herbivory

For the evaluation of the phenology and frequency of herbivory, 45 individuals of *Trifolium repens* (Figure 2) were selected at different altitudes of the PNI (Table 1 and Figure 1). Monthly observations were carried out from June to August 2021. For each individual, each phenophase of leaf budding, open leaves, flowering, fructification (ripe fruit), abscission (leaves in senescence and petiole without leaf), besides leaves burned by frost and leaves damaged by herbivory were registered and quantified.

Figure 2. *Trifolium repens* in Itatiaia National Park. (**A**) inflorescence; (**B**) leaf composed of three leaflets (photos: Alessandra Pinto) and (**C**) Stolon (photo: Amanda Alencar).

2.3. Data Analysis

To assess whether altitude had an effect on phenology, herbivory and damage, linear mixed-effect models were performed. Six models were generated with different response variables (number of shoots, number of open leaves, number of leaves burned by frost, number of leaves with herbivory damage, number of leaves in the process of abscission and inflorescence) with the function "lmer" from the "lme4" package [23]. Altitudes (three levels: 1.700 (L), 2.000 (M) and 2.400 m (H)) were used as the predictor variable in all models. For the number of shoots and number of leaves models, the individual was use as a random variable, and for the other models, the number of leaves of each individual was use as a random variable. The adequacy of the models was evaluated by visual inspection of the residues using the "qqnorm" function and the ANOVA (type II) of the models was performed using the "Anova" function of the "car" package [24]. Differences between altitudes were also calculated using the "emmeans" package [25]. All analyzes were performed in R v. 3.5.3 [26].

3. Results

Altitude affected vegetative phenophases (Figure 3 and Table 2) and damage caused by herbivory (Figure 3 and Table 2). Regarding the number of leaves, the individuals of region H (2.400 m) differed from those of regions L (1.700 m) and M (2.000 m), having a smaller number of leaves. Region M had more shoots than region H, but there was no difference in shoots between region L and the others (Figure 3 and Table 2). Regarding the abscission, the M region had a greater number of abscised leaves per individual than the L region; H did not differ from L or M. Altitude H had a lower herbivory rate than the others. It was not possible to observe an influence of altitude on the percentage of leaves burned by frost. Finally, altitude H was the only one that did not present any flowering individual in the months of observation, however the low intensity of this phenophase at other altitudes was not possible to determine whether the altitude is in fact affecting flowering.

