Norwegian University of Life Sciences
Faculty of Environmental Sciences
and Natural Resource Management

Philosophiae Doctor (PhD)
Thesis 2019:28

Herbivory and climate effects on plant defenses: an ecological and molecular approach

Effekter av beiting og klima på planteforsvar: en studie med økologiske og molekylære metoder

Rafael Fonseca Benevenuto

Herbivory and climate effects on plant defenses: an ecological and molecular approach

Effekter av beiting og klima på planteforsvar:
en studie med økologiske og molekylære metoder

Philosophiae Doctor (PhD) Thesis

Rafael Fonseca Benevenuto

Norwegian University of Life Sciences
Faculty of Environmental Sciences and Natural Resource Management

Ås (2019)

Thesis number 2019:28
ISSN 1894-6402
ISBN 978-82-575-1588-1

PhD supervisors

Associate Professor Dr. Stein Joar Hegland – Main supervisor
Department of Environmental Sciences
Faculty of Engineering and Science
Western Norway University of Applied Sciences
NO-6851 Sogndal, Norway

Professor Dr. Stein Ragnar Moe
Faculty of Environmental Sciences and Natural Resources Management
Norwegian University of Life Sciences
NO-1432 Ås, Norway

Head of Department Dr. Tarald Seldal
Department of Environmental Sciences
Faculty of Engineering and Science
Western Norway University of Applied Sciences
NO-6851 Sogndal, Norway

Associate Professor Dr. Cesar Rodriguez-Saona
Department of Entomology
School of Environmental and Biological Sciences
Rutgers, The State University of New Jersey
NJ 08901 New Brunswick, USA

Evaluation committee

Assistant Professor Dr. Sergio Rasmann
Institute of Biology
Faculty of Science
University of Neuchâtel
CH-2000 Neuchatel, Switzerland

Professor Dr. Laura Jaakola
Department of Arctic and Marine Biology
UiT, The Arctic University of Norway
NO-9037 Tromsø, Norway

Committee coordinator:
Professor Dr. Line Nybakken
Faculty of Environmental Sciences and Natural Resource Management
Norwegian University of Life Sciences
NO-1432 Ås, Norway

Table of Contents

ACKNOWLEDGEMENTS

First of all, I would like to thank my big team of supervisors, Stein Joar, Tarald, Stein and Cesar. I'm so grateful for being chosen as your PhD student to work in such interesting project. Working with all of you has been a huge pleasure. You were always available for advising me, and open to great discussions with your different background, always in a lighter way. Thank you for showing me that a PhD can be fun!

I want to thank the extended people who has contributed, in different ways, to the conclusion of these PhD project. I had the opportunity to live and work in completely different environments and research groups, in Norway (HVL and NMBU) and the USA (Rutgers University), which I believe added much on my formation. People at the Høgskulen på Vestlandet (Western Norway University of Applied Sciences), in Sogndal, warmly received me in the cold winter of January 2016, the only South American in the department at this time. I had the lucky of spend great time conducting my field work in the inner of Sognefjord, one of the most beautiful landscapes I've ever seen. A special thanks to Mark and Joachim for all the statistics help. I also spent a wonderful time at NMBU, where I've made good friends and had the opportunity to learn a lot with my ecologist colleagues. I can't say that living between Oslo and Drøbak was a bad experience. Finally, I had the great opportunity of spending almost a year at Rutgers, The State University of New Jersey in New Brunswick. I want to thank Cesar and his research group, as well as all the other entomologist and molecular biologist researchers, from the Philip E. Marucci Center for Blueberry and Cranberry Research and Extension, that I had the opportunity of work with during this wonderful time. Thank you very much!

There are some special people that I've met over these three years that for sure turned this time much special. My Portuguese crew in Sogndal made me few at home even during the cold Norwegian winter in Sogndal, in special my good friend Victor Aguiar. *Saudades meu*

amigo, você vai longe! I want to thank you Karoline, my good friend since South African times, for opening the doors of your house in Ås, and share good and hard times with me. I'm waiting for your visit in Brazil! My good German friend Sylvia, thank you for the good conversations and lunch times at MINA. My Brazilian friends, I mean BrasileirÅs, always ready to party and have a good laugh. Thank you all for the good times! Tati, my partner in crime, you were for sure one of the most special persons that this time in Norway gave me. Thank you for the relaxing times drinking a beer, the hard times complaining about the cold and dark winter, and of course for the epic parties. I will miss you! Michele, my Italian friend, thank you for your friendship and to have made my social life in Oslo much more interesting. Keep strong in your PhD, and I'm waiting for your promise of moving to Brazil for a postdoc. Vini, my research partner, even in distance you know how important your friendship and advises were, inside and outside the lab. Finally, I want to thank you Moa, for being such a wonderful person. Thank you for your partnership and patience at all times, always giving me forces to go ahead. Just you know how important you were during this last year, and how essential you are in my life.

Thanks to my lovely family, you are the best. Father and mother, you know how difficult was spend long times far from you, but I did it for us, you know that. I love you so much. Thank you for giving me all the support I needed until now. Cado and Gabi, my siblings, thank you for being my best friends, for understanding me, and loving me unconditionally. Thank you also to my friends from life for being always there, so enthusiastic giving me support during this trajectory, in special: Bastos, Tomé, Gui, Pri, Julia, Julinha, Ruth, Carol, Gabi, Marina, Mila, Camilinha, Marijn and Cris. I love you.

List of papers

This thesis consists of the following papers that are referred to by the roman numerals (I-IV):

Paper I

Multiannual effects of induced plant defenses: Are defended plants good or bad neighbors?

Benevenuto RF, Hegland SJ, Töpper JP, Rydgren K, Moe SR, Rodriguez-Saona C, Seldal T. (2018). *Ecology and Evolution*, 8(17), 8940-8950.

Paper II

Transcriptional profiling of methyl jasmonate-induced defense responses in bilberry (*Vaccinium myrtillus* L.)

Benevenuto RF, Seldal T, Hegland SJ, Rodriguez-Saona C, Kawash J, Polashock J. (2019). *BMC Plant Biology*, 19(1), 70

Paper III

Molecular and ecological plant defense responses along an elevational gradient in a boreal ecosystem.

Benevenuto RF, Seldal T, Polashock J, Moe SR, Rodriguez-Saona C, Gillespie MA, Hegland SJ. (2019). *Manuscript*.

Paper IV

Neighborhood effects of herbivore-induced plant resistance vary along an elevational gradient.

Benevenuto RF, Moe SR, Rodriguez-Saona C, Seldal T, Hegland SJ. (2019). *Submitted.*

Summary

Knowledge of plant defense responses and how they interact with biotic (e.g., herbivores) and abiotic (e.g., temperature) factors is of fundamental importance for understanding ecosystem functioning. Increases in temperature can dramatically weaken plant immune systems, and thereby cause elevated herbivory rates. On the other hand, rising temperatures can make plants more alert, and consequently result in investment in more effective defense strategies. Such adaptation can potentially influence plant-animal and plant-plant interactions. The effects of climate change on plant defenses can have particularly strong impacts on biodiversity and ecosystem functioning of vulnerable high-latitude systems. The boreal forest is the dominant biome in Fennoscandia (the Scandinavian peninsula and Finland), where bilberry (*Vaccinium myrtillus* L.), a widely distributed dwarf shrub, is considered to be a key food source for many species, and an excellent model organism for ecological studies in the boreal system.

The main objective of this thesis is to explore bilberry defense responses through plant-herbivore and plant-plant interactions, as well as investigate how these relationships are affected by environmental variation. To investigate this, we experimentally treated bilberry ramets with methyl jasmonate (MeJA) to simulate herbivory and induce plant defenses. Subsequently we observed ecological and molecular responses in relation to growth, reproduction and defense along an elevational gradient in a boreal system in Western Norway. The elevational gradient design mainly reflected variation in temperature and snow cover, and included optimal growing conditions for bilberry (ca. 500 m a.s.l.; mid-montane zone), as well as the plants' range limits at low (ca. 100 m a.s.l.; submontane zone) and high (ca. 900 m a.s.l.; subalpine zone) altitudes.

We observed that MeJA-induced defenses in bilberry plants effectively reduced insect and mammalian herbivory, as well as growth and reproduction across the three years of study. Such findings provide evidence for an effective ecological trade-off between growth/reproduction and defense over multiple seasons. Interestingly, bilberry induced defense systems occurred in a delayed manner, with the strongest effects from one (insect herbivores) to two (mammalian herbivores) years after induction. We also provide evidence of long-term effects on plant-plant communication, indicating that MeJA-induced bilberry plants are "good" neighbors, due to ecological facilitation with conspecifics in the boreal system (*Paper I*). Subsequently, we reported such a trade-off at transcriptional level, which I termed a 'genetic trade-off', highlighting molecular mechanisms underpinning and supporting our previous field observations (*Paper II*).

We found that bilberry defense responses are modulated by the altitudinal variation in climate and herbivory at both molecular and ecological levels. High-altitude plants living under cooler temperatures and limiting resource availability invest more in constitutive defenses, whereas low-altitude plants growing under higher temperatures and herbivory pressure rely strongly on induced defenses (*Paper III*). Environmental variation along the elevational gradient also affected plant-plant communication. Suboptimal lower (warmer submontane zone) and optimal medium-altitude (mid-montane zones) environments in the boreal system favored the facilitation of defense-induced plants with long distanced conspecifics; as compared to the suboptimal upper elevation (colder subalpine zone) (*Paper IV*). The greater prominence of this inducible plant defense system at lower altitudes may presage the kinds of change expected in plant-plant communication systems and associated VOC emissions in a warmer world, which will likely feedback to further influence ecological functioning. Such findings support previous observations that under increasingly warmer conditions and higher herbivory

pressure, bilberry plants are more responsive to herbivory by strongly relying on induced defenses against future attacks.

In this thesis we document that multiannual induced defenses in bilberry plants cause resource allocation from growth and reproduction to defense (trade-off), also seen at gene level, with its strongest effect delayed. We also report that altitudinal variation modulated defense capacity, where plants from warmer low/medium altitudes were more responsive to induced defenses in terms of plant-herbivore and plant-plant interactions. Due to rapid global warming, higher elevations are likely to become the new optimal growing environment for bilberry in the near future. Therefore, if low-altitude plants migrate to higher elevations, they will need to re-adapt to novel biotic (e.g. less diversity and herbivory pressure) and abiotic (e.g. harsher environment and less resource availability) conditions. Furthermore, abundance of 'more-defended' plants in subalpine zones can affect herbivore population dynamics, by limiting cycle amplitude due to higher levels of defense during the growing season. Such a combination of events in response to climate change might catalyze new ecological and co-evolutionary dynamics in the boreal system.

Synopsis

1. INTRODUCTION

Climate is changing at an unprecedent way due to human-induced climate change (IPCC 2018). Atmospheric temperature, precipitation, snow cover and distribution, are all changing faster than expected (Loarie et al. 2009). Evidences from across the globe shows that many ecosystems are being affected by such changes in climate (IPCC 2018). As a consequence, one of the biggest challenges for human society today is to slow the rate of global warming and adapt to climate change. The distribution, abundance, and phenology of plants and animals are already changing by environmental changes, of which climate warming is one of the most important (Parmesan 2006). For example, over the next century, climate change is projected to considerably alter soil and air temperatures in temperate forest ecosystems, where high-latitude forests are particularly at risk (IPCC 2018; Serreze et al. 2000). It is generally accepted that the largest temperature increases are currently found in the Northern Hemisphere upper latitudes, where the boreal forest resides (Serreze et al. 2000). Increases in mean annual air temperature during the growing season will reduce snow depth and alter timing of snow melt in high-latitude ecosystems (Fan et al. 2014; Hayhoe et al. 2007).

For many species, the main impact of climate change may be mediated through effects on synchrony with that species' food and habitat resources (Parmesan 2006). The most crucial is the potential disruption of coordination in timing between the life cycle of predators and their prey, herbivorous insects and their host plants, parasitoids and their host insects, and insect pollinators with flowering plants (Harrington et al. 1999; Visser and Both 2005). Small changes in climate can have profound impacts for the ecosystem if species that depend on one another, react differently to an increase in temperature, thus leading to asynchrony (Hegland et al. 2009). Furthermore, altered environments are also expected to influence plant phenology (e.g., differences in growth and maturation), physiological factors (e.g., physicochemical properties of volatiles compounds and stomatal function in plants) and allocation of resources (e.g., trade-off between growth, reproduction and defense) (Bale et al. 2002; Stenseth and Mysterud 2002; Visser and Both 2005). Estimate possible consequences of current and further climate change in ecosystems dynamics and species interactions is becoming increasingly urgent (Schroter et al. 2005; Wu et al. 2011). For instance, increases in temperature can dramatically affect the ecosystem by weakening plant immune systems and thereby cause spreading of plant diseases, elevate herbivory rates, and reduce pollinator populations (Cheng et al. 2013; Velásquez et al. 2018). On the other hand, temperature rising can also make plants more alert, so that they can invest in effective defense strategies and better defend themselves (Bidart-Bouzat and Imeh-Nathaniel 2008). Despite all information that exist on global climate change, still little is known about how these changes may affect ecosystem functioning, particularly interactions among living organisms. If plant defense systems, consequently plant-herbivore and plant-plant interactions are substantially affected by global warming, important indirect consequences on ecosystem functioning can occur in the near future. Moreover, studies on ecological consequences of climate change in ecosystem dynamics must be dependent on how dominant and widespread species is affected, and how many species that live on them may be affected.

1.1. Plant defense responses: plant-herbivore and plant-plant interactions

Knowledge on plant defense responses and how these are affected by biotic (e.g., herbivores, pathogens, disease) and abiotic factors (e.g., temperature) is of fundamental importance for understanding ecosystem functioning. Plants protect themselves against herbivory by spreading a wide range of chemical defenses (Agrawal 1999). Green and Ryan (1972) were the first to demonstrate that plants under attack from herbivores produce chemical defense compounds that help to protect them from further damage. Wounding-induced plant responses can directly target herbivores by stimulating the synthesis of toxic or antifeedant metabolites, as well as by activating antinutrient enzymes such as proteinase inhibitors or polyphenol oxidases (Felton et al. 1994; Stout et al. 1996). The role of chemical defenses is mainly regulated by jasmonic acid (JA) and related hormones, which is a key compound on the activation of plant defense response process. The involvement of JA in regulating gene activation subsequent to wounding has been established in several plant species (Staswick and Lehman 1999; Wasternack et al. 1998). There are evidences that plant defense systems can be also activated experimentally by exogenous application of methyl jasmonate (MeJA), a ubiquitous defense signal in plants released in response to tissue damages (Koo and Howe 2009; Pieterse et al. 2012).

As part of chemical defense system, plants are known to emit volatile organic compounds (VOCs) from both above ground vegetative and reproductive tissues (i.e., leaves, flowers and stems) and below-ground roots (Dudareva et al. 2004). Once emitted by the plant, VOCs can be also perceived by surrounding organisms, which play various ecological roles in interactions between plants and other co-existing organisms (i.e., pollinators, pathogens, predators, herbivores and parasitoids). Indeed, constitutive (that are always expressed) and induced (expressed in response to herbivory) plant volatiles play important ecological roles in plant-plant and plant-animal interactions. Constitutive VOCs serve as constant barriers to herbivore attack by deterring colonization through their antixenotic function, as well as inhibiting growth, reproduction, development, and survival through their antibiotic function (Paiva 2000; Walling 2000). These characteristics are referred as ‘bottom-up’ defenses, as they directly impact herbivore performance. On the other hand, induced VOCs is the activation of chemical defense system in response to attack or perceived herbivore presence. Induced VOCs can serve as direct defense, affecting the herbivore through immediate toxicity (‘bottom-up’ defense) or as an indirect (‘top-down’) defense, affecting the herbivore via recruiting its natural enemies (Kessler and Baldwin 2001).

The role of VOCs in mediating signaling between plants has also been explored, and these interactions are frequently referred to as plant-plant interaction or communication. Plants are capable of detecting and responding to neighboring plants, generating consequences for plant performance and playing important roles in ecosystem functioning (Callaway 2002; de Wit et al. 2015; Genung et al. 2012). Plant–plant interactions can occur both above and belowground and are mediated through internal signals driven by specific compounds moving within interconnected ramets in some clonal species (Gomez et al. 2010), or by external VOCs emitted by neighboring plants (Rodriguez-Saona et al. 2009). Karban (2008) proposed three criteria for recognizing that plants "communicate": First, the cue (signal) should cause a rapid response in the receiving plant; second, the cue needs to be plastic and the response to be conditional on receiving the cue; and third, the emission of a cue needs to be "intentional" and beneficial. Some plants "eavesdrop" VOCs emitted by other plants; that is, neighboring plants receive and utilize chemical signals that may have originally been released to repel herbivores (direct defense) or attract natural enemies (indirect defense) by nearby infested plants (Bruin and Dicke 2001). This phenomenon suggests that undamaged plants, utilize VOCs from damaged neighbors as cues to prepare for future herbivore attacks (Kobayashi and Yamamura 2003). Furthermore, some studies suggest that undamaged eavesdropper plants turn into signalers, referred as "cooperative signal", because undamaged and damaged individuals signal together to call natural enemies (Chamberlain et al. 2001; Dicke and Bruin 2001; Kobayashi and Yamamura 2003).

Although plant response to external signals from damaged neighboring plants have been discussed during the last years (Arimura et al. 2002; Farag and Pare 2002; Ruther and Kleier 2005), it's still not clear whether amounts of airborne signals emitted from damaged plants are abundant enough to efficiently trigger defense responses from neighbors at long distances, as well as how abiotic factors can affect this interaction under natural system. Moreover, if plant defense systems, as well as plant-herbivore and plant-plant interactions are affected by environmental variation, important indirect consequences on ecosystem functioning (e.g., outbreaks in herbivore populations) can occur in further changes in climate of high-latitude environments.

1.2. Climate effects on plant defenses

Plant defense responses to herbivory are dependent on the plants' life history (e.g., past exposure to herbivory), as well as the environment, affecting plant-herbivore interactions (Tollrian and Harvell 1999). Human-induced changes in important environmental factors, such as atmospheric CO_2, ultraviolet (UV) light, precipitation patterns and temperature may directly affect the concentration of secondary metabolites in plants, which in turn may influence herbivory rates or pathogen attack. Variation in plant chemical induction caused by changes in climate can have significant ecological and evolutionary implications for plants and their interactions with herbivores. Bidart-Bouzat et al. (2005) were the first to report that herbivore induction of plant secondary metabolites (glucosinolates) can be affected by changes in climatic factors, like CO_2 concentrations. This has been corroborated by Himanen et al. (2008) showing that inducibility of secondary metabolites can be altered not only by elevated CO_2 but also by changes in O_3 levels. Exposure to elevated temperature stimulates the plant defense hormone JA, as well as ethylene (ET), and salycilic acid (SA), enhancing defenses (DeLucia et al. 2012). Temperature increase has also been associated with increases in VOCs production (Helmig et al. 2007; Loreto et al. 2006), which could in turn affect their own ecological functions (Pinto et al. 2007). Higher VOC production and volatility under elevated temperatures are expected to change plant-herbivore interactions by altering the ability of specialist herbivores to locate their hosts, and increasing antiherbivore defense responses and/or altering plant-plant interactions (Yuan et al. 2009).

