The effect of experimental warming on insect herbivory in an alpine plant community

Effekten av eksperimentell oppvarming på insektherbivori i et alpint plantesamfunn

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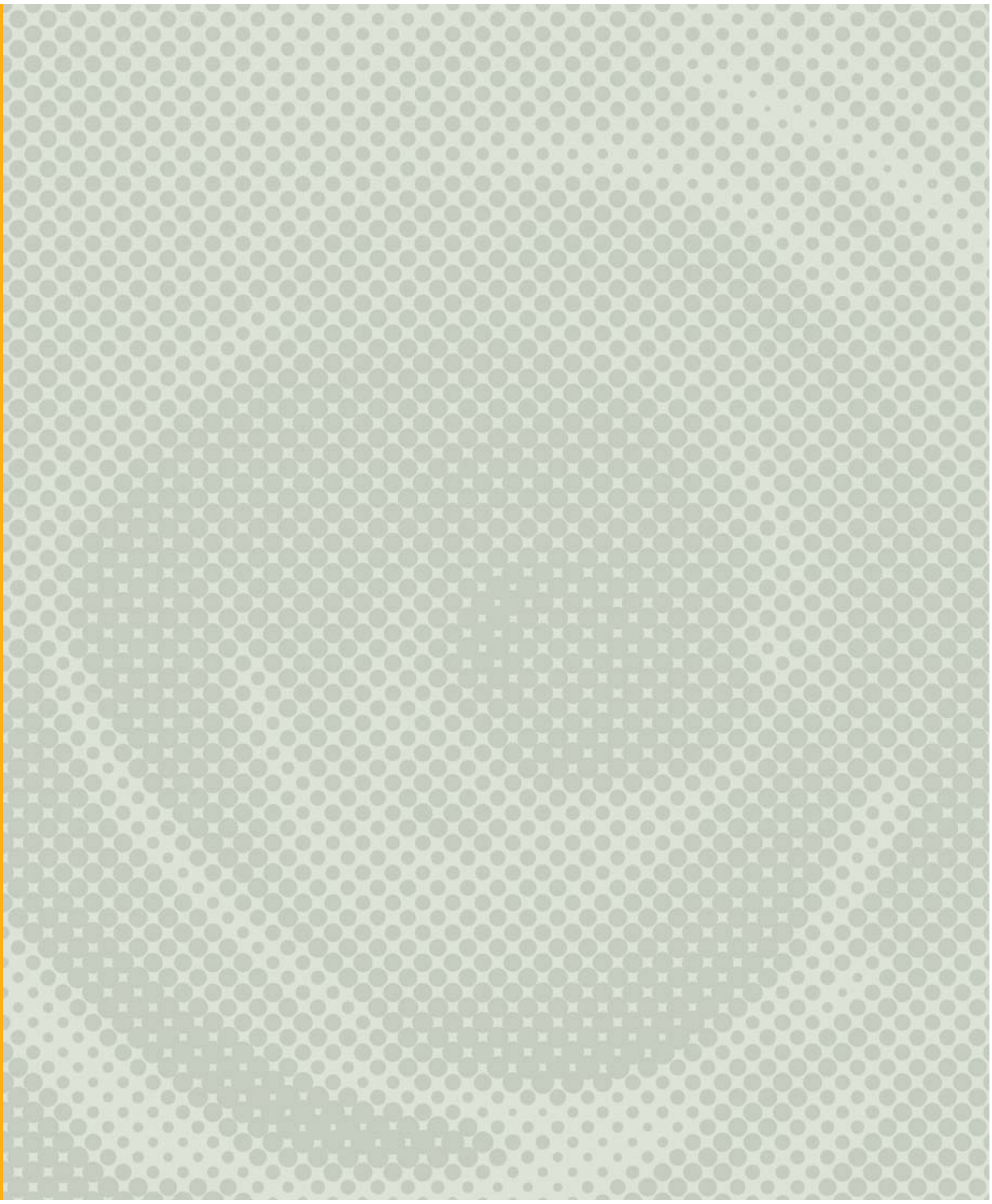

Preface

This thesis has been written at the Department of Ecology and Natural Resource Management (INA), Norwegian University of Life Sciences (UMB). It completes my Teacher Education for Scientific and Mathematical Subjects (lektorutdanning) with a master's degree in biology. I chose the subject of the thesis because I am interested in global change research and how species and ecosystems respond to global warming. I was fortunate to do my field work in the alpine area of Finse and had an enjoyable time there, both in the laboratory with the larvae and outdoors.

I wish to thank

- My supervisors, Tone Birkemoe and Kari Klanderud, for help and guidance in the field, with analysis and in the writing process
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Abstract

Climate warming is predicted to affect species and trophic interactions worldwide, and alpine ecosystems are expected to be especially sensitive to changes. There are few studies on how insect herbivory respond to warming. Therefore, the aim of this study was to examine if experimental warming had an effect on herbivory by leaf-chewing insects in an alpine plant community. To manipulate the climate I used open-top chambers (OTCs) from an ongoing long-term experiment at Finse, Norway. By recording feeding damages on the vascular plants in the OTCs and in control plots, I found that warming increased the herbivory pressure on *Dryas octopetala*, but not on *Bistorta vivipara* and *Salix reticulata*. The increase was significant both in early and late season. Species-specific responses suggest that warming might have caused changes in herbivore activity or plant quality. A feeding preference experiment with the larvae of the moth *Zygaena exulans*, a common herbivore at the study site, showed a strong dislike for *Saussurea alpina*. However, no significant difference between the preference for *D. octopetala*, *B. vivipara*, *S. reticulata*, and *S. herbacea* was found. There was little consistence between the feeding experiment and the field registrations. This discrepancy might be caused by the presence of additional insect herbivore species in the field or the fact that the feeding experiment was based on an introductory no-choice test. Even with the limitations in using OTCs for herbivory research, the present study indicates that some species, such as *D. octopetala*, will be more susceptible to insect herbivory than others when temperatures increase. Although the impact of increased herbivory on plant performance has not been assessed in this study, the increase in damages on *D. octopetala* suggests that it is important to take insect herbivory into consideration when predicting changes in alpine plant communities due to climate warming.

Table of contents

Preface I

Abstract II

1 Introduction 1

2 Materials and methods 2

  2.1 Study site and study species 2
  2.2 Warming experiment 3
  2.3 Feeding preference experiment 4
  2.4 Statistics 6

3 Results 7

  3.1 Feeding damages 7
  3.2 Presence of insects and feeding preference experiment 10

4 Discussion 11

  4.1 Feeding damages 11
  4.2 The limitations of OTCs for predicting insect herbivory 15
  4.3 Feeding preferences of *Z. exulans* 15

5 Conclusion 16

References 17

1 Introduction

Global mean temperatures have already increased due to climate change and the warming is predicted to continue (IPCC 2007). Worldwide, this is affecting species and trophic interactions (Parmesan & Yohe 2003; Parmesan 2006; Tylianakis et al. 2008; Walther 2010). Alpine ecosystems are expected to be especially sensitive to changes (Shen & Harte 2000) and climate change is predicted to be the major driver of shifts in biodiversity and species composition (Sala et al. 2000).

Most of the research on the effects of climate warming, both in alpine and other ecosystems, has focused on plants (Arft et al. 1999; Elmendorf et al. 2012; Pieper et al. 2011; Walker et al. 2006). Some focus has been directed to the effects on insects, for example studies showing range shifts to higher latitudes or altitudes (e.g. Dieker et al. 2011; Drees et al. 2011; Hickling et al. 2006) or studies examining abundance in response to experimental warming (e.g. Adler et al. 2007; Dollery et al. 2006). Less studies have focused on the plant-insect interaction in general and insect herbivory in particular (but see Richardson et al. 2002; Roy et al. 2004), even though this may be important for understanding how ecosystems respond to climate warming (Roy et al. 2004; Tylianakis et al. 2008).

How insect herbivory will change due to warming is difficult to predict (Richardson et al. 2002), therefore scientists ask for research on interactions between herbivores and plants under climate warming in various ecosystems (Li et al. 2011). Especially, long-term warming experiments in the field are considered to be important (Adler et al. 2007). Warming experiments studying insect herbivory in alpine and arctic areas have included various plant species and different guilds of insect herbivores such as leaf-chewing, leaf-mining, and sap-sucking insects. These experiments found that some plants experienced decreased herbivory, some experienced no change, whereas others experienced increased herbivory with warming (Richardson et al. 2002; Roy et al. 2004).

The above mentioned studies indicate that the response varies between plant species. Therefore it is likely that some species will be more susceptible to increased insect herbivory than others when the temperatures increase. In order to predict which species that will experience increased herbivory, it is essential to gain knowledge about the feeding preferences of insect herbivores. At present, this information is incomplete or missing for many species. A common way to study feeding preferences is to conduct feeding preference experiments in the laboratory (e.g. Hernandez et al. 2011; Hågvar 1976; Pérez-Harguindeguy et al. 2003).

The aim of this study was to examine if experimental warming had an effect on herbivory by leaf-chewing insects in an alpine plant community. To manipulate the climate, I used open-top chambers (OTCs) from an ongoing long-term warming experiment at Finse, Norway. I also examined the food preference of the larvae of the diurnal moth *Zygaena exulans* (Hohenwart 1792), a common species at Finse and one of the main insect herbivores in the study area (Hågvar 1976). I ask the specific questions:

(1) Does warming have an effect on the amount of insect herbivory?
(2) Does the effect of warming on the amount of insect herbivory vary between the plant species?
(3) Does warming have an effect on the amount of insect herbivory both early and late in the season?
(4) What is the preferred food plant of *Z. exulans*?

2 Materials and methods

2.1 Study site and study species

Field work was performed during the summer 2012 at Finse, situated in the northern part of Hardangervidda mountain plateau, southwest Norway. The study site was located in the midalpine zone at 1450 m a.s.l. on the southwest facing slope of Sanddalsnuten (60º36.8’N-7º31.2’ E). Finse has an alpine-oceanic climate with 6.3ºC as mean temperature June - August (Aune 1993), and 89 mm as mean monthly precipitation during the same period (Førland 1993).

The dominant plant in the study area is the wintergreen dwarf shrub *Dryas octopetala* L., which often forms heath communities on dry calcareous soils in arctic-alpine environments (Elkington 1971; Mossberg & Stenberg 2007). Alpine *Dryas* heaths are biodiversity hotspots with common species like *Bistorta vivipara* (L.) Delarbre, *Salix reticulata* L., *Saussurea alpina* L., and *Tofieldia pusilla* (Michx.) Pers. Important leaf-chewing insects feeding on vascular plants at Finse are Lepidoptera (e.g. *Z. exulans*, *Boloria napaea* (Hoffmannsegg 1804), *Erebia pandrose* (Borkhausen 1788)), beetles (e.g. Curculionidae, Chrysomelidae), sawflies (e.g. Tenthredinidae) and a grasshopper (*Melanoplus frigidus* (Boheman 1846)) (Hågvar 1975; Hågvar 1976; Seglen 1967; Solhøy 1997).

2.2 Warming experiment

Open-top chambers (OTCs) were established in the study area in 2003 (Fig. 1). OTCs are frequently used to simulate regional climate warming and are considered to have few undesired side effects (Arft et al. 1999; Hollister & Webber 2000; Marion et al. 1997). During the growth season, the mean air temperature in the OTCs compared to ambient temperature is 1.6°C warmer at 5 cm above ground (Sandvik & Eide 2009). This is in accordance with an expected warming of 0.2-0.5°C per decade until 2050 for the Norwegian mainland (Hanssen-Bauer & Førland 2001). Further details about the OTCs can be found in Sandvik and Eide (2009).

Figure 1. OTCs at the slope of Sanddalsnuten, Finse, Norway, 2012. Photo: T. Hasle

For the registrations I used a subset of 10 out of the 32 OTCs in the study area. I selected the OTCs to use randomly, but made sure most of them contained *D. octopetala* so their conditions were as similar as possible and comparable to the research done by another master student, S. Bergmann, at a higher elevation site at Sanddalsnuten (1550 m a.s.l.). I established 10 control plots randomly around the OTCs, but made sure most of them also contained

D. octopetala to make their conditions similar to the OTCs. The control plots were placed at least 1 m from OTCs to avoid being influenced by my registration activity.

I did registrations inside a 50 x 50 cm frame in each OTC and control plot. The frame was divided into subplots to make registrations easier. To avoid edge effects from the walls, I put the frame in the middle of the OTCs. I recorded the feeding damages made by leaf-chewing insects on all living vascular plants inside the frame in each plot. I also recorded all leaf-chewing insects known to feed on vascular plants (ca one hour of searching per plot). If there were insects in an OTC, but not inside the frame, I recorded them too because the insects move fast and might as well have been inside the frame. To obtain comparable data, I also recorded insects close to the control plots (< 15 cm). In order not to disturb the experiment, no insects were removed from the plots. Therefore, all species identification was done on site or later from photographs. Because of the limited scope of a 30 credits master thesis, I did no other insect collections. I estimated the percentage cover separately for *D. octopetala, B. vivipara, and S. reticulata* inside the frame in each plot. To detect differences in herbivory between early and late season, I did the same field registrations twice during the summer (early: 19.-28.06.12 and late: 01.-04.08.12).

2.3 Feeding preference experiment

I conducted a feeding preference experiment together with S. Bergmann in late June 2012 to identify the preferred food plants of the polyphagous *Z. exulans* larvae (Naumann et al. 1999). This species was selected because we observed it in high abundances in the study area. Based on a combination of literature on preferred food plants (Hågvar 1976; www.lepidoptera.no s.a.), own observation of common plants species, and own observation of species with feeding damages, we decided to use *D. octopetala*, *B. vivipara*, *S. reticulata*, *S. herbacea*, and *S. alpina* in the experiment. First we did an introductory no-choice test (Schoonhoven et al. 2005) to see which plant species to exclude from the main test, a paired-choice test (Horton 1995). In the paired-choice test each larva was given two plant species in each trial to observe which one it preferred. However, feeding marks were found in only 17% of the trials as the larvae became inactive after ca one week in the laboratory. Therefore, the data from the paired-choice test are not presented, and I used the data from the no-choice test to approach the feeding preferences of *Z. exulans.*

We collected larvae in vegetation similar to the study area 20-100 m away from the OTCs and brought them back to the laboratory at Finse Research Station. Each larva was put in a separate plastic box with a ca 1 cm thick layer of plaster of Paris at the bottom (Fig. 2). To prevent the larvae from escaping, but allow air circulation, we covered the boxes with netting. We used lids with a hole in the middle to secure the netting. Large windows in the laboratory ensured similar day and night cycles as outside. Room temperature was ca 21ºC during the day and ca 16ºC during the night. To keep the boxes moist, we added a bit of water to the plaster of Paris every morning. The plant material used was undamaged leaves collected one or two days before feeding and kept fresh in plastic boxes with water in a cool room. The leaves were collected in the same area as the larvae.

Figure 2. Box with *Z. exulans* larva used in the feeding preference experiment. Photo: T. Hasle.

We starved the larvae overnight to make conditions for all individuals as similar as possible and to make sure they were motivated to eat during the feeding trials (Jogesh et al. 2008; Pérez-Harguindeguy et al. 2003). We fed the larvae in the morning by putting leaves in the center of the boxes and placing the larvae next to the leaves to make sure they all had the same chance to discover the food. In the no-choice test each larva was fed two leaves, both from the same plant species, in each trial. After ca 11 hours, we removed the leaves. Because of variation in interspecific and intraspecific leaf size, we classified the leaves as either *not eaten* (less than 10% eaten) or *eaten* for the statistical analysis. Each larva was used in several trials and we randomized which larvae that were fed which plant species. Dead or molting larvae were removed from the experiment and recordings on these individuals were excluded if they did not feed in earlier trials. This was done because *Z. exulans* larvae do not eat for 5-6 days before molting (Hågvar 1976). We did new trials to replace the ones excluded so that we had 20 replicates of each plant species.

2.4 Statistics

The field data were not normally distributed and it was not possible to transform them to normality. Therefore I used nonparametric tests. I used R version 2.14.2 (R Development Core Team 2012) in all statistical analyses.

I used Mann Whitney U tests in all analyses except for the feeding preference experiment. In most Mann Whitney U tests, Treatment (OTC or control) was used as explanatory variable. The response variable was either Number of damaged leaves, Relative amount of damages, Number of all Lepidoptera larvae, or Number of *Z. exulans* larvae. Relative amount of damages was calculated as the number of damaged leaves divided by the percentage cover of the damaged plant species. To determine the relationship between feeding damages and presence of all Lepidoptera larvae or presence of *Z. exulans* larvae alone, I used Mann Whitney U tests with Number of damaged leaves as response variable and Presence of all larvae (1 or 0) or Presence of *Z. exulans* larvae (1 or 0) as explanatory variables. These analysis included data from both the OTCs and the control plots. Data from early and late season were always combined in analyses with all Lepidoptera larvae. Only early season data were used in analyses with *Z. exulans*, because these larvae were only recorded in this period. To compare preferences of *Z. exulans* in the feeding experiment, I tested whether number of replicates with feeding damages differed between plant species by using Fisher's exact test. Number of replicates with and without damages was used as response variable, and each plant species as explanatory variable. I performed tests for all possible combinations of plant species.

3 Results

3.1 *Feeding damages*

Based on the difference in medians, experimental warming increased the total number of damaged leaves by 115% late in the season, whereas early in the season warming had no significant effect (Fig. 3). Feeding damages were recorded on *D. octopetala*, *B. vivipara*, *S. reticulata*, *S. alpina*, *T. pusilla*, *Vaccinium myrtillus* L., *V. uliginosum* L., *Parnassia palustris* L., *Thalictrum alpinum* L., and *Carex* species. Other vascular plants, for example *S. herbacea*, were observed in the plots as well, but none of these had feeding damages.

Combined, *D. octopetala*, *B. vivipara*, and *S. reticulata* had more than 95% of the recorded damages. Early in the season none of these species showed a significant difference in number of damaged leaves in OTCs compared to control plots, whereas late in the season warming increased the number of damaged leaves on *D. octopetala* by 310% and on *B. vivipara* by 153%, based on the medians (Table 1). However, when the cover of *D. octopetala*, *B. vivipara*, and *S. reticulata* in the plots was accounted for in the analyses, *D. octopetala* was the only species that showed an increase in amount of damages with warming (Fig 4). In contrast to the increase in Number of damaged leaves, this increase in Relative amount of damages for *D. octopetala* was significant both early and late in the season. Based on the medians, the increase was 168% early and 458% late. For late season the increase was significant even when the outlier with the highest value was removed (Mann Whitney U tests: W = 62.0, $p < 0.001$, control: n = 9, OTC: n = 7, Fig. 4 d).

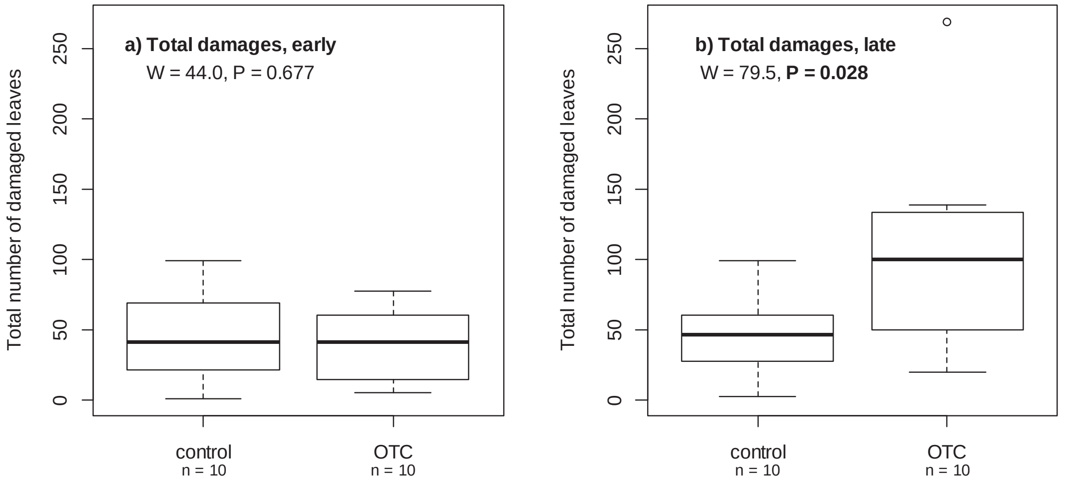


Figure 3. Total number of damaged leaves in OTCs and control plots for early and late season, Finse, Norway, 2012. Box plots show the median, boxes mark the interquartile range. Whiskers show the minimum and maximum values that fall within 1.5x the length of the box away from the interquartile range, data further away are shown as outliers. P values in bold are significant at 5% level according to Mann Whitney U tests. Without the outlier in Fig b; W = 69.5, p = 0.050.

Table 1. Number of damaged leaves on *B. vivipara*, *D. octopetala*, and *S. reticulata* in OTCs and control plots for early and late season, Finse, Norway, 2012. Medians for each group are shown, in parenthesis are the 1st and 3rd quartiles. P values in bold are significant at 5% level according to Mann Whitney U tests.

		Control		OTC			
Species	Season	Number of damaged leaves	n	Number of damaged leaves	n	W	P
B. vivipara	Early	14 (7, 31)	10	7 (1, 15)	10	41.5	0.543
	Late	9 (4, 18)	10	22 (13, 25)	10	78.0	**0.037**
D. octopetala	Early	26 (10, 49)	9	30 (9, 42)	8	44.0	0.677
	Late	26 (11, 56)	9	107 (34, 120)	8	79.5	**0.028**
S. reticulata	Early	0 (0, 2)	9	0 (0, 0)	5	21.0	0.867
	Late	1 (0, 2)	8	0 (0, 2)	10	30.5	0.379

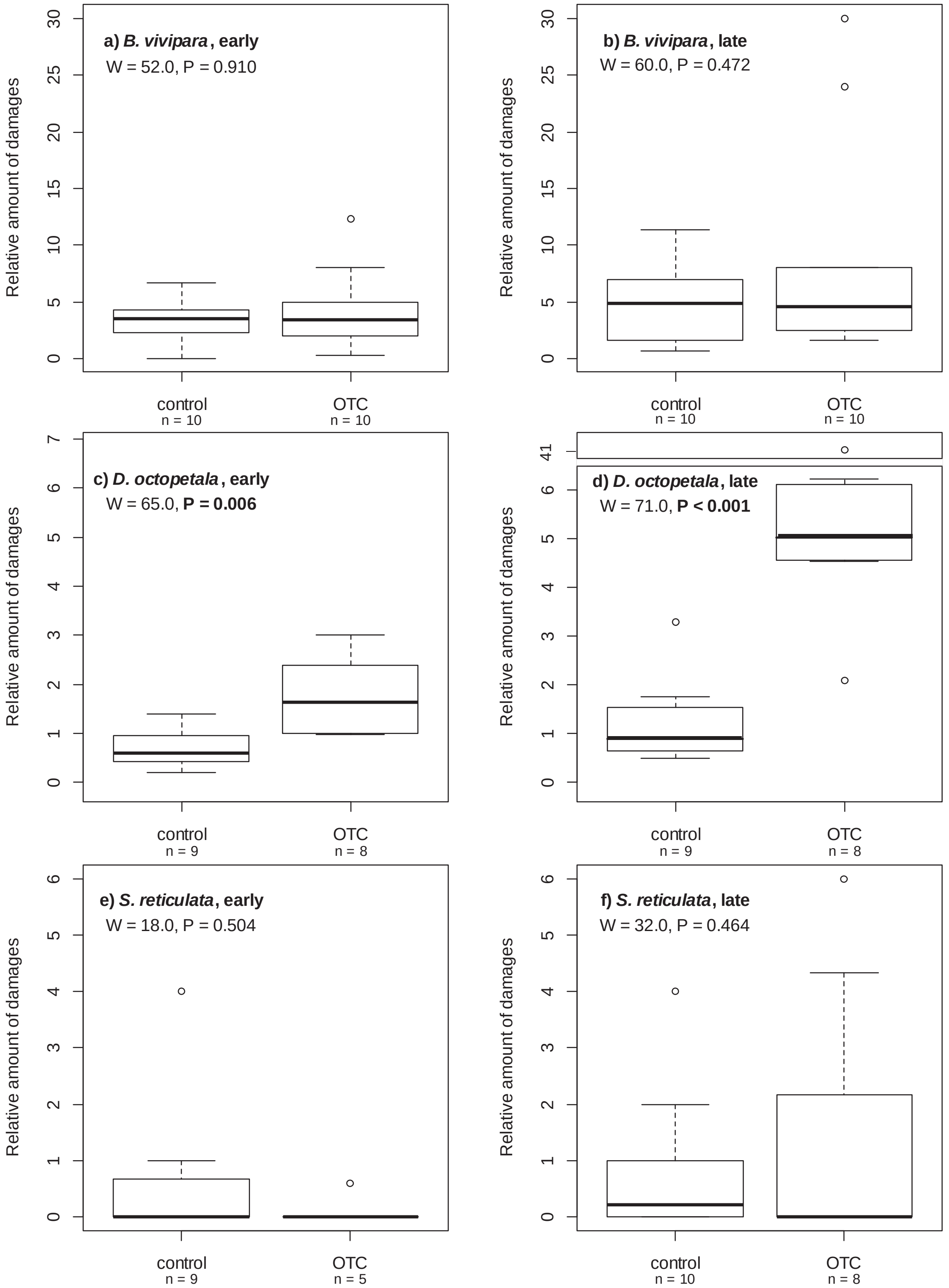


Figure 4. Relative amount of damages calculated as number of damaged leaves divided by percentage cover of the damaged plant species in OTCs and control plots for early and late season, Finse, Norway, 2012. Box plots show the median and boxes mark the interquartile range. Whiskers show the minimum and maximum values that fall within 1.5x the length of the box away from the interquartile range, data further away are shown as outliers. P values in bold are significant at 5% level according to Mann Whitney U tests. Note the axis break in Fig. d and the differences in scale.

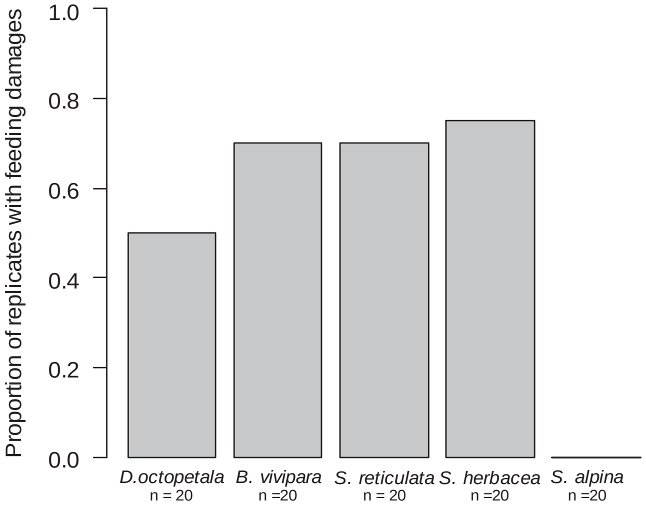
3.2 Presence of insects and feeding preference experiment

Few Lepidoptera larvae were recorded in the plots (Table 2) and the ones found were *Z. exulans, E. pandrose,* and Noctuidae species. No beetles feeding on vascular leaves were recorded. There was no significant effect of warming on occurrence of Lepidoptera larvae or *Z. exulans* larvae only (Mann Whitney U tests: W = 183.5, P = 0.486, n = 40 and W = 51.5, P = 0.914, n = 20, respectively). Furthermore, there were no significant differences in the number of feeding damages between plots with and without Lepidoptera larvae (Mann Whitney U test: W = 127.0, P = 0.353, larvae present: n = 6, larvae absent: n= 34) or with and without *Z. exulans* larvae (Mann Whitney U tests: W = 48.0, P = 0.142, *Z. exulans* present: n = 4, *Z. exulans* absent: n = 16).

The no-choice test showed that the *Z. exulans* larvae preferred *S. alpina* less than *D. octopetala*, *B. vivipara*, *S. reticulata*, and *S. herbacea* (Fisher's exact test: P < 0.001 for all combinations) (Fig. 5). However, there were no significant differences between the preference for *D. octopetala*, *B. vivipara*, *S. reticulata*, and *S. herbacea* (Fisher's exact test: P > 0.1 for all combinations).

Table 2. Number of plots with Lepidoptera larvae early and late season, Finse, Norway, 2012. Number of larvae in each plot is given in parenthesis.

	All Lepidoptera larvae		*Z. exulans* larvae only	
	Early	Late	Early	Late
Control plots	2 (1 + 2)	2 (1+1)	2 (1 + 2)	0
OTCs	2 (2 + 3)	0	2 (2 + 3)	0



Figur 5. The preference of *Z. exulans* larvae for *D. octopetala*, *B. vivipara*, *S. reticulata*, *S. herbacea*, and *S. alpina* in a laboratory no-choice feeding experiment.

4 Discussion

4.1 Feeding damages

Warming increased the total number of damaged leaves in an alpine *Dryas* heath at Finse late in the season. This is consistent with Bergmann (manuscript) who showed that there were more damaged leaves in the OTCs than in the control plots at a higher elevation site at Sanddalsnuten. The results from Finse are in accordance with Roy and colleagues (2004) who found that experimental warming increased the herbivory by leaf-chewing insects in a montane meadow in the Rocky Mountains. Furthermore, Kozlov (2008) found that the herbivory by leaf-chewing and leaf-mining insects increased with warmer climate along a gradient in Fennoscandia. Studies of fossil leaves from the Paleocene-Eocene global warming period also show that the amount of damages increased with warming (Wilf & Labandeira 1999).

The increase in total number of damaged leaves late in the season was mainly due to feeding marks on *D. octopetala* and *B. vivipara*. However, when the cover of the plant species was accounted for, only *D. octopetala* showed a significant increase. This indicates that the increase in number of damaged leaves on *D. octopetala* was due to increased herbivore pressure in the OTCs, whereas the increase in damaged leaves on *B. vivipara* probably was due to greater cover of the species in the OTCs. This is consistent with Bergmann (manuscript) who found that warming increased the cover of *B. vivipara* at the higher elevation site at Sanddalsnuten. The increase in number of damaged leaves in some species, but not others, is in accordance with Richardson and colleagues (2002). They found that warming of a subarctic dwarf shrub heath in Scandinavia increased the herbivory on *V. myrtillus* by 600%, but had no effect on *V. uliginosum* or *V. vitis-idaea* L. The effect of warming on the amount of insect herbivory in a montane meadow in the Rocky Mountains also varied between the plant species (Roy et al. 2004).

The increase in herbivory on some plant species, but not others, may have several explanations. For example, warming might have changed the abundance, species composition, feeding amount, or food preference of the insects (Bale et al. 2002; Liu et al. 2011; Niziolek et al. 2012; Schoonhoven et al. 2005). Warming might also have altered the chemical composition the of plants (DeLucia et al. 2012) or the occurrence and population densities of the insects' parasitoids, competitors, or predators (Ayres & Lombardero 2000; Both et al. 2009), and thereby influenced the amount of herbivory.

Warming might have had a direct species-specific effect on insect abundance. If this was the case, the species that preferred *D. octopetala* should be the ones that increased the most in abundance in order to explain why warming only increased the amount of herbivory on *D. octopetala*, and not on the other species. However, neither total number of Lepidoptera larvae, nor *Z. exulans* larvae only, were more abundant in the OTCs than in the control plots. Furthermore, there was no relationship between the presence of Lepidoptera or *Z. exulans* larvae and the amount of feeding damages in my study. However, I only did recordings once early and once late in the season, and I recorded very few larvae. Recordings of larvae every day for a longer period might have detected a more correct diversity and abundance of insects. Analyses combining the data from my study with the data from the higher elevation site at Sanddalsnuten show that there were more *Z. exulans* larvae in the OTCs than in the control plots, but for the total number of Lepidoptera larvae there was no significant difference (Bergmann manuscript). Accordingly, studies from other areas show that the effect of warming on abundance of leaf-chewing insects varies between the species; experimental warming increased the density of the larvae of the moth *Melanchra pisi* (Linnaeus 1758) by a factor of 10 in a Tibetan meadow (Liu et al. 2011), whereas it decreased the density of Symphyta larvae and weevils in a *D. octopetala* heath at Svalbard (Dollery et al. 2006).

Another possible reason for the increased amount of damages on *D. octopetala* in the OTCs is that the amount eaten per insect of this species increased. Increased feeding can happen because insects are poikilotherms and up to a certain limit, warming stimulates their metabolism (DeLucia et al. 2012; Ipekdal & Caglar 2012; Niziolek et al. 2012; Speight et al. 2008). Niziolek and colleagues (2012) found that beetles of the herbivorous species *Popillia japonica* (Newman 1841) fed more under warmed compared to ambient conditions. In contrast, Johns and colleagues (2003) found that this was not the case for two other herbivorous beetles, *Octotoma championi* (Baly 1886) and *O. scabripennis* (Guérin-Méneville 1844). This indicates that the effect of warming on consumption per insect is species-specific. To my knowledge, no similar studies have been done for the insect herbivores at Finse. However, it is possible that the species preferring *D. octopetala* in my study site increased feeding as a response to warming and ate more per individual than the species preferring other plants.

Phenological changes in insects or plants due to warming causing longer time for feeding might also have increased the amount of biomass eaten per insect. Warming can lead to earlier hatching or emergence of insects (Masters et al. 1998; Miles et al. 1997; Woiwod 1997, but see Liu et al. 2011). If warming advances the time of snowmelt, the early emerging insects can start feeding on the wintergreen *D. octopetala*. However, not all plant species will advance their phenology. The initiation of growth in deciduous species and herbs like *B. vivipara* is often determined by photoperiod and not by temperature (Welker et al. 1997 and references therein). This might be another reason why warming only increased the amount of herbivory on *D. octopetala*, and not on the other species.

Furthermore, it is possible that changed food preference of the insects can explain the increase in damages on *D. octopetala*. Some studies report that the preferred food plant of insect herbivores changed with warming, although it is not known if this was mediated by changes happening to the insects themselves or to their food plants (Ipekdal & Caglar 2012; Schalk et al. 1969; Schoonhoven et al. 2005). To my knowledge, this has not been studied for the species at Finse.

The differences in herbivory between the plant species might have been induced by changes in concentration of antiherbivory compounds due to warming (DeLucia et al. 2012; Hansen et al. 2006). However, an earlier study at Sanddalsnuten found that in both *D. octopetala,* *B. vivipara*, and *S. reticulata* the concentration of carbon-based secondary compounds (CBSCs) was hardly affected by experimental warming (Nybakken et al. 2008). Therefore, if the explanation for the increase in damages on *D. octopetala* is that the concentration of antiherbivory compounds had decreased, it might have been caused by other compounds than CBSCs.

Plant quality can also change if warming leads to altered nutrient concentration (DeLucia et al. 2012; Niziolek et al. 2012). The research of Richardson and colleagues (2002) showed that for *Vaccinium* species, shoot nitrogen concentration was more important than phenolics in explaining the amount of herbivory. Nitrogen concentration in *D. octopetala* has shown contrasting responses to warming, both negative (Welker et al. 1993; Welker et al. 1997), positive (Rustad et al. 2001), and no response (Welker et al. 2005; Wookey et al. 1995). To my knowledge, no studies have been done on nitrogen content in *D. octopetala* at Finse, therefore it is uncertain whether increased nitrogen concentration might explain the increased herbivory in the present study.

Most of the damages seen on the leaves in the field looked similar to those made by *Z. exulans* in the feeding preference experiment. However, there were other insect herbivores feeding in the area as well, both Lepidoptera larvae and, according to pitfall traps placed ca 40 m from the OTCs, Chrysomelidae (Bergmann manuscript). I was unable to separate the feeding damages made by the different insect species. This complicates the interpretation of which insect species that were influenced by the warming and caused the increase in number of damaged leaves on *D. octopetala*. Early in the season, the insect recordings from the site in the present study, the higher elevation site at Sanddalsnuten, and the pitfall traps (Bergmann manuscript) indicate that *Z. exulans* is the most abundant insect herbivore. This suggests that the increase in herbivory on *D. octopetala* in the OTCs early in the season mostly was caused by *Z. exulans*. *Z. exulans* has a life cycle that lasts for at least two years and overwinter as larvae (Hågvar 1976; Tremewan 1985). In the feeding experiment most of the *Z. exulans* larvae stopped eating in the end of June, after about a week in the laboratory (pers. obs.), even though they live in habitats with a long snow-free period and can be observed throughout the summer at Finse (Hågvar 1976). However, the larvae used in the present experiment started feeding again after an experimental cooling period in August (Birkemoe pers. comm. 2013). This indicates that young *Z. exulans* larvae feed mostly early in the season and then go into a diapause before they continue feeding the next spring. The older larvae pupate in early July (Hågvar 1976). The early season feeding pattern of *Z. exulans* and the occurrence of other Lepidoptera larvae in late season in my study site, the higher elevation site at Sanddalsnuten, and in the pitfall traps (Bergmann manuscript), indicate that the increased amount of damages on *D. octopetala* in the OTCs late in the season probably was caused by additional insect herbivore species as well as *Z. exulans*.

4.2 The limitations of OTCs for predicting insect herbivory

Based on my data, I do not know whether the insects aggregated in the OTCs when feeding and moved out when not feeding, or if they mainly stayed in the OTCs. Gaps under the walls of the OTCs allowed the insects to move in and out, although the movement might have been restricted. Free movement of insects into warmer areas to feed can cause unrealistically high densities in the OTCs (Moise & Henry 2010). If that happened in the present study the results do not show a realistic response to climate warming, because when the whole region is warm the insects cannot escape from the heat when not feeding. Another source of error in the experiment was that the insects might have preferred to stay in the OTCs because there they were more protected from wind (pers. obs.; Richardson et al. 2002). This effect can be avoided by using overhead heaters instead of OTCs (e.g. Roy et al. 2004). This can be an alternative for further herbivory research, but is more expensive than OTCs, and is not suited for remote and powerless areas such as the site in the present study (Shen & Harte 2000).

4.3 Feeding preferences of Z. exulans

The feeding preference experiment showed no significant difference between *Z. exulans* larvae's preference for *D. octopetala*, *B. vivipara*, *S. reticulata*, and *S. herbacea*. This is in contrast to an earlier experiment with *Z. exulans* at Finse by Hågvar (1976). He found that they preferred *S. herbacea* and other *Salix* species and hardly fed on *B. vivipara* (*D. octopetala* and *S. reticulata* were not included in the experiment). The differences in results might be caused by induced preference, a preference for the plant fed on earlier (Bernays & Weiss 1996; Jermy et al. 1968), because the larvae from the two experiments were collected in different vegetation types. *S. herbacea* was common in the site of Hågvar (1976), whereas it was almost absent from my study site (pers. obs.). Induced preference is quite common among larvae of Lepidoptera species (Bernays & Chapman 1994; Schoonhoven et al. 2005), but to my knowledge, not studied for *Z. exulans*.

In contrast to the feeding experiment, in the field I found more damaged leaves on *D. octopetala* and *B. vivipara* than on *S. reticulata* and *S. herbacea*. This might be because the two latter species were less abundant in the field (pers. obs.). As noted by Pérez-Harguindeguy and colleagues (2003), feeding preference experiments are most useful when comparing feeding on plant species that have the same accessibility in the field. Furthermore, one should be careful comparing the results from the experiment and the field because the data came from a no-choice test that tested which of the species the larvae accepted to eat, not the preference from a choice between alternatives. The difference between the results in the feeding experiment and in the field may also have been caused by the other insect herbivore species feeding in the study site. *Z. exulans* never fed on *S. alpina* in the experiment, so the damages on this species in the field were likely caused by other insect herbivores.

5 Conclusion

Even with the limitations in using OTCs for herbivory research, the present study provides an indication of how the herbivory by leaf-chewing insects might respond to climate warming. It indicates that some species, such as *D. octopetala*, will be more susceptible to insect herbivory than others when the temperatures increase due to global warming. I have discussed possible explanations for why the effect of warming on herbivory varied between the plant species, however, research is needed to determine the causal relations. Furthermore, the impact of increased herbivory on plant performance has not been assessed in this study, and would be interesting to examine. Climate change includes more than just warming, and is predicted to cause differences in precipitation, snow regime, and availability of nutrients (Hobbie 1996; IPCC 2007). It would be interesting to examine the combined effect of these factors on insect herbivory to get more reliable predictions following future climate change. More research is needed to determine the preferred food plant of *Z. exulans* larvae, both because the no-choice test in the present study did not show a pronounced preference and because the results from the present and earlier experiments at Finse diverged. I suggest doing a paired-choice test early in the season to avoid inactive larvae.

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