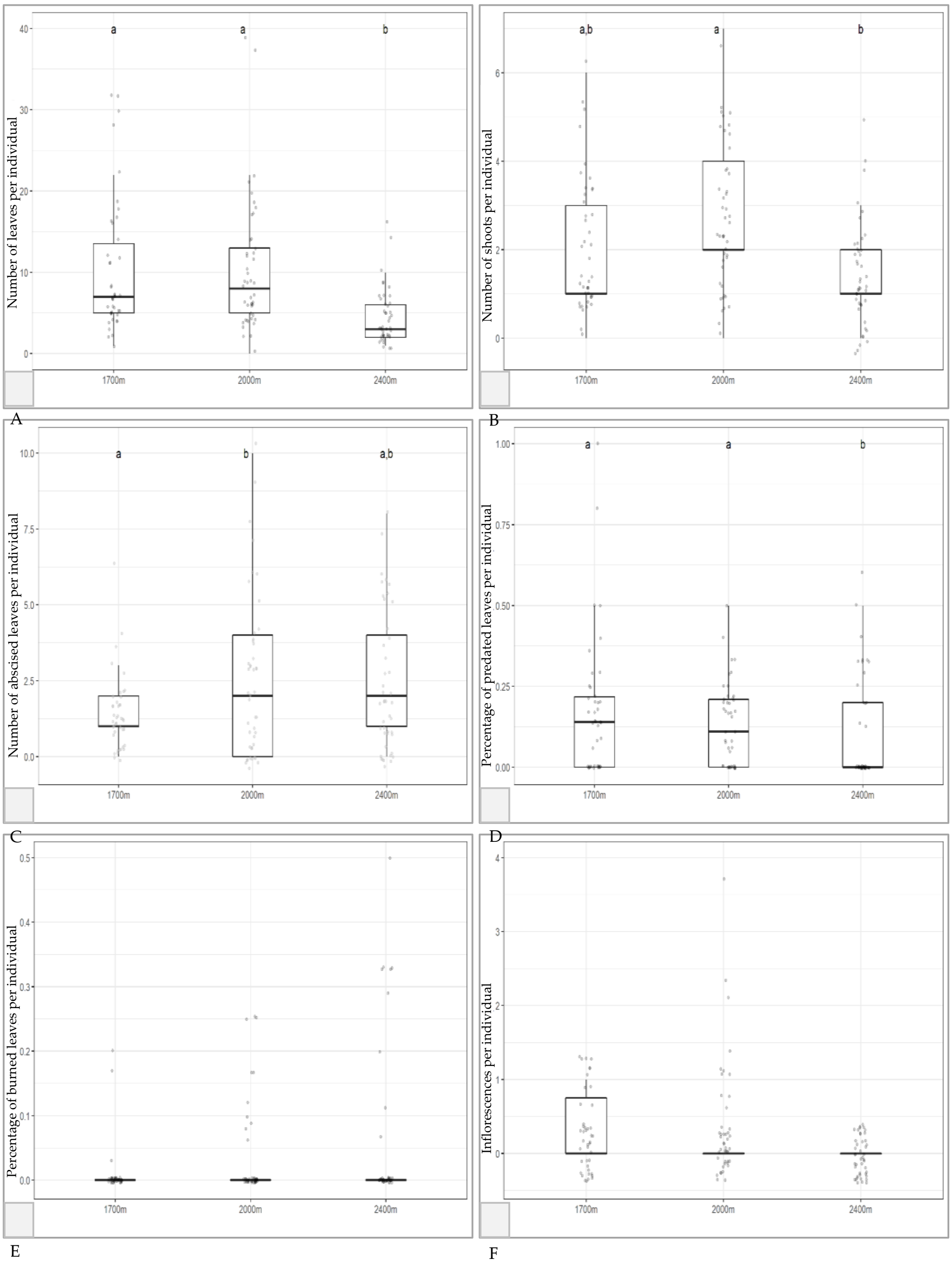

Figure 3. Effect of altitude on: (**A**) Number of open leaves, (**B**) Number of shoots, (**C**) Number of abscised leaves, (**D**) Predated leaves, (**E**) Burnt leaves and (**F**) Inflorescence.

Table 2. ANOVA results showing the effects of altitude on phenological data and on herbivory damage to *T. repens* leaves in Itatiaia National Park.

Variable	***f***	***df***	***p***
Leaves	5.94	2	<0.01
Sprout	5.96	2	<0.01
Abscission	3.29	2	<0.05
Predated leaves	4.99	2	<0.01
Burnt leaves	2.20	2	0.12

Inflorescence	2.96	2	0.06

4. Discussion

Initial analyzes of the three months of collected morphological data revealed that the number of leaves, sprouting, abscission and predated leaves per individual were affected by altitude. Altitude M had the highest number of shoots and abscissions and altitude H had the lowest number of open leaves, this fact can be explained by the influence of climate at high altitudes. More than any other bioclimatic zone, phenological events at high altitudes are limited by a short growing season bounded by cold temperatures [27]. The influence of climate on the growing season and therefore on phenology can be observed in transplant experiments. Plants transplanted to lower altitudes usually develop earlier than those left in their native high elevation locations [28]. As for the damage caused by herbivores, altitude H had a lower predation rate compared to the others. This result was already expected, ever since it is widely known that high altitudes can reduce the occurrence of various insects and herbivores [8,11,29], among them herbivores such as molluscs that usually feed on clover leaves [30,31].

5. Conclusions and Future Perspectives

In this preliminary study, the three-month results were satisfactory with significant indices. Showing that the phenological data and the damage caused by the herbivore in the white clover are related to the altitude. However, it is necessary to increase the sampling (data collection) to achieve robustness in the study. To that end, monthly data collection fields are planned until June 2022 to include one year of morphological data in the analyses.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available because the data still generation.

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