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THE EFFECT OF HERBIVORY ON DESCHAMPSIA ANTARCTICA FROM TWO DIFFERENT GEOGRAPHICAL REGION

Parinaz Asadnejad

M.Sc. thesis in Evolutionary Biology (MDP in Biosciences) – Department of Biology

University of Turku

Faculty of Science
Department of Biology
Master Degree Programme in Biosciences, Evolutionary Biology track

Supervised by

Ph.D. Kari Saikkonen
University of Turku, Biodiversity Unit

Ph.D. Aino Kalske
University of Turku, Biology Department

Reviewed by

Ph.D. Satu Ramula
University of Turku, Biology Department

Ph.D. Tero Klemola
University of Turku, Biology Department

Faculty of Science
Department of Biology
MDP in Bioscience, Evolutionary Biology track
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This thesis investigates the effect of herbivory on *Deschampsia antarctica*, a grass species found in two geographically distinct regions: the Antarctic peninsula and Patagonia, South America. This study aims to understand how these different populations from 2 different regions respond to herbivores. Global warming causes the species shift polewards and occupy new habitats. Climate change and human activity has notably affected Antarctica and there is a possible introduction of invasive herbivore species in this region, which has been empty of any herbivores until now. In this experiment growth rates (tiller number and dry mass) and aphid numbers were used to assess the susceptibility of plants to herbivores, particularly the aphid *Rhopalosiphum padi*. Statistical analyses suggest significant differences in plant responses between the two regions. Patagonian plants exhibited significantly fewer aphids on them than Antarctic plants. The plant growth (dry mass) was not effected significantly by herbivores, and the plants had more tillers in the beginning, weighed more than other plants with less tillers. The variation in defense mechanisms between these geographical groups likely stems from genetic diversity in genes responsible for defense responses, since Patagonian populations were more exposed to herbivores, they have evolved a stronger mechanism to cope with insect herbivores. It is also possible that the underlying cause is the defense induction, since Patagonian plants had long term herbivore exposure during their lifetime. This study suggests that Antarctic populations of *D. antarctica* are more susceptible to herbivores than the Patagonian populations. This findings raise concerns around protecting Antarctica and mitigating effects of global warming since climate change might alter the species distribution and threaten the wildlife there.

KEYWORDS: Deschampsia antarctica, Antarctic grass, herbivory, insect herbivores, biodiversity, invasive species, climate change, plant defense mechanism

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1 Introduction

1.1 Effects of climate change in South America and Antarctica

The current rate of global warming is the greatest in the past 1000 years. Human activities, particularly the release of greenhouse gases, have undeniably been the primary driver of global warming [1]. It is expected that the increase in greenhouse gases will alter the Earth's climate, impacting species physiology, distribution, and phenology [2]. Since 1950, some of the highest near-surface air temperatures have been recorded on the Antarctic Peninsula, leading to the expansion of vegetation in this region [3]. Two crucial effects of global warming are changes in temperature and precipitation, which directly impact glaciers. The glaciers and permafrost in South America are receding, particularly in the Patagonia and Tierra del Fuego regions [4]. This recession has caused the expansion of lakes and fjords, altering the ecosystem in the Patagonian region. Glacial fluctuations can change the floristic composition and affect the frequency of disturbances such as insect attacks and fires [5].

Antarctica is divided to three major biogeographic regions of sub-, Continental and Maritime Antarctica region. The Maritime Antarctica includes South Shetland, South Orkney and South Sandwich Islands (Scotia Arc) and west coast of Antarctica Peninsula to South Georgia. South Georgia is considered as a part of sub-Antarctic region [6]. There are several plant species in maritime Antarctic, such as liverworts and mosses. The only two native vascular plant species, *Deschampsia antarctica* É.Desv. 1854 (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. 1831 (Caryophyllaceae), also can be found in the maritime Antarctica [7]. Warming trends in maritime Antarctica has caused a significant expansion of these two species on Signy Island. The number of sites that *D. antarctia* occupies, has doubled during the warming periods in Antarctica first from 1960 to 2009 and again from 2009 to 2018 [8].

Antarctica is a pristine area and vulnerable to invasion of non-native species. Therefore, establishment of any non-indigenous species there, can be harmful for the native species of this region and most likely leads to loss of endemic wildlife there 7/30/2024 11:59:00 AM.

1.2 Herbivores in Patagonia and Antarctica

Herbivory is a crucial ecological interaction which shapes the ecosystem by affecting plant community dynamics and evolution of plant defense system [10]. There are no terrestrial vertebrate herbivores or predators that occurs naturally in Antarctica. Species richness is low, and several taxonomic groups are absent [6]. In ecological communities, the absence of natural enemies reduces or eliminates the vulnerability of species to predation or herbivory, creating what is known as "enemy-free space"[11].

Guanaco (*Lama guanicoe* Müller, 1776 (Camelidae)) is the only large native mammal herbivore of Patagonia region; their population in the region had decreased previously due to habitat fragmentation, but the population has been revived in the past 20 years. They mainly graze on Patagonia’s grasslands and their primary competitors are the livestock [12], [13].

Grazing intensification has a huge impact on central grasslands in Chile; the plant communities which are overgrazed have more native plants than the introduced species probably due to superior defense mechanisms of native species against herbivory [14]. In addition to large herbivores like guanacos, Patagonia is home to a variety of herbivorous insects. These insects play a huge role in the ecosystem by interacting with native flora [15].

Insect pests cause significant damage to crop yields every year, and warmer climates favor them, as they can reproduce more rapidly and feed more easily at higher temperatures [16]. Aphids (Hemiptera: Aphididae) are an important group of insect pests which are distributed worldwide except in Antarctica and have wild and domesticated hosts [17].

Several long-distance migrations of aphids have been reported in different locations globally; they can migrate up to 2000 km distances [18].Aphids can also be introduced to new regions due to anthropogenic activity and causing damage not only to crops but also to native plants that already exist in those areas [19]. A new

species of aphid parasitoid *Aphidius matricariae* Halliday 1834 (Hymenoptera: Braconidae) has been introduced on Marion Island, in subantarctic region, which required the prior introduction of aphids, specifically *Rhopalosiphum padi* [20].
This aphid also lives in Patagonia and attacks grasses there [21].

Despite Antarctica being a pristine area, free of any natural herbivores, it is at risk of invasion by new species including herbivores moving there due to anthropogenic activities and climate change [22]. There are slugs (Mollusca) and aphids herbivores that has been recorded in Antarctica recently [6].
Researchers are continually recording non-indigenous species in the sub-Antarctic and Antarctic regions despite strict biosecurity measures in place to prevent their introduction [20], [23].

1.3 Plant defense mechanisms toward herbivores

Plants have evolved several defense strategies to protect themselves from herbivores. They have evolved chemical defenses and physical barriers to minimize the damage they might receive from herbivores [24]. They produce secondary metabolites such as phenols, alkaloids and cyanogenic glucosides to improve their resistance; some secondary metabolites such as tannins cause unpalatable taste in plant tissues or others might produce odors that are unpleasant for herbivores [25]. Plants have also evolved morphological traits to defend against herbivores which includes trichomes, tough leaves, thorn and spines [26].

Prior exposure to herbivores leads to induction of defense which makes the plant more resistant to recurrence of herbivores attack [27]. Resistance to insects and other pathogens can differ in a plant species from different geographical populations [28]. Different populations of a plant species might differ in composition of defensive chemical compounds and morphological traits based on their distribution range [29]. The selection pressure of herbivores might alter the defense mechanism relative genes in different geographical populations of plants [30].

1.4 Aims of the study

In this study I investigate the response of *D. antarctica* from two different geographical origins of Antarctica and Patagonia to the insect herbivore, aphid *Rhopalosiphum padi* Linnaeus, 1758 (Hemiptera: Aphididae). The main concern is

that the warming trends influences species distribution to shift towards higher latitudes (poleward) and higher elevation (upward)[31].
As a result, I want to examine how Antarctic populations of *D. antarctica* respond to herbivores, in case of invasion of insect herbivores there.
As it was said earlier, resistance to herbivore in plants might differ in different geographical populations [28] so it is likely that populations of *D. antarctica* in these two different regions have variations in defense mechanisms towards herbivores. In this experiment, I am going to have two groups of control and aphid treatment for the plants from Patagonia and Antarctica .I am going to put aphids on the aphid treatment group plants then I will wait for plants' response to herbivores. My hypothesis is that Patagonian plants will have less aphids on them comparing to the Antarctica populations. Since Patagonian plants were more exposed to insect herbivores during their evolution, as a result they have evolved a stronger defense mechanism towards herbivores. However, *D. antarctica* is a perennial grass [32] and I have used the whole plant individuals in the experiment that were living for a period of time in the nature; therefore, the better resistance of Patagonian plants than the Antarctic plants to herbivores might be due to long term exposure to insect herbivores which has caused induced defense later.

2 Materials and Methods

2.1 Study species

2.1.1 *Deschampsia antarctica*

In this study I investigated the variation in herbivore defenses in the perennial grass species *Deschampsia antarctica* (Poaceae). It is one of the only two native flowering plant species that grows in the Antarctica [33]. This plant is distributed from northern Patagonia to Tierra del Fuego and in Maritime Antarctica [34]. The origin of this species in Antarctica is still unknown. There is no direct evidence of how this species reached Antarctica, but most likely it has been transported through birds with long distance dispersal during Holocene or late Pleistocene [35]. Antarctic populations of *D. antarctica* have less genetic variations comparing to Patagonian populations due to bottle neck effect [34]. *Deschampsia antarctica* has a facilitative interaction with moss carpets in Antarctica; mosses play a pivotal role by providing more moisture and favourable temperature, for the growth of this grass in Antarctica [36]. Studies also have suggested that the population of vascular plants of Antarctica is increasing due to global warming [37].

2.1.2 *Rhopalosiphum padi*

In this herbivory experiment I used the aphid called *Rhopalosiphum padi* as my herbivore species. This aphid is also called Bird cherry-oat aphid; this is a polyphagous aphid, and feeds on many species from the Poaceae family [38]. It has a worldwide distribution and targets mostly cereal farms [39]. However, there is no record of alive *R. padi* in Antarctica yet but some mummified samples of this insect has been collected from subantarctic region in Marion Island [20].
R. padi is a holocyclic aphid that undergoes host-alternation during its life cycle. Holocyclic means that there are many cyclical parthenogenesis and a single annual sexual reproduction.

The life cycle of *R. padi*, has several stages and can vary depending on environmental conditions, particularly temperature. The life cycle begins with overwintering eggs laid on the bark of bird cherry trees (*Prunus padus*) in the autumn. In spring when the temperature rises, these eggs hatch into nymphs that develop into wingless females known as *fundatrices*, which reproduce parthenogenetically, giving birth to virginoparous *fundatrigeniae* (wingless). After two or three generations, winged aphids which are also called spring migrants, migrate to their secondary hosts (Poaceae, Cyperaceae e.g.). On these secondary hosts, they continue to reproduce parthenogenetically throughout the summer, producing multiple generations. As autumn approaches winged parthenogenetic females (*gynoparae*) production starts, winged aphids migrate back to the primary host, where they produce first female (*oviparae*) and then male sexual forms. These sexual forms mate, and females lay eggs on the bark of the bird cherry trees, which then overwinter and hatch in the following spring, completing the cycle [40], [41], [42].

Winged morph variation is a response to environmental changes [43]. Winged morph is considered a crucial stage in their life cycle, since they will be able to fly to their secondary hosts which helps them to cope with uncertain environment and expand their population on their alternative hosts [44].

2.2 Field sampling

Samples of *Deschampsia antarctica* were collected during two expeditions between the year 2022 and 2023 in January and February. In the first expedition seven population of plants were collected from Patagonia and only two populations were collected from Antarctica, due to travel restriction to Antarctica during COVID-19 pandemic. In the next expedition six populations of plants were collected from each region. 10 to 20 individuals were collected from each population during the sampling.

The individuals were imported to Finland under the permission of Chilean Antarctic institute (INACH) and then they were taken to the University of Turku Botanic Garden at Ruissalo.

The collected plants were kept in quarantine about 3 months at the University of Turku Botanic Garden in order to prevent release of any alien species into the Finland nature. After the quarantine plants were examined by a representative from Finnish Food Authority to check if they are safe to release. After the authorization the plants were taken out of the quarantine and then potted in 50 % normal potting soil & 50 % sand. They were watered weekly and fertilized every other week.

In this experiment I have used the populations from 2023 expedition and only one Patagonia population from 2022.

I meant to choose 4 populations out of each region and 5 individuals (genotype) from each population. Each genotype had two replicates (number of plants=2 regions x 4 populations x 5 genotypes x 2 replicates per genotype=80). At first were aiming to have 5 populations and 10 individuals in total in each cage number of plants=2 regions x 5 populations x 5 genotypes x 2 replicates per genotype=100). However, the size of the plants of some individuals limited the choice of the suitable samples for the experiment. At the end I understood that one of the populations of Patagonia from 2022 expedition in Tierra del Fuego is the same as one of the chosen populations from 2023 expedition and they belong to same geographical coordinate, therefore the collected plants are the same in populations but different individuals. I eventually did the experiment with 3 populations from Patagonia and 4 from Antarctica as a result.

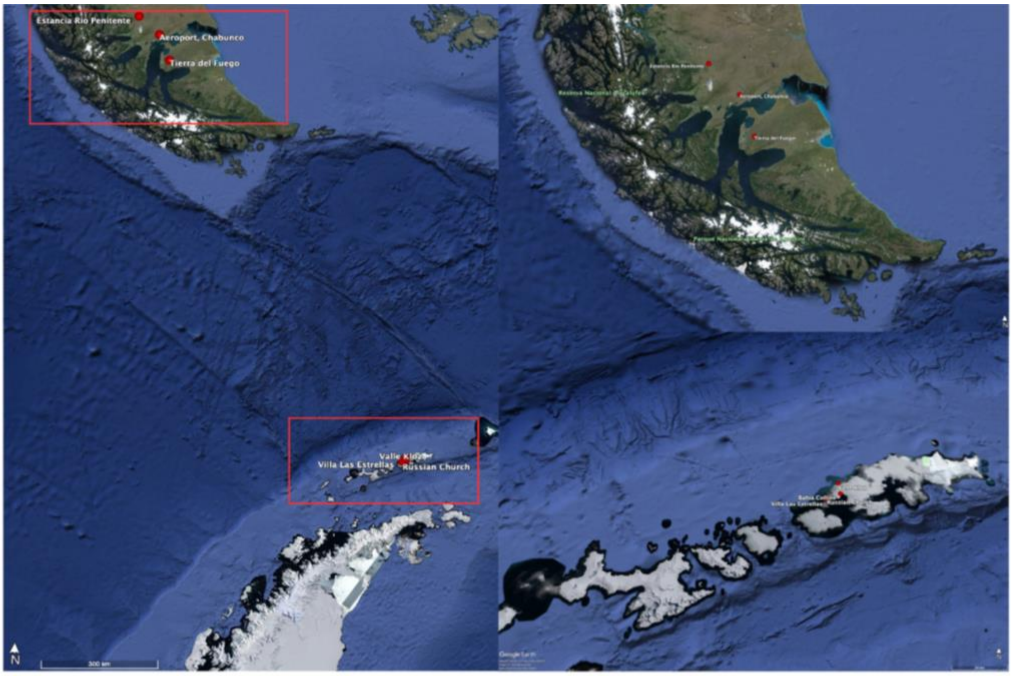

Figure 1. In the map above the regions that plants from Patagonia and Antarctica that have been collected are indicated.

2.3 Experiment setup

The experiment was done at the research green house at the Botanic Garden of University of Turku in Ruissalo from 28th of August until the 25th of September 2023. The room that the experiment was conducted in, is a quarantine room with one corridor between the main room and the greenhouse main corridor to prevent any aphid escape to other rooms in the green house. I used 10 insect rearing cages (BugDorm-4M3074 Insect Rearing Cage) with the dimension width 32.5 x length 32.5 x height 77.0 cm. In each cage there were 8 plants and 4 of them were from Patagonia and 4 from Antarctica. The plants were chosen randomly from different population and then their replicates were chosen for the control cage. In the next step, I divided the cages into two groups of aphid treatments (5 cages) and controls (5 cages). Each individual was repeated in one control and one aphid cage; so, they were pairs in every two treatment and control groups.

The order of plants placement within each cage was in a shape of 3x3 square; however, the center is empty since there are 8 plants in each cage. The plants were placed in each cage in a checkered order; so, an Antarctic individual was placed next to a Patagonian one. I did the tiller number counting as the first measurement of my study before the experiment started. In the first week I put 4 aphids in each aphid cage, two on an Antarctic individual and two on the Patagonian plants. The plants that were put aphids on were chosen randomly. I checked and watered the plants every day and fertilized them each week by the fertilizer (Kekkilä Kastelulannoite). In the first the activity of aphids was low in the aphid cages, therefore next week I added 1 aphid in each cage with the same procedure. In the third week the activity of aphids was more noticeable, but unfortunately all the control cages were also infested with aphids. I started counting the aphids for the first time. I counted them once again in the next week. On the start of the fifth week, I ended the experiment. I cut all the plants shoots and put them in the labelled paper bags.

All the aphid treatment plants in addition to one control cage (since it was infested with the aphids thoroughly) were frozen in a freezer at Botanic Garden of University of Turku for three days to kill all the aphids to count them for the third time under stereomicroscope.

The control plants were taken to the university to dry in the oven for dry weight measurement. The samples were dried in 60°C for three days in the oven (Isotherm® Forced Convection Laboratory Oven). The dry weight measurement was chosen as an indicator of the plant growth.

I counted all the aphids under stereomicroscope. I included also one of the control groups in my counting since it was highly infested with the aphids, but I did not use them in the analysis at the end since the time of infestation was unspecified.

Once I finished aphid counting, I dried the plants in the oven and let them to be there for three days, I also repeated the drying again for control samples to avoid errors in case of absorbing any moisture from the environment. I weighed all of them using a digital scale (AT261 DeltaRange®).

2.4 Statistical analysis

I used the R version 4.2.3 of Rstudio [45] to analyze the data. I tested if the tiller numbers of the experimental plants are any different across the region and treatment in the beginning of the experiment using the package glmmTMB [46]. The difference in the region tiller numbers does not affect the next statistical analysis and the results are the same with and without including this factor in the analysis. I ran a generalized mixed model using the tiller number as the response variable and region and treatment as fixed effects. The random the intercept were population, sample ID and block. This model was fitted with Poisson distribution and logarithmic link function because of count-based nature of the tiller number variable. I tested the model significancy with Type II Wald X^2 tests using the car package [47].
I used the same package to run a zero-inflated generalized mixed-effect model for aphid numbers with time and region, population, block, and sample ID. Aphid number was the response variable and Time, and region were the fixed effects. In this linear model I used population, sample ID and block as the random intercepts.
I ran Wald-test type II using the same package to check the significancy of the fixed effects. I did a pairwise comparison with Tukey correction for multiple comparisons of aphid number differences of Antarctic and Patagonia samples during different times levels by using the package emmeans [48].
To examine if the plant growth was affected by the aphid treatment and/or region, I made a linear mixed-effect model using lmer function of lmerTest package [49]. The response variable was plant growth (plant dry biomass), and the fixed effect were region, treatment, and tiller number. Population and block were used as the random effects. I tested the significancy of model using Type III Analysis of Variance Table with Satterthwaite's method from lmerTest package [50].
I assessed the assumptions of the repeated linear mixed-effect models with DHARMa [51] package in R and I used residual plots for the non-repeated models. The generalized mixed models followed the Poisson distribution and linear-mixed effect model followed the normal distribution.

3 Results

3.1 Plant size

I analyzed if the plant size in the beginning (Tiller number) is significantly different across all the samples. There was a difference in tiller numbers between Antarctic and Patagonia plants (Figure 2). The Patagonia plants have more tillers than Antarctic plants (mean tiller number± SE: Antarctic= 7.57±0.26, Patagonia= 9.67±0.45); However, according to the statistical analysis this difference is not significant across the treatment and control groups (Table 1). This result does not effect the next statistical analysis and the result is the same with and without including this factor.

Table 1. The association between tiller numbers in samples at the beginning of the experiment with treatment & region factors (Analysis of Deviance Table (Type II Wald chi-square tests)) .

EXPLANOTORY VARIABLES	DF	CHI-SQ	PR(>CHI-SQ)
REGION	1	7.0750	0.02835 *
TREATMENT	1	15.0566	0.54331
REGION X TREATMENT	1	4.6039	0.28197

* Significant codes: 0 ‘***’ ,0.001 ‘**’ ,0.01 ‘*’ ,0.05 ‘.’ ,0.1 ‘ ’

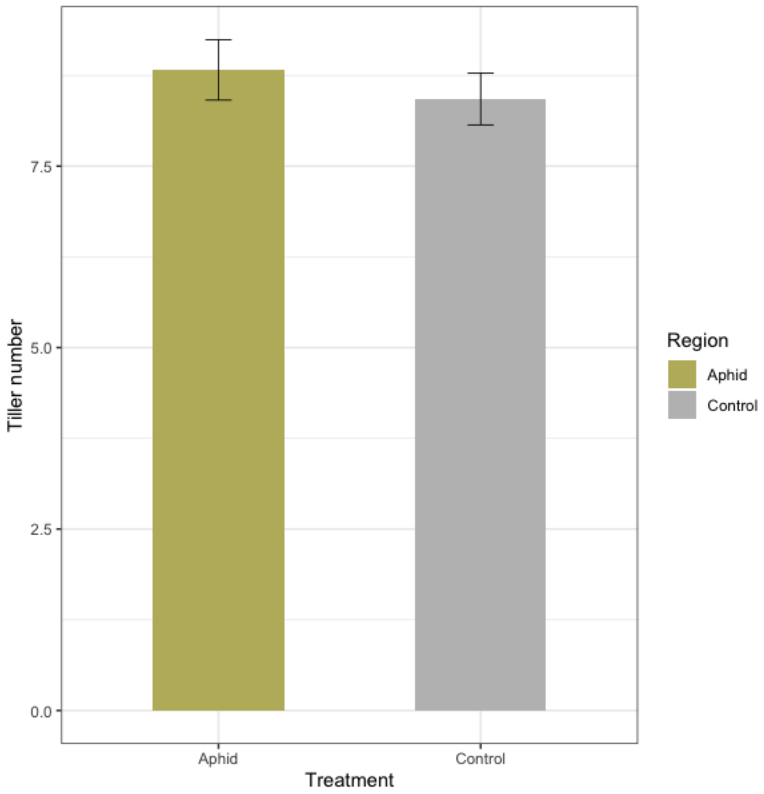

Figure 2. The difference in tiller number at the beginning of the experiment (mean tiller number± SE) of Antarctic and Patagonian samples of treatment and control groups is not significant.

3.2 Aphid numbers on plant individuals

I analyzed the association of aphid numbers and the *D. antarctica* samples' origin. I found the interaction of time & region statistically significant, indicating that the number of aphids on the plants has impacted by the time and the individuals' region. As a result, as the time goes by aphids tend to grow more on Antarctic plants than the Patagonian ones. In the first week the mean number of aphids on Antarctic plants was 14.85 ±2.67 while Patagonia plants had only 0.80 ±0.18 in total. In second week, aphid number for Antarctic plants was 16.80 ±2.97 and Patagonia 6.30 ±1.4. In the last time of counting Antarctic plants had 53.62 ±10.96 and Patagonia 19.16 ±2.94.

Table 2. The analysis results of generalized mixed effect model of number of association of aphids on plant samples with time & region (Analysis of Deviance Table (Type II Wald chi-square tests)).

EXPLANOTORY VARIABLES	DF	CHI-SQ	PR(>CHI-SQ)
REGION	1	1.7087	0.1912
TIME	2	616.9115	< 2e-16 ***
REGION X TIME	2	55.9471	7.1e-13 ***

* Significant codes: 0 '***' ,0.001 '**' ,0.01 '*' ,0.05 '.' ,0.1 ' '

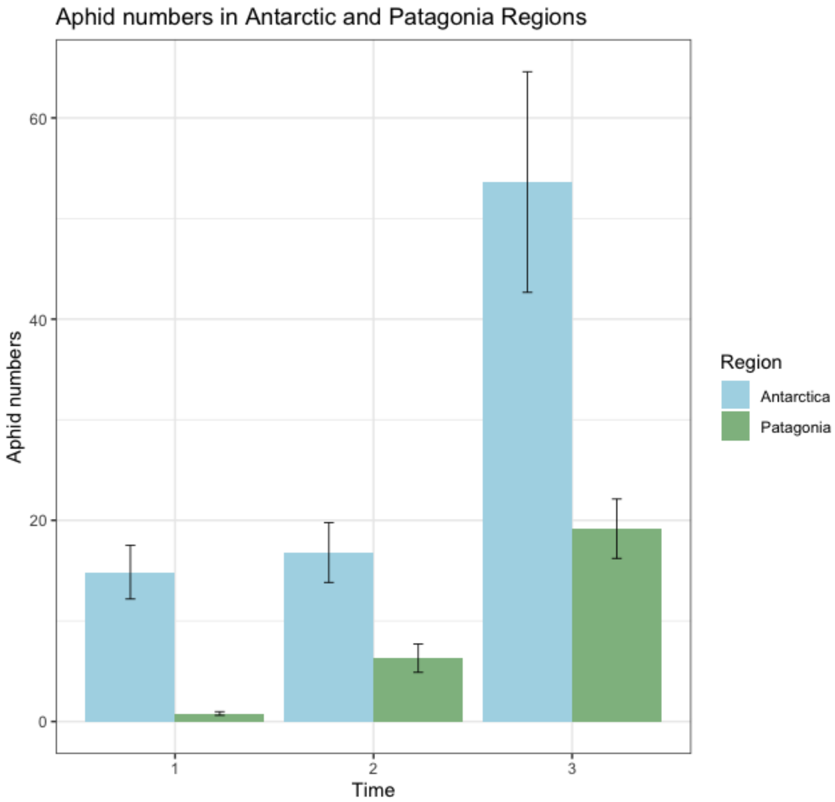

Figure 3. The bar chart demonstrates the number of aphids on Patagonia & Antarctica regions' samples (mean aphid numbers ±SE). Number of aphids was higher on the samples from the Antarctica than the Patagonia.

3.3 Plant Growth

I tested if the aphids had any impacts on plant growth at the end of the experiment. As a result, I used dry weight of the plants as an indicator of growth. The analysis suggested that aphids did not have any significant impacts on plant growth as the p-values for treatment and the interaction between region and treatment were not significant (Table 3). It can be implied from the Figure 3 the Patagonian plants have a larger dry mass than the Antarctic plants (mean dry weight ±SE: Patagonia biomass = 0.21 ±0.024, Antarctic biomass = 0.11 ±0.012).

Table 3. The table below demonstrates the analysis of linear mixed-effect model of the effect of aphids on plant growth(Type III Analysis of Variance Table with Satterthwaite's method).

EXPLANOTORY VARIABLES	DF	F VALUE	PR(>F)
REGION	1	5.8801	0.04936 *
TREATMENT	1	0.3670	0.54664
TILLER NUMBER	1	31.8642	2.886e-07 ***
REGION X TREATMENT	1	0.3748	0.54241

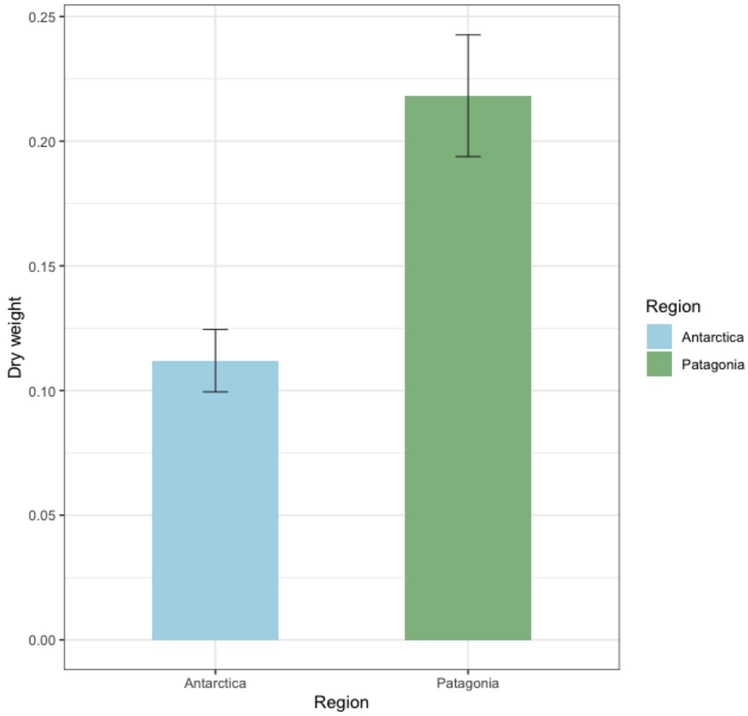

Figure 4. Dry weight used as an indicator of growth in this experiment. The weight measurement (mean dry weight ±SE) indicates that plants from Patagonia have a larger biomass than the Antarctic plants.

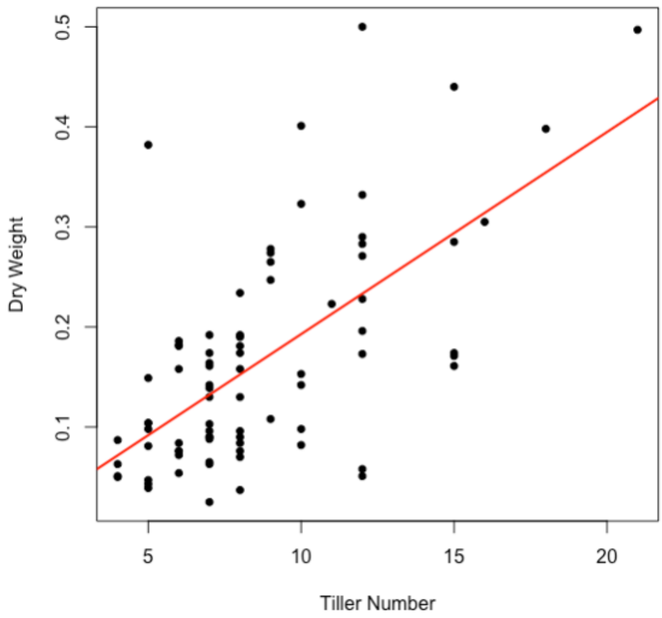

Figure 5. This plot depicts the association of dry weight and tiller number. The plants that had more tillers, weighed heavier at the end of the experiment.

4 Discussion

In this study I investigated the differences in susceptibility to herbivores of *D. antarctica* from two different geographical regions: the Antarctic Peninsula and Patagonia, South America. In this study we used the *Rhopalosiphum padi* as the insect herbivore. The aim of this study is to demonstrate how climate change and human activities might endanger the biodiversity in pristine areas such as Antarctica which are empty of non-indigenous species. Global warming causes species to shift towards milder temperature zones and occupy new habitat [31]. There is this chance that Antarctica be invaded by new herbivore species which might attack the vegetation there [6]. In this experiment I found a significant interaction between aphid numbers and plant origin over time; Antarctic plants exhibited more susceptibility to herbivores attack than the Patagonian plants. I measured tiller number, and the impact of aphid herbivory on plant growth of Antarctic and Patagonian regions. The plants had more tillers at the beginning of the experiment were heavier at the end. However, aphid herbivory did not significantly affect the final plant biomass.

4.1 Plant size differences between two regions

The analysis of initial tiller numbers indicated a non-significant difference between Antarctic and Patagonian plants. This difference was not significant across the treatment groups as well. Specifically, Patagonian plants had a higher number of tillers compared to Antarctic plants (Table 1). There were a few characteristics which were different between plants of these two regions. In general Patagonian plants looked firmer and darker (higher concentration of chlorophyll) than the Antarctic plants. Antarctic plants leaves were flatter and softer. The Patagonian individuals were in a better health condition than the Antarctic plants in total. The reason that Antarctic plants were weaker than their Patagonian counterparts is likely due to temperature. Antarctic plants have more vegetative growth under 5° to 20°C fluctuated temperature rather than sustained temperature of 18° to 20°C [52]. In this experiment the average temperature of the greenhouses exceeded this range, as a result it inhibited the growth in Antarctic plants.

4.2 Effect of genotype on response to herbivores

In this study I found that Antarctic plants were more susceptible to aphids than the Patagonian plants as Patagonian plants had less aphids on them than the Antarctic ones in general.

I examined the association between aphid numbers and plant origin over time. The results revealed a significant interaction between time and region ($p < 2e-16$ for time; $p = 7.1e-13$ for region x time interaction, Table 2). This interaction suggests that aphid populations increased more rapidly on Antarctic plants compared to Patagonian plants over the experimental period. For instance, in the first week, the mean number of aphids on Antarctic plants was 14.85, which increased to 53.62 by the final week. In contrast, Patagonian plants showed a slower increase from 0.80 to 19.16 over the same period.

Resistance of Patagonian plants can be due to induction of defense responses since they were more exposed to herbivores in Patagonia. Plant performance can be improved if herbivory responses are induced in advance [27]. Induction of defense responses causes the accumulation of secondary metabolites in plants which makes them unfavorable to consume for herbivores [53]. Additionally, the resistance of Patagonian plants towards herbivores can be due to the accumulation of foliar silica. Grasses use silicas as the first barrier of their defense towards herbivores [54]. In Poaceae family, plants accumulate silica in their leaves more than usual in the presence of mammal herbivores [54]. As result, since Patagonian plants were more exposed to them, they probably had a foliar Si induction already and they are more resilient.

My other hypothesis is that Patagonian plants are genetically more resistant to herbivores since they have been more exposed to them during their evolution. Geographical variations in plant defense mechanisms can occur due to exposure to different types of herbivores, with selection pressure altering the genetic code related to these defense mechanisms [30].

4.3 Impact of herbivory on plant growth

Despite the significant differences in aphid infestation rates, the impact of aphids on overall plant growth, as measured by dry biomass, was not significant. The analysis showed no significant effect of treatment or the interaction between region and treatment on plant growth (Table 3). This suggests that aphid presence did not drastically affect the final biomass of the plants, although Patagonian plants exhibited significantly higher biomass compared to Antarctic plants (Patagonian biomass = 0.21 ± 0.024; Antarctic biomass = 0.11 ± 0.012).

The statistical analysis shows that the effect of tiller number on plant biomass ($p = 2.886e-07$) is significant. Plants with higher initial tiller numbers generally had greater biomass at the end of the experiment, regardless of aphid infestation. However, *D. antarctica* growth rate is usually noticeable after 2 months of growth [52] and this experiment was done over one month; As a result, the plant size (measured by tiller number and dry mass) was not significantly different at the beginning and end of the experiment most likely due to the short duration of the study. Another factor worthy of mention is that all the control cages were infested with aphids at some point. Consequently, even if the experiment had been longer, it is possible that there would not have been any significant differences in growth measurements between the treatment and control groups.

5 Summary & Conclusion

During my master's thesis, I investigated the effect of herbivory on two different populations of Antarctic grass, *Deschampsia antarctica*. The study suggested that Antarctic populations are more susceptible to insect herbivores compared to their Patagonian counterparts. This finding highlights the potential threat to Antarctic biodiversity due to the introduction of invasive species, particularly in the context of climate change and human activities.

Future research can be a combination of exploring the chemical composition of the grasses and genetic analysis to determine if susceptibility to herbivores is related to specific genes and genotypes. In addition, growing the plants from seeds rather than using whole plants could provide a more controlled way to study the influence of genetic factors and environmental conditions on plant susceptibility to herbivores; growing plants from seeds helps us to avoid gaining results which are related to induced defense.

Implementing more detailed growth measurements can help in understanding the physiological responses of plants to herbivory. Measuring height in this experiment could help us to understand the effect of herbivory on growth better. Ensuring a more regulated experimental environment, including precise control of factors such as temperature is crucial for obtaining more accurate and reproducible results.

In conclusion, this study has revealed the impact of herbivores on *D. antarctica*, one of the main vegetation species of Antarctica region. These results can have broader implications to understand how organisms are going to response to consequences of climate change. Additionally, it emphasizes on the importance of protecting the biodiversity in fragile and isolated ecosystems such as Antarctica.

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List of References

[1] K. Calvin *et al.*, “IPCC, 2023: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland.,” Intergovernmental Panel on Climate Change (IPCC), Jul. 2023. doi: 10.59327/IPCC/AR6-9789291691647.

[2] I. I. Hughes, “Biological consequences of global warming: is the signal already apparent?,” *Trends Ecol. Evol.*, vol. 15, no. 2, pp. 56–61, Feb. 2000, doi: 10.1016/s0169-5347(99)01764-4.

[3] J. Turner *et al.*, “Absence of 21st century warming on Antarctic Peninsula consistent with natural variability,” *Nature*, vol. 535, no. 7612, pp. 411–415, Jul. 2016, doi: 10.1038/nature18645.

[4] J. Rabassa, “Impact of Global Climate Change on Glaciers and Permafrost of South America, with Emphasis on Patagonia, Tierra del Fuego, and the Antarctic Peninsula,” in *Developments in Earth Surface Processes*, vol. 13, E. M. Latrubesse, Ed., Elsevier, 2009, pp. 415–438. doi: 10.1016/S0928-2025(08)10019-0.

[5] A. Rivera, J. C. Aravena, A. Urra, and B. Reid, “Chilean Patagonian Glaciers and Environmental Change,” in *Conservation in Chilean Patagonia: Assessing the State of Knowledge, Opportunities, and Challenges*, J. C. Castilla, J. J. Armesto Zamudio, M. J. Martínez-Harms, and D. Tecklin, Eds., Cham: Springer International Publishing, 2023, pp. 393–407. doi: 10.1007/978-3-031-39408-9_15.

[6] P. Convey and R. I. L. Smith, “Responses of terrestrial Antarctic ecosystems to climate change,” in *Plants and Climate Change*, J. Rozema, R. Aerts, and H. Cornelissen, Eds., Dordrecht: Springer Netherlands, 2006, pp. 1–12. doi: 10.1007/978-1-4020-4443-4_1.

[7] C. Colesie, C. V. Walshaw, L. G. Sancho, M. P. Davey, and A. Gray, “Antarctica’s vegetation in a changing climate,” *WIREs Clim. Change*, vol. 14, no. 1, p. e810, Jan. 2023, doi: 10.1002/wcc.810.

[8] N. Cannone, F. Malfasi, S. E. Favero-Longo, P. Convey, and M. Guglielmin, “Acceleration of climate warming and plant dynamics in Antarctica.,” *Curr. Biol. CB*, vol. 32, no. 7, pp. 1599-1606.e2, Apr. 2022, doi: 10.1016/j.cub.2022.01.074.

[9] K. A. Hughes *et al.*, “Invasive non-native species likely to threaten biodiversity and ecosystems in the Antarctic Peninsula region,” *Glob. Change Biol.*, vol. 26, no. 4, pp. 2702–2716, Apr. 2020, doi: 10.1111/gcb.14938.

[10] F. Hernández, C. Ríos, and H. L. Perotto-Baldivieso, “Evolutionary history of herbivory in the Patagonian steppe: The role of climate, ancient megafauna, and guanaco,” *Quat. Sci. Rev.*, vol. 220, pp. 279–290, Sep. 2019, doi: 10.1016/j.quascirev.2019.07.014.

[11] M. J. Jeffries and J. H. Lawton, “Enemy free space and the structure of ecological communities,” *Biol. J. Linn. Soc.*, vol. 23, no. 4, pp. 269–286, Dec. 1984, doi: 10.1111/j.1095-8312.1984.tb00145.x.

[12] E. C. Iranzo *et al.*, "Niche Segregation between Wild and Domestic Herbivores in Chilean Patagonia," *PLOS ONE*, vol. 8, no. 3, p. e59326, Mar. 2013, doi: 10.1371/journal.pone.0059326.
[13] G. Oliva, P. Paredes, D. Ferrante, C. Cepeda, and J. Rabinovich, "Are Patagonia grasslands being overgrazed? A response to Marino et al. (2020)," *J. Appl. Ecol.*, vol. 57, no. 12, pp. 2399–2405, Dec. 2020, doi: 10.1111/1365-2664.13753.
[14] A. del Pozo, C. Ovalle, M. A. Casado, B. Acosta, and J. M. de Miguel, "Effects of grazing intensity in grasslands of the Espinal of central Chile," *J. Veg. Sci.*, vol. 17, no. 6, pp. 791–798, 2006, doi: 10.1111/j.1654-1103.2006.tb02502.x.
[15] R. A. Chorbadjian, M. I. Ahumada, F. Urra, M. Elgueta, and T. M. Gilligan, "Biogeographical Patterns of Herbivore Arthropods Associated with Chenopodium quinoa Grown along the Latitudinal Gradient of Chile.," *Plants Basel Switz.*, vol. 10, no. 12, Dec. 2021, doi: 10.3390/plants10122811.
[16] C. C. Figueroa, E. Fuentes-Contreras, M. A. Molina-Montenegro, and C. C. Ramírez, "Biological and genetic features of introduced aphid populations in agroecosystems," *Ecol. • ParasitesParasitoidsBiological Control*, vol. 26, pp. 63–68, Apr. 2018, doi: 10.1016/j.cois.2018.01.004.
[17] M. E. O'Neal, A. J. Varenhorst, and M. C. Kaiser, "Rapid evolution to host plant resistance by an invasive herbivore: soybean aphid (Aphis glycines) virulence in North America to aphid resistant cultivars," *Ecol. • ParasitesParasitoidsBiological Control*, vol. 26, pp. 1–7, Apr. 2018, doi: 10.1016/j.cois.2017.12.006.
[18] K. J. Finlay and J. E. Luck, "Response of the bird cherry-oat aphid (Rhopalosiphum padi) to climate change in relation to its pest status, vectoring potential and function in a crop–vector–virus pathosystem," *Agric. Ecosyst. Environ.*, vol. 144, no. 1, pp. 405–421, Nov. 2011, doi: 10.1016/j.agee.2011.08.011.
[19] R. H. Messing, M. N. Tremblay, E. B. Mondor, R. G. Foottit, and K. S. Pike, "Invasive aphids attack native Hawaiian plants," *Biol. Invasions*, vol. 9, no. 5, pp. 601–607, Jul. 2007, doi: 10.1007/s10530-006-9045-1.
[20] J. E. Lee, S. Slabber, B. Jansen Van Vuuren, S. Van Noort, and S. L. Chown, "Colonisation of sub-Antarctic Marion Island by a non-indigenous aphid parasitoid Aphidius matricariae (Hymenoptera, Braconidae)," *Polar Biol.*, vol. 30, no. 9, pp. 1195–1201, Aug. 2007, doi: 10.1007/s00300-007-0277-z.
[21] M. E. Rubio-Meléndez, J. Barrios-SanMartin, F. E. Pina-Castro, C. C. Figueroa, and C. C. Ramirez, "Asexual reproduction of a few genotypes favored the invasion of the cereal aphid Rhopalosiphum padi in Chile," *PeerJ*, vol. 7, p. e7366, 2019.
[22] Y. Frenot *et al.*, "Biological invasions in the Antarctic: extent, impacts and implications," *Biol. Rev.*, vol. 80, no. 1, pp. 45–72, Feb. 2005, doi: 10.1017/S1464793104006542.
[23] M. Houghton, P. B. McQuillan, D. M. Bergstrom, L. Frost, J. van den Hoff, and J. Shaw, "Pathways of alien invertebrate transfer to the Antarctic region," *Polar Biol.*, vol. 39, no. 1, pp. 23–33, Jan. 2016, doi: 10.1007/s00300-014-1599-2.
[24] G. S. FRAENKEL, "The raison d'ětre of secondary plant substances; these odd chemicals arose as a means of protecting plants from insects and now guide insects to food.," *Science*, vol. 129, no. 3361, pp. 1466–1470, May 1959, doi: 10.1126/science.129.3361.1466.
[25] R. N. BENNETT and R. M. WALLSGROVE, "Secondary metabolites in plant defence mechanisms," *New Phytol.*, vol. 127, no. 4, pp. 617–633, Aug. 1994, doi: 10.1111/j.1469-8137.1994.tb02968.x.
[26] A. R. War *et al.*, "Mechanisms of plant defense against insect herbivores.," *Plant Signal. Behav.*, vol. 7, no. 10, pp. 1306–1320, Oct. 2012, doi: 10.4161/psb.21663.

[27] A. A. Agrawal, "Induced Responses to Herbivory and Increased Plant Performance," *Science*, vol. 279, no. 5354, pp. 1201–1202, Feb. 1998, doi: 10.1126/science.279.5354.1201.

[28] S. Christensen, C. Heimes, N. Agerbirk, V. Kuzina, C. E. Olsen, and T. P. Hauser, "Different Geographical Distributions of Two Chemotypes of Barbarea vulgaris that Differ in Resistance to Insects and a Pathogen," *J. Chem. Ecol.*, vol. 40, no. 5, pp. 491–501, May 2014, doi: 10.1007/s10886-014-0430-4.

[29] P. Medina-van Berkum *et al.*, "Plant geographic distribution influences chemical defences in native and introduced Plantago lanceolata populations," *Funct. Ecol.*, vol. 38, no. 4, pp. 883–896, Apr. 2024, doi: 10.1111/1365-2435.14535.

[30] T. Züst, C. Heichinger, U. Grossniklaus, R. Harrington, D. J. Kliebenstein, and L. A. Turnbull, "Natural Enemies Drive Geographic Variation in Plant Defenses," *Science*, vol. 338, no. 6103, pp. 116–119, Oct. 2012, doi: 10.1126/science.1226397.

[31] G.-R. Walther *et al.*, "Ecological responses to recent climate change," *Nature*, vol. 416, no. 6879, pp. 389–395, Mar. 2002, doi: 10.1038/416389a.

[32] O. I. Yudakova, V. S. Tyrnov, V. A. Kunakh, I. A. Kozeretskaya, and I. Yu. Parnikoza, "Adaptation of the seed reproduction system to conditions of Maritime Antarctic in Deschampsia antarctica E. Desv.," *Russ. J. Dev. Biol.*, vol. 47, no. 3, pp. 138–146, May 2016, doi: 10.1134/S1062360416030073.

[33] M. Alberdi, L. A. Bravo, A. Gutiérrez, M. Gidekel, and L. J. Corcuera, "Ecophysiology of Antarctic vascular plants," *Physiol. Plant.*, vol. 115, no. 4, pp. 479–486, Aug. 2002, doi: 10.1034/j.1399-3054.2002.1150401.x.

[34] M. Fasanella, A. C. Premoli, J. D. Urdampilleta, M. L. González, and J. O. Chiapella, "How did a grass reach Antarctica? The Patagonian connection of Deschampsia antarctica (Poaceae)," *Bot. J. Linn. Soc.*, vol. 185, no. 4, pp. 511–524, Nov. 2017, doi: 10.1093/botlinnean/box070.

[35] S. L. Mosyakin, L. G. Bezusko, and A. S. Mosyakin, "Origins of native vascular plants of antarctica: Comments from a historical phytogeography viewpoint," *Cytol. Genet.*, vol. 41, no. 5, pp. 308–316, Oct. 2007, doi: 10.3103/S009545270705009X.

[36] M. Angélica Casanova-Katny and L. A. Cavieres, "Antarctic moss carpets facilitate growth of Deschampsia antarctica but not its survival," *Polar Biol.*, vol. 35, no. 12, pp. 1869–1878, Dec. 2012, doi: 10.1007/s00300-012-1229-9.

[37] J. A. Fowbert and Ronald I. Lewis Smith, "Rapid Population Increases in Native Vascular Plants in the Argentine Islands, Antarctic Peninsula," *Arct. Alp. Res.*, vol. 26, no. 3, pp. 290–296, 1994, doi: 10.2307/1551941.

[38] S. G.K. and V. Karuppaiah, "Aftermath of climate change on insect migration: A review," *Agric. Rev.*, vol. 37, Sep. 2016, doi: 10.18805/ag.v37i3.3537.

[39] X. Peng *et al.*, "The Survival and Reproduction of Rhopalosiphum padi (Hemiptera: Aphididae) on Different Plants: Exploring the Possible Host Range for a Serious Wheat Pest," *J. Econ. Entomol.*, vol. 113, no. 1, pp. 185–193, Feb. 2020, doi: 10.1093/jee/toz263.

[40] A. Halarewicz and B. Gabryś, "Probing behavior of bird cherry-oat aphid Rhopalosiphum padi (L.) on native bird cherry Prunus padus L. and alien invasive black cherry Prunus serotina Erhr. in Europe and the role of cyanogenic glycosides," *Arthropod-Plant Interact.*, vol. 6, no. 4, pp. 497–505, Dec. 2012, doi: 10.1007/s11829-012-9228-x.

[41] A. F. G. DIXON, "The life-cycle and host preferences of the bird cherry-oat aphid, Rhopalosiphum padi L., and their bearing on the theories of host alternation in aphids," *Ann. Appl. Biol.*, vol. 68, no. 2, pp. 135–147, Jul. 1971, doi: 10.1111/j.1744-7348.1971.tb06450.x.

[42] G. Powell and J. Hardie, "The chemical ecology of aphid host alternation: How do return migrants find the primary host plant?," *Appl. Entomol. Zool.*, vol. 36, no. 3, pp. 259–267, 2001, doi: 10.1303/aez.2001.259.

[43] S. J. Simpson, G. A. Sword, and N. Lo, "Polyphenism in insects.," *Curr. Biol. CB*, vol. 21, no. 18, pp. R738-749, Sep. 2011, doi: 10.1016/j.cub.2011.06.006.

[44] R.-J. Zhang, J. Chen, L.-Y. Jiang, and G.-X. Qiao, "The genes expression difference between winged and wingless bird cherry-oat aphid Rhopalosiphum padi based on transcriptomic data," *Sci. Rep.*, vol. 9, no. 1, p. 4754, Mar. 2019, doi: 10.1038/s41598-019-41348-1.

[45] R Core Team, *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing, 2023. [Online]. Available: https://www.R-project.org/

[46] M. E. Brooks *et al.*, "glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling," *R J.*, vol. 9, no. 2, pp. 378–400, 2017, doi: 10.32614/RJ-2017-066.

[47] J. Fox and S. Weisberg, *An R Companion to Applied Regression*, Third. Thousand Oaks CA: Sage, 2019. [Online]. Available: https://socialsciences.mcmaster.ca/jfox/Books/Companion/

[48] R. V. Lenth, *emmeans: Estimated Marginal Means, aka Least-Squares Means*. 2024. [Online]. Available: https://CRAN.R-project.org/package=emmeans

[49] D. Bates, M. Mächler, B. Bolker, and S. Walker, "Fitting Linear Mixed-Effects Models Using lme4," *J. Stat. Softw.*, vol. 67, no. 1, pp. 1–48, 2015, doi: 10.18637/jss.v067.i01.

[50] A. Kuznetsova, P. B. Brockhoff, and R. H. B. Christensen, "lmerTest Package: Tests in Linear Mixed Effects Models," *J. Stat. Softw.*, vol. 82, no. 13, pp. 1–26, 2017, doi: 10.18637/jss.v082.i13.

[51] F. Hartig, *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*. 2022. [Online]. Available: https://CRAN.R-project.org/package=DHARMa

[52] A. Holtom, S. W. Greene, and J. E. Smith, "The growth and reproduction of Antarctic flowering plants," *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, vol. 252, no. 777, pp. 323–337, Jan. 1997, doi: 10.1098/rstb.1967.0021.

[53] R. Karban, "The ecology and evolution of induced resistance against herbivores," *Funct. Ecol.*, vol. 25, no. 2, pp. 339–347, Apr. 2011, doi: 10.1111/j.1365-2435.2010.01789.x.

[54] O. Huitu *et al.*, "Silicon, endophytes and secondary metabolites as grass defenses against mammalian herbivores," *Front. Plant Sci.*, vol. 5, 2014, [Online]. Available: https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2014.00478