Plants and insect herbivores together comprise more than half of the terrestrial macro-biodiversity (Strong et al. 1984) and display a complex network of interactions that are expected to be affected by changes in climate (Visser and Both 2005). For instance, plant defense strategies against herbivores (i.e., direct and indirect; constitutive and induced) have been shown to vary along ecological gradients in different ecosystems (Moles et al. 2011; Pellissier et al. 2012; Rasmann and Agrawal 2011) and to be adapting to climate change (DeLucia et al. 2012). Classical predictions indicate an overall decrease in plant defenses at higher altitudes due to a reduction of herbivory pressure along elevational gradients (Coley and Barone 1996; Schemske 2009). However, contrasting findings have suggested that reduction in herbivory pressure in harsher environment does not necessarily lead to decrease in plant defenses (Pellissier et al. 2012; Rasmann et al. 2014a; Scheidel and Bruelheide 2001), which demonstrate that variation of plant defense strategies along elevational gradients is complex and not exclusively a result of herbivory pressure. Indeed, several abiotic factors may contribute to adaptation and selection of specific plant traits which in turn will can be used as part of defense strategy against herbivore attack (Rasmann et al. 2014b). If this adaptation process occurs, the effects (additive or synergistic) of climate change on plant defense systems, consequently on plant-herbivore and plant-plant interactions, can have strong impacts on ecosystem functioning. A deeper understanding of how variation in environmental factors, singly or in combination, modulate plant defense mechanisms and strategies, promises to increase our knowledge of how climate change will affect interactions between plant and herbivores at vulnerable biomes.

Elevational gradient has often been used to investigate the effects of climate change on plant-herbivore interactions (Beier et al. 2012). It serve as ‘natural experiments’ that surrogates for inferring global change-driven effects (Garibaldi et al. 2011; Rasmann et al. 2014a) because it provides variation in abiotic factors under which its effects on interactions between living organisms can be evaluated (Rasmann et al. 2014b; Salmore and Hunter 2001; Schemske 2009;

Yarnes and Boecklen 2005). Unlike latitudinal gradients, elevational gradients avoid several confounding effects that occur over a much larger spatial scale of latitude, such as plants and herbivores species pools (Hodkinson 2005; Moreira et al. 2018). Besides that, elevational gradients are independent of regional variation in other abiotic factors such as day length and inter-annual climatic variation. By recapitulating much of the latitudinal variation in abiotic conditions over much smaller geographical scale, elevational gradients provide research opportunities for understanding how environmental changes drives variation in species composition, traits and interactions (Moreira et al. 2018).

1.3. MeJA and its application in induced defense studies

Jasmonic acid (JA) and its derivatives are cyclic fatty acid-derived regulators which function as phytohormones important for plant defense against herbivorous insects and necrotrophic pathogens (Zhu and Zhu 2013). They also participate in the regulation of plant growth and development, including fertility of reproductive organs, root growth, anthocyanin accumulation, and trichome initiation (Wasternack and Hause 2013). Induced plant defense system depends on a complex signaling and regulatory network of plant hormones where jasmonic acid (JA) and its derivate methyl jasmonate (MeJA) are one of the important elicitors against biotic and abiotic stresses (Moreira et al. 2018; Pieterse et al. 2012). Once the plant perceives JA signals, a considerable reprogramming of gene expression occurs. Consequently, changes in the regulation of important pathways are made, including the induction of defensive genes and their associated biosynthetic pathways (Pauwels et al. 2009).

Specifically, MeJA plays central role in plant defense systems, which through exogenous application can trigger the allocation of energy from plant primary metabolism to the activation of plant defense genes (Howe and Jander 2008). Plant defense responses to exogenous MeJA include production of a range of toxic metabolites and anti-digestive proteins, such as proteinase inhibitors, which harm both specialist and generalist herbivores (Baldwin 1999). Also, activation of octadecanoid pathway by MeJA treatment have been shown to affect herbivore performance and preference on plants (Bruinsma et al. 2008; Kessler and Baldwin 2001; Thaler et al. 2001). MeJA also showed to induce the emission of plant volatiles that affecting the same and other trophic levels, such as attracting predators and interacting with neighboring plants (Dicke et al. 1999; Gols et al. 2003; Rodriguez-Saona et al. 2013). As MeJA-induced responses are generally similar to those induced by insect herbivory (Van Dam and Baldwin 2001; Yang et al. 2013), the application of exogenous MeJA is a useful tool to stimulate plant resistance in studies of plant-herbivore interactions at multiple organismal levels.

2. OBJECTIVES

The main goal of this thesis is to explore bilberry (*Vaccinium myrtillus* L.) defense response system through plant-herbivore and plant-plant interactions, as well as investigate how these relationships are affected by environmental variation on this key boreal plant species. This main goal will be achieved through the following specific objectives:

2.1. Explore multiannual plant defense responses, through plant-herbivore and plant-plant interactions in wild bilberry plants (***Paper I***).

2.2. Study the transcriptional profiling of induced defense responses in bilberry plants (***Paper II***).

2.3. Investigate the effects of altitudinal variation in climate (e.g., temperature and timing of snow melt) on plant defense response system at:

- Ecological level: resource allocation from growth and reproduction to defense means (***Paper III***);
- Molecular level: changes in expression of key target genes involved in defense, photosynthesis, and nitrogen metabolism (***Paper III***).
- Neighborhood level: effects on neighboring bilberry ramets through plant-plant communication (***Paper IV***).

3. MATERIAL & METHODS

3.1. Study species and system

Bilberry (*Vaccinium myrtillus* L.), our study species, is a long-lived deciduous dwarf shrub with evergreen aerial shoots (stems) that are usually between 10-60 cm in height, arising from underground rhizome (Flower-Ellis 1971). New ramets are produced through clonal reproduction by the spreading rhizome, which is a ramet being a primary stem and its associated branches. Its clone size and distribution can vary according to the area of occurrence, rhizome length can reach from 90 cm to 200 cm depending on age, and the proportion of genetic variation within population is usually high (Albert et al. 2003, 2004; Flower-Ellis 1971). Although vegetative reproduction predominates, sexual reproduction involves small, pink, campanulate flowers that produce nectar (Fig. 1). The main pollinators of bilberry are bumblebees, honeybees, and syrphid flies (Jacquemart 1993; Jacquemart and Thompson 1996). Mature fruits are fleshy berries (Fig. 2) with a variable number of seeds, which are dispersed via ingestion by birds and mammals (Pato and Obeso 2012). Bilberry occurs throughout Europe on acid soils (Flower-Ellis 1971; Ritchie 1956) and have an important ecological role as food source for many vertebrate and invertebrate herbivores in the boreal ecosystem (Hegland et al. 2010). Its shoots, leaves, and fruits are among the most important food resources for insects and large mammals, since it is an abundant species in many forest areas which produces large amounts of flowers and subsequently berries. The most common insect herbivores are Geometridae larvae (Atlegrim 1989), whereas the main mammalian herbivores feeding on bilberry in the Norwegian part of the boreal forest are red deer (*Cervus elaphus* L.) and various rodent species (Hegland and Rydgren 2016). Because it is abundant, widely distributed and have interactions with many groups of species, bilberry is an ideal organism for studies on plant defense responses, as well as how it is possibly affected by environmental changes.

The boreal forest is the dominant biome in Fennoscandia (the Scandinavian peninsula and Finland), and has a latitudinal extension from 56°N to 69°N (Esseen et al. 1997). The total forested area amounts to more than 50 million ha, of which about 13% occurs in Norway, 40% in Finland, and 47% in Sweden. The Norwegian boreal forest is relatively homogenous due to low plant species diversity, mainly composed by conifer and shrub species. The field experiments for this study were conducted within a pine-bilberry boreal forest in Kaupanger, inner Sognefjord, western Norway (61.2°N, 007.2°E) (Fig. 3). The area has annual precipitation of 700–900 mm, and a mean summer temperature range of 12–16°C (Moen et al. 1999). The most abundant vascular plant species in the field area are bilberry, Scots pine (*Pinus sylvestris* L.), birch (*Betula pendula* Roth and *B. pubescens* Ehrh.), lingonberry (*Vaccinium vitis-idaea* L.), and crowberry (*Empetrum nigrum* L.) (Rydgren K., unpublished data). The area has a dense winter population of red deer (*Cervus elaphus* L.) (pers. obs. S.J. Hegland), which is the most abundant wild ungulate in Norway (SSB 2018). The inner part of Sognefjord offers marked topographical variation representing climatic gradients within rather short distances that are ideal for climate research, making it practical and convenient for ecological experiments.

Figure 1. Photo of a bilberry plant flowering from our study area in Kaupanger, Western Norway. Photo: R. F. Benevenuto

Figure 2. Photo of a bilberry plant with mature fruits, from our study area in Kaupanger, Western Norway. Photo: R. F. Benevenuto

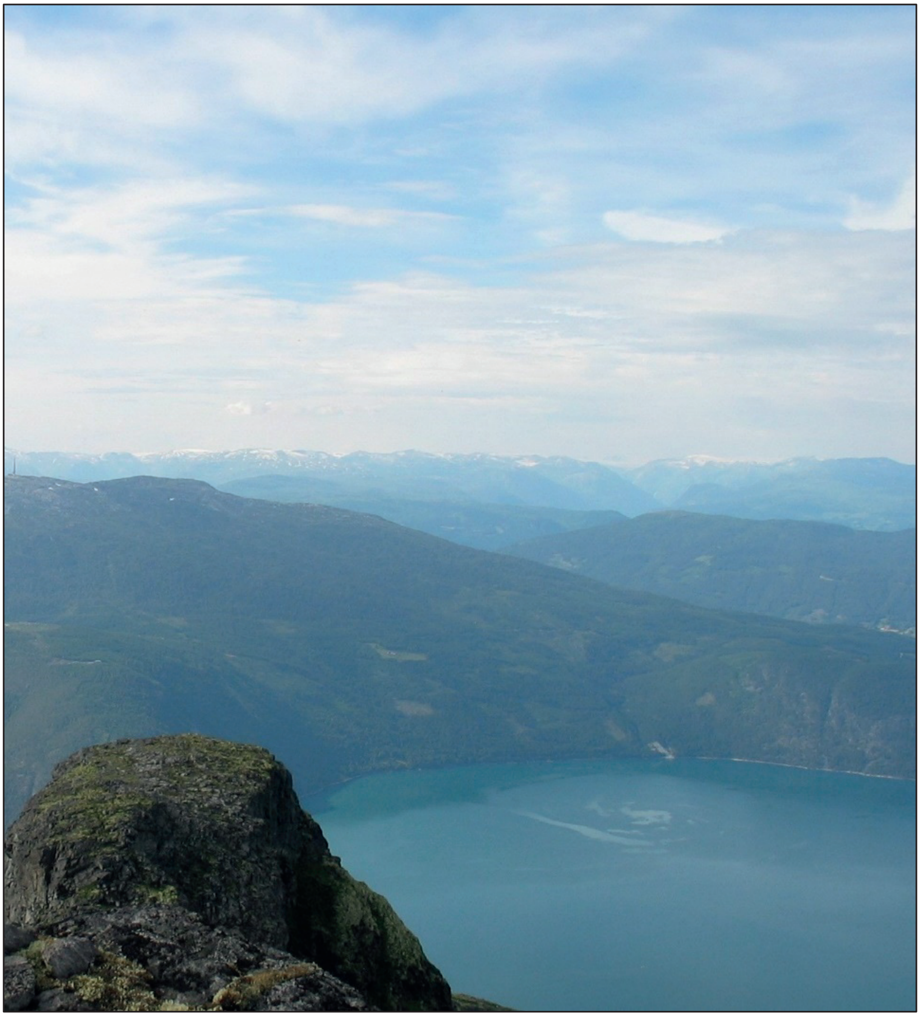


Figure 3. Photo of the study area, Kaupanger, Western Norway, taken from the other side of the Sognefjord. The tower (middle left) at the top of the Storehaugen mountain is at 1169 meters above sea level. All studies in this thesis where done in open sites in the area that is dominated by pine-bilberry forest (e.g., the middle of the picture). Photo: S. J. Hegland.

3.2. Experiment I: Multiannual effects of plant defense responses in bilberry

A start-up experiment was conducted over three consecutive years aiming to explore multiannual effects of plant defense activation in bilberry plants and its possible effects on untreated neighboring ramets under natural field conditions (***Paper I***). We performed a study of induced plant defense from 2013 to 2015 in a ca. 20-year old clear-cut in a pine forest at 350 m above sea level (m a.s.l.), in Kaupanger, Western Norway. In June 2013, we established ten blocks of 350 m^2 (10 m × 35 m), containing four transects, with five individually marked bilberry ramets in each. The five ramets were located 10–40 cm (dist. 1), 40–80 cm (dist. 2), 80–150 cm (dist. 3), and 400–530 cm (dist. 4) from ramet one (dist. 0) in each of the transects (Fig. 4). Transects were subsequently randomly assigned and exposed to two treatments with two replicates in each block. In 2013, we treated the first ramet (dist. 0) in each transect with either 10 mM MeJA (experimental transects) or water (control transects). The ramets were not exposed to further treatments in 2014 and 2015 to evaluate possible multiannual effects on growth, reproduction, and herbivory. We treated only the first ramet in each transect to evaluate possible effects of plant–plant interaction between MeJA-treated and its untreated neighboring ramets.

In each transect, before the start of the treatments, we measured ramet height from the ground to crown with a ruler and stem diameter at ground with digital calipers. We also counted the number of annual shoots, flowers, leaves, browsed shoots, and insect-chewed leaves. We repeated recordings of these variables 30 and 72 days after the initial treatment in 2013 (Fig. 5). In these subsequent recordings, we also counted the number of berries. We repeated the measurements in 2014 and 2015.

We analyzed how exogenous MeJA application of bilberry ramets affected growth (dry mass), reproduction (number of flowers and berries), and herbivory (proportion of grazed leaves by insect herbivores; number of browsed shoots by large herbivores) by comparing untreated control ramets at dist. 0 with corresponding ramets in the experimental transects (MeJA dist. 0 = MeJA-treated ramets; MeJA dist. 1–4 = untreated ramets with increasing distances from the treated ramet). For each response variable, we parameterized a generalized linear mixed-effects model under Bayesian inference with intercepts and seasonal time slopes (three seasonal censuses) for each treatment (control, MeJA dist. 0–4) in every year (2013–2015). All models were run using the "rjags" library (Plummer 2013) in R (RDevelopment 2012).

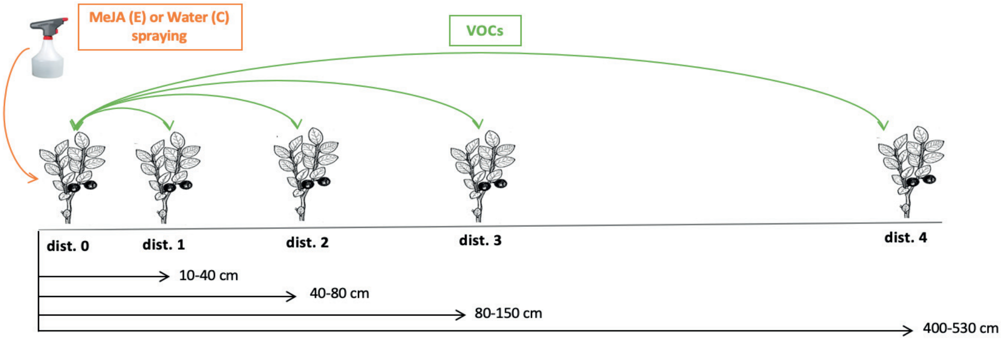


Figure 4. Experiment I: Transect design with the distances between the treated bilberry ramet and its untreated neighbors. C: control transect; E: experimental transect; MeJA: methyl jasmonate.

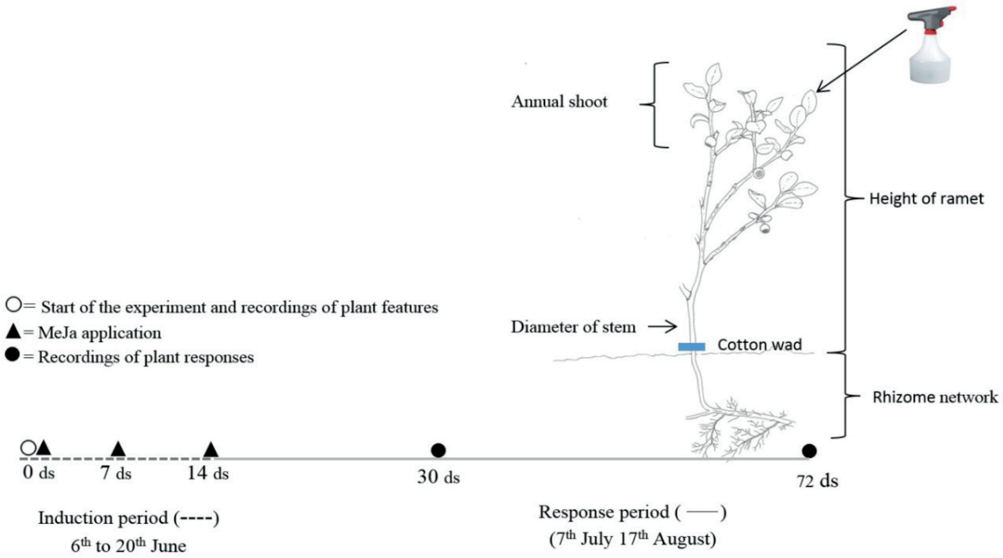


Figure 5. Experiment I: A bilberry ramet including size recordings, and timeline for the induction and response period when the measurements were recorded. MeJA = Methyl jasmonate; ds = days.

3.3. Experiment II: Transcriptomic profiling of defense-induced bilberry

A second experiment was conducted aiming to report a comprehensive transcriptome profile of MeJA-induced bilberry plants, as well as provide a catalog of the primary genes, involved in growth, reproduction and plant defense pathways (***Paper II***). This experiment was conducted in a ca. 20-year old clear-cut area at 500 m a.s.l., in Kaupanger, Western Norway. In June 2016, two groups of 15 bilberry plants each were randomly selected and exposed to two treatments: 10 mM MeJA application (treated) and water/ethanol application (control). The applications were repeated three times at one-week intervals (Fig. 6) to simulate attack by herbivores, following the protocol used in previous studies on bilberry in the field (Benevenuto et al. 2018; Seldal et al. 2017). One day after the last treatment application, leaves from the apical part of all plants were collected and immediately frozen in liquid nitrogen and stored at

-80°C. Samples were then transferred to RNA*later*-ICE (Life Technologies, Carlsbad, CA) and allowed to thaw at 20°C before RNA isolation and further transcriptomic analysis (Fig. 6). The 15 plants in each treatment were randomly separated into five groups of three plants each. The three plants of each group were pooled and considered as one biological replicate (sample) for a total of five biological replicates per treatment for the RNA sequencing analysis. Methods used for RNA isolation, library construction, sequencing and *de novo* assembly are detailed in ***Paper II***. The assembled transcriptome was annotated using BLAST for NR, NT, SwissProt, and KOG databases. Differentially expressed genes (DEGs) between MeJA-treated and untreated control bilberry plants were identified using DESeq (Anders and Huber 2010).

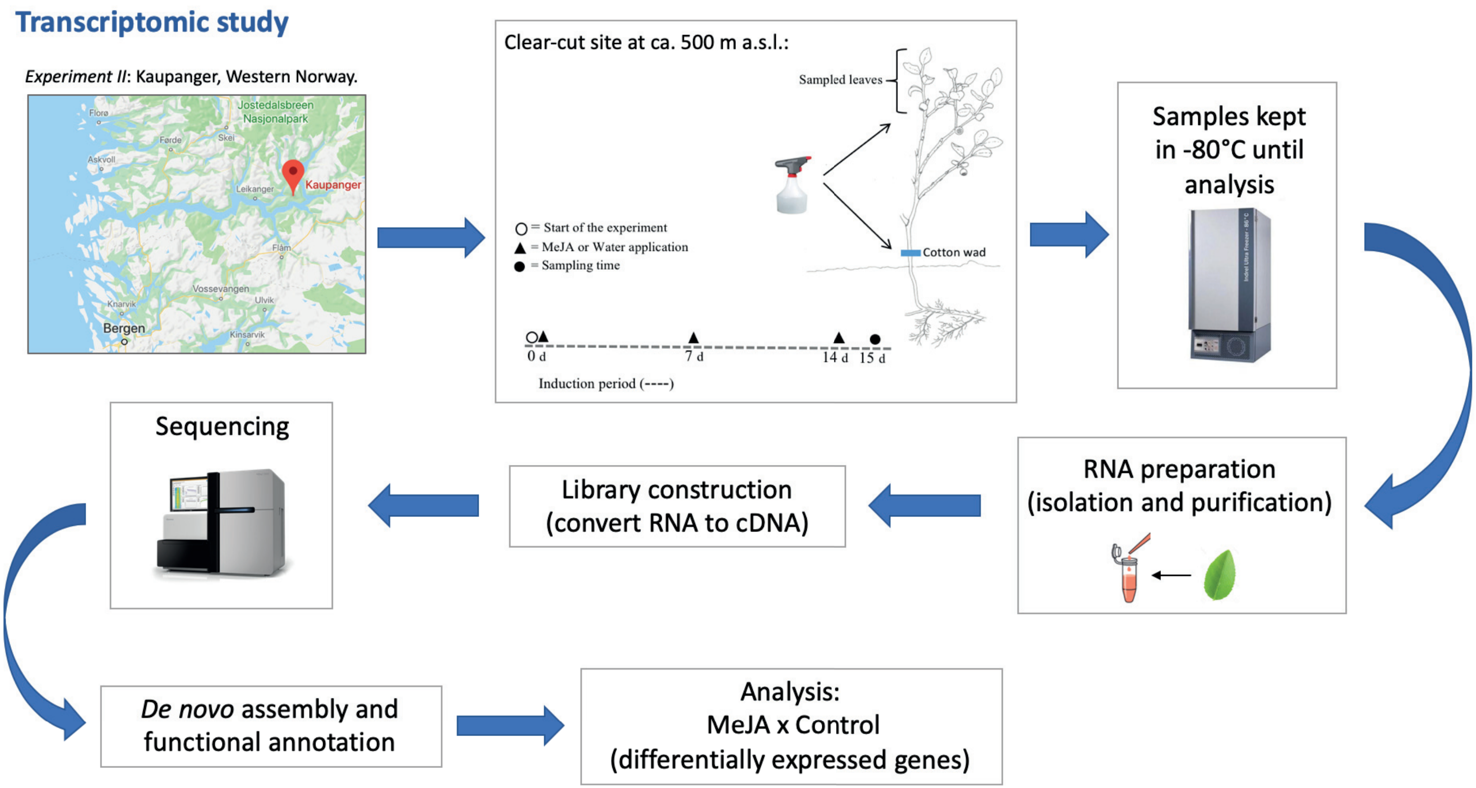


Figure 6. Experiment II: Workflow of our transcriptomic profiling study of defense-induced bilberry plants.

3.4. Experiment III: Climate effects on plant-herbivore and plant-plant interactions

In the third field experiment, a natural elevational gradient ranging from just above sea level to subalpine areas was used to investigate the effects of environmental variation, mainly climate variables (e.g., temperature and time of snowmelt), on plant-herbivore (***Paper III***) and plant-plant interactions (***Paper IV***). Our system spanned the boreal zone and the experiment was conducted at three different elevational sites: 'Low' elevation = ca. 100 m a.s.l. (submontane zone); 'Medium' elevation = ca. 500 m a.s.l. (mid-montane zone); 'High' elevation = ca. 900 m a.s.l. (subalpine zone) (Fig. 7C). The middle part of the range, at mid-montane zone, is considered as the optimal elevation for bilberry growing conditions. The submontane zone is considered as suboptimal conditions because of the warmer temperatures and heavy herbivory, while the subalpine zone mainly because of the cooler average temperatures and exposure. Low and Medium sites consisted of more than 15-year-old clear-cuts with small pines, birches and alders that produced negligible shadow effects. The High site was a naturally open subalpine area just below the tree line. Vegetation structure was similar among the sites, with a field layer and scattered tree layer up to ca. 5 m. Anorthosite bedrock and moraine material dominate the whole gradient (NGU 2019) and there was no latitudinal or longitudinal influence. The study area and the study sites, comprising the elevational gradient, was selected to increase the possibility to link findings to climate effects, and to reduce the probability for confounding effects. Unpublished data from 2016 showed that there was relatively small variation in pH (mean, 4.19) and slope (16.4 degrees) among sites, although soil organic matter (mean, 60.4%) was slightly lower at the low altitude (Rydgren K., unpublished data). Aiming to avoid variation in light, the experiment was conducted in clear-cut sites across all elevations with almost no shading effects. Information provided by the weather report services indicate little variation in UV-radiation index between Norwegian low-altitude (~25 m a.s.l.) and high-altitude locations (~1200 m a.s.l.) during the summer (NILU 2019). Based on the careful study design, the recordings taken, and additional data available, I believe that the elevational gradient used here reflects a gradient in climate, mainly through changes in temperature and snow cover (Fig. 7C). Experimental design and sampling methodology for *Experiment III* was conducted in a similar manner as *Experiment I*, with minor modifications regarding untreated neighboring ramet distances and sampling times (Fig. 7 and 8).

- **Ecological responses**

We investigated how environmental variation along a natural elevational gradient, affected plant defense activation of MeJA-treated bilberry plants (***Paper III***), as well as untreated neighboring bilberry ramets at different distances from the induced plant (***Paper IV***). Following similar sampling procedure of ecological variables conducted in *Experiment I*, before the start of treatment in all elevational sites, in May/June 2016, sampling time 1 (ST1), we measured: ramet height, stem diameter, number of annual shoots, flowers, leaves, browsed shoots, and insect-chewed leaves for all experimental ramets. We recorded the same variables 6 weeks (42 days) later, at sampling time 2 (ST2). At this subsequent recording, we also counted the number of berries. The same sampling procedure was repeated one year later (2017). These ecological measurements were used to analyze how MeJA induction of wild bilberry affected the seasonal changes in herbivory (proportion of insect-chewed leaves and proportion of browsed shoots), growth (changes in biomass through dry mass calculation), and fruit set (proportion of berries to flowers), of treated and untreated neighboring ramets at different distances, across an elevational gradient over two consecutive seasons (2016 and 2017). For each response variable, we used the *lme4* (Bates et al. 2014) and *mixlm* (Liland and Sæbø 2014) libraries in R (RDevelopment 2012) to fit generalized linear mixed effect models with Gaussian error distribution and identity link and to perform posterior ANOVA, respectively. For all models, we entered Treatment (MeJA-treated vs. Control [***Paper III***]; MeJA dist. 0-500 vs. Control [***Paper IV***]), Site (Low, Medium and High), and Year (2016 and 2017), with interaction terms, as fixed effects. Detailed data analyses description can be found in the respective papers.

- **Molecular responses**

In ***Paper III***, we also investigated effects of environmental variation on plant defense responses of MeJA-induced bilberry plants at gene expression level, aiming to find molecular mechanisms underlying ecological responses. To analyze gene expression of key target genes involved in plant defense responses, one day after the last treatment application, at 10:00 am, we collected 10 leaves from the apical part of MeJA-treated and Control ramets in each block, and they were immediately frozen in liquid nitrogen and stored at −80°C to avoid degradation. One year after treatment (June, 2017), leaf samples were collected in the same manner at the three elevational sites. Samples were subsequently transferred to RNAlater-ICE (Life Technologies, Carlsbad, CA) and allowed to thaw at −20°C prior to RNA isolation. For each treatment (MeJA and control), samples were collected along the elevational gradient in 2016 and 2017, and randomly separated into three biological groups, each containing a pool of five plants. Total RNA from each biological group was extracted using the RNeasy plant mini kit (Qiagen, Valencia, CA, USA) for posterior real-time PCR reactions aiming to assess relative expression level of the target genes.

A selection of eight target genes involved in bilberry defense and growth-related pathways was based on results from our previous transcriptomic profiling study (***Paper II***). The selected defense target genes, involved in circadian rhythm, phenylpropanoid, tyrosine, flavonoid, and anthocyanin biosynthesis pathways were: shikimate O-hydroxycinnamoyltransferase (*SHIKIMATE*); tyrosine aminotransferase (*TYR*);

leucoanthocyanidin dioxygenase (*LDOX*); UDP-glycosyltransferase (*UDP*); MYB-related transcription factor LHY (*LHY*); and flavonoid 3′,5′-hydroxylase (*FLAV*). Growth-related target genes involved in photosynthesis and nitrogen metabolism were: photosystem II PsbW (*PHOTO*); and glutamine synthetase chloroplastic (*GLU*).

To analyze gene expression levels, normalized relative expression values were log transformed and fitted to linear models with the *lme4* package (Bates et al. 2014) in R. Subsequently, factorial ANOVA was conducted with the *mixlm* package (Liland and Sæbø 2014). Differences and means within each target gene were analyzed by Tukey *post hoc* comparisons ($p < 0.05$). We presented fold change values as a measure of describing how much the expression of specific gene changes from control to MeJA-treated plants. It is defined as the ratio of MeJA-treated expression value in relation to control expression value. Fold changes are presented in logarithm base 2 (Log2FC) for comparisons of MeJA-treated and control plants in the same site and year. A positive Log2FC value indicates that the respective gene was significantly up-regulated for the MeJA-treated plant. A negative Log2FC value means that the respective gene was significantly down-regulated for the MeJA-treated plant. Detailed data analyses description can be found in ***Paper IV***.

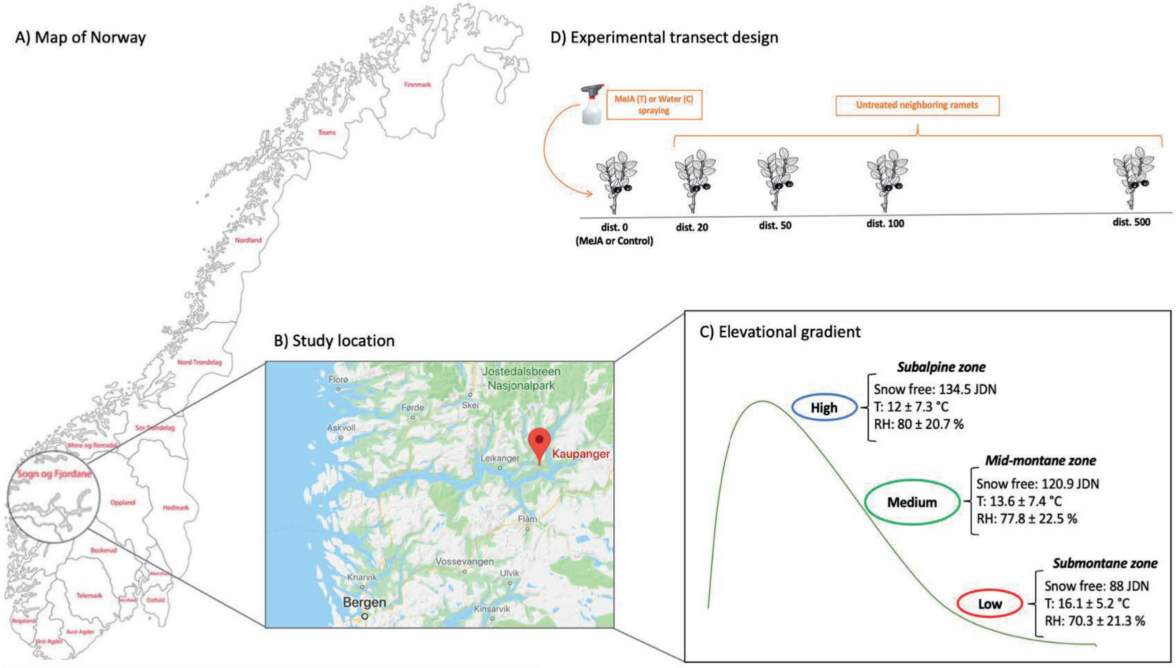


Figure 7. Experiment III: Climate effects on plant-herbivore and plant-plant interactions. A: Map of Norway with the highlighted Sogn og Fjordane region, Western Norway. B: Location where the study was conducted: Sognefjord and Kaupanger. C: Elevational gradient design with average timing of snow melt (Julian Day Number [JDN] for complete snow melt), temperature (T) and relative humidity (RH) of respective study sites. D: Transect design for Experiment III with the treated plant (MeJA or Water) at dist. 0 and its untreated neighboring ramets from dist. 20 to 500 cm.

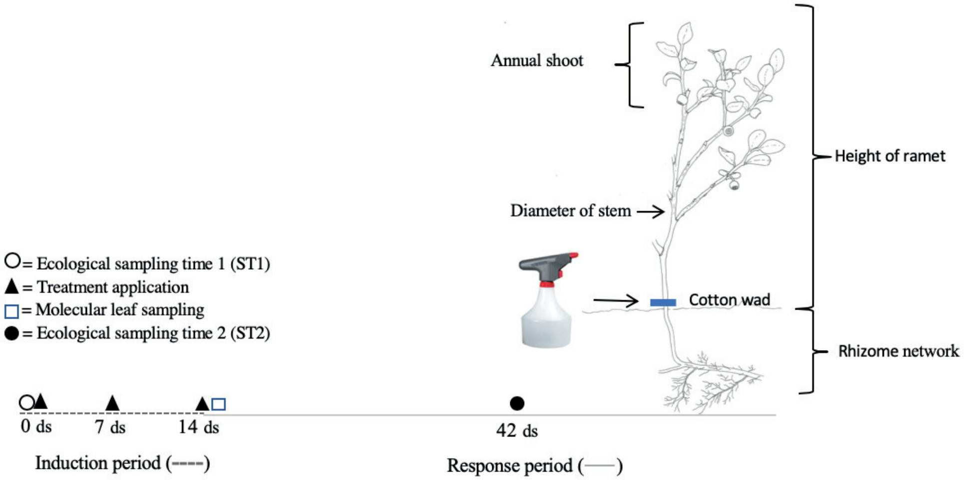


Figure 8. Experiment III: A bilberry ramet including size recordings, and timeline for the induction and response period when the measurements were recorded. ds: days**.**

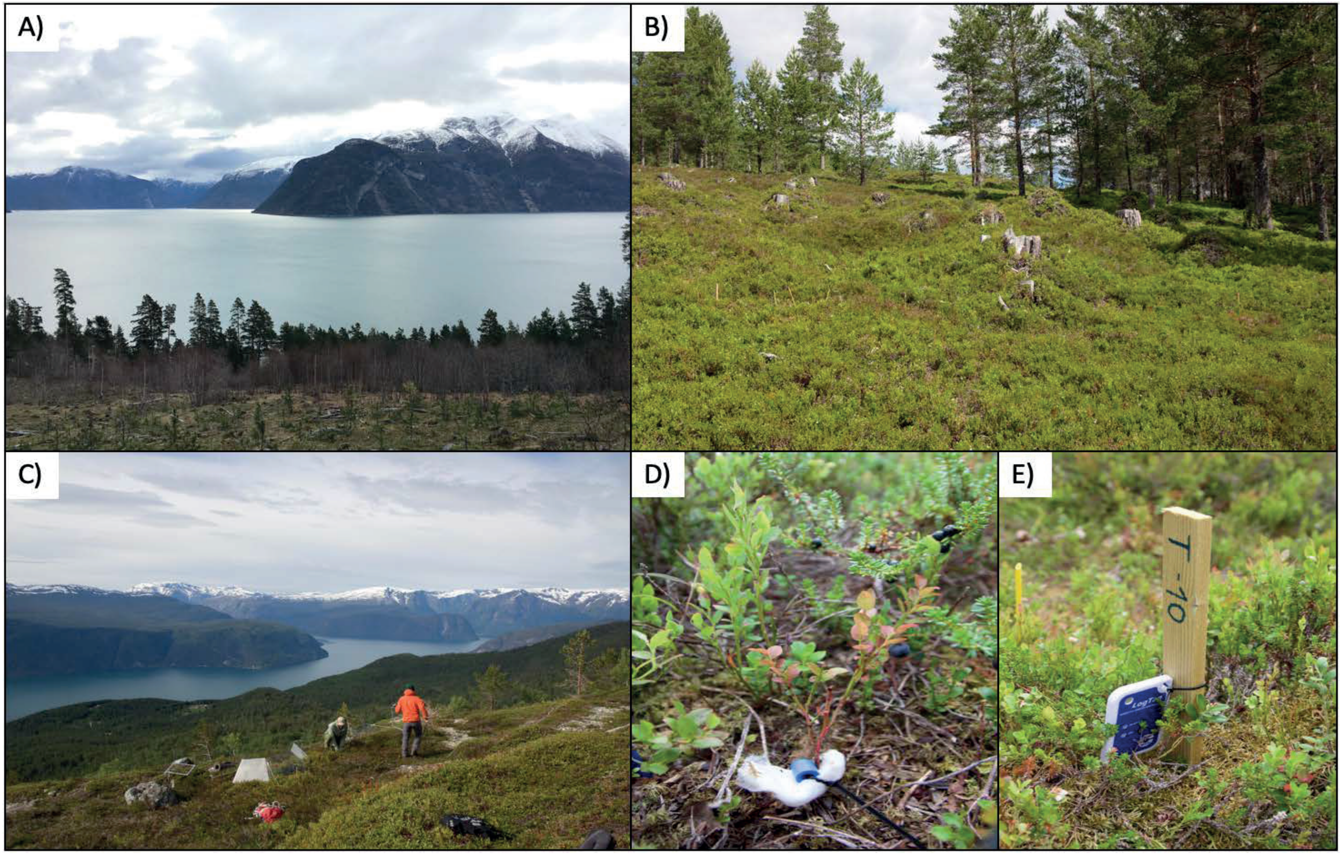


Figure 9. Photos of the sites and along the elevational gradient in Kaupanger, Western Norway. A: Low site (ca. 100 m a.s.l.); B: Medium site (ca. 500 m a.s.l.); C: High site (ca. 900 m a.s.l.); D: Bilberry treated ramet; E: Datalogger in a random transect. Photos: R. F. Benevenuto.

4. RESULTS & DISCUSSION

4.1. Multiannual effects of plant defense responses in bilberry (*Paper I*)

In this study we explored multiannual plant defense activation in MeJA-treated bilberry plants and on untreated neighboring ramets under natural field conditions. We found significant reductions in growth and herbivory rates of MeJA-treated ramets, suggesting a rapid and efficient allocation of resources from growth to defense during the first growing season (2013), when the treatments were conducted (*Paper I*: Fig. 3). These results agree with previous studies showing that MeJA-induced bilberry ramets reduces herbivory rates at the expense of growth and reproduction (Hegland et al. 2016; Seldal et al. 2017).

We also showed that this defense state varies in strength and is capable to persist across subsequent years, what we called 'multiannual effect'. Total insect herbivory was significantly reduced in MeJA-treated ramets for two subsequent years (2014 and 2015). Besides that, we found that induced defense responses occurred in a delayed manner with strongest effects one and two years after induction, against insect and mammalian herbivores respectively (*Paper I*: Table 1). Generally, rapidly induced plant defenses affect the performance of short-lived invertebrate herbivores, whereas delayed induced defense responses affect the next generation of short-lived invertebrate and long-lived vertebrate herbivores (Haukioja and Hanhimaki 1985). In addition, delayed induced resistance involves 'quantitative defenses,' which are effective against both specialists and generalists, in contrast to rapid induced resistance which involves 'qualitative defenses' that are more efficient against generalists but not specialists (Rhoades 1979). According to the plant apparency theory, plants that are easily found by herbivores, such as trees and shrubs, should invest heavily in quantitative defenses that are effective against a broad spectrum of herbivores (Feeny 1976). Bilberry is an ‘apparent’ deciduous shrub which store reserves of carbon in stems and roots, enabling it to produce quantitative carbon-based defenses (e.g., flavonoids and tannins), which are efficient against specialist mammalian herbivores present in the boreal forest, such as red deer (Gallet 1994). As a result, we hypothesize that bilberry plants may use multiple induced defensive tactics against herbivores: some are rapidly induced and more efficient against insect herbivores (likely qualitative defenses), while others are delayed induced and more efficient against mammalian herbivores (likely quantitative).

Our plant-plant interactions results indicated the effective long-distance signaling transfer between MeJA-treated ramets and its untreated neighbors in distanced between ten centimeters to five meters. These findings are consistent with results from studies involving other species where above- and belowground signaling activate the defense system and reduce herbivory of untreated neighbor plants (Baldwin et al. 2002; Dicke and Bruin 2001; Heil and Karban 2010; Pickett et al. 2003). The elevated resistance to herbivory found in untreated neighbor ramets lasted for several growth seasons, although this varied in time and space. Consistent with the patterns found in MeJA-treated ramets, effects on untreated neighboring ramets were also multiannual with its highest effect delayed. We found the strongest resistance against herbivorous insects in untreated neighbor ramets one year after the MeJA treatment (*Paper I*: Table 2; 2014), where ramets distanced from 10 to 150 cm showed on average four times less insect herbivory compared to control ramets. However, browsing by large mammalian herbivores was lowest in untreated neighbor ramets in 2015, two years after the MeJA treatment (*Paper I*: Table 2; 2015).

We documented that induced defense in bilberry reduces both insect and mammalian herbivory, as well as growth, over multiple seasons. Additionally, our findings provide evidence for long-term effects of plant–plant signaling mediated by jasmonate-induced responses in bilberry, indicating that induced plants are “good” neighbors due to ecological facilitation with conspecifics under natural conditions. In summary, this study shows that induced defenses are important ecological strategies not only for the induced individual plant but also for neighboring plants across multiple years. Such findings may have important implications of our understanding on the effects of bilberry defenses on the dynamics of herbivore populations in the boreal ecosystem.

4.2. Transcriptomic profiling of defense-induced bilberry (*Paper II*)

As shown in ***Paper I*** and previous studies (Benevenuto et al. 2018; Seldal et al. 2017), because defense-induced bilberry plants display an effective ecological trade-off between growth/reproduction and defense, we predict finding supporting evidences at the transcriptional level. In ***Paper II*** we conducted an RNA sequencing study aiming to report, for the first time, a comprehensive transcriptome profile of wild MeJA-induced bilberry plants, as well as understand molecular mechanisms that underpin ecological responses previously found.

We found significant transcriptional changes in MeJA induced bilberry and provided evidence for allocation of resources from growth, development, and reproduction, to defense related pathways. Transcripts of receptor and response-related genes of important plant hormone signal transduction responded to MeJA treatment through cross talk, and regulation of genes involved in growth/development (e.g., ABA and AUX) and defense (e.g., ET and SA). Our data also showed significantly up-regulation of genes encoding key enzymes in metabolic pathways involved in biosynthesis of flavonoids (e.g., anthocyanins, flavones/flavonols), lignin compounds (e.g., syringyl, guaiacyl and p-hydroxyphenyl), and deterrent/repellent VOCs (e.g., phenylpropenes, sesquiterpenes) in MeJA-treated plants.

We showed that defended bilberry plants synthesized a range of phenolic compounds, from lignin to more complex flavonoids, aiming to interfere in the digestion and palatability of potential herbivores. The activation of defense-related pathways appeared to be costly as evidenced by the significant down-regulation of important genes involved in primary metabolism, such as those associated with photosynthesis, circadian rhythm, carbohydrate and nitrogen metabolism, presumably to optimize the allocation of resources towards defense. For instance, genes involved in carbohydrate anabolism were repressed, while the ones linked to carbohydrate catabolism were induced, as a possible means to allocate C resources from primary to secondary metabolism (Broeckling et al. 2005; Cheng et al. 2011; Frenkel et al. 2009; Sanchez-Sampedro et al. 2007). Genes responsible for the remobilization of N sources were up-regulated, while key enzymes playing crucial roles in glutamate metabolism through ammonium assimilation, mostly invested in photosynthesis (Makino et al. 2000; Makino et al. 2003; Nunes-Nesi et al. 2010), were down-regulated. Circadian clock genes were also affected by MeJA, inducing arrhythmicity in bilberry plants via altering the expression of clock-regulated genes related to flowering time.

Some variation in gene expression among replicates was evident (*Paper II*: Fig. 5-9). This was expected since the experiment was conducted with wild plants under natural environment. Nonetheless, the changes in the transcription regulation associated with MeJA treatment highlighted in this paper are statistically significant and support the concept of 'genetic trade-off'. While previous studies (including our results from ***Paper I)*** provided ecological evidences of resource allocation from growth and reproduction to defense in bilberry (Benevenuto et al. 2018; Seldal et al. 2017), our molecular results presented in this study highlights the occurrence of 'genetic trade-off' at the transcriptional level, which supports previous published field observations.

4.3. Climate effects on plant-herbivore and plant-plant interactions (*Paper III and IV*)

Finally, we studied the effects of variation in climate (e.g., temperature and timing of snow melt), using an elevational gradient, on plant defense responses at ecological and molecular levels in MeJA-treated bilberry plants (***Paper III***), as well as effects on untreated neighboring bilberry ramets from short (20 cm) to long distance (500 cm) (***Paper IV***). To investigate this, we performed a study across an elevational gradient from submontane to subalpine zones (100-900 m a.s.l.) in a boreal ecosystem. The gradient used in this study included optimal growing conditions for bilberry in a medium altitude (ca. 500 m a.s.l.), and the plant's range limits at high (ca. 900 m a.s.l.) and low (ca. 100 m a.s.l.) altitudes.

- **Plant-herbivore interactions**

In ***Paper III*** we tested whether the allocation of resources from growth and reproduction to defense in herbivore-induced bilberry plants were affected by altitudinal variation in climate, at ecological and molecular levels. We found that bilberry defense systems varied over the elevational gradient. Induced defenses were strongly activated for plants growing at the optimum (Medium site) and suboptimal lower altitude (Low site), suggesting that these plants have more resources available to invest in defense against herbivore attack, compared with high-altitude plants growing under colder and resource-limiting conditions. Although MeJA activated induced defenses in plants growing at both elevations, these effects were even stronger at the lowest altitude of the elevational gradient. Such ecological responses were supported by molecular mechanisms found: low-altitude plants effectively reduced seasonal growth, fruit set, and insect and mammalian herbivory, while significantly down-regulated growth-related genes and up-regulated defense-related genes in response to MeJA treatment. In a previous transcriptomic profile study of MeJA-induced defenses in bilberry (***Paper II***), we proposed the term ‘genetic trade-off,’ which is the up-regulation of genes involved in important defense-related pathways and the corresponding down-regulation of genes related to growth and nitrogen metabolism pathways (Benevenuto et al. 2019). In summary, these combined responses indicate a shift in resource allocation from growth and reproduction to induced defenses (ecological and genetic trade-offs), especially for plants growing at the warmest low-altitude site. Although relaxed, such effects of ecological and genetic trade-offs persisted for one year after induction in the lowest altitude. This result corroborates with our previous ecological study showing that the induced defense system in bilberry plants functions in a multiannual manner (Benevenuto et al. 2018). Also, induced defenses is dependent of plant species, herbivory pressure, and life history characteristics (Haukioja and Hanhimaki 1985; Karban and Baldwin 1997), which in turn are affected by abiotic factors, such as variation in temperature, potentially affecting the speed, magnitude and costs of resource allocation to induced defenses (Trussell and Smith 2000).

High-altitude bilberry plants invested more in constitutive than induced defenses. This was supported by the small effect sizes of changes in growth, fruits set, and herbivory resistance, and the consistently high expression levels of defense-related genes in both MeJA-treated and untreated plants. Furthermore, we observed that general herbivory pressure was less intense (e.g., lower total proportion of chewed leaves) at the high-altitude than the low-altitude site of the elevational gradient, consistent with recent studies (Pellissier et al. 2012; Rasmann et al. 2014a). Such findings suggest that bilberry plants growing in suboptimal upper elevations are under a constant state of ‘alert’ (constitutive defenses), possibly because of the resource-limiting and stressful environmental conditions (i.e., low nutrients, cool average temperatures, late timing of snow melt, and consequent short-growing season); as well as lower herbivory pressure in subalpine habitats of the boreal forest (Moreira et al. 2018). The combination of these conditions likely affects the type of defense strategy expressed in bilberry plants from a reliance on constitutive defenses instead of inducible defenses. Moreira et al. (2014); Pellissier et al. (2016) found similar ecological responses among pine species, where constitutive and induced levels of defensive chemicals traded off along elevation. Bilberry plants from higher elevations may have developed consistent levels of plant defenses to ecologically fit on severe climatic conditions of alpine environments, which indirectly confer increased resistance to herbivores.

- **Neighborhood effects**

In ***Paper IV*** we focused on bilberry neighborhood effects of herbivore-induced resistance through plant-plant communication along the elevational gradient. Our results showed that treated plants and untreated neighbors up to 500 cm away appear to have activated their defense systems in response to treatment with MeJA, as shown by reductions in both insect and mammalian herbivory, relative to control plants in the same area. This in turn resulted in reduced growth and reproduction in these MeJA-treated plants and their untreated neighbors, presumably due to the reallocation of resources from growth and reproduction to defense. Such effects were observed at all elevations, but especially at Low and Medium altitude, where the effect persisted into a second year. At these sites, effects were more strongly induced and persistent, including among untreated conspecific ramets from intermediate to long distances (ca. 100-500 cm), suggesting that plants growing under medium to low altitudes are better enabled to communicate. Conversely, at the High site, where temperatures are lower and the growing season is shorter, only MeJA-treated and untreated neighboring ramets from short to intermediate distances (ca. 20–100 cm) showed reduced herbivory and fruit set, and this effect did not persist into the next year.

In summary, our findings provide evidence for climate modulation of plant-plant communication as part of the induced plant defense system in bilberry. Low (submontane zone) and Medium altitude (mid-montane zone) environments in the boreal system, with higher average temperatures and longer growth seasons, favored induced defenses in the ramets of neighboring conspecifics, at least within ca. 5 m away from the focal MeJA-treated plant. The elevational gradient used in our study parallels a natural climatic gradient. Overall, climate can be an important abiotic factor in determining the intensity and efficacy of induced plant defenses, for example, by affecting the release of plant volatiles (Gouinguene and Turlings 2002) (Gouinguené and Turlings 2002). For instance, elevated temperature increases biogenic VOC emission (Gouinguene and Turlings 2002; Guenther et al. 1993; Tigney 1991), which in turn can directly affect signaling efficacy in diverse ecological interactions: e.g., enhance plant-pollinator interactions by increasing flower visitation rates; alter the ability of specialist herbivores to locate their hosts; increase direct and indirect antiherbivore defenses; and consequently alter plant-plant interactions (Yuan et al. 2009). Although induced defenses, and consequently plant-plant communication, appeared to be improved under warmer environments, increased levels of VOCs in the atmosphere can contribute to climate change through aerosol formation and enhanced greenhouse effects (Calfapietra et al. 2007; Peñuelas and Llusià 2003; Penuelas and Staudt 2010; Rosenstiel et al. 2003), which in turn could mitigate their own ecological function (Pinto et al. 2007). Given the function of some VOCs, such as terpenoids, in plant-plant communication, changes in volatile abundance as a result of climate change could therefore affect plant-herbivore relationships by either weakening or strengthening signaling between plants (Yuan et al. 2009).

5. CONCLUSIONS

We documented that induced defenses in bilberry plants effectively reduced herbivory, as well as growth and reproduction, a trade-off between growth/reproduction to defense over multiple seasons. Supporting such field observations, we highlighted the occurrence of trade-off at transcriptional level, what we called ‘genetic trade-off’. Bilberry induced defense responses occurred in a delayed manner, with strongest effects from one (insect herbivores) to two (mammalian herbivores) years after induction. Besides that, we provided evidences for long-term effects on plant-plant interactions, indicating that MeJA-induced bilberry plants are “good” neighbors due to ecological facilitation with conspecifics in the boreal system. As previous studies have shown close correlations between bilberry and local herbivores population size (Selås 1997, 2000. 2006; Selås et al. 2013), such multiannual effect and the delay of the highest level of induced resistance on MeJA-treated and untreated neighboring bilberry ramets may have important ecological implications for our understanding on herbivore population dynamics in the boreal system.

Bilberry defense strategies are modulated by the altitudinal variation in climate and herbivory pressure at both molecular and ecological levels: high-altitude plants living under cooler temperatures and limiting resource availability invest more in constitutive defenses; whereas low-altitude plants growing under higher temperatures and herbivory pressure, rely strongly on induced defenses. Environmental variation along the elevational gradient also affected plant-plant communication as part of the induced plant defense system: suboptimal lower (warmer submontane zone) and optimal medium-altitude (mid-montane zones) environments in the boreal system favored the facilitation of defense-induced plants with long distanced conspecifics; as compared to the suboptimal upper elevation environment (colder subalpine zone). The greater prominence of this inducible plant defense system at these lower altitudes may presage the kinds of change expected in plant-plant communication systems and associated VOC emissions in a warmer world, which will likely feedback to further influence ecological functioning.

This thesis contains relevant results on how bilberry defense systems works at ecological level, as well as molecular mechanisms underlying such defense responses, and how it is affected by environmental variation along an elevational gradient in the boreal system. By identifying consistent trends in changes of plant-herbivore and plant-plant interactions along elevational gradients, as performed here, we might be able to extrapolate our findings to future climate change effects on ecosystem functioning. Under the current scenario of global warming, how would we expect the defense systems of key plant species in the boreal system to change? And what is the possible consequences of it? In the near future, higher elevations in the subalpine and alpine zones likely to become the new optimal growing environment for most species in the boreal system. Therefore, if the offspring of low-altitude plants migrate to such higher elevations, they might encounter novel abiotic (i.e. harsher environment and less resource availability) and biotic (i.e. less diversity and herbivory pressure) conditions to re-adapt. Concomitantly, rapid shifts of herbivore range to higher elevation due to increasing temperatures may find novel defense levels and strategies of host species, or result in limited damage to alpine plants not adapted to such herbivory pressure. On the other hand, increases in plant quality (i.e., more 'defended' plants) and quantity (i.e., abundance) due to global warming effects in the boreal system can indirectly cause outbreaks in herbivore populations by affecting cycle amplitude.

Previous studies have shown evidences for climate influencing cyclic herbivore population dynamics by changes in plant metabolism (Schmidt et al. 2018). In doing so, herbivore population dynamics in these subalpine zones can be affected by limiting cycle amplitude driven by limited resources, with high peak of herbivore densities occurring together with high levels of plant defenses (during the growing season), when food resources are more abundant.

The combination of such ecological events in response to global warming will possibly accelerate new ecological and co-evolutionary dynamics in the boreal system. Although challenging to do, we now have a basis for future long-term comparative studies fitting ecological and molecular approaches together aiming to monitor species migration and possibly changes in plant-herbivore relationships. These kinds of studies are important to help us predicting possible ecological consequences of a changing climate on vulnerable ecosystems, especially high-latitude systems where biodiversity is low and there are marked dependences among species, such as bilberry and most insect and mammalian herbivores.

6. FUTURE PERSPECTIVES

In fact, elevational gradients have been effective tools for climate change studies on plant-herbivore interactions. However, one of the barriers that limits the use of natural elevational gradients to infer effects of abiotic factors, isolated or in combination, is the complex of its interactions with biotic factors under natural systems. In doing so, other interesting possibilities of manipulative experiments can be done aiming to isolate the effect of specific environmental factors as predictors. For instance, comparing experimentally warmed plants (e.g., by using open-top chambers) with not warmed plants would be useful to understand more about increasing temperature effects on plant defenses. As another possibility, by experimentally adding or removing snow we could test effects of snow cover on plant defenses. By combining such experiments with elevational gradients, aiming to account for differences in nutrients, disturbance and other relevant factors for plant defenses, although more challenging, would bring us much new knowledge.

Integrating ecological and molecular studies to understand plant defense systems under natural conditions is complex, and a range of additional analysis could help us to better understand our current findings. In the context of plant-plant interactions results, we have also collected samples of untreated neighboring plant in the transect for future gene expression analysis aiming to complement ecological findings. Besides that, complementary lab analysis of other defense parameters, such as: collection and analysis of volatiles; other secondary metabolites (e.g., flavonoids and phenolic acids), could help us to better interpret current findings. Measures in photosynthetic activity and chlorophyll content would be a good complement to understand reductions in gene expression of photosynthesis and nitrogen-related genes and seasonal growth. Also, since bilberry has interconnected ramets with extensive underground rhizomes that vary on size, a side experiment in greenhouse with transplanted MeJA-treated and untreated neighboring bilberry plants in pots, would help us to interpret the actual type of signaling strategy of our plant-plant communication results.

7. REFERENCES

Agrawal AA (1999) Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. Ecology 80 (5):1713-1723. doi:Doi 10.1890/0012-9658(1999)080[1713:Irthiw]2.0.Co;2

Albert T, Raspe O, Jacquemart AL (2003) Clonal structure in Vaccinium myrtillus L. revealed by RAPD and AFLP markers. International Journal of Plant Sciences 164 (4):649-655. doi:Doi 10.1086/375373

Albert T, Raspe O, Jacquemart AL (2004) Clonal diversity and genetic structure in Vaccinium myrtillus populations from different habitats. Belgian Journal of Botany 137 (2):155-162

Anders S, Huber W (2010) Differential expression analysis for sequence count data. Genome Biology 11 (10):R106. doi:10.1186/gb-2010-11-10-r106

Arimura G, Ozawa R, Nishioka T, Boland W, Koch T, Kuhnemann F, Takabayashi J (2002) Herbivore-induced volatiles induce the emission of ethylene in neighboring lima bean plants. The Plant Journal 29 (1):87-98

Atlegrim O (1989) Exclusion of birds from bilberry stands: impact on insect larval density and damage to the bilberry. Oecologia 79 (1):136-139. doi:10.1007/BF00378251

Baldwin IT (1999) Inducible nicotine production in native Nicotiana as an example of adaptive phenotypic plasticity. Journal of Chemical Ecology 25 (1):3-30. doi:Doi 10.1023/A:1020880931488

Baldwin IT, Kessler A, Halitschke R (2002) Volatile signaling in plant–plant–herbivore interactions: what is real? Current Opinion in Plant Biology 5 (4):351-354

Bale JS, Masters GJ, Hodkinson ID, Awmack C, Bezemer TM, Brown VK, Butterfield J, Buse A, Coulson JC, Farrar J, Good JEG, Harrington R, Hartley S, Jones TH, Lindroth RL, Press MC, Symrnioudis I, Watt AD, Whittaker JB (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology 8 (1):1-16. doi:DOI 10.1046/j.1365-2486.2002.00451.x

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R package version 1 (7):1-23

Beier C, Beierkuhnlein C, Wohlgemuth T, Penuelas J, Emmett B, Körner C, de Boeck H, Christensen JH, Leuzinger S, Janssens IA (2012) Precipitation manipulation experiments–challenges and recommendations for the future. Ecology Letters 15 (8):899-911

Benevenuto RF, Hegland SJ, Topper JP, Rydgren K, Moe SR, Rodriguez-Saona C, Seldal T (2018) Multiannual effects of induced plant defenses: Are defended plants good or bad neighbors? Ecology and Evolution 8 (17):8940-8950. doi:10.1002/ece3.4365

Benevenuto RF, Seldal T, Hegland SJ, Rodriguez-Saona C, Kawash J, Polashock J (2019) Transcriptional profiling of methyl jasmonate-induced defense responses in bilberry (Vaccinium myrtillus L.). BMC Plant Biology 19 (1):70. doi:10.1186/s12870-019-1650-0

Bidart-Bouzat MG, Imeh-Nathaniel A (2008) Global change effects on plant chemical defenses against insect herbivores. Journal of Integrative Plant Biology 50 (11):1339-1354. doi:10.1111/j.1744-7909.2008.00751.x

Bidart-Bouzat MG, Mithen R, Berenbaum MR (2005) Elevated CO 2 influences herbivory-induced defense responses of Arabidopsis thaliana. Oecologia 145 (3):415-424

Broeckling CD, Huhman DV, Farag MA, Smith JT, May GD, Mendes P, Dixon RA, Sumner LW (2005) Metabolic profiling of Medicago truncatula cell cultures reveals the effects of biotic and abiotic elicitors on metabolism. Journal of Experimental Botany 56 (410):323-336. doi:10.1093/jxb/eri058

Bruin J, Dicke M (2001) Chemical information transfer between wounded and unwounded plants: backing up the future. Biochemical Systematics and Ecology 29 (10):1103-1113. doi:Doi 10.1016/S0305-1978(01)00053-9

Bruinsma M, IJdema H, van Loon JJA, Dicke M (2008) Differential effects of jasmonic acid treatment of Brassica nigra on the attraction of pollinators, parasitoids, and butterflies. Entomologia Experimentalis Et Applicata 128 (1):109-116. doi:10.1111/j.1570-7458.2008.00695.x

Calfapietra C, Wiberley AE, Falbel TG, Linskey AR, Mugnozza GS, Karnosky DF, Loreto F, Sharkey TD (2007) Isoprene synthase expression and protein levels are reduced under elevated O3 but not under elevated CO2 (FACE) in field-grown aspen trees. Plant, Cell and Environment 30 (5):654-661

Callaway RM (2002) The detection of neighbors by plants. Trends in Ecology & Evolution 17 (3):104-105. doi:Doi 10.1016/S0169-5347(01)02438-7

Chamberlain K, Guerrieri E, Pennacchio F, Pettersson J, Pickett JA, Poppy GM, Powell W, Wadhams LJ, Woodcock CM (2001) Can aphid-induced plant signals be transmitted aerially and through the rhizosphere? Biochemical Systematics Ecology 29 (10):1063-1074

Cheng C, Gao X, Feng B, Sheen J, Shan L, He P (2013) Plant immune response to pathogens differs with changing temperatures. Nature Communications 4:2530. doi:10.1038/ncomms3530

Cheng Z, Sun L, Qi T, Zhang B, Peng W, Liu Y, Xie D (2011) The bHLH transcription factor MYC3 interacts with the Jasmonate ZIM-domain proteins to mediate jasmonate response in Arabidopsis. Molecular Plant 4 (2):279-288. doi:10.1093/mp/ssq073

Coley PD, Barone JA (1996) Herbivory and plant defenses in tropical forests. Annual Review of Ecology and Systematics 27 (1):305-335. doi:DOI 10.1146/annurev.ecolsys.27.1.305

de Wit M, Ljung K, Fankhauser C (2015) Contrasting growth responses in lamina and petiole during neighbor detection depend on differential auxin responsiveness rather than different auxin levels. New Phytologist 208 (1):198-209

DeLucia EH, Nabity PD, Zavala JA, Berenbaum MR (2012) Climate change: resetting plant-insect interactions. Plant Physiology 160 (4):1677-1685. doi:10.1104/pp.112.204750

Dicke M, Bruin J (2001) Chemical information transfer between plants: back to the future. Biochemical Systematics and Ecology 29 (10):981-994. doi:Doi 10.1016/S0305-1978(01)00045-X

Dicke M, Gols R, Ludeking D, Posthumus MA (1999) Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in lima bean plants. Journal of Chemical Ecology 25 (8):1907-1922. doi:Doi 10.1023/A:1020942102181

Dudareva N, Pichersky E, Gershenzon J (2004) Biochemistry of plant volatiles. Plant Physiology 135 (4):1893-1902. doi:10.1104/pp.104.049981

Esseen P-A, Ehnström B, Ericson L, Sjöberg K (1997) Boreal forests. Ecological bulletins:16-47

Fan FX, Bradley RS, Rawlins MA (2014) Climate change in the northeastern US: regional climate model validation and climate change projections. Climate Dynamics 43 (1-2):145-161. doi:10.1007/s00382-014-2198-1

Farag MA, Pare PW (2002) C-6-green leaf volatiles trigger local and systemic VOC emissions in tomato. Phytochemistry 61 (5):545-554. doi: 10.1016/S0031-9422(02)00240-6

Feeny P (1976) Plant apparency and chemical defense. In: Biochemical interaction between plants and insects. Springer, Boston, MA. 1-40

Felton GW, Summers CB, Mueller AJ (1994) Oxidative responses in soybean foliage to herbivory by bean leaf beetle and three-cornered alfalfa hopper. Journal of Chemical Ecology 20 (3):639-650. doi:10.1007/BF02059604

Flower-Ellis J (1971) Age, structure and dynamics in stands of bilberry (Vaccinium myrtillus L.). Department of Forest Ecology and Forest Soils. Research Note 9. Royal College of Forestry, Stockholm, Frenkel M, Kulheim C, Jankanpaa HJ, Skogstrom O, Dall'Osto L, Agren J, Bassi R, Moritz T, Moen J, Jansson S (2009) Improper excess light energy dissipation in Arabidopsis results in a metabolic reprogramming. BMC Plant Biology 9 (1):12. doi:10.1186/1471-2229-9-12

Gallet C (1994) Allelopathic potential in bilberry-spruce forests: Influence of phenolic compounds on spruce seedlings. Journal of Chemical Ecology 20 (5):1009-1024. doi:10.1007/BF02059738

Garibaldi LA, Kitzberger T, Chaneton EJ (2011) Environmental and genetic control of insect abundance and herbivory along a forest elevational gradient. Oecologia 167 (1):117-129. doi:10.1007/s00442-011-1978-0

Genung MA, Bailey JK, Schweitzer JA (2012) Welcome to the neighbourhood: interspecific genotype by genotype interactions in Solidago influence above- and belowground biomass and associated communities. Ecology Letters 15 (1):65-73. doi:10.1111/j.1461-0248.2011.01710.x

Gols R, Roosjen M, Dijkman H, Dicke M (2003) Induction of direct and indirect plant responses by jasmonic acid, low spider mite densities, or a combination of jasmonic acid treatment and spider mite infestation. Journal of Chemical Ecology 29 (12):2651-2666

Gomez S, van Dijk W, Stuefer JF (2010) Timing of induced resistance in a clonal plant network. Plant Biology 12 (3):512-517. doi:10.1111/j.1438-8677.2009.00234.x

Gouinguene SP, Turlings TCJ (2002) The effects of abiotic factors on induced volatile emissions in corn plants. Plant Physiology 129 (3):1296-1307. doi:10.1104/pp.001941

Green TR, Ryan CA (1972) Wound-Induced Proteinase Inhibitor in Plant Leaves: A Possible Defense Mechanism against Insects. Science 175 (4023):776-777. doi:10.1126/science.175.4023.776

Guenther AB, Zimmerman PR, Harley PC, Monson RK, Fall R (1993) Isoprene and Monoterpene Emission Rate Variability - Model Evaluations and Sensitivity Analyses. Journal of Geophysical Research-Atmospheres 98 (D7):12609-12617. doi:Doi 10.1029/93jd00527

Harrington R, Woiwod II, Sparks T (1999) Climate change and trophic interactions. Trends in Ecology and Evolution 14 (4):146-150

Haukioja E, Hanhimaki S (1985) Rapid Wound-Induced Resistance in White Birch (Betula, Pubescens) Foliage to the Geometrid Epirrita-Autumnata, a Comparison of Trees and Moths within and Outside the Outbreak Range of the Moth. Oecologia 65 (2):223-228. doi:Doi 10.1007/Bf00379221

Hayhoe K, Wake CP, Huntington TG, Luo LF, Schwartz MD, Sheffield J, Wood E, Anderson B, Bradbury J, DeGaetano A, Troy TJ, Wolfe D (2007) Past and future changes in climate and hydrological indicators in the US Northeast. Climate Dynamics 28 (4):381-407. doi:10.1007/s00382-006-0187-8

Hegland SJ, Jongejans E, Rydgren K (2010) Investigating the interaction between ungulate grazing and resource effects on Vaccinium myrtillus populations with integral projection models. Oecologia 163 (3):695-706. doi:10.1007/s00442-010-1616-2

Hegland SJ, Nielsen A, Lázaro A, Bjerknes AL, Totland Ø (2009) How does climate warming affect plant-pollinator interactions? Ecology letters 12 (2):184-195

Hegland SJ, Rydgren K (2016) Eaten but not always beaten: winners and losers along a red deer herbivory gradient in boreal forest. Journal of Vegetation Science 27 (1):111-122

Hegland SJ, Seldal T, Lilleeng MS, Rydgren K (2016) Can browsing by deer in winter induce defence responses in bilberry (Vaccinium myrtillus)? Ecological Research 31 (3):441-448. doi:10.1007/s11284-016-1351-1

Heil M, Karban R (2010) Explaining evolution of plant communication by airborne signals. Trends in Ecology and Evolution 25 (3):137-144. doi:10.1016/j.tree.2009.09.010

Helmig D, Ortega J, Duhl T, Tanner D, Guenther A, Harley P, Wiedinmyer C, Milford J, Sakulyanontvittaya T (2007) Sesquiterpene emissions from pine trees--identifications, emission rates and flux estimates for the contiguous United States. Environmental Science and Technology 41 (5):1545-1553

Himanen SJ, Nissinen A, Auriola S, Poppy GM, Stewart CN, Holopainen JK, Nerg A-M (2008) Constitutive and herbivore-inducible glucosinolate concentrations in oilseed rape (Brassica napus) leaves are not affected by Bt Cry1Ac insertion but change under elevated atmospheric CO 2 and O 3. Planta 227 (2):427

Hodkinson ID (2005) Terrestrial insects along elevation gradients: species and community responses to altitude. Biological Reviews 80 (3):489-513

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu. Rev. Plant Biol. 59:41-66. doi:10.1146/annurev.arplant.59.032607.092825

IPCC (2018) IPCC report 2018: Global warming of 1.5 °C: an IPCC special report on the impacts of global warming of 1.5 °C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. IPCC report.

Jacquemart AL (1993) Floral Visitors of Vaccinium Species in the High Ardennes, Belgium. Flora 188 (3):263-273

Jacquemart AL, Thompson JD (1996) Floral and pollination biology of three sympatric Vaccinium (Ericaceae) species in the Upper Ardennes, Belgium. Canadian Journal of Botany-Revue Canadienne De Botanique 74 (2):210-221. doi:DOI 10.1139/b96-025

Karban R (2008) Plant behaviour and communication. Ecology Letters 11 (7):727-739. doi:10.1111/j.1461-0248.2008.01183.x

Karban R, Baldwin I (1997) Induced Responses to Herbivory. University of Chicago Press. Chicago, IL, USA.

Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Science 291 (5511):2141-2144. doi:10.1126/science.291.5511.2141

Kobayashi Y, Yamamura N (2003) Evolution of signal emission by non-infested plants growing near infested plants to avoid future risk. Journal of Theoretical Biology 223 (4):489-503

Koo AJ, Howe GA (2009) The wound hormone jasmonate. Phytochemistry 70 (13-14):1571-1580. doi:10.1016/j.phytochem.2009.07.018

Liland KH, Sæbø S (2014) mixlm: Mixed Model ANOVA and Statistics for Education. R package version 1 (7)

Loarie SR, Duffy PB, Hamilton H, Asner GP, Field CB, Ackerly DD (2009) The velocity of climate change. Nature 462 (7276):1052

Loreto F, Barta C, Brilli F, Nogues I (2006) On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. Plant, Cell and Environment 29 (9):1820-1828

Makino A, Nakano H, Mae T, Shimada T, Yamamoto N (2000) Photosynthesis, plant growth and N allocation in transgenic rice plants with decreased Rubisco under CO2 enrichment. Journal of Experimental Botany 51 Spec No (suppl_1):383-389

Makino A, Sakuma H, Sudo E, Mae T (2003) Differences between maize and rice in N-use efficiency for photosynthesis and protein allocation. Plant and Cell Physiology 44 (9):952-956. doi:DOI 10.1093/pcp/pcg113

Moen A, Lillethun A, Odland A (1999) Vegetation. National atlas of Norway. Norwegian Mapping Authority, Hønefoss, Norway

Moles AT, Wallis IR, Foley WJ, Warton DI, Stegen JC, Bisigato AJ, Cella-Pizarro L, Clark CJ, Cohen PS, Cornwell WK, Edwards W, Ejrnaes R, Gonzales-Ojeda T, Graae BJ, Hay G, Lumbwe FC, Magana-Rodriguez B, Moore BD, Peri PL, Poulsen JR, Veldtman R, von Zeipel

H, Andrew NR, Boulter SL, Borer ET, Campon FF, Coll M, Farji-Brener AG, De Gabriel J, Jurado E, Kyhn LA, Low B, Mulder CP, Reardon-Smith K, Rodriguez-Velazquez J, Seabloom EW, Vesk PA, van Cauter A, Waldram MS, Zheng Z, Blendinger PG, Enquist BJ, Facelli JM, Knight T, Majer JD, Martinez-Ramos M, McQuillan P, Prior LD (2011) Putting plant resistance traits on the map: a test of the idea that plants are better defended at lower latitudes. New Phytologist 191 (3):777-788. doi:10.1111/j.1469-8137.2011.03732.x

Moreira X, Mooney KA, Rasmann S, Petry WK, Carrillo-Gavilán A, Zas R, Sampedro L (2014) Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. Ecology Letters 17 (5):537-546

Moreira X, Petry WK, Mooney KA, Rasmann S, Abdala-Roberts L (2018) Elevational gradients in plant defences and insect herbivory: recent advances in the field and prospects for future research. Ecography 41 (9):1485-1496. doi:10.1111/ecog.03184

NGU (2019) Norges Geologiske Undersøkelse. http://geo.ngu.no/kart/minkommune/?kommunenr=1420. Accessed 08/01/2019

NILU (2019) Norwegian Institute for Air Research - NILU. http://uv.nilu.no/index.cfm?fa=uv.showtext&id=86. Accessed 15/01/2019

Nunes-Nesi A, Fernie AR, Stitt M (2010) Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. Molecular Plant 3 (6):973-996. doi:10.1093/mp/ssq049

Paiva NL (2000) An introduction to the biosynthesis of chemicals used in plant-microbe communication. Journal of Plant Growth Regulation 19 (2):131-143

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annual Review of Ecology Evolution and Systematics 37:637-669. doi:10.1146/annurev.ecolsys.37.091305.110100

Pato J, Obeso JR (2012) Growth and reproductive performance in bilberry (Vaccinium myrtillus) along an elevation gradient. Ecoscience 19 (1):59-68. doi:10.2980/19-1-3407

Pauwels L, Inzé D, Goossens A (2009) Jasmonate-inducible gene: what does it mean? Trends in Plant Science 14 (2):87-91

Pellissier L, Fiedler K, Ndribe C, Dubuis A, Pradervand JN, Guisan A, Rasmann S (2012) Shifts in species richness, herbivore specialization, and plant resistance along elevation gradients. Ecology and Evolution 2 (8):1818-1825. doi:10.1002/ece3.296

Pellissier L, Moreira X, Danner H, Serrano M, Salamin N, van Dam NM, Rasmann S (2016) The simultaneous inducibility of phytochemicals related to plant direct and indirect defences against herbivores is stronger at low elevation. Journal of Ecology 104 (4):1116-1125. doi:10.1111/1365-2745.12580

Peñuelas J, Llusià J (2003) BVOCs: plant defense against climate warming? Trends in Plant Science 8 (3):105-109

Penuelas J, Staudt M (2010) BVOCs and global change. Trends in Plant Science 15 (3):133-144. doi:10.1016/j.tplants.2009.12.005

Pickett J, Rasmussen H, Woodcock C, Matthes M, Napier J (2003) Plant stress signalling: understanding and exploiting plant–plant interactions. Portland Press Limited,

Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC (2012) Hormonal modulation of plant immunity. Annu. Rev. of Cell and Developmental Biology 28:489-521. doi:10.1146/annurev-cellbio-092910-154055

Pinto DM, Blande JD, Nykänen R, Dong W-X, Nerg A-M, Holopainen JK (2007) Ozone degrades common herbivore-induced plant volatiles: does this affect herbivore prey location by predators and parasitoids? Journal of Chemical Ecology 33 (4):683-694

Plummer M (2013) rjags: Bayesian graphical models using MCMC. R package version 3

Rasmann S, Agrawal AA (2011) Latitudinal patterns in plant defense: evolution of cardenolides, their toxicity and induction following herbivory. Ecology Letters 14 (5):476-483. doi:10.1111/j.1461-0248.2011.01609.x

Rasmann S, Alvarez N, Pellissier L (2014a) The altitudinal niche-breadth hypothesis in insect-plant interactions. Annual Plant Reviews 47:339-359

Rasmann S, Pellissier L, Defossez E, Jactel H, Kunstler GJFE (2014b) Climate-driven change in plant–insect interactions along elevation gradients. Functional Ecology 28 (1):46-54

RDevelopment C (2012) TEAM 2009: R: A language and environment for statistical computing. Vienna, Austria Internet: http://www.R-project org

Rhoades DF (1979) Evolution of plant chemical defense against herbivores. Herbivores: their interaction with secondary plant metabolites. 3-54

Ritchie JC (1956) Vaccinium-Myrtillus L. Journal of Ecology 44 (1):291-299. doi:Doi 10.2307/2257181

Rodriguez-Saona CR, Polashock J, Malo EA (2013) Jasmonate-Mediated Induced Volatiles in the American Cranberry, Vaccinium macrocarpon: From Gene Expression to Organismal Interactions. Frontiers in Plant Science 4:115. doi:10.3389/fpls.2013.00115

Rodriguez-Saona CR, Rodriguez-Saona LE, Frost CJ (2009) Herbivore-Induced Volatiles in the Perennial Shrub, Vaccinium corymbosum, and Their Role in Inter-branch Signaling. Journal of Chemical Ecology 35 (2):163-175. doi:10.1007/s10886-008-9579-z

Rosenstiel TN, Potosnak MJ, Griffin KL, Fall R, Monson RK (2003) Increased CO 2 uncouples growth from isoprene emission in an agriforest ecosystem. Nature 421 (6920):256

Ruther J, Kleier S (2005) Plant–plant signaling: ethylene synergizes volatile emission in Zea mays induced by exposure to (Z)-3-hexen-1-ol. Journal of Chemical Ecology 31 (9):2217-2222

Salmore AK, Hunter MD (2001) Elevational trends in defense chemistry, vegetation, and reproduction in Sanguinaria canadensis. Journal of Chemical Ecology 27 (9):1713-1727

Sanchez-Sampedro A, Kim HK, Choi YH, Verpoorte R, Corchete P (2007) Metabolomic alterations in elicitor treated Silybum marianum suspension cultures monitored by nuclear magnetic resonance spectroscopy. Journal of Biotechnology 130 (2):133-142. doi:10.1016/j.jbiotec.2007.03.007

Scheidel U, Bruelheide H (2001) Altitudinal differences in herbivory on montane Compositae species. Oecologia 129 (1):75-86. doi:10.1007/s004420100695

Schemske D (2009) Biotic interactions and speciation in the tropics. Speciation Patterns of Diversity:219-239

Schmidt JH, Rexstad EA, Roland CA, McIntyre CL, MacCluskie MC, Flamme MJ (2018) Weather-driven change in primary productivity explains variation in the amplitude of two herbivore population cycles in a boreal system. Oecologia 186 (2):435-446

Schroter D, Cramer W, Leemans R, Prentice IC, Araujo MB, Arnell NW, Bondeau A, Bugmann H, Carter TR, Gracia CA, de la Vega-Leinert AC, Erhard M, Ewert F, Glendining M, House JI, Kankaanpaa S, Klein RJ, Lavorel S, Lindner M, Metzger MJ, Meyer J, Mitchell TD, Reginster I, Rounsevell M, Sabate S, Sitch S, Smith B, Smith J, Smith P, Sykes MT, Thonicke K, Thuiller W, Tuck G, Zaehle S, Zierl B (2005) Ecosystem service supply and vulnerability to global change in Europe. Science 310 (5752):1333-1337. doi:10.1126/science.1115233

Selås V (1997) Cyclic population fluctuations of herbivores as an effect of cyclic seed cropping of plants: the mast depression hypothesis. Oikos 80 (2):257-268. doi:Doi 10.2307/3546594

Selås V (2000) Population dynamics of capercaillie Tetrao urogallus in relation to bilberry Vaccinium myrtillus production in southern Norway. Wildlife Biology 6 (1):1-11

Selås V, Kobro S, Sonerud GA (2013) Population fluctuations of moths and small rodents in relation to plant reproduction indices in southern Norway. Ecosphere 4 (10):1-11. doi:Unsp 12310.1890/Es13-00228.1

Selås V (2006) Explaining bank vole cycles in southern Norway 1980–2004 from bilberry reports 1932–1977 and climate. Oecologia 147 (4):625-631

Seldal T, Hegland SJ, Rydgren K, Rodriguez-Saona C, Töpper JP (2017) How to induce defense responses in wild plant populations? Using bilberry (Vaccinium myrtillus) as example. Ecology and Evolution 7 (6):1762-1769

Serreze MC, Walsh JE, Chapin FS, Osterkamp T, Dyurgerov M, Romanovsky V, Oechel WC, Morison J, Zhang T, Barry RG (2000) Observational evidence of recent change in the northern high-latitude environment. Climatic Change 46 (1-2):159-207. doi:Doi 10.1023/A:1005504031923

SSB (2018) Statistics Norway 2018. https://www.ssb.no/jord-skog-jakt-og-fiskeri/faktaside/jakt. Accessed 15/01/2019

Staswick PE, Lehman CC (1999) Jasmonic acid-signaled responses in plants. Inducible Plant Defenses Against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture:117-136

Stenseth NC, Mysterud A (2002) Climate, changing phenology, and other life history traits: nonlinearity and match–mismatch to the environment. Proceedings of the National Academy of Sciences 99 (21):13379-13381

Stout MJ, Workman KV, Duffey SS (1996) Identity, spatial distribution, and variability of induced chemical responses in tomato plants. Entomologia Experimentalis Et Applicata 79 (3):255-271. doi:DOI 10.1111/j.1570-7458.1996.tb00834.x

Strong DR, Lawton JH, Southwood SR (1984) Insects on plants. Community patterns and mechanisms. Blackwell Scientific Publicatons.

Thaler JS, Stout MJ, Karban R, Duffey SS (2001) Jasmonate-mediated induced plant resistance affects a community of herbivores. Ecological Entomology 26 (3):312-324

Tigney D (1991) Factors controlling the emissions of monoterpenes and other volatile organic compounds. Trace Gas Emission by Plants:93-119

Tollrian R, Harvell CD (1999) The ecology and evolution of inducible defenses. Princeton University Press, 306:321

Trussell GC, Smith LD (2000) Induced defenses in response to an invading crab predator: an explanation of historical and geographic phenotypic change. Proceedings of the National Academy of Sciences 97 (5):2123-2127. doi:10.1073/pnas.040423397

Van Dam N, Baldwin I (2001) Competition mediates costs of jasmonate-induced defences, nitrogen acquisition and transgenerational plasticity in Nicotiana attenuata. Functional Ecology 15 (3):406-415

Velásquez AC, Castroverde CDM, He SY (2018) Plant–Pathogen Warfare under Changing Climate Conditions. Current Biology 28 (10):R619-R634

Visser ME, Both C (2005) Shifts in phenology due to global climate change: the need for a yardstick. Proceedings of the Royal Society B: Biologial Sciences 272 (1581):2561-2569. doi:10.1098/rspb.2005.3356

Walling LL (2000) The Myriad Plant Responses to Herbivores. Journal of Plant Growth Regulation 19 (2):195-216

Wasternack C, Hause B (2013) Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Annals of Botany 111 (6):1021-1058. doi:10.1093/aob/mct067

Wasternack C, Miersch O, Kramell R, Hause B, Ward J, Beale M, Boland W, Parthier B, Feussner I (1998) Jasmonic acid: biosynthesis, signal transduction, gene expression. Lipid/Fett 100 (4-5):139-146

Wu Z, Dijkstra P, Koch GW, Peñuelas J, Hungate BA (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. Global Change Biology 17 (2):927-942

Yang SY, Wu HH, Xie JC, Rantala MJ (2013) Depressed performance and detoxification enzyme activities of Helicoverpa armigera fed with conventional cotton foliage subjected to methyl jasmonate exposure. Entomologia Experimentalis Et Applicata 147 (2):186-195. doi:10.1111/eea.12060

Yarnes CT, Boecklen WJ (2005) Abiotic factors promote plant heterogeneity and influence herbivore performance and mortality in Gambel's oak (Quercus gambelii). Entomologia Experimentalis et Applicata 114 (2):87-95

Yuan JS, Himanen SJ, Holopainen JK, Chen F, Stewart CN, Jr. (2009) Smelling global climate change: mitigation of function for plant volatile organic compounds. Trends in Ecology and Evolution 24 (6):323-331. doi:10.1016/j.tree.2009.01.012

Zhu X, Zhu JK (2013) Double repression in jasmonate-mediated plant defense. Molecular Cell 50 (4):459-460. doi:10.1016/j.molcel.2013.05.009

Paper I

Received: 23 March 2018 | Revised: 30 May 2018 | Accepted: 17 June 2018

DOI: 10.1002/ece3.4365

ORIGINAL RESEARCH

WILEY Ecology and Evolution Open Access

Multiannual effects of induced plant defenses: Are defended plants good or bad neighbors?

Rafael Fonseca Benevenuto[1,2] | Stein Joar Hegland[1] | Joachim Paul Töpper[3] | Knut Rydgren[1] | Stein R. Moe[2] | Cesar Rodriguez-Saona[4] | Tarald Seldal[1]

[1]Faculty of Engineering and Science, Western Norway University of Applied Sciences, Sogndal, Norway

[2]Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Ås, Norway

[3]Norwegian Institute for Nature Research, Bergen, Norway

[4]Department of Entomology, Rutgers University, New Brunswick, New Jersy

Correspondence
Rafael Fonseca Benevenuto, Faculty of Engineering and Science, Western Norway University of Applied Sciences, Sogndal, Norway.
Email: rafaelb@hvl.no

Funding information
Norwegian Research Council, Grant/Award Number: 204403/E40; Norwegian Environment Agency

Abstract

Defenses induced by herbivore feeding or phytohormones such as methyl jasmonate (MeJA) can affect growth, reproduction, and herbivory, not only on the affected individual but also in its neighboring plants. Here, we report multiannual defense, growth, and reproductive responses of MeJA-treated bilberry (*Vaccinium myrtillus*) and neighboring ramets. In a boreal forest in western Norway, we treated bilberry ramets with MeJA and water (control) and measured responses over three consecutive years. We observed the treatment effects on variables associated with herbivory, growth, and reproduction in the MeJA-treated and untreated ramet and neighboring ramets distanced from 10 to 500 cm. MeJA-treated ramets had fewer grazed leaves and browsed shoots compared to control, with higher effects in 2014 and 2015, respectively. In 2013, growth of control ramets was greater than MeJA-treated ramets. However, MeJA-treated ramets had more flowers and berries than control ramets 2 years after the treatment. The level of insect and mammalian herbivory was also lower in untreated neighboring ramets distanced 10–150 cm and, consistent with responses of MeJA-treated ramets, the stronger effect was also one and 2 years delayed, respectively. The same neighboring ramets had fewer flowers and berries than untreated ramets, indicating a trade-off between defense and reproduction. Although plant–plant effects were observed across all years, the strength varied by the distance between the MeJA-treated ramets and its untreated neighbors. We document that induced defense in bilberry reduces both insect and mammalian herbivory, as well as growth, over multiple seasons. The defense responses occurred in a delayed manner with strongest effects one and 2 years after the induction. Additionally, our results indicate defense signaling between MeJA-treated ramets and untreated neighbors. In summary, this study shows that induced defenses are important ecological strategies not only for the induced individual plant but also for neighboring plants across multiple years in boreal forests.

KEYWORDS

bilberry, boreal forest, delayed response, methyl jasmonate, multiannual effects, plant–plant interactions, *Vaccinium myrtillus*

1 | INTRODUCTION

Plants have evolved a diversity of structural, constitutive, and inducible defenses to protect tissues, seeds, and fruits from attacking herbivores, fungi, and pathogens (Agrawal, 1999). Green and Ryan (1972) were the first to demonstrate that plants under attack from herbivores produce chemical defense compounds that help to protect them from further damage. For example, the emission of volatile organic compounds (VOCs) functions as warning signals to deter herbivores and attract beneficial predatory insects (Dicke, 1999; Macel & Vrieling, 2003; Melis et al., 2006; Nieminen, Suomi, Van Nouhuys, Sauri, & Riekkola, 2003; Paré & Tumlinson, 1999). Moreover, plant VOCs are key signals in plant–plant interactions, and work as external signals in the activation of plant defense systems (Arimura et al., 2002; Dolch & Tscharntke, 2000; Heil & Karban, 2010; Karban, 2001; Ruther & Kleier, 2005). Plant–plant interactions can occur both above and belowground and are mediated through internal signals driven by specific compounds moving within interconnected ramets in clonal species (Gómez, Van Dijk, & Stuefer, 2010), or by external VOCs emitted by neighboring plants (Rodriguez-Saona, Rodriguez-Saona, & Frost, 2009). The role of these VOCs is mainly regulated by the hormone jasmonic acid (JA) and related compounds, which perform a key role in the activation of plant defense responses (Staswick & Lehman, 1999; Wasternack et al., 1998). Laboratory and field studies have shown that plant chemical defense systems can be elicited experimentally by exogenous application of methyl jasmonate (MeJA), a VOC derivate of jasmonic acid, known as an omnipresent defense signal in plants (Koo & Howe, 2009; Pieterse, Van der Does, Zamioudis, Leon-Reyes, & Van Wees, 2012).

In the boreal forest, bilberry (*Vaccinium myrtillus* L.) is a key food plant for many insects, birds, and mammals (Hjältén, Danell, & Ericson, 2004; Jacquemart, 1993; Selås, 2001; Welch, Keay, Kendall, & Robbins, 1997). As a result of its ecological importance, bilberry is an ideal organism for studies on inducible plant defense responses, as well as plant–plant and plant–animal interactions under natural field conditions. In previous studies, defenses induced by herbivore feeding or MeJA treatment were shown to reduce herbivory and increase reproduction of the damaged or treated bilberry plants (Hegland, Seldal, Lilleeng, & Rydgren, 2016; Seldal, Hegland, Rydgren, Rodriguez-Saona, & Töpper, 2017). However, little is known from natural systems about the multiannual effects of induced plant defenses. Similarly, the extent to which such effects are transferred to neighboring plants is unknown (Karban, Ishizaki, & Shiojiri, 2012; Karban & Maron, 2002). Such studies may improve our understanding of the ecological consequences of induced defenses and plant–plant interactions on herbivore population dynamics.

Over three consecutive years, we explored plant defense activation in response to exogenous MeJA application in bilberry and its effects on untreated neighboring bilberry ramets under natural field conditions. Inducible plant defense responses are assumed to be energetically costly due to the allocation of resources from growth and reproduction to defense (Karban, Yang, & Edwards, 2014; Rodriguez-Saona, Polashock, & Malo, 2013; Sampedro, Moreira, & Zas, 2011; Seldal et al., 2017). Thus, in the first year after treatment, we predicted decreased insect and mammalian herbivory and reduced plant size (growth) and reproduction in MeJA-treated bilberry ramets compared to untreated controls (prediction I). Based on the role of VOCs in the detection of induced defenses in neighboring plants (Arimura et al., 2002; Dolch & Tscharntke, 2000; Farag & Pare, 2002; Hare, 2011; Heil & Karban, 2010; Karban, 2001; Karban, Baldwin, Baxter, Laue, & Felton, 2000; Ruther & Kleier, 2005), we also predicted less herbivory and reduced growth and reproduction of untreated neighboring ramets at short distances from the induced plant (i.e. 10–500 cm; prediction II). Finally, because bilberry is a relatively slow-growing perennial and deciduous shrub (Flower-Ellis, 1971; Jacquemart & Thompson, 1996; Ritchie, 1956), we predicted a 1-year delay of the largest resource allocation effects, and possible long-term (multiannual) reduction in growth, reproduction, and insect and mammalian herbivory of MeJA-treated and untreated neighboring ramets (prediction III) (Haukioja, Suomela, & Neuvonen, 1985; Zvereva, Kozlov, Niemelä, & Haukioja, 1997).

2 | MATERIAL AND METHODS

2.1 | Study system

We conducted a study of induced plant defense from 2013 to 2015 in a ca. 20-year old 50 × 200 m clear-cut in a pine forest at 350 m above sea level. The study area, Kaupanger in western Norway (61.2°N, 007.2°E), has annual precipitation of 700–900 mm and a mean summer temperature range of 12–16° C (Moen, 1999). Pine (*Pinus sylvestris* L.), bilberry (*V. myrtillus*), lingonberry (*Vaccinium vitis-idaea* L.), and crowberry (*Empetrum nigrum* L.) are the most abundant plant species in the field layer. The area has a dense winter population of red deer (*Cervus elaphus* L.), which is the most abundant wild ungulate in Norway (pers. obs. S.J. Hegland). Bilberry, our study species, is a long-lived deciduous clonal dwarf shrub, with evergreen stems usually 10–60 cm high (Flower-Ellis, 1971; Ritchie, 1956). Although we do not have specific information regarding clone size and distribution for the study area, we have based our work on the assumption that rhizomes can reach around 200 cm in length, depending on age, and the proportion of genetic variation within population is high (Albert, Raspé, & Jacquemart, 2003, 2004; Flower-Ellis, 1971). Bilberry is also a key species in boreal and alpine ecosystems because of its ecological role as a food source for many invertebrate and vertebrate species (Dahlgren, Oksanen, Sjödin, & Olofsson, 2007; Hegland, Jongejans, & Rydgren, 2010). The main mammalian herbivores feeding on bilberry in the study area are red deer and various rodent species, whereas the most common insect herbivores are Geometridae larveae (pers. obs. S.J. Hegland). Bumblebees, honeybees, and syrphid flies are the main pollinators for this species (Jacquemart, 1993; Jacquemart & Thompson, 1996).

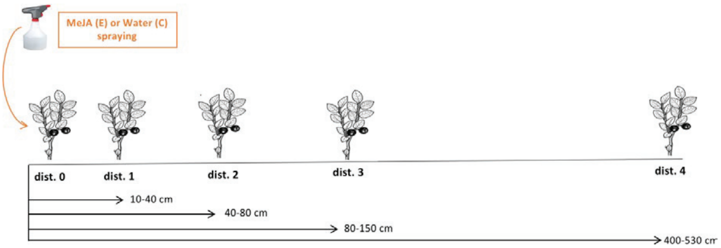


FIGURE 1 Transect design with the distances between the treated bilberry ramet and its untreated neighbors. C: control transect; E: experimental transect; MeJA: methyl jasmonate

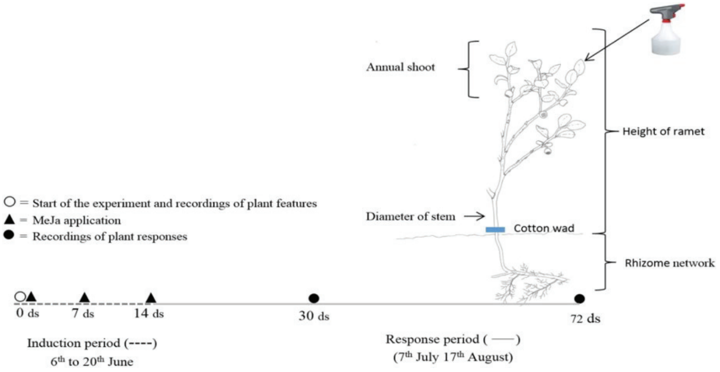


FIGURE 2 A bilberry ramet including size recordings, and timeline for the induction and response period when the measurements were recorded. ds: days MeJA: Methyl jasmonate

2.2 | Experimental design and treatments

In June 2013, we established ten blocks of 350 m^2 (10 m × 35 m), leaving a minimum of ten meters between each block to avoid interaction. To reduce variation in light conditions, humidity, and snow cover, we established the ten blocks oriented in the same direction in a uniform clear-cut facing southwest. Within each block, four transects were established at least ten meters apart with five individually marked bilberry ramets in each, ranging in height from 10 to 25 cm. The five ramets were located 10–40 cm (dist. 1), 40–80 cm (dist. 2), 80–150 cm (dist. 3), and 400–530 cm (dist. 4) from ramet one (dist. 0) in each of the transects (Figure 1). Transects were subsequently randomly assigned and exposed to two treatments with two replicates in each block. In 2013, we treated the first ramet (dist. 0) in each transect with either 10 mM MeJA (experimental transects) or water (control transects). Spraying was repeated three times within 2 weeks in the first year (2013). Prior to treatment application, MeJA was diluted with 95% (v/v) ethanol and then with water to provide 10 mM MeJA (Seldal et al., 2017). To avoid rapid evaporation of MeJA, a wad of cotton wool was attached to the stem of the ramet at ground level and saturated with the assigned treatment until the point of runoff (Seldal et al., 2017). The ramets were not exposed to further treatments in 2014 and 2015 to evaluate possible multiannual effects on growth, reproduction, and herbivory. We treated only the first ramet in each transect to evaluate possible effects of plant–plant interaction between MeJA-treated and its untreated neighboring ramets.

2.3 | Sampling procedure

Before the start of the MeJA treatments (6 June, 2013), we measured ramet height from the ground to crown with a ruler and stem diameter at ground with digital calipers. We also counted the number of annual shoots, flowers, leaves, browsed shoots, and leaves grazed by chewing insects (Figure 2). We repeated recordings of these variables 30 and 72 days after the initial treatment in 2013. In these subsequent recordings, we also counted berries. We repeated the measurements in 2014 and 2015. Plant height (H), stem diameter (DS), and number of shoots (AS) were used to calculate dry mass (DM) of each ramet as a nondestructive estimation of plant size using the formula of Hegland et al. (2010): $\log_2(\mathrm{DM}) = 1.41700 \times \log_2(\mathrm{DS}) + 0.97104 \times \log_2(H) + 0.44153 \times \log_2(\mathrm{AS} + 1) - 7.52070$.

2.4 | Data analysis

We analyzed how exogenous MeJA application of bilberry ramets affected growth (dry mass), reproduction (number of flowers and berries), and herbivory (ratio of grazed leaves by insect herbivores;

number of browsed shoots by large herbivores) by comparing untreated control ramets at dist. 0 with corresponding ramets in the experimental transects (MeJA dist. 0 = MeJA-treated ramets; MeJA dist. 1–4 = untreated ramets with increasing distances from the treated ramet). For each response variable, we parameterized a generalized linear mixed-effects model under Bayesian inference with intercepts and seasonal time slopes (three seasonal censuses) for each treatment (control, MeJA dist. 0–4) in every year (2013–2015), that is, we adopt a "means parameterization" approach (see also Section 2.4.2 for interpretation) (Kéry, 2010). In these models, differences in intercepts between control and MeJA treatment ramets represent differences in the respective responses at the first census in each year (intercept effect). Differences in slopes reflect differences in the temporal development during the season (slope effect). Effects on reproduction (flowers and berries) were analyzed yearly, based on records from the last census in each year. All models included random intercepts for each individual to account for the repeated measurements through time. For models of dry mass, we used a Gaussian error distribution with an identity link; for models of "ratio of insect grazed leaves," we used a binomial error distribution with a logit-link; and for models of "number of browsed shoots," "number of flowers," and "number of berries," we used a Poisson error distribution with a log-link. The binomial and Poisson models were specified to account for overdispersion by extending the error structure with an observation-level random intercept effect (modeling of errors drawn from a normal distribution extra to the implicit deviation in the Poisson family). Furthermore, the Poisson models were specified to account for zero-inflation (did not apply to flower/berry models) by modeling an observation-level Bernoulli process (Kéry, 2010). Upon inclusion of "block" as a random effect, the models failed to converge, which likely was due to increasingly uneven sampling size in 2014 & 2015 following the death of some plants. Therefore, we decided to focus on modeling individual random effects, overdispersion, and zero-inflation. All models were run using the "rjags" library (Plummer, 2013) in R (R Core Team, 2016).

2.4.1 | Specifications of statistical models under Bayesian inference

We used uninformative priors for the MCMC runs: For the treatment intercepts and time slopes, a normal distribution with a mean of 0 and a standard deviation of 0.001 was used, and for the (random) individual intercepts, we used a normal distribution with a mean of 0 and a standard deviation which was randomly drawn from a uniform distribution between 0 and 100. The treatment precisions (in the Gaussian models), the observation precisions (in Poisson's models with overdispersion), and individual precisions (random intercepts for individual) were specified as the inverse of a uniform distribution between 0 and 100. We specified four Markov chains with 200,000 samples each from which the first 100,000 iterations were discarded as an adaptation phase. From the remaining 100,000 posterior samples in each of the four chains, every 20th value was retained to save disk space; this resulted in a total of 20,000 final posterior samples per model. We assessed model convergence by visually checking trace plots of the Markov chains and by applying the Gelman and Rubin's convergence diagnostic (values below 1.1 were accepted). Model performance was checked visually through posterior density plots (only unimodal distributions without shoulders were accepted).

2.4.2 | Interpretation of model results under Bayesian inference

Bayesian analyses result in posterior distributions for every model parameter, and this subsequently allows us to deduce the significance of differences in intercepts and slopes between controls and all distances in the MeJA transects in all years. We assessed this significance by subtracting the respective control posterior distributions from their corresponding MeJA posterior distributions and calculating the ratio of the resulting values below and above zero. Positive numbers mean that MeJA-treated ramets had a higher value for the respective variable or had a greater time slope value than did control ramets. Negative numbers mean that MeJA-treated ramets had lower values for the respective variable or had smaller time slope values than did the control ramets.

3 | RESULTS

3.1 | Inducible defense responses

Methyl jasmonate-treated ramets showed significantly less insect herbivory than control ramets through the growth season in the first year (slope effect; Table 1; Figure 3), as well as on average (intercept effect and absence of slope effect; Table 1) in the following years, with a particularly strong effect of about ten times fewer grazed leaves in 2014 (Table 1). In 2014 and 2015, there was no significant reduction in insect herbivory through the growth season for the MeJA-treated ramets (slope effect; Table 1; Figure 3). We did not observe differences in the number of browsed shoots by large herbivores between MeJA-treated and control ramets in 2013 or 2014, but in 2015, MeJA treatment resulted in four times fewer browsed shoots compared to the control (negative intercept effect; Table 1). In 2013, the growth of untreated bilberry ramets (control) was slightly higher compared to MeJA-treated ramets (marginally significant slope effect; Figure 3; Table 1), but in 2014 and 2015, we did not find any growth differences between control and MeJA-treated ramets (Table 1). The numbers of flowers and berries did not differ between MeJA-treated and untreated ramets in 2013 or 2014 (Table 1). However, 2 years after treatments (2015), MeJA-treated ramets carried 2.6 more flowers and three times as many berries on average, in comparison with untreated control ramets (Table 1).

3.2 | Ramet interactions

In 2013, we did not find any differences in insect herbivory between MeJA-treated and control ramets at dist. 1 (10–40 cm; Table 2). However,

TABLE 1 Effects of methyl jasmonate (MeJA) on inducible defense responses in treated bilberry ramets over three consecutive years

	2013		2014		2015	
	Intercept	Slope	Intercept	Slope	Intercept	Slope
Insect grazed leaves	0.56	−0.79**	−2.67**	0.35	−0.92^(*)	−0.16
Browsed shoots	−0.10	−0.14	−0.13	−0.10	−1.97**	0.60*
Dry mass	−0.09	−0.15^(*)	−0.30	−0.030	−0.18	−0.15
Flowers	−0.37	NA	−0.39	NA	1.71^(*)	NA
Berries	−0.28	NA	−0.29	NA	1.87*	NA

Notes. This table shows the effect values of MeJA on intercepts (mean) and time slopes for the MeJA-treated ramets in relation to the control at distance 0 over three consecutive years. Effects on the intercept reflect general differences on average in each year. Effects on the slope reflect differences in the temporal development during the respective season. Positive numbers mean that MeJA-treated ramets had higher values for the respective variable or had a higher time slope than the controls. Negative numbers mean that MeJA-treated ramets had lower values for the respective variable or had lower time slope than the controls. For reproduction variables (flowers and berries), seasonal slopes are not applicable (NA) as they are measured once per season, and only annual means per treatment (intercept effect) are reported.

Significance is indicated by: ***<0.001, **<0.01, *<0.05, (*) <0.1.

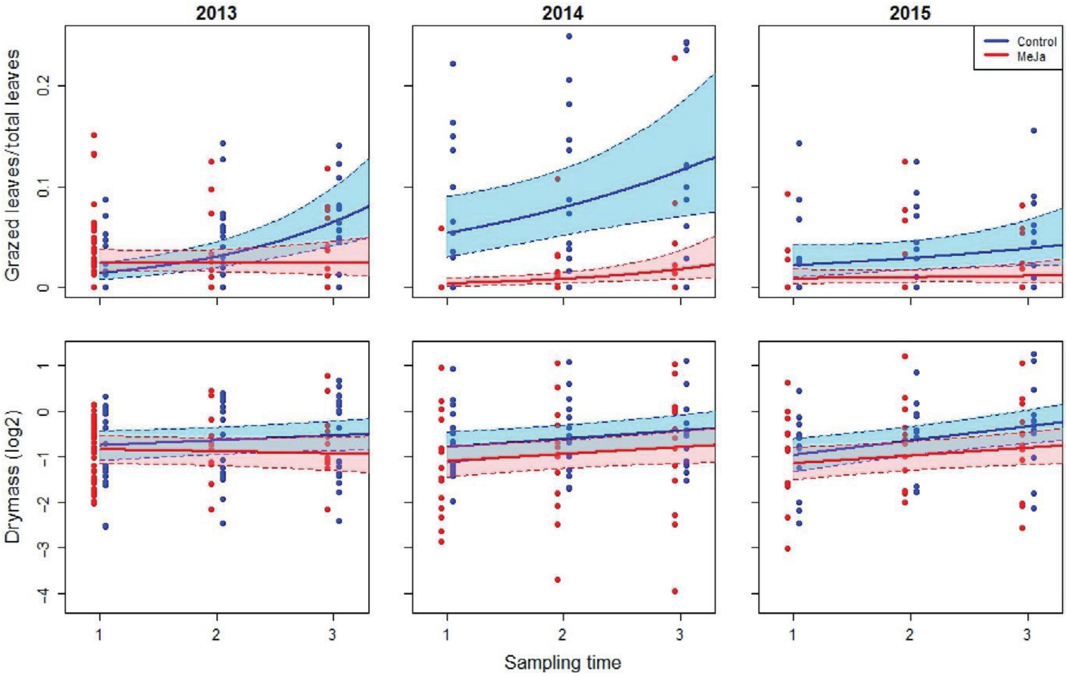


FIGURE 3 Development in time slope of insect herbivory (ratio of grazed leaves) and growth (dry mass) of bilberry for methyl jasmonate-treated (dist. 0) and control ramets. Data points were jittered around the three sampling times (6 June, 7 July and 17 August) in order to promote readability of the plots

a significant reduction in insect herbivory was found for ramets at dist. 2 (40–80 cm; $p < 0.1$), dist. 3 (80–150 cm; $p < 0.1$), and dist. 4 (400–530 cm; $p < 0.05$) through the growth season (slope effect; Table 2). In the two following years, we found reduced insect herbivory on ramets at dist. 1, 2, and 3 (10–150 cm; $p < 0.05$) in 2014, and at dist. 1 (10–40 cm; $p < 0.01$) and 3 (80–150 cm; $p < 0.05$) in 2015 (intercept effects; Table 2).

We did not find any difference in the number of browsed shoots through the first growing season for any of the ramets in the MeJA transects (no slope effect in 2013; Table 2). However, in 2013 at dist. 1 (10–40 cm; $p < 0.05$), 2 (40–80 cm; $p < 0.05$), and 4 (400–530 cm; $p < 0.1$), we found significantly fewer browsed shoots on average in MeJA transects compared to control transects (intercept effects in 2013; Table 2). In both 2014 and 2015, ramets at distance 1 (10–40 cm) had significantly fewer browsed shoots compared with control ramets (intercept effects in 2014 and 2015; Table 2).

In 2013, we did not find growth differences through the growth season for any of the untreated ramets in the MeJA transects (no slope effect in 2013; Table 2). No slope effect was observed in 2014 and 2015 either (Table 2). However, from the start of the experiment, neighboring ramets in the MeJA transects had significantly smaller dry mass compared to ramets in the control transects (intercept effects in 2013; Table 2). These size differences were maintained in the MeJA transects in both 2014 and 2015 (Table 2).

In 2013, we found significantly fewer flowers and berries in ramets at distances 1–3 (10–150 cm; $p < 0.01$ and $p < 0.05$) in the MeJA transects (Table 2). In 2014, only ramets at distance 1 (10–40 cm; $p < 0.01$) had significantly fewer berries compared to control ramets (intercept effect in 2014; Table 2), whereas in 2015 only ramets at distance 3 (80–150 cm) had significantly fewer flowers ($p < 0.05$) and berries ($p < 0.1$) compared to control ramets (intercept effects in 2015; Table 2).

4 | DISCUSSION

We found significant changes in herbivore resistance, growth, and reproduction after MeJA application to wild bilberries under natural environmental conditions over three consecutive seasons. Induced ramets showed significant reductions in growth and herbivory, indicating an efficient strategy of allocating resources from growth to defense over the growing season in the first year (prediction I). Moreover, our findings also show that this state of defense varies in

TABLE 2 Effects of methyl jasmonate (MeJA) on inducible plant defense response of untreated neighboring bilberry ramets at different distances over three consecutive years

	dist. 1		**dist. 2**		**dist. 3**		**dist. 4**	
	Interc.	**Slope**	**Interc.**	**Slope**	**Interc.**	**Slope**	**Interc.**	**Slope**
2013								
Insect grazed leaves	−0.29	−0.28	0.66	−0.55$^{(*)}$	0.32	−0.51$^{(*)}$	0.84	−0.60*
Browsed shoots	−0.70*	0.09	−0.76*	0.14	−0.52	−0.03	−0.64$^{(*)}$	0.12
Dry mass	−0.84**	−0.05	−0.74**	0.06	−0.73*	−0.04	−0.51*	−0.03
Flowers	−1.22$^{(*)}$	NA	−1.25$^{(*)}$	NA	−1.60*	NA	−0.20	NA
Berries	−0.82$^{(*)}$	NA	−1.11*	NA	−1.11*	NA	0.07	NA
2014								
Insect grazed leaves	−1.04*	−0.17	−1.44**	−0.18	−1.35**	−0.17	−0.46	−0.11
Browsed shoots	−1.22*	−0.12	−0.08	−0.49**	−0.70	−0.37$^{(*)}$	−0.76	−0.16
Dry mass	−1.03***	−0.01	−0.68*	−0.12	−0.63*	−0.03	−0.40	−0.03
Flowers	−0.32	NA	−0.35	NA	−0.06	NA	−0.40	NA
Berries	−0.45**	NA	−0.17	NA	−0.09	NA	−0.14	NA
2015								
Insect grazed leaves	−1.55**	0.48	0.81	0.03	−1.64*	0.05	−0.71	−0.04
Browsed shoots	−2.18***	0.27	−1.50*	0.43$^{(*)}$	−0.92	−0.09	−1.88*	0.27
Dry mass	−0.90**	−0.21	−0.62*	−0.07	−0.34	−0.38	−0.08	−0.34
Flowers	−0.54	NA	−0.24	NA	−0.84*	NA	−0.11	NA
Berries	−0.40	NA	−0.28	NA	−0.60$^{(*)}$	NA	−0.08	NA

Notes. This table shows the effect of MeJA on intercepts (mean) and time slopes for untreated neighbor ramets at different distances (experimental transect) in relation to the control at dist. 0 (control transect) over three consecutive years. Effects on intercept reflect general differences on average in each year. Effects on slope reflect differences in the temporal development during the respective season. Positive numbers mean that neighbor ramets at respective distance had higher values for the respective variable or had higher time slope than the controls. Negative numbers mean that neighbor ramets at respective distance had lower values for the respective variable or had lower time slope than the controls. For reproduction variables (flowers and berries), seasonal slopes are not applicable (NA) as they are measured once per season, and only annual means per treatment (intercept effect) are reported.

Significance is indicated by: ***<0.001, **<0.01, *<0.05, (*) <0.1.

strength and persists across subsequent years (multiannual effect). As predicted, there was a delay of 1 year for the strongest resistance effect to insect herbivores in the MeJA-treated ramets (prediction III). However, this delayed effect was even longer (2 years) for resistance to herbivory by large animals. In the context of plant–plant interactions, our results indicate long-distance signaling transfer related to defense between MeJA-treated ramets and its untreated neighbors under natural conditions (prediction II). The effects of this signaling process on untreated neighboring ramets were multiannual, and its highest effect was delayed, consistent with the patterns found on the MeJA-treated ramets (prediction III).

4.1 | Inducible defense responses

The responses related to plant growth (dry mass) and insect herbivory to MeJA application in the first year (2013) suggest that treated plants rapidly allocate resources from growth to defense. These results corroborate previous studies showing that MeJA application on bilberry ramets reduces insect and mammalian herbivory at the expense of growth and reproduction (Hegland et al., 2016; Seldal et al., 2017).

Insect herbivory was significantly reduced in the MeJA-treated ramets for two subsequent growing seasons (2014 and 2015), indicating a multiannual defense response in bilberry. This multiannual allocation of resources from growth to defense after MeJA treatment in 2013 may explain the lack of a seasonal reduction in insect herbivory in 2014 and 2015 because these plants may already be in a state of "alert" from the previous year. Consistent with our prediction (III), the largest effect in resistance to insect herbivory was found 1 year after the treatment (2014), where MeJA-treated ramets exhibited about ten times fewer insect grazed leaves compared to controls, followed by reduced herbivory 2 years after treatment (2015). For long-lived plants, the defense system can be active across multiple growing seasons, a phenomenon referred to as "delayed induced resistance," depending on the life history of the plant and previous grazing pressure (Haukioja et al., 1985; Zvereva et al., 1997). Induced plant defense responses can persist over a large range of time intervals from rapid (e.g. a few hours or days) to annually delayed induced responses (Agrawal, 1999; Karban & Baldwin, 1997). A study of MeJA-treated Norway spruce (*Picea abies*) showed less bark beetle colonization and higher terpene content soon after treatment and a relaxation of the defense in the next growing season (Erbilgin, Krokene, Christiansen, Zeneli, & Gershenzon, 2006). Conversely, studies of deciduous trees report more delayed defense responses, which can last for years after the induction (Haukioja, 1982; Haukioja, Ruohomäki, Senn, Suomela, & Walls, 1990; Neuvonen, Haukioja, & Molarius, 1987; Schultz & Baldwin, 1982; Tuomi, Niemelä, Haukioja, Sirén, & Neuvonen, 1984; Valentine, Wallner, & Wargo, 1983). In a meta-study, Nykänen and Koricheva (2004) showed that induced defense responses in woody plants have the strongest negative impact on the performance of the next generation of herbivores, suggesting a strong delayed defense response in such species.

Two years after treatment (2015), we found reduced herbivory by large mammalian herbivores, where MeJA-treated ramets had on average four times fewer browsed shoots compared to control ramets. These results suggest that there is a long-term buildup of defense against large vertebrate herbivores such as red deer, which are abundant in the study area. Generally, rapidly induced plant defenses affect the performance of short-lived invertebrate herbivores, whereas delayed induced defense responses affect the next generation of short-lived invertebrate and long-lived vertebrate herbivores (Haukioja & Hanhimäki, 1985). In addition, delayed induced resistance involves "quantitative defenses," which are effective against both specialists and generalists, in contrast to rapid induced resistance which involves "qualitative defenses" that are more efficient against generalists but not specialists (Rhoades, 1979). Although costly, quantitative defenses provide better protection against specialized and polyphagous herbivores because they act in a dosage-dependent manner (Price, Denno, Eubanks, Finke, & Kaplan, 2011). According to the plant apparency theory, plants that are easily found by herbivores, such as trees and shrubs, should invest heavily in quantitative defenses that are effective against a broad spectrum of herbivores (Feeny, 1976). Bilberry is an "apparent" deciduous shrub which store reserves of carbon in stems and roots, enabling it to produce quantitative carbon-based defenses (e.g. flavonoids and tannins), which are efficient against specialist mammalian herbivores present in the boreal forest, such as red deer (Gallet, 1994). As a result, we hypothesize that bilberry plants may use multiple induced defensive tactics against herbivores: Some are rapidly induced and more efficient against insect herbivores (likely qualitative defenses), while others are delayed induced and more efficient against mammalian herbivores (likely quantitative).

We found that the MeJA treatment led to a reduction in growth (dry mass) in the year of treatment (2013). In contrast to our last prediction (III), this allocation of resources from growth to defense was not significant in the years following treatment. Interestingly, 2 years after treatment (2015), the numbers of flowers and berries of MeJA-treated plants increased significantly (2.6 and three times, respectively) compared to control plants, suggesting that the defense system reduces herbivory and increases long-term reproductive success of bilberry. These results indicate that defense mobilization in bilberry lasts for years and thus increases the fitness of defended plants. Although jasmonate-induced responses function as defenses, this is considered costly for the plant as it has to allocate important resources from growth, reproduction, or other functions. Therefore, as inducible defense responses are considered to be a cost-saving strategy, plants have the capacity to time the production of these chemicals according to the current environmental conditions, and hence avoid using resources on defenses when they are not needed (Baldwin, 1998). Taking into account the existing competition for limited resources in the boreal forest system, as well as considering the relatively low MeJA effect on treated plants after 2 years, we suggest that induced bilberry plants used a cost-saving strategy in 2015 by foregoing the excessive costs to allocate resources from reproduction to defenses when these are considered ecologically unnecessary.

4.2 | Multiannual ramet interactions

Untreated bilberry ramets growing at distances of between ten centimeters to five meters from MeJA-treated ramets showed reduced insect herbivory compared to untreated control ramets. These findings indicate that the MeJA itself or the emission of VOCs from MeJA-treated ramets can activate the defense system of untreated neighbor ramets at distances of up to five meters. Our results are consistent with results from studies involving other species where above- and belowground signaling activate the defense system and reduce herbivory of untreated neighbor plants (Baldwin, Kessler, & Halitschke, 2002; Dicke & Bruin, 2001; Heil & Karban, 2010; Pickett, Rasmussen, Woodcock, Matthes, & Napier, 2003).

Bilberry has interconnected ramets with extensive belowground rhizomes (Tolvanen & Laine, 1997). Therefore, both airborne and belowground signaling probably contributed to the activation of the defense system of untreated ramets in this study (Chen, Lei, & Liu, 2011; Gómez, Latzel, Verhulst, & Stuefer, 2007; Gomez & Stuefer, 2006; Gómez et al., 2010). Regardless of the type of signaling strategy, the evidence here and in other studies supports two types of responses by the neighboring “eavesdropping” plants: The induction of a direct defense mechanisms that makes them resistant to subsequent herbivory (e.g. altering palatability and/or toxicity of leaf tissues) and an indirect defense strategy, such as the recruitment of natural enemies as “bodyguards” (Dicke, Agrawal, & Bruin, 2003).

The elevated resistance to herbivory of untreated neighbor ramets lasted for several growth seasons, although this varied in time and space. In 2013, we found reduced insect herbivory of untreated ramets growing at distances of up to five meters from the MeJA-treated ramets. In the subsequent growing season (2014), however, only ramets growing close to the MeJA-treated ramets showed less insect herbivory compared to control ramets. Consistent with the results found among the MeJA-treated ramets, untreated neighbor ramets were most resistant to herbivory in 2014. Two years after the MeJA treatment (2015), the effects of the defense system started to relax in some of the neighbor ramets at greater distances from the MeJA-treated ramet. Previous studies that resurrected interplant communication in the last decade have shown similar results by conducting laboratory and field experiments and exploring molecular, physiological, and ecological data. For instance, Dolch and Tscharntke (2000) demonstrated that experimental defoliation of single trees in different sites in Germany caused natural herbivory to increase with distance from the defoliated tree, and the authors attributed this effect to above- or belowground signaling. Similar to our study, another field experiment conducted over three consecutive years showed that experimentally damaged sagebrush plants led to herbivory resistance in neighboring tobacco plants compared to those neighboring undamaged sagebrush (Karban et al., 2000). This plant–plant interaction process was correlated with induced emissions of MeJA in damaged sagebrush and increased production of an important defense chemical (polyphenol oxidase) in the neighboring tobacco plants.

An unexpected situation occurred regarding our growth-related results on untreated neighboring ramets. Untreated neighboring ramets from the experimental transects were significantly smaller than the ramets from the control transects already at the onset of the experiment in 2013 (i.e. significant intercept effect in 2013). Because of this bias in plant size, we cannot imply that the observed differences in dry mass are a result of a trade-off between growth and defense caused by plant–plant interaction. However, this bias likely remains without any effect for our interpretation, as neither our data or analysis show any signs of differences for dry mass during the season (slope effects) between controls and MeJA neighboring ramets.

We found the strongest resistance against herbivorous insects in untreated neighbor ramets 1 year after the MeJA treatment (2014; Insect grazed leaves; Table 2), where ramets at dist. 1, 2, and 3 (10–150 cm) showed on average four times less insect herbivory compared to control ramets. However, browsing by large mammalian herbivores was lowest in untreated neighbor ramets in 2015, 2 years after the MeJA treatment (2015; Browsed shoots; Table 2). Both results are consistent with the responses of MeJA-treated ramets in 2014 and 2015 (Insect grazed leaves; Browsed shoots; Table 1). As a result, our findings on untreated neighboring ramets appear to be consistent with the results found for the MeJA-treated ramets, suggesting that both defensive strategies of induced plants and its neighbors are effectively multiannual and that the strongest effects are delayed.

For inducible resistance to cause cyclic fluctuations in herbivore populations, the intensity of the rapid inducible resistance has to be weaker than the long-term resistance in the subsequent years (Haukioja & Hanhimäki, 1985). However, as indicated by several authors (Fox, 1981; Högstedt, Seldal, & Breistøl, 2005; Lundberg, Järemo, & Nilsson, 1994; Myers, 1988; Seldal, Andersen, & Högstedt, 1994; Spencer, 2013; Underwood, 1999), more studies of the delayed action of plant defense responses under natural field conditions are necessary to better understand how these systems affect herbivore populations. However, due to the large scale (i.e. space, time) and complexity of ecosystems, such studies are challenging to design (Underwood, 1999). Nevertheless, there are some studies that show close correlations between bilberry production and local population sizes of both insects and large herbivores known to feed on bilberry (Selås, 1997, 2000, 2006; Selås, Kobro, & Sonerud, 2013).

In summary, our findings provide evidence for long-term effects of plant–plant signaling mediated by jasmonate-induced responses in bilberry, indicating that induced plants are “good” neighbors due to ecological facilitation with conspecifics under natural conditions. The demonstrated effects of below- and aboveground plant–plant interactions, especially related to herbivore resistance, varied in efficacy according to time (seasons after induction) and distance from the induced plant emitting the chemical information to its neighbors. Moreover, the documented multiannual effect and the delay of the highest level of induced resistance on MeJA-treated and untreated neighbor bilberry ramets may have important implications for our understanding of outbreaks of insect and mammalian herbivore populations in the boreal ecosystem.

AKNOWLEDGMENTS

We thank Tine Tande Seldal, Helge Hustveit, and Torbjørn Stokke for their help with establishment and execution of the field experiment. We also thank the landowner Nils Knagenhjelm, Kaupanger Hovedgård for letting us use their land and roads for this study. This research was financially supported by the Norwegian Research Council (project no. 204403/E40) and the Norwegian Environment Agency.

CONFLICT OF INTEREST

None declared.

AUTHORS' CONTRIBUTIONS

T.S., S.J.H., and K.R. conceived the initial ideas and designed methodology and collected the data; J.P.T. and R.F.B analyzed the data; R.F.B led the writing of the manuscript. All authors contributed equally to the drafts and gave final approval for the publication.

DATA ACCESSIBILITY

All the data generated for this study are publicly available at Dryad Digital Repository.

ORCID

Rafael Fonseca Benevenuto http://orcid.org/0000-0002-4287-4721

Knut Rydgren http://orcid.org/0000-0001-8910-2465

Stein R. Moe http://orcid.org/0000-0003-1005-3192

Stein Joar Hegland http://orcid.org/0000-0001-7039-5966

Joachim Paul Töpper http://orcid.org/0000-0002-6996-7223

Cesar Rodriguez-Saona http://orcid.org/0000-0001-5888-1769

Tarald Seldal http://orcid.org/0000-0002-2636-4035

REFERENCES

Agrawal, A. A. (1999). Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. *Ecology*, *80*, 1713–1723. https://doi.org/10.1890/0012-9658(1999)080[1713:IRTHIW]2.0.CO;2

Albert, T., Raspé, O., & Jacquemart, A.-L. (2003). Clonal structure in *Vaccinium myrtillus* L. revealed by RAPD and AFLP markers. *International Journal of Plant Sciences*, *164*, 649–655. https://doi.org/10.1086/375373

Albert, T., Raspé, O., & Jacquemart, A.-L. (2004). Clonal diversity and genetic structure in *Vaccinium myrtillus* populations from different habitats. *Belgian Journal of Botany*, *137*, 155–162.

Arimura, G. I., Ozawa, R., Nishioka, T., Boland, W., Koch, T., Kühnemann, F., & Takabayashi, J. (2002). Herbivore-induced volatiles induce the emission of ethylene in neighboring lima bean plants. *The Plant Journal*, *29*, 87–98. https://doi.org/10.1046/j.1365-313x.2002.01198.x

Baldwin, I. T. (1998). Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 8113–8118. https://doi.org/10.1073/pnas.95.14.8113

Baldwin, I. T., Kessler, A., & Halitschke, R. (2002). Volatile signaling in plant–plant–herbivore interactions: What is real? *Current Opinion in Plant Biology*, *5*, 351–354. https://doi.org/10.1016/S1369-5266(02)00263-7

Chen, J.-S., Lei, N.-F., & Liu, Q. (2011). Defense signaling among interconnected ramets of a rhizomatous clonal plant, induced by jasmonic-acid application. *Acta Oecologica*, *37*, 355–360. https://doi.org/10.1016/j.actao.2011.04.002

Dahlgren, J., Oksanen, L., Sjödin, M., & Olofsson, J. (2007). Interactions between gray-sided voles (*Clethrionomys rufucanus*) and bilberry (*Vaccinium myrtillus*), their main winter food plant. *Oecologia*, *152*, 525–532. https://doi.org/10.1007/s00442-007-0664-8

Dicke, M. (1999). Plant-carnivore interactions: Evolutionary and ecological consequences for plant, herbivore and carnivore. In H. Olff, V. K. Brown, & R. H. Drent (Eds.), *Herbivores: Between plants and predators* (pp. 483–520). London, UK: Blackwell Science.

Dicke, M., Agrawal, A. A., & Bruin, J. (2003). Plants talk, but are they deaf? *Trends in Plant Science*, *8*, 403–405. https://doi.org/10.1016/S1360-1385(03)00183-3

Dicke, M., & Bruin, J. (2001). Chemical information transfer between plants: Back to the future. *Biochemical Systematics and Ecology*, *29*, 981–994. https://doi.org/10.1016/S0305-1978(01)00045-X

Dolch, R., & Tscharntke, T. (2000). Defoliation of alders (*Alnus glutinosa*) affects herbivory by leaf beetles on undamaged neighbours. *Oecologia*, *125*, 504–511. https://doi.org/10.1007/s004420000482

Erbilgin, N., Krokene, P., Christiansen, E., Zeneli, G., & Gershenzon, J. (2006). Exogenous application of methyl jasmonate elicits defenses in Norway spruce (*Picea abies*) and reduces host colonization by the bark beetle Ips typographus. *Oecologia*, *148*, 426–436. https://doi.org/10.1007/s00442-006-0394-3

Farag, M. A., & Pare, P. W. (2002). C 6-Green leaf volatiles trigger local and systemic VOC emissions in tomato. *Phytochemistry*, *61*, 545–554. https://doi.org/10.1016/S0031-9422(02)00240-6

Feeny, P. (1976). Plant apparency and chemical defense. In J. W. Wallace, & R. L. Mansell (Eds.), *Biochemical interaction between plants and insects* (pp. 1–40). Berlin, Germany: Springer.

Flower-Ellis, J. G. K. (1971). Age structure and dynamics in stands of bilberry (Vaccinium myrtillus L.), PhD-thesis. Royal College of Forest Ecology and Forest Soils, Research Notes 9: 1–108.

Fox, L. R. (1981). Defense and dynamics in plant-herbivore systems. *American Zoologist*, *21*, 853–864. https://doi.org/10.1093/icb/21.4.853

Gallet, C. (1994). Allelopathic potential in bilberry-spruce forests: Influence of phenolic compounds on spruce seedlings. *Journal of Chemical Ecology*, *20*, 1009–1024. https://doi.org/10.1007/BF02059738

Gómez, S., Latzel, V., Verhulst, Y. M., & Stuefer, J. F. (2007). Costs and benefits of induced resistance in a clonal plant network. *Oecologia*, *153*, 921–930. https://doi.org/10.1007/s00442-007-0792-1

Gomez, S., & Stuefer, J. F. (2006). Members only: Induced systemic resistance to herbivory in a clonal plant network. *Oecologia*, *147*, 461–468. https://doi.org/10.1007/s00442-005-0293-z

Gómez, S., Van Dijk, W., & Stuefer, J. F. (2010). Timing of induced resistance in a clonal plant network. *Plant Biology*, *12*, 512–517. https://doi.org/10.1111/j.1438-8677.2009.00234.x

Green, T., & Ryan, C. A. (1972). Wound-induced proteinase inhibitor in plant leaves: A possible defense mechanism against insects. *Science*, *175*, 776–777. https://doi.org/10.1126/science.175.4023.776

Hare, J. D. (2011). Ecological role of volatiles produced by plants in response to damage by herbivorous insects. *Annual Review of Entomology*, *56*, 161–180. https://doi.org/10.1146/annurev-ento-120709-144753

Haukioja, E. (1982). *Inducible defences of white birch to a geometrid defoliator, Epirrita autumnata. 5th International Symposium in Insect-Plant Relationships* (pp. 199–203). Wageningen, the Netherland: Pudoc.

Haukioja, E., & Hanhimäki, S. (1985). Rapid wound-induced resistance in white birch (*Betula pubescens*) foliage to the geometrid *Epirrita autumnata*: A comparison of trees and moths within and outside the outbreak range of the moth. *Oecologia*, *65*, 223–228. https://doi.org/10.1007/BF00379221

Haukioja, E., Ruohomäki, K., Senn, J., Suomela, J., & Walls, M. (1990). Consequences of herbivory in the mountain birch (*Betula pubescens* ssp tortuosa): Importance of the functional organization of the tree. *Oecologia*, *82*, 238–247. https://doi.org/10.1007/BF00323540

Haukioja, E., Suomela, J., & Neuvonen, S. (1985). Long-term inducible resistance in birch foliage: Triggering cues and efficacy on a defoliator. *Oecologia*, *65*, 363–369. https://doi.org/10.1007/BF00378910

Hegland, S. J., Jongejans, E., & Rydgren, K. (2010). Investigating the interaction between ungulate grazing and resource effects on *Vaccinium myrtillus* populations with integral projection models. *Oecologia*, *163*, 695–706. https://doi.org/10.1007/s00442-010-1616-2

Hegland, S. J., Seldal, T., Lilleeng, M. S., & Rydgren, K. (2016). Can browsing by deer in winter induce defence responses in bilberry (*Vaccinium myrtillus*)? *Ecological Research*, *31*, 441–448. https://doi.org/10.1007/s11284-016-1351-1

Heil, M., & Karban, R. (2010). Explaining evolution of plant communication by airborne signals. *Trends in Ecology and Evolution*, *25*, 137–144. https://doi.org/10.1016/j.tree.2009.09.010

Hjältén, J., Danell, K., & Ericson, L. (2004). Hare and vole browsing preferences during winter. *Acta Theriologica*, *49*, 53–62. https://doi.org/10.1007/BF03192508

Högstedt, G., Seldal, T., & Breistøl, A. (2005). Period length in cyclic animal populations. *Ecology*, *86*, 373–378. https://doi.org/10.1890/02-0561

Jacquemