BACHELOR’S ASSIGNMENT

The response of Vaccinium myrtillus to simulated warming and external application of a hormonal regulator at different altitudes.

Anne Steuer

Environmental Protection
External Student
Mark Andrew Gillespie
28.05.2018

I confirm that the work is self-prepared and that references/source references to all sources used in the work are provided, cf. Regulation relating to academic studies and examinations at the Western Norway University of Applied Sciences (HVL), § 10.
University of Applied Sciences Bingen

Faculty 1 – Life Sciences and Engineering

Environmental Protection

The response of *Vaccinium myrtillus* to simulated warming and external application of a hormonal regulator at different altitudes.

Bachelor thesis

28.05.2018

Steuer, Anne

External supervision:

Western Norway University of Applied Sciences
Acknowledgments

I am grateful for the great support of my supervisor Mark Andrew Gillespie, who introduced me to the work with R and guided me through the working process of this bachelor thesis with constructive comments and helpful suggestions. Furthermore I like to thank Prof. Dr. Elke Hietel, as my supervisor from the University of Applied Sciences in Bingen, for the successful cooperation despite the distance, which enabled me to write this thesis abroad and gather several unique experiences.

In this regard, I also want to thank Famke Geißler, who inspired me to stay in Sogndal, strengthened my power of endurance during the writing and who I experienced physically as well as mentally challenging trips with. Moreover special thanks to my family for proofreading several reports and always providing me with food and love. Finally I want to express my gratitude to Valérie Laschet, who not only assisted with the research of this work, but moreover accompanied me during my whole studies as a roommate and a true friend because we are like birds of a feather.
# Table of content

Acknowledgments.......................................................................................................................................... I

List of abbreviations...................................................................................................................................... IV

List of figures................................................................................................................................................ V

List of tables................................................................................................................................................ VI

Abstract......................................................................................................................................................... VII

1 Introduction ................................................................................................................................................ 1

2 Study method and equipment .......................................................................................................................... 5

   2.1 Terrain and environmental conditions .......................................................................................................... 5

   2.2 Experimental design and treatments ............................................................................................................... 5

   2.3 Sampling procedure and plant measurements .......................................................................................... 8

3 Statistical data analysis ................................................................................................................................. 9

   3.1 Data set ..................................................................................................................................................... 9

   3.2 Methods of analysis ................................................................................................................................ 10

4 Results ......................................................................................................................................................... 12

   4.1 Growth ...................................................................................................................................................... 14

      4.1.1 Effect of simulated warming ............................................................................................................... 14

      4.1.2 Effect of MeJa...................................................................................................................................... 15

   4.2 Grazing .................................................................................................................................................... 16

      4.2.1 Leaf grazing insects .............................................................................................................................. 16

      4.2.2 Shoot grazing by deer .......................................................................................................................... 17

   4.3 Reproduction .......................................................................................................................................... 18

   4.4 Weather data of 2016 and 2017 ................................................................................................................ 19

5 Discussion .................................................................................................................................................... 20

   5.1 Influence of MeJa application ................................................................................................................... 20

      5.1.1 Reproduction ................................................................................................................................... 20

      5.1.2 Grazing ............................................................................................................................................ 22

      5.1.3 Growth .......................................................................................................................................... 25

   5.2 Influence of simulated warming .................................................................................................................. 28

      5.2.1 Reproduction ................................................................................................................................... 28

      5.2.2 Grazing ............................................................................................................................................ 29

      5.2.3 Growth .......................................................................................................................................... 30
6 Conclusion ................................................................................................. 32

List of Appendices .................................................................................... 34
  List of Supplementary Figures ............................................................... 34
  List of Supplementary Maps .................................................................. 34
  List of Supplementary Tables ............................................................... 35
Appendix .................................................................................................... 36
  Supplementary Figures .......................................................................... 36
  Supplementary Maps ........................................................................... 38
  Supplementary Tables .......................................................................... 39
References ................................................................................................ 42
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. myrtillus</td>
<td><em>Vaccinium myrtillus</em></td>
</tr>
<tr>
<td>Id.</td>
<td>Idem</td>
</tr>
<tr>
<td>Ibid.</td>
<td>ibidem</td>
</tr>
<tr>
<td>Jas</td>
<td>Jasmonates</td>
</tr>
<tr>
<td>MeJa</td>
<td>methyl-jasmonate</td>
</tr>
<tr>
<td>VOC</td>
<td>Volatile Organic Compound</td>
</tr>
<tr>
<td>OTC</td>
<td>Open-top chamber</td>
</tr>
<tr>
<td>DM</td>
<td>Dry mass</td>
</tr>
<tr>
<td>DS</td>
<td>Stem diameter</td>
</tr>
<tr>
<td>H</td>
<td>Height</td>
</tr>
<tr>
<td>AS</td>
<td>Amount of shoots</td>
</tr>
<tr>
<td>GLM</td>
<td>Generalized linear model</td>
</tr>
<tr>
<td>GLMM</td>
<td>Generalized linear mixed model</td>
</tr>
<tr>
<td>DF</td>
<td>Degrees of freedom</td>
</tr>
<tr>
<td>F</td>
<td>F-value, Fisher’s value</td>
</tr>
<tr>
<td>P</td>
<td>P – Value, Probability value</td>
</tr>
<tr>
<td>W</td>
<td>Water treatment</td>
</tr>
<tr>
<td>M</td>
<td>methyl-jasmonate treatment 30</td>
</tr>
<tr>
<td>°C</td>
<td>Grad Celsius</td>
</tr>
<tr>
<td>mM</td>
<td>millimolar</td>
</tr>
<tr>
<td>Dev</td>
<td>Deviance</td>
</tr>
</tbody>
</table>
List of figures

Figure 1: Significant impact on the gain in dry mass in 2017 by the temperature treatment (ambient, OTC).......................................................................................................................... 14

Figure 2: Significant impact on the gain in dry mass in 2017 by the interaction between site (low, medium, high) and temperature (ambient, OTC)...................... 14

Figure 3: Significant impact on the increase of the amount of leaves in 2016 by the treatment with MeJa or water ......................................................................................... 15

Figure 4: Significant impact on the increase of the amount of leaves in 2016 by the interaction between site (low, medium, high) and treatment (MeJa, water)........... 15

Figure 5: Significant impact on the increase of grazed leaves in 2016 by site (low, medium, high) .................................................................................................................. 16

Figure 6: Significant impact on the increase of grazed leaves in 2017 by site (low, medium, high) .................................................................................................................. 16

Figure 7: Significant impact on the increase of grazed leaves in 2016 by the interaction between site (low, medium, high) and treatment (MeJa, water) .............. 16

Figure 8: Significant impact on the increase of grazed leaves in 2017 by the interaction between site (low, medium, high) and temperature (ambient, OTC).... 16

Figure 9: Significant impact on the total amount of flowers in 2017 by the treatment with MeJa or water) ................................................................................................. 18

Figure 10: Significant impact on the total amount of flowers in 2017 by the interaction between site (low, medium, high) and treatment (MeJa, water)........... 18

Figure 11: Significant impact on the total amount of flowers in 2017 by temperature (ambient, OTC) ................................................................................................. 18

Figure 12: Significant impact on the total amount of flowers in 2017 by the bilberry coverage ............................................................................................................... 18
List of tables

Table 1: Overview of statistical responses after running the maximal linear mixed effect models fitted for all response variables including the three explanatory variables (site, temperature, treatment) and all two-way interactions. (Part 1) .... 12

Table 2: Overview of statistical responses after running the maximal linear mixed effect models fitted for all response variables including the three explanatory variables (site, temperature, treatment) and all two-way interactions. (Part 2) ... 13
Abstract

*Vaccinium myrtillus* fulfils an essential role as a primary producer in boreal ecosystems as it provides shelter and nutritive food for several invertebrate and vertebrate species. The study investigates responses of *V. myrtillus* in a pine-bilberry ecosystem to simulated warming with open-top chambers (OTC) and the application of the hormonal regulator methyl-jasmonate (MeJa) along an elevational gradient over a period of two years (2016 – 2017). Aiming at providing additional information about the direct impacts on the plants itself and improving the understanding of associated indirect effects on other constituents in the ecosystem that may occur in the course of climate change.

Plant defences against herbivory induced by the hormonal regulator were assumed to increase the resistance against herbivores while restricting the growth and reproduction of the plants due to the reallocation of resources. Whereas simulated warming was suggested to favour the plant development and emphasizing the response to the hormonal regulator. A uniform trend of significantly reduced amount of flowers was found in 2017, when bilberry were treated with MeJa. Moreover, in consequence of MeJa application and natural grazing, a negative impact on the growth of plants at the lowest elevation was observed in 2016, since plants suffered a significant loss of leaves and were restricted in their vertical growth. Also the resistance against invertebrate herbivores increased in that case, indicating the upregulation of defence genes at the expense of growth and reproduction. The study could not confirm whether simulated warming affects the responses of bilberry to the application of MeJa.
1 Introduction

Warmer temperatures, as they are predicted in prospective climate scenarios, are likely to have complex impacts on the abundance and distribution of plant species in boreal ecosystems\(^1\). Former studies indicate, that besides increasing temperature itself locally changing weather phenomena will also cause shifts in prevailing and dominating flora and fauna species. Some shrubs for instance are anticipated to gain more benefits from climate-induced alterations in the ecosystems compared to other plant species, as they may take advantage of shifts in nutrient availability or soil disturbances\(^2\). However too little is known about the responses of shrubs and their interactions with other species to evaluate how changing climatic conditions may affect the communities and ecosystems. For example vegetal shifts have direct and indirect impacts on the vertebrate and invertebrate herbivores that rely on the plants for food\(^3\).

Thus, this study aims at improving the understanding of the dominant species *Vaccinium myrtillus* in a boreal alpine ecosystem, since it fulfils an essential role in the food chain as a primary producer. *Vaccinium myrtillus*, also known as European blueberry or bilberry, is a deciduous dwarf shrub with evergreen stems and usually reaches a height of 15 – 60 cm.\(^4\) Its maximum vegetative and reproductive development can be observed in low to moderately fertile woodlands\(^5\), especially in pine forests due to its relatively high shade-tolerance\(^6\).

---

Its early spring bloom as well as its deciduous leaves in summer are beneficial for several species within the ecosystem feeding on bilberry\(^7\). Moreover the berry formation 2 – 4 weeks after the pollination\(^8\) and the evergreen stems in winter provide essential nutrient supply for e.g. birds, mammals and invertebrates\(^9\). Additionally its ecological importance can be highlighted by its shelter function within a habitat (ibid.). Altogether \textit{V. myrtillus} can be determined as a key species in boreal and alpine ecosystems and hence is well suited to represent possible intra and inter species reactions in the investigated mountain area (Hegland, Seldal, Lilleeng and Rydgren, 2016). For instance as Hegland et al. states, correlations between population sizes of invertebrate as well as vertebrate herbivores feeding on bilberry and the abundance of the plant itself have been proven in several former studies (ibid.). However only little information is available about if and to which extent warmer temperatures in combination with accompanying changes in grazing patterns may affect bilberry and its role in the ecosystem. Gaining more information about how key species, such as \textit{V. myrtillus}, respond to those external forces is crucial to understand the effects that changing climatic conditions may have on the earth’s ecosystem. Therefore this study investigates how \textit{Vaccinium myrtillus} in a pine-bilberry ecosystem responds at different altitudes to simulated warming and external application of a hormonal regulator in order to simulate herbivory induced defence responses.

---

\(^7\) Benevenuto, Rafael Fonseca, Hegland, Stein Joar, Töpper, Joachim Paul, Rydgren, Knut, Moe Stein R., Rodriguez-Saona, Cesar and Seldal, Taral. Multiannual effects of induced plant defenses: Are defended plants good or bad neighbors? (unpublished).


Hormonal regulators in plants, such as Jasmonates (Jas) control several developmental processes (Bennett and Wallsgrove, 1994) including seed germination, root growth, flowering, fruit ripening and senescence\textsuperscript{10,11,12}. For instance the volatile compound methyl-jasmonate (MeJa) is not only known to regulate physiological processes but also to be involved in the signal transduction in order to activate plant defence mechanisms\textsuperscript{13}. Those processes can be induced by external stimuli\textsuperscript{14} such as herbivore or insect-driven wounding and are expressed by a rise in the responsible compounds followed by the mobilization of various defence genes (Cheong and Choi, 2003).

Previous laboratory and field studies have shown, that the defence genes of Vaccinium plants are activated by the exogenous application of methyl-jasmonate (MeJa), subsequently deterring insect attacks (Benevenuto et al., in press). Thus in this study MeJa has been used to elicit bilberry defence responses, which are expected to be reflected in the plant growth and reproduction, as the hormonal regulator may also affect diverse physiological processes\textsuperscript{15}, such as growth inhibition or promotion of senescence and abscission (Farmer and Ryan, 1990).


Following the findings of previous studies, the hormonal regulator is expected to initiate defence responses and lower the plant’s attractiveness to potential herbivores and concurrently negatively affect growth and reproduction indicating an allocation of the plant resources. Additionally one can assume, that simulated warming favours the plant development, since the reproductive performance of bilberry is especially closely related to temperature (Ritchie, 1956). Moreover the increase in temperature in combination with the application of methyl-jasmonate is expected to emphasize the plant responses to the hormonal regulator, since plants are expected to be more alert to react to herbivory during a warmer climate.\footnote{Cornelissen, T., 2011. Climate change and its effects on terrestrial insects and herbivory patterns, vol. 40. \textit{Neotrop. entomol.} (2), 155–163. 10.1590/S1519-566X2011000200001.}

Furthermore plant growth and the occurrence of reproductive traits are also anticipated to differ along altitudinal gradients, since the plant performance may vary within its habitat range as it is dependent on environmental conditions, such as local climate or nutrient availability.\footnote{Nestby, Rolf, Percival, David, Martinussen, Inger, Opstad, Nina and Rohloff, Jens, 2011. The European Blueberry (Vaccinium myrtillus L.) and the Potential for Cultivation.: Global Science Books. \textit{European Journal of Plant Science and Biotechnology} (Volume 5), 5–16. Accessed 12 April 2018.} In summary the investigation of \textit{V. myrtillus} responses to climate alterations and hormonal regulation in a pine-bilberry ecosystem will improve the understanding of the direct impacts on the plant itself and the associated indirect effects on other surrounding biotic constituents.
2 Study method and equipment

The performance of a field study has been favoured, since it is likely to display more valuable information about the plant responses within the ecosystem than laboratory studies. The plants are exposed to several environmental stressors and the group of sampled species represents a broader genetical variability, when investigated within their natural habitat. However the results of a field study may be less distinct compared to a laboratory study due to its higher level of complexity (Hegland, Seldal, Lilleeng and Rydgren, 2016).

2.1 Terrain and environmental conditions

The three study sites used in this project are located within a pine-bilberry ecosystem on the south facing hillside of Storehaugfjellet near Kaupanger, Western Norway (61°9'52"N 7°8'5"E, Supplementary Map 1). The sampling locations are situated in recently cut clearings within pine forest in order to minimize variations in the micro climate, for instance caused by differing insolation and shadowing effects. As depicted in the Supplementary Figure 1, the study sites are established at three different altitudes (~100 m, 450 m and 900 m) to achieve a range of temperature and snow melt timing regimes. Furthermore these altitudes are likely to represent the elevational range of the focal plant species, Vaccinium myrtillus, with the 450m site providing optimal growing conditions and the lower and upper sites at the edges of the range. These sites will be referred to as Low, Medium and High throughout this thesis.

2.2 Experimental design and treatments

In May 2016, six experimental blocks were established at each of the three different elevated locations in order to consider the impact of altitude on plant processes during the study. They are placed within relatively homogenous vegetation ensuring a good representation of the target plant V. myrtillus. At the low (100 masl) and middle (450 masl) sites the blocks measured 10 x 10 m and were at least 10 m apart from each other.
In the four corners of each block, 2 x 2 m grids of 16 squares (50 x 50 cm) were marked out and then one square was randomly selected using a random number generator. Each chosen square became an established treatment plot (2.5 m²) if it contained a good cover of *V. myrtillis*. If this could not be ensured, for example due to a tree stump or bare rock, an adjacent plot was selected. This selection procedure was conducted to provide semi-random selection of plots within blocks and to ensure that plots were at least 4 m apart. The square block design was not possible at the high site due to the sparsity of vegetation, hence the plots were most often arranged linearly.

In order to prevent the activation of defence mechanism through plant communication, each plot was at least 4 m away from its nearest neighbour, based on the findings of Benevenuto et al. (in press). Each plot within the study blocks was randomly assigned to one of the four different plant treatments:

1) Ambient temperature and water application (Control)
2) Ambient temperature and methyl jasmonate application
3) Elevated temperature and water application
4) Elevated temperature and methyl jasmonate application

Water and MeJa (10mM), based on the study of Benevenuto et al. (in press), were sprayed on assigned plots with a 5 liter knapsack sprayer three times during the summer 2016 (Low site: 26 May, 3 and 14 June; Middle site: 2, 10 and 19 June; High site: 10, 19 and 30 June). Each application involved steadily spraying the liquid over the plot twice to ensure complete coverage. In this study, the exogenous application of MeJa has been favoured over the clipping method, which attempts to simulate herbivory related stress by imitating the grazing process. Even though the latter might evoke defence responses as well, it is known that herbivore specific cues, transmitted by their salvia, are involved and required to fully activate the activation of defence mechanisms (Agrawal, Tuzun and Bent, 2000, 126–127; Hegland, Seldal, Lilleeng and Rydgren, 2016). The chemical method using MeJa may generate plant defence responses, yet this method is not able to differ between specific herbivory types as invertebrates or vertebrates (ibid.).
In order to increase the temperature and simulate a warmer climate open-top chambers (OTCs) were installed on the same day as the first exogenous water and MeJa applications in 2016. Previous studies revealed, that the hexagonal miniature greenhouses, constructed of transparent polycarbonate panels, elevate the temperature on plots by average of 1°C\textsuperscript{18}. The OTCs were removed at the end of the growing seasons (28 October 2016 and 11 October 2017) and replaced in spring the subsequent year to prevent the accumulation of snow during the winter. Open-top chambers were preferred over other simulated warming techniques, such as soil heating cables or infrared heaters, in order to minimize costs and effort regarding the study set-up.

Four ramets of *V. myrtillus* were selected within each plot by placing a metal quadrat (50 x 50 cm) featuring strings to create a grid of 16 squares (12.5 x 12.5 cm) over the plot. Marker sticks were then inserted into the soil at the grid cross-points closest to the four corners. The nearest *V. myrtillus* ramet to each stick was then marked with a cable tie and small coloured bead, so that the same ramets could be measured repeatedly.

2.3 Sampling procedure and plant measurements

In advance of the first spraying (26 May 2016 (Low), 2 June 2016 (Medium) and 10 June 2016 (High)), growth and herbivory impact on the four individual ramets in each plot were recorded. Each ramet was measured for 1) stem diameter at surface level (using digital calipers), 2) ramet height, 3) number of leaves, 4) number of annual shoots, 5) number of leaves grazed by insects, 6) number of shoots browsed by deer.

Approximately 8 – 9 weeks after the initial recording, when *V. myrtillus* was assumed to have ceased growing for the season, the measurements were repeated (Low: 17 July 2016, Medium: 25 July 2016, High: 1 August 2016). In 2017 the measurements were conducted in the same way, first on 22 May 2017 (Low), 5 June 2017 (Medium) and 26 June 2017 (High), and then on 29 July 2017 (Low), 3 August 2017 (Medium) and 24 August 2017 (High). Additionally the dry mass data was calculated with the equation from Hegland et al. as non-destructive estimation:\(^{19}\):

\[
\log_2(DM) = 1.41700 \times \log_2(DS) + 0.97104 \times \log_2(H) + 0.44153 \\
\times \log_2(AS + 1) - 7.52070
\]

Since this model exclusively uses the stem diameter (DS), height (H) measurements and the number of annual shoots (AS) for its calculation, it allows the estimation of biomass data without detaching the whole plant from its habitat. The differences in measurements between the two measuring dates for each parameter, are used as response variables in the subsequent analysis.

Furthermore in 2017, the number of flowers occurring in each plot was counted every week during the flowering season as a measure of reproductive effort. The maximum number of flowers per plot was then used as a response variable in the analysis.

---

3 Statistical data analysis

The statistical analysis was performed using the software programme R, Version 3.4.3 (2017-11-30)\textsuperscript{20} in order test the impacts of the different treatment combinations on the plant responses. Predominately linear mixed effect models were chosen to compare the treatment plots with the untreated control ramets due to the block design of the experiment.

3.1 Data set

The compacted data set comprises the explanatory variables 1) block, 2) site (Low, Medium, High), 3) temperature (Ambient, OTC), and 4) treatment (Water or MeJa), with the effects emanating from the three latter are of most interest. Therefore the modelling considered the impact of these variables on all of the response variables listed below.

- Stem
- Height
- Leaves
- Grazed leaves
- Shoots
- Grazed shoots
- Dry mass (calculated)
- Total amount of flowers (only in 2017)

Each response variable (except for flowers) describes the difference between the initial and the second recording in each year, as described in the prior chapter (3.3). To simplify the analysis, the values of the four sampled ramets in each plot are averaged to gain a single value for each plot, rather than four non-independent values. The response variables were initially inspected visually for their distribution and it was found that all approximate a normal distribution except for the flower data, which approximately displays a Poisson distribution.

3.2 Methods of analysis

Initially maximal linear mixed effects models\textsuperscript{21} were fitted for all response variables with the three categorical (id., 411) explanatory variables 1) site (three levels), 2) temperature (two levels), 3) treatment (two levels) and all two-way interactions as fixed effects and block as a random effect (id., 682) to take into account the block design of the study. After simplifying the models by stepwise deletion of non-significant variables, the minimal adequate model was checked for validity by visual inspection for constancy of variance and normality of residuals. There were no validity issues, except that the removal of outliers was performed for the models of the number of leaves (removal of one outlier) and shoots (removal of two outliers) for 2016 in order to gain normally distributed residuals. The removal of outliers did not qualitatively affect the results of the analysis, and the results presented here are from models with the outliers excluded.

The model for grazed leaves for the same year (2016) also contained four outliers, but in this case, a log transformation of the data was favoured over the removal of the outliers in order to avoid diluting the data set. Due to the presence of negative values in this variable, the transformation was achieved by adding the absolute value of the smallest number and adding the constant 1 to all values to guarantee a valid logarithmic transformation all values. This method was not applicable for the previous mentioned variables, because the log transformation caused a non-normal distribution of the data. The flower data from 2017 represents count data and hence requires the use of a generalized linear model (GLM) with poisson distributed errors. The initial and final models for this response variable suffered from overdispersion\textsuperscript{22}, so this was taken into account by specifying “quasipoisson” errors (id., 561). In order to operate a valid analysis the cover data of the corresponding year (2017) was also included in that model, since the coverage ratio of \textit{V. myrtillus} is likely to have an impact on the total amount of flowers in the equivalent plot.


\textsuperscript{22} If the residual deviance is greater than the residual degrees of freedom in the minimal adequate model, it is called overdispersion. Since this is contravening the assumptions of the model it is necessary to correct this by specifying the errors, as mentioned above (Crawley, Michael J., 2013).
The cover of bilberry within each plot was estimated by visual judgement. The blocking design was not taken into account in the analysis of the flower data, since this would require the specification of a generalized linear mixed model (GLMM, beyond the scope of this study). However, in previous work, the blocking factor was found to have a negligible effect on this variable\textsuperscript{23}.

4 Results

Table 1: Overview of statistical responses after running the maximal linear mixed effect models fitted for all response variables including the three explanatory variables (site, temperature, treatment) and all two-way interactions.\(^{24}\) (Part 1)

<table>
<thead>
<tr>
<th>Response</th>
<th>Treatment</th>
<th>DF</th>
<th>2016 F</th>
<th>2016 P</th>
<th>2017 F</th>
<th>2017 P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry mass</td>
<td>Treatment</td>
<td>1</td>
<td>2.394</td>
<td>0.129</td>
<td>1.365</td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>0.367</td>
<td>0.548</td>
<td>4.503</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>1.780</td>
<td>0.180</td>
<td>3.243</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>1.515</td>
<td>0.230</td>
<td>0.099</td>
<td>0.906</td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>2.266</td>
<td>0.115</td>
<td>6.747</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Temperature:Treatment</td>
<td>1</td>
<td>2.043</td>
<td>0.160</td>
<td>0.807</td>
<td>0.373</td>
</tr>
<tr>
<td>Leaves</td>
<td>Treatment</td>
<td>1</td>
<td>40.855</td>
<td>&lt;0.001</td>
<td>2.372</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>0.017</td>
<td>0.897</td>
<td>1.625</td>
<td>0.208</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>2.430</td>
<td>0.097</td>
<td>0.766</td>
<td>0.470</td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>2.932</td>
<td>0.062</td>
<td>0.417</td>
<td>0.661</td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>0.034</td>
<td>0.966</td>
<td>1.680</td>
<td>0.196</td>
</tr>
<tr>
<td></td>
<td>Temperature:Treatment</td>
<td>1</td>
<td>0.087</td>
<td>0.769</td>
<td>1.250</td>
<td>0.268</td>
</tr>
<tr>
<td>Grazed leaves</td>
<td>Treatment</td>
<td>1</td>
<td>1.024</td>
<td>0.316</td>
<td>0.115</td>
<td>0.736</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>1.139</td>
<td>0.290</td>
<td>2.277</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>11.771</td>
<td>7.00E-04</td>
<td>4.347</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>4.726</td>
<td>0.013</td>
<td>2.578</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>0.096</td>
<td>0.098</td>
<td>4.077</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>Temperature:Treatment</td>
<td>1</td>
<td>0.809</td>
<td>0.372</td>
<td>0.076</td>
<td>0.784</td>
</tr>
<tr>
<td>Grazed shoots</td>
<td>Treatment</td>
<td>1</td>
<td>0.196</td>
<td>0.660</td>
<td>2.372</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>0.400</td>
<td>0.530</td>
<td>1.625</td>
<td>0.208</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>1.898</td>
<td>0.159</td>
<td>0.766</td>
<td>0.470</td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>1.012</td>
<td>0.370</td>
<td>0.417</td>
<td>0.661</td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>0.595</td>
<td>0.555</td>
<td>1.680</td>
<td>0.196</td>
</tr>
<tr>
<td></td>
<td>Temperature:Treatment</td>
<td>1</td>
<td>0.021</td>
<td>0.885</td>
<td>1.250</td>
<td>0.268</td>
</tr>
</tbody>
</table>

\(^{24}\) The minimal model p-values can be found in the supplementary table 2, whereas some differ slightly from the maximal model p-values displayed in this table. The interaction of the site and MeJa treatment for the amount of leaves variable was found to be significant after model simplification, whereas in the maximal model only the single MeJa treatment caused a significant response.
Table 2: Overview of statistical responses after running the maximal linear mixed effect models fitted for all response variables including the three explanatory variables (site, temperature, treatment) and all two-way interactions. (Part 2)

<table>
<thead>
<tr>
<th>Response</th>
<th>Treatment</th>
<th>DF</th>
<th>2016</th>
<th>2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amount of flowers</td>
<td>Treatment</td>
<td>1</td>
<td>-</td>
<td>1,094.490</td>
</tr>
<tr>
<td></td>
<td>Cover</td>
<td>1</td>
<td>-</td>
<td>229.330</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>-</td>
<td>14.100</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>-</td>
<td>37.690</td>
</tr>
<tr>
<td></td>
<td>Treatment:Cover</td>
<td>1</td>
<td>-</td>
<td>11.670</td>
</tr>
<tr>
<td></td>
<td>Treatment:Site</td>
<td>2</td>
<td>-</td>
<td>67.370</td>
</tr>
<tr>
<td></td>
<td>Treatment:Temperature</td>
<td>1</td>
<td>-</td>
<td>11.610</td>
</tr>
<tr>
<td></td>
<td>Cover:Site</td>
<td>2</td>
<td>-</td>
<td>1.630</td>
</tr>
<tr>
<td></td>
<td>Cover:Temperature</td>
<td>1</td>
<td>-</td>
<td>0.590</td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>-</td>
<td>30.810</td>
</tr>
</tbody>
</table>

The statistically significant effects differ in the two study years, yet the comprehensive data set allows to subdivide the results into detected impact on plant growth, herbivory and reproduction. The stem diameter, plant height, amount of leaves and new shoots, as well as the resultant dry mass data represent the progress of growth. In terms of simplification the single variables height, stem diameter and new shoots are not listed and discussed explicitly below, because they are integrated in the dry mass estimation. However they can be found in the appendix (Supplementary Table 1, Supplementary Table 2). The impact of herbivory is supposed to be demonstrated by the amount of leaves attacked by insects and the number of shoots grazed by deer.

Table 1 and Table 2 display the responses of the tested variables after running the maximum model and compares both study years. The red coloured values highlight statistically significantly values (p < 0.05). A similar structured table, exclusively including significant responses after model simplification can be found in the appendix (Supplementary Table 2).
4 Results

4.1 Growth

4.1.1 Effect of simulated warming

The response of dry mass in 2017 demonstrates accelerated growth within the tested period for plots equipped with open-top chambers (Figure 1). However, this phenomenon cannot be supported by the data of the previous year. Even though the ramets in 2016 show a positive impact of simulated warming on plant growth of *V. myrtillus* at high altitude (Supplementary Figure 5), this effect is not visible in the calculated dry mass data. This may be due to the lack of significant impacts on the variables stem and shoots.

Considering the interaction between site and temperature and its influence on the gain in dry mass, a distinct difference between the temperature treatments can neither be detected at low site nor at the high site. At the middle site though, the gain in dry mass is significantly higher within plots exposed to simulated warming compared to ramets grown at ambient temperature (Figure 2).

*Figure 1: Significant impact on the gain in dry mass in 2017 by the temperature treatment (ambient, OTC)*

*Figure 2: Significant impact on the gain in dry mass in 2017 by the interaction between site (low, medium, high) and temperature (ambient, OTC)*
4.1.2 Effect of MeJa

The amount of leaves of plants treated with MeJa drastically reduced within the sampling period, whereas the plants sprayed with water were not affected by this defoliation process (Figure 3) \(^{25}\). This impact is visible at all study sites, while it is most pronounced in the lower and higher altitudes than at the medium site, where no significant p-value could be registered in the statistical testing (Figure 4).

The responses of other growth variables to the methyl-jasmonate treatment are limited in both study years. The data set of 2016 exhibits significantly reduced vertical growth of plants at the low site, when treated with MeJa compared to plants sprayed with water. However no similar effects can be detected for the medium or high site during the same growing period (Supplementary Figure 6).

Controversial are the results for the same response variable height in the second year (2017), regarding to the MeJa impact on *V. myrtillus*. Opposite to the expectations, the sampled ramets experienced an increased growth when treated with MeJa in the second year of the study (1 year after MeJa application, Supplementary Figure 7). Since no other statistically significant impact on growth variables could be proven during the modelling, it is challenging to gain information about the influence of the methyl-jasmonate application on the plant development from the data.

\(^{25}\) The term “treatment” in the figures and their captions refers to the application of either water or methyl jasmonate.
### 4.2 Grazing

#### 4.2.1 Leaf grazing insects

Figure 5: Significant impact on the increase of grazed leaves in 2016 by site (low, medium, high)

Figure 6: Significant impact on the increase of grazed leaves in 2017 by site (low, medium, high)

Figure 7: Significant impact on the increase of grazed leaves in 2016 by the interaction between site (low, medium, high) and treatment (MeJa, water)

Figure 8: Significant impact on the increase of grazed leaves in 2017 by the interaction between site (low, medium, high) and temperature (ambient, OTC)

Figure 5\(^{26}\) displays significantly more grazing at the low site during the study period in 2016 compared to both other sites, independently from temperature (ambient or OTC). Whereas in 2017 grazing pressure was most intense at the medium site (Figure 6) with significantly higher grazing levels compared to the high site. The tendency at the lowest location however, was decreasing after grazing was most dominantly there the previous year (2016), especially within water treated plots (Figure 7).

---

\(^{26}\) The statistical testing of the grazed leaves variable (2016) is based on a logarithmic transformation after addition of a constant to the raw data values in order to valid the modelling. However the depicted graphs display the values before transformation to simplify comparison and ensure a better understanding.
Furthermore the low site is the only site demonstrating a significant difference between its MeJa and water treated plots in 2016, because the data exhibits significantly reduced grazing for the MeJa applied plots. While the graph demonstrates a similar tendency at medium site, the grazing at high site even appears to be slightly increased after MeJa application. Nevertheless none of the latter trends provide significant differences between water and MeJa treated plots based on the change in insect herbivory.

Little variation between the different temperature treatments can be detected in 2017. However significantly more insect grazed leaves can be found inside OTCs at medium site, underlining the tendency of increased grazing (Figure 8). Additionally less grazing (compared to OTC plots at the medium site) was found at the low site grown at ambient temperature reflecting the trend of decreasing grazing at that location during the second year of the study (2017).

4.2.2 Shoot grazing by deer

There were no significant differences in grazed shoots for any of the explanatory variables.
4 Results

4.3 Reproduction

The most distinct effect on the amount of flowers as a measure for the reproductive effort, can be assigned to the treatment with the hormonal regulator (MeJa). The number of flowers is significantly reduced within the plots treated with methyl-jasmonate (Figure 9). In addition the effect can clearly be seen for all three sites \((p<0.05)\), with the biggest difference at the medium site, (Figure 10). It should also be noted, that not a single flower could be found at the high site within the plots where methyl-jasmonate has been applied. Additionally a positive correlation could be found for the amount of flowers and the coverage of bilberry in the study plots.
Hence Figure 12 demonstrates, that with an increasing percentage cover of *V. myrtillus*, the number of flowers tends to increase as well. Less distinct but still significant were the responses towards the temperature treatments. On average, slightly less flowers could be counted inside OTC plots compared to plots without the simulated temperature increase, but this was only weakly significant (p=0.035) (Figure 11).

4.4 Weather data of 2016 and 2017

Due to the likely importance of weather in interpreting the results from the two years, weather data for monthly precipitation, temperature and the snow depth were collected from the Norwegian Meteorological Institute. The average temperatures of both years differ only slightly, yet the data reports higher monthly average values for 2016 in the study period (Beginning of May – End of July). In particular in June 2016, the mean temperature exceeds that of 2017. Moreover the average temperature reached in March 2016 was already a positive value, but still below zero in 2017, indicating a warmer spring in 2016.

The precipitation was generally higher in 2017 and the monthly values exceed the generally expected amount of precipitation in every month of the study period. The data for May and June 2016 are close to the estimated standards, while July of that year was affected by increased rainfall. (Supplementary Table 3) Low precipitation values in October and November 2016 are reflected in the low snow depth values of the following winter (Supplementary Figure 2).27

5 Discussion

The results of this study are relatively heterogenic, leading to accept some of the previous stated hypotheses and reject others. Some observed plant reactions may affirm the predictions regarding an inhibiting effect on growth and reproduction initiated by the application of MeJa, as well as the positive effect of simulated warming on plant growth. Moreover only in 2016 a uniform trend was found at low site regarding to responses of the growth variables and the insect grazing after MeJa application. However the data does not provide consistent information about the combined impact of simulated warming and MeJa application on bilberry. Varying responses of bilberry were found for interactions between the temperature or MeJa treatment and the site, suggesting that the sensibility of the plants to the different treatments varies along an elevational gradient.

5.1 Influence of MeJa application

5.1.1 Reproduction

The most distinct responses of wild bilberries under natural environment to the methyl-jasmonate application can be found, when considering the total amount of flowers in the different treatment plots. The data provides a strongly negative effect between the MeJa treatment and the amount of flowers in the second study year and hence supports the statement of constrained reproduction of *V. myrtillus*. This phenomenon may be explained by the hypothesis of resource allocation, which suggests, that the costs associated with the activation of defence mechanism restrict the available resources for other plant functions such as reproduction. Considering the combined impact of the MeJa treatment and the site, all studied sites provide the uniform trend of reduced flower development of ramets applied with MeJa. This leads to the conclusion that the statement of hormonal triggered restriction of reproductive mechanisms can be reached.

Generally, growth favouring conditions at the medium site, as it is suggested to be the optimal habitat for bilberry (Ritchie, 1956; Pato and Obeso, 2012), may be the reason why the highest amount of flowers could be found there within the control plots. Since this value is exceeding the number of flowers of the water treated ramets at both other sites, the most pronounced difference can be observed at medium altitude. Contrariwise Pato et al. (2012) observed the reproductive maximum at higher elevation and refers to the required cold temperatures to successfully initiate the formation of floral buds. However, the benefit of cold conditions for bilberry flowering may be counteracted by the increasing severity of abiotic forces, such as reduced air temperature and atmospheric pressure or intensified solar radiation\textsuperscript{29} and the necessity of warmth in spring to initiate the actual flowering\textsuperscript{30}. Asides those climatic factors, the nutrient limitation in the highlands (Pato and Obeso, 2012) may be a reason for the inhibition of the flower development of MeJa treated plants at the high site. Fabbro and Körner (2004) found that at high elevation the reallocation of biomass to reproductive structures, such as the development of flowers, is prioritised over maximisation of growth in order to compensate reduced pollinator diversity and abundance\textsuperscript{31}. Consequently the resources attributed to the plant growth may already be minimized and the investment in defence mechanisms restricts the available amount of resources, which can be devoted to reproductive traits.

The similar effect on low site may also be attributed to external forces, whereas there especially moisture is a limiting factor due to lower precipitation (Pato and Obeso, 2012). However during the study period in 2017, the amount of rain even exceeded the expected average values and hence is unlikely to be responsible for the reproductive restriction (Supplementary Table 3).


In fact Selås claims, that some species, such as bilberry, require a period of cold temperatures to initiate the successful development of floral buds. Regarding to that, the relatively mild winter in advance of the growing period in 2017 may have inhibited the vernalization at low site. This would conform to the findings of Selås, which demonstrate a correlation between the depression of bilberry reproduction and high temperatures in winters with thin snow cover. Hence, in this study, the effort of upregulating defence genes (Cheong and Choi, 2003) induced by MeJa affects particularly already resource restricted plants dealing with challenging environmental conditions.

5.1.2 Grazing

In general, one can expect variations in herbivory sort and intensity along altitudinal gradients, since the diversity of faunal species is strongly correlated to vegetal distribution due to their mutual dependency, as e.g. plant-pollinator or food chain correlations. This study found a relatively little increase in attacked leaves at high elevation, whereas the invertebrate grazing was most dominant in 2016 at the low site while this peak shifted to the middle site in the subsequent year. Generally, the abundance and diversity of species decreases with increasing elevation or peaks at intermediate altitudinal level Speed et al. (2013), since vertical distribution of insects currently is limited by temperature and the length of the growing season, which both declines along an altitudinal gradient (Hallinger, Manthey and Wilmking, 2010).

Moreover, according to White (1984)\textsuperscript{36}, weather stressed plant may accumulate an increased amount of nitrogen in their tissues, which consequently provide nutritious food for invertebrates. Based on this theory, potentially drought stressed plants at the low site affected by the warm and relatively dry weather in 2016, could be responsible for the attraction of invertebrate herbivores there\textsuperscript{37}, since their population dynamics are highly dependent on the food palatability\textsuperscript{38}. Also the deer grazing was most dominant at low site in 2016, even though the variations in ungulate shoot grazing along the elevational gradient were not statistically significant. Hegland et al. (2005) found that ungulate grazing may have a negative impact on invertebrate population dynamics due to the reduced biomass and degraded food quality. Since according to the plant vigour hypothesis (Price, 1991) herbivore species tend to prefer the most nutritive plants, mammal herbivory at the low site in 2016 may have influenced the shift in insect grazing peak.

Moreover the grazing pressure at the low site in 2016 may have contributed to the activation of induced plant defences, as bilberry are known to accumulate secondary metabolites in terms of resistance against herbivory\textsuperscript{39}. Since only at that site MeJa treated plants showed an increased resistance against insect herbivory, conforming to findings of former studies, which indicate that methyl-jasmonate can lower the attraction of the plant to herbivores (Agrawal, Tuzun and Bent, 2000, 323). In contrast plants at high site exhibit even an opposite trend (yet not significant), which may be attributed to measurement errors. Additionally the absent resistance against herbivores, may be due to stronger winds at the less sheltered site and lower air pressure due to increased altitude.

Those conditions favour evaporation and thus may lower the concentration of methyl-jasmonate\(^{40}\). However the sparsity of responses in the subsequent year (2017) does not allow to determine a pattern of insect grazing patterns in MeJa treated plots. Especially because the defence mechanisms may be expected to be the strongest in 2017 based on the findings of Benevenuto et al. (in press). They found the strongest resistance of bilberry against insect grazing one year after MeJa application due to delayed responses of the relatively slow growing perennial and deciduous shrub (ibid.). The study of Benevenuto et al. (in press) included a different technique to apply the MeJa to the plants, as cotton wool soaked with MeJa was attached to the stem of the treated ramet at the ground (ibid.). Thus the bilberry may have been exposed to a higher dose of the hormonal regulator, whereby the more distinct responses, such as significantly reduced insect grazing, compared to the findings of this study could be readily explained. Additionally referring to the cost hypothesis (Agrawal, Tuzun and Bent, 2000, 233), defence mechanisms are only activated if the effort associated with the resource allocation is beneficial for the plant. For instance if the plants are exposed to stresses as for instance herbivory, which may reduce their biomass and threaten their survival. Furthermore the internal constraint hypothesis (Agrawal, Tuzun and Bent, 2000, 233-234, 237) implies that this exterior impact must exceed a certain threshold to activate defence mechanisms. Accordingly to this, the concentration of methyl-jasmonate applied to the plants may have been too little to initiate the cost expensive defence mechanisms against insect grazing.

Opposite to the assumptions based on previous studies, the MeJa application did not prevent the plants from being grazed on by deer, since no distinct difference in grazing pressure could be determined compared to the control plants. Benevenuto et al. (in press) observed a significantly reduced amount of bilberry shoots for MeJa treated plants two years after the application.

Regarding the delayed response, they hypothesise the activation of an induced long-term defence system against large vertebrate herbivores, which are suggested to be more costly to the plant (ibid.). Hence responses regarding to the shoot grazing may be absent in this study, because the activation of those long-term defences to the plant and has not yet developed its full potential. In that case a regulating effect should be visible in the third year of the study.

5.1.3 Growth

The distinct loss of leaves in MeJa plots may be a further side effect of the hormonal compound. Since opposite to that, the water treated plots provide steady numbers of leaves over the sampling period. Due to the influence on physiological processes (Bennett and Walls Grove, 1994) the MeJa treatment may cause alterations in the plant morphology as it is known to promote leaf senescence and abscission. Herbivory grazing as a reason for the reduced numbers of leaves can most likely be excluded, based on findings of several previous studies proving a decline in grazing intensities on plants in contact with MeJa. Furthermore the observation of defoliation as a response to methyl-jasmonate induced stress, can be confirmed by the study of Percival and MacKenzie.

The trend of defoliation in MeJa plots is visible on all sites, though not significant at medium altitude. Since there the growing conditions are assumed to favour the plant development, the plants may be able to devote resources to the activation of defence mechanisms while reducing the abortion of leaves to a minimum.

---

The inverse phenomenon of reduced plant resistance to insect herbivory has been described by the plant stress hypothesis\textsuperscript{44,45}. According to this, the food palatability is altered by environmental stresses influencing biochemical source-sink relationships in the plants\textsuperscript{46}.

Moreover the peak of leaf abscission at the low site may support the assumption of a local reallocation of plants resources in 2016 induced by the MeJa application and high local grazing pressure (Cheong and Choi, 2003). Coherent with former findings, also the vertical plant growth inside those plots was restricted. This may affirm the thesis of a negative impact on the plant growth due to the costly production of secondary metabolites in terms of defences against herbivory\textsuperscript{47}.

Moreover the magnitude of defoliation at the low site may also be associated with local weather conditions, since \textit{V. myrtillus} is known to avoid drought by shedding primarily mature leaves to maintain a sufficient water balance\textsuperscript{48}. Especially plants at lower altitude are little flexible to deal with drought stresses\textsuperscript{49} when exposed to direct insolation often followed by a drying-out of the soil. The Norwegian Meteorological Institute registered higher monthly average temperatures for the study period in 2016 compared to the subsequent year (Supplementary Table 3), supporting the hypothesis of increased vulnerability of bilberry due to additionally drought stress.

The lack of a similar behaviour in 2017 does not support this, because an increased impact on the plant was expected in the second year of the study due to the previous mentioned delay in responses. However based on the statement that moisture is a limiting factor for the plant development (Taulavuori, Tahkokorpi, Laine and Taulavuori, 2010), this may be attributed to the high amount of precipitation during the study period in 2017 (Supplementary Table 3). Thereby sufficient water supply may have created favourable growing conditions for bilberry, avoiding the necessity of leaf abscission. Generally warmer temperature can lengthen the growing season for *V. myrtillus* by initiating premature flowering\(^5^0\). However bilberry are known to be sensitive to frost damage\(^5^1\) and hence may be negatively affected by an earlier spring bloom induced by changing climate, as they are endangered to suffer a loss of flowers during a proximate cold period.


5.2 Influence of simulated warming

5.2.1 Reproduction

Minor responses to the simulated warming could be determined regarding to the reproductive effort, as the amount of flowers is reduced inside OTCs. Little research is conducted regarding to the effect of temperature and the flower abundance for shrubs species. However studies investigating the influence of climate change on the reproductive traits of other plant species, state alterations in flowering phenology and/or a reduction in flower production\(^{52}\). For instance Karapanos et al. (2008) documented higher abundance of flowers for tomato plants during lower temperatures\(^{53}\) and refers to Dinar and Rudich (1985), who found flower abscission under heat stress due to the restricted supply of assimilates to the flower buds\(^{54}\).

The study results though, may be affected by increased grazing pressure inside OTCs at the medium site in 2017, whereby the lower amount of flowers may be a direct impact of grazing in terms of reduced biomass\(^{55}\) or induced plant defences\(^{56}\). Moreover the intensified insect grazing could affect reproductive traits indirectly, as herbivory is known to reduce the plant fitness and to have a detrimental effect on growth and reproduction\(^{57}\).

---

56 Stange, Erik E. and Ayres, Matthew P. Climate Change Impacts: Insects, vol. 262, 75. 10.1002/9780470015902.a002555.
Generally warmer temperature can lengthen the growing season for *V. myrtillus* by initiating premature flowering\(^{58}\). However bilberry are known to be sensitive to frost damage\(^{59}\) and hence may be negatively affected by an earlier spring bloom induced by changing climate, as they are endangered to suffer a loss of flowers during a proximate cold period.

### 5.2.2 Grazing

In 2017 the insect grazing was most dominant at mid elevation, whereas primarily plants inside OTCs were attacked. This may be attributed to the favourable growing conditions and the resulting abundant and vigorous biomass there\(^{60,61}\). Consequently, sufficient and nutritious bilberry plants appear to attract invertebrate herbivores. This behaviour conforms to the plant vigour hypothesis\(^{62}\), according to which herbivores prefer to feed on vigorous plants.

Furthermore some species preferring the local conditions in the open-top chambers can benefit from the sheltered environment and congregate within OTCs, whereas on the contrary other insects may rather avoid those\(^{63}\). Hence Moise et al. points out the risk of inaccurate interpretation, since possible community interactions may be overlooked when only considering the local response. Therefore to define a direct positive effect of the simulated warming on the grazing intensity, may not be completely correct due to the hypothetically manipulating effect of open-top chambers.

---


5.2.3 Growth

The larger increase in stem diameter and dry mass within plots equipped with OTCs in 2017 allow the assumption of enhanced bilberry growth at warmer temperatures. Similar results were also exhibited in previous studies proving boosted growth and spread of *V. myrtillus* as a response to simulated warming. Rinnan et al. (2009) for instance found that simulated warming triggered an extended cover of bilberry in a subarctic tundra heath in Finland after 10–13 years of exposure. In that study other plant groups were not affected positively by the temperature treatment and the shrub was able to outcompete adjacent species (Rinnan, Stark and Tolvanen, 2009). This observation can also be confirmed by the findings of Dawes et al.64, who found enhanced shoot growth of *V. myrtillus* influenced by simulated warming65. Other investigated dwarf shrubs (*Vaccinium gaultherioides* and *Empetrum hermaphroditum*) were excluded from the adolescence stimulating effect. Hence warmer climatic conditions66 may have a beneficial impact on the growth of *V. myrtillus*, especially due to its advantage over coexisting plant species (Dawes, Hagedorn, Zumbrunn, Handa, Hättenschwiler, Wipf and Rixen, 2011).

Considering the interaction between site and temperature, the thesis of enhanced growth at elevated temperature can be underlined to a limited extent. The significant responses there are restricted to the medium site and again only visible in 2017. The lacking impacts on growth in 2016 may be attributed to less optimal weather conditions due to the former stated high temperatures and the corresponding drought potential. Moreover the assumption of optimal conditions for plant development at mid site (Pato and Obeso, 2012) and sufficient water supply because of extensive precipitation in 2017 may have caused the significant increase in dry mass in OTCs there, while no trend is visible at low or high elevation.

---


65 Simulated warming there was effectuated with heating cables on the ground surface causing a soil warming instead of OTC usage.

66 The study also tested the influence of enriched CO₂ of bilberry, as its atmospheric concentration is predicted to continue rising in future climate scenarios. The combination of elevated CO₂ levels and increased temperature reinforced the positive effect on bilberry.
This also confirms the previous stated hypothesis of sufficient and high quality food supply within the OTC plots attracting invertebrate herbivores. Even though not significant in the general dry mass data, the increase in plant height was positively affected by OTCs in 2016 at high elevation, where the simulated warming appears to have the strongest advantageous impact on the vertical plant growth. The OTC plots at the high site depict an enhanced growth not only compared to the ambient temperate plots at the same elevation, but also compared to the average growth in OTC plots of both other sites (Low, Medium). The particular sensitivity may be attributed to the fact, that temperature is one of the growth restricting parameters at the high site (Grace, 1977). Therefore, a slight increase in temperature and protection from wind (Marion et al., 1997) seems to have enabled a boost in growth, compared to medium or low site plots. There the prevailing ambient temperature is already close to optimal growing conditions and hence a larger temperature increase may be necessary to enhance the plant growth at medium site.

Even though the observed responses to the temperature treatment appear to be coherent with former findings, the single results of the growth variables lack a consistent trend. Despite its statistical significance, the trustworthiness of the single responses may be limited due to possible measurement inaccuracies especially for the height or stem variables.
6 Conclusion

This study discovered a significantly reduced amount of flowers after the application of the hormonal regulator methyl-jasmonate. This trend could be observed at all investigated sites (circa 100, 450 and 900 masl) indicating a reallocation of the plants resources from reproduction to defence mechanisms (Cheong and Choi, 2003), whereas already resource restricted plants were affected the most. However no significant impact on the plant growth was observed in the same year (2017), suggesting that reproduction is the first to lose out in case of limited resources during herbivore attacks.

Moreover a significant defoliation process (Curtis, 1984) could be registered for MeJa treated bilberry in 2016, whereas the most significant difference compared to the control plots could be found at the lowest elevation. At the same site the vertical growth was negatively affected by the MeJa treatment, whereas the resistance against invertebrate herbivores significantly increased. Leading to the assumption that plant defence genes were successfully mobilized, whereby less resources were devoted to the plant growth. However this consistent behaviour was only observed at the low site, where the increase in invertebrate grazed leaves was the highest in 2016. Hence one can assume that the natural grazing pressure contributed to the local activation of defence mechanisms against herbivores. To sum up, those uniform trends may confirm the hypothesis that the upregulation of defence genes induced by MeJa and/ or natural grazing increase the protection of V. myrtillus against herbivory at the expense of growth and reproduction.

On the other hand the MeJa treatment did not prevent bilberry to be grazed on by ungulates, since no significant differences to the control plots could be discovered in both years. The lack of responses may be attributed to an underdose of the VOC concentration or the delay in responses as observed by Benevenuto et al. (in press). Additionally the gains in dry mass were increased inside OTCs in the second study year, supporting the assumption of favourable growing conditions at elevated temperature. Whereas this single response to the simulated warming is little meaningful, since this effect was not underlined by other growth variables.
Moreover the combined effect of MeJa and simulated warming can not be evaluated, since the data analysis did not provide significant responses regarding to this interaction. Former findings claim that bilberry may benefit from climate change and outcompete other species (Rinnan, Stark and Tolvanen, 2009), which may cause a negative feedback on the plant as the increased food supply may favour the populations of several herbivore species grazing on bilberry. Referring to the findings of this study, especially plants under poor conditions may suffer a loss of reproductive traits, when activating defences against herbivores. Further research is necessary to gain more information about how the changing climate may affect key species, such as the shrub *V. myrtillus*, as it fulfils an essential role as primary producer within ecosystems.
List of Appendices

List of Supplementary Figures

Supplementary Figure 1: Sketch of the study design including three different elevated sites and the schematical set-up of the treatment plots..........................36

Supplementary Figure 2: Monthly average snow depth [cm] from July 2014 until July 2018 measured in Hafslo (246 masl) and Selseng (421 masl). The weather stations differ almost 200 meters in altitude and both have a distance of approximately 30 km to the study area. Selseng is located north-west of the study area, while the meteorological station in Hafslo is situated in the northwards. This data was collected from the Norwegian Meteorological Institute..............................36

Supplementary Figure 3: Significant impact on the gain in stem diameter in 2017 by the temperature treatment (ambient, OTC).................................................................37

Supplementary Figure 4: Significant impact on the gain in stem diameter in 2017 by the site (low, medium, high) .................................................................................37

Supplementary Figure 5: Significant impact on the increase in height in 2016 by the interaction between site (low, medium, high) and temperature treatment (ambient, OTC) .................................................................37

Supplementary Figure 6: Significant impact on the increase in height in 2016 by the interaction between site (low, medium, high) and treatment (MeJa, water) ........................................37

Supplementary Figure 7: Significant impact on the increase in height in 2017 by the treatment (MeJa, water) .........................................................................................38

List of Supplementary Maps

Supplementary Map 1: Map of study location situated in Western Norway and a close-up view displaying the positions of the three study sites ..........................38
List of Supplementary Tables

Supplementary Table 1: Overview of statistical responses after running the maximal linear mixed effect models fitted for the supplementary response variables (stem diameter, height, amount of annual shoots) including the three explanatory variables (site, temperature, treatment) and all two-way interactions..................39

Supplementary Table 2: Overview of statistical responses after model simplification for all response variables.................................................................................................................40

Supplementary Table 3: Monthly average temperature and precipitation values during both study periods from MET Norway ..................................................................................................................41
Supplementary Figures

**Supplementary Figure 1**: Sketch of the study design including three different elevated sites and the schematical set-up of the treatment plots.

**Supplementary Figure 2**: Monthly average snow depth [cm] from July 2014 until July 2018 measured in Hafslo (246 masl) and Selseng (421 masl). The weather stations differ almost 200 meters in altitude and both have a distance of approximately 30 km to the study area. Selseng is located north-west of the study area, while the meteorological station in Hafslo is situated in the northwards. This data was collected from the Norwegian Meteorological Institute.
Supplementary Figure 3: Significant impact on the gain in stem diameter in 2017 by the temperature treatment (ambient, OTC)

Supplementary Figure 4: Significant impact on the gain in stem diameter in 2017 by the site (low, medium, high)

Supplementary Figure 5: Significant impact on the increase in height in 2016 by the interaction between site (low, medium, high) and temperature treatment (ambient, OTC)

Supplementary Figure 6: Significant impact on the increase in height in 2016 by the interaction between site (low, medium, high) and treatment (MeJa, water)
Supplementary Figure 7: Significant impact on the increase in height in 2017 by the treatment (MeJa, water)

Supplementary Maps

Supplementary Map 1: Map of study location situated in Western Norway and a close-up view displaying the positions of the three study sites
Supplementary Tables

*Supplementary Table 1: Overview of statistical responses after running the maximal linear mixed effect models fitted for the supplementary response variables (stem diameter, height, amount of annual shoots) including the three explanatory variables (site, temperature, treatment) and all two-way interactions.*

<table>
<thead>
<tr>
<th>Response</th>
<th>Treatment</th>
<th>DF</th>
<th>2016</th>
<th>2017</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>Treatment</td>
<td>1</td>
<td>2.208</td>
<td>0.144</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>0.029</td>
<td>0.865</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>2.429</td>
<td>0.099</td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>0.690</td>
<td>0.507</td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>0.390</td>
<td>0.680</td>
</tr>
<tr>
<td></td>
<td>Temperature:Treatment</td>
<td>1</td>
<td>1.642</td>
<td>0.206</td>
</tr>
<tr>
<td>Height</td>
<td>Treatment</td>
<td>1</td>
<td>1.753</td>
<td>0.191</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>0.115</td>
<td>0.736</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>2.236</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>4.069</td>
<td><strong>0.022</strong></td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>7.013</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td></td>
<td>Temperature:Treatment</td>
<td>1</td>
<td>2.765</td>
<td>0.102</td>
</tr>
<tr>
<td>Shoots</td>
<td>Treatment</td>
<td>1</td>
<td>0.022</td>
<td>0.884</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>0.257</td>
<td>0.614</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>1.464</td>
<td>0.240</td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>3.943</td>
<td><strong>0.025</strong></td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>0.695</td>
<td>0.503</td>
</tr>
<tr>
<td></td>
<td>Temperature:Treatment</td>
<td>1</td>
<td>0.080</td>
<td>0.779</td>
</tr>
</tbody>
</table>
**Supplementary Table 2: Overview of statistical responses after model simplification for all response variables**

<table>
<thead>
<tr>
<th>Response</th>
<th>Treatment</th>
<th>DF</th>
<th>2016</th>
<th>2017</th>
<th></th>
<th>2016</th>
<th>2017</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem diameter shoots</td>
<td>Temperature</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>7.864</td>
<td>0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>7.571</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>1</td>
<td>0.023</td>
<td>0.881</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>1.518</td>
<td>0.227</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>4.089</td>
<td>0.021</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>Treatment</td>
<td>1</td>
<td>1.706</td>
<td>0.196</td>
<td>5.840</td>
<td>0.018</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>0.112</td>
<td>0.739</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>2.175</td>
<td>0.122</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>3.958</td>
<td>0.024</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>6.822</td>
<td>0.002</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry mass</td>
<td>Temperature</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>4.326</td>
<td>0.042</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2.8555</td>
<td>0.06512</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>6.334</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>Treatment</td>
<td>1</td>
<td>42.745</td>
<td>1.13E-08</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>2.5805</td>
<td>0.08348</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>2.999</td>
<td>0.025</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grazed leaves</td>
<td>Treatment</td>
<td>1</td>
<td>1.5266</td>
<td>0.221</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2.229</td>
<td>0.140</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>10.868</td>
<td>8.32E-05</td>
<td>3.423</td>
<td>0.039</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>4.921</td>
<td>0.010</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>3.954</td>
<td>0.024</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Response</th>
<th>Treatment</th>
<th>DF</th>
<th>Dev</th>
<th></th>
<th>P</th>
<th>Dev</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amount of flowers</td>
<td>Cover</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2.04E-07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>&lt; 2.2E-16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>0.035</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>0.436</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment:Site</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>0.010</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Supplementary Table 3: Monthly average temperature and precipitation values during both study periods from MET Norway.

<table>
<thead>
<tr>
<th>Date</th>
<th>Average temperature [°C]</th>
<th>Normal Temperature [°C]</th>
<th>Total precipitation [mm]</th>
<th>Normal precipitation [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>-5.6</td>
<td>-5.2</td>
<td>169</td>
<td>133</td>
</tr>
<tr>
<td>February</td>
<td>-2.9</td>
<td>-4.7</td>
<td>105</td>
<td>96</td>
</tr>
<tr>
<td>March</td>
<td>0.3</td>
<td>-1.7</td>
<td>49</td>
<td>171</td>
</tr>
<tr>
<td>April</td>
<td>2.1</td>
<td>1.7</td>
<td>53</td>
<td>110</td>
</tr>
<tr>
<td>May</td>
<td>8.6</td>
<td>7.5</td>
<td>70</td>
<td>85</td>
</tr>
<tr>
<td>June</td>
<td>13.0</td>
<td>11.2</td>
<td>77</td>
<td>96</td>
</tr>
<tr>
<td>July</td>
<td>12.5</td>
<td>12.5</td>
<td>131</td>
<td>99</td>
</tr>
<tr>
<td>August</td>
<td>11.7</td>
<td>11.7</td>
<td>177</td>
<td>105</td>
</tr>
<tr>
<td>September</td>
<td>11.4</td>
<td>7.6</td>
<td>204</td>
<td>125</td>
</tr>
<tr>
<td>October</td>
<td>4.2</td>
<td>4.4</td>
<td>93</td>
<td>258</td>
</tr>
<tr>
<td>November</td>
<td>-1.4</td>
<td>-0.7</td>
<td>140</td>
<td>284</td>
</tr>
<tr>
<td>December</td>
<td>1.0</td>
<td>-3.1</td>
<td>313</td>
<td>253</td>
</tr>
<tr>
<td>Mean</td>
<td>4.6</td>
<td>3.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>-</td>
<td>1582</td>
<td>1814</td>
</tr>
</tbody>
</table>

Temperature data collected from weather station “Sogndal Lufthamn” (located 479 masl on the mountain investigated in this study). Precipitation data is not available for the same location, and hence is listed for the weather station “Selseng” (app. 406 masl, 30km distanced from the study area). Normal temperature and precipitation are averaged over the period from 1961 – 1990.
References


Benevenuto, Rafael Fonseca, Hegland, Stein Joar, Töpper, Joachim Paul, Rydgren, Knut, Moe Stein R., Rodriguez-Saona, Cesar and Seldal, Tarald. *Multiannual effects of induced plant defenses: Are defended plants good or bad neighbors?* (unpublished)


Stange, Erik E. and Ayres, Matthew P. *Climate Change Impacts: Insects*.


List of Appendices

List of Supplementary Figures

Supplementary Figure 1: Sketch of the study design including three different elevated sites and the schematical set-up of the treatment plots .................. 36

Supplementary Figure 2: Monthly average snow depth [cm] from July 2014 until July 2018 measured in Hafslo (246 masl) and Selseng (421 masl). The weather stations differ almost 200 meters in altitude and both have a distance of approximately 30 km to the study area. Selseng is located north-west of the study area, while the meteorological station in Hafslo is situated in the northwards. This data was collected from the Norwegian Meteorological Institute ........................ 36

Supplementary Figure 3: Significant impact on the gain in stem diameter in 2017 by the temperature treatment (ambient, OTC) ........................................................................ 37

Supplementary Figure 4: Significant impact on the gain in stem diameter in 2017 by the site (low, medium, high) ........................................................................................................ 37

Supplementary Figure 5: Significant impact on the increase in height in 2016 by the interaction between site (low, medium, high) and temperature treatment (ambient, OTC) ........................................................................................................................................ 37

Supplementary Figure 6: Significant impact on the increase in height in 2016 by the interaction between site (low, medium, high) and treatment (MeJa, water) . 37

Supplementary Figure 7: Significant impact on the increase in height in 2017 by the treatment (MeJa, water) .................................................................................................................. 38

List of Supplementary Maps

Supplementary Map 1: Map of study location situated in Western Norway and a close-up view displaying the positions of the three study sites .......................... 38
List of Supplementary Tables

Supplementary Table 1: Overview of statistical responses after running the maximal linear mixed effect models fitted for the supplementary response variables (stem diameter, height, amount of annual shoots) including the three explanatory variables (site, temperature, treatment) and all two-way interactions. .................................................. 39

Supplementary Table 2: Overview of statistical responses after model simplification for all response variables ............................................................................................................. 40

Supplementary Table 3: Monthly average temperature and precipitation values during both study periods from MET Norway .................................................................................. 41
Appendix

Supplementary Figures

**Supplementary Figure 1:** Sketch of the study design including three different elevated sites and the schematic set-up of the treatment plots.

**Supplementary Figure 2:** Monthly average snow depth [cm] from July 2014 until July 2018 measured in Hafslo (246 masl) and Selseng (421 masl). The weather stations differ almost 200 meters in altitude and both have a distance of approximately 30 km to the study area. Selseng is located north-west of the study area, while the meteorological station in Hafslo is situated in the northwards. This data was collected from the Norwegian Meteorological Institute.
Supplementary Figure 3: Significant impact on the gain in stem diameter in 2017 by the temperature treatment (ambient, OTC)

Supplementary Figure 4: Significant impact on the gain in stem diameter in 2017 by the site (low, medium, high)

Supplementary Figure 5: Significant impact on the increase in height in 2016 by the interaction between site (low, medium, high) and temperature treatment (ambient, OTC)

Supplementary Figure 6: Significant impact on the increase in height in 2016 by the interaction between site (low, medium, high) and treatment (MeJa, water)
Supplementary Figure 7: Significant impact on the increase in height in 2017 by the treatment (MeJa, water)

Supplementary Maps

Supplementary Map 1: Map of study location situated in Western Norway and a close-up view displaying the positions of the three study sites
**Supplementary Tables**

**Supplementary Table 1:** Overview of statistical responses after running the maximal linear mixed effect models fitted for the supplementary response variables (stem diameter, height, amount of annual shoots) including the three explanatory variables (site, temperature, treatment) and all two-way interactions.

<table>
<thead>
<tr>
<th>Response</th>
<th>Treatment</th>
<th>DF</th>
<th>2016 F</th>
<th>2016 P</th>
<th>2017 F</th>
<th>2017 P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem diameter</td>
<td>Treatment</td>
<td>1</td>
<td>2.208</td>
<td>0.144</td>
<td>0.010</td>
<td>0.922</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>0.029</td>
<td>0.865</td>
<td>7.864</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>2.429</td>
<td>0.099</td>
<td>7.571</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>0.690</td>
<td>0.507</td>
<td>1.880</td>
<td>0.162</td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>0.390</td>
<td>0.680</td>
<td>1.394</td>
<td>0.257</td>
</tr>
<tr>
<td></td>
<td>Temperature:Treatment</td>
<td>1</td>
<td>1.642</td>
<td>0.206</td>
<td>0.145</td>
<td>0.705</td>
</tr>
<tr>
<td>Height</td>
<td>Treatment</td>
<td>1</td>
<td>1.753</td>
<td>0.191</td>
<td>5.375</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>0.115</td>
<td>0.736</td>
<td>0.433</td>
<td>0.513</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>2.236</td>
<td>0.116</td>
<td>0.175</td>
<td>0.840</td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>4.069</td>
<td>0.022</td>
<td>0.453</td>
<td>0.638</td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>7.013</td>
<td>0.002</td>
<td>0.884</td>
<td>0.419</td>
</tr>
<tr>
<td></td>
<td>Temperature:Treatment</td>
<td>1</td>
<td>2.765</td>
<td>0.102</td>
<td>0.376</td>
<td>0.542</td>
</tr>
<tr>
<td>Shoots</td>
<td>Treatment</td>
<td>1</td>
<td>0.022</td>
<td>0.884</td>
<td>0.369</td>
<td>0.546</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>1</td>
<td>0.257</td>
<td>0.614</td>
<td>0.126</td>
<td>0.724</td>
</tr>
<tr>
<td></td>
<td>Site</td>
<td>2</td>
<td>1.464</td>
<td>0.240</td>
<td>0.087</td>
<td>0.917</td>
</tr>
<tr>
<td></td>
<td>Site:Treatment</td>
<td>2</td>
<td>3.943</td>
<td>0.025</td>
<td>0.935</td>
<td>0.399</td>
</tr>
<tr>
<td></td>
<td>Site:Temperature</td>
<td>2</td>
<td>0.695</td>
<td>0.503</td>
<td>0.903</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td>Temperature:Treatment</td>
<td>1</td>
<td>0.080</td>
<td>0.779</td>
<td>0.011</td>
<td>0.916</td>
</tr>
</tbody>
</table>
**Supplementary Table 2: Overview of statistical responses after model simplification for all response variables**

<table>
<thead>
<tr>
<th>Response</th>
<th>Treatment</th>
<th>DF</th>
<th>2016</th>
<th>2017</th>
<th>2016</th>
<th>2017</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>diameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shoots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grazed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>amount</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>of flowers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Response**

- **Stem diameter**
  - Temperature: 1 - 7.864 0.007
  - Site: 2 - 7.571 0.001

**Shoots**

- Treatment: 1 - 0.023 0.881
- Site: 2 - 1.518 0.227
- Site:Temperature: 2 - 4.089 0.021

**Height**

- Treatment: 1 - 1.706 0.196 5.840 0.018
- Temperature: 1 - 0.112 0.739
- Site: 2 - 2.175 0.122
- Site:Temperature: 2 - 3.958 0.024

**Dry mass**

- Temperature: 1 - 4.326 0.042
- Site: 2 - 2.855 0.06512
- Site:Temperature: 2 - 6.334 0.003

**Leaves**

- Treatment: 1 - 42.745 1.13E-08
- Site: 2 - 2.5805 0.08348
- Site:Temperature: 2 - 2.999 0.025

**Grazed leaves**

- Treatment: 1 - 1.5266 0.221
- Temperature: 1 - 2.229 0.140
- Site: 2 - 10.868 8.32E-05 3.423 0.039
- Site:Temperature: 2 - 4.921 0.010
- Site:Treatment: 2 - 3.954 0.024

**Total amount of flowers**

- Cover: 1 - 2.04E-07
- Treatment: 1 - < 2.2E-16
- Temperature: 1 - 0.035
- Site: 2 - 0.436
- Treatment:Site: 2 - 0.010
**Supplementary Table 3: Monthly average temperature and precipitation values during both study periods from MET Norway**

<table>
<thead>
<tr>
<th>Date</th>
<th>2016 Average temperature [°C]</th>
<th>Normal Temperature [°C]</th>
<th>2016 Total precipitation [mm]</th>
<th>2017 Total precipitation [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>-5.6</td>
<td>-5.2</td>
<td>169</td>
<td>133</td>
</tr>
<tr>
<td>February</td>
<td>-2.9</td>
<td>-4.7</td>
<td>105</td>
<td>96</td>
</tr>
<tr>
<td>March</td>
<td>0.3</td>
<td>-1.7</td>
<td>49</td>
<td>171</td>
</tr>
<tr>
<td>April</td>
<td>2.1</td>
<td>1.7</td>
<td>53</td>
<td>110</td>
</tr>
<tr>
<td>May</td>
<td>8.6</td>
<td>7.5</td>
<td>70</td>
<td>85</td>
</tr>
<tr>
<td>June</td>
<td>13.0</td>
<td>11.2</td>
<td>77</td>
<td>96</td>
</tr>
<tr>
<td>July</td>
<td>12.5</td>
<td>12.5</td>
<td>131</td>
<td>99</td>
</tr>
<tr>
<td>August</td>
<td>11.7</td>
<td>11.7</td>
<td>177</td>
<td>105</td>
</tr>
<tr>
<td>September</td>
<td>11.4</td>
<td>7.6</td>
<td>204</td>
<td>125</td>
</tr>
<tr>
<td>October</td>
<td>4.2</td>
<td>4.4</td>
<td>93</td>
<td>258</td>
</tr>
<tr>
<td>November</td>
<td>-1.4</td>
<td>-0.7</td>
<td>140</td>
<td>284</td>
</tr>
<tr>
<td>December</td>
<td>1.0</td>
<td>-3.1</td>
<td>313</td>
<td>253</td>
</tr>
<tr>
<td>Mean</td>
<td>4.6</td>
<td>3.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>-</td>
<td>1582</td>
<td>1814</td>
</tr>
</tbody>
</table>

Temperature data collected from weather station “Sogndal Lufthamn” (located 479 masl on the mountain investigated in this study). Precipitation data is not available for the same location, and hence is listed for the weather station “Selseng” (app. 406 masl, 30km distanced from the study area). Normal temperature and precipitation are averaged over the period from 1961 – 1990.
References


Benevenuto, Rafael Fonseca, Hegland, Stein Joar, Töpper, Joachim Paul, Rydgren, Knut, Moe Stein R., Rodriguez-Saona, Cesar and Seldal, Tarald. Multiannual effects of induced plant defenses: Are defended plants good or bad neighbors? (unpublished)


Stange, Erik E. and Ayres, Matthew P. *Climate Change Impacts: Insects.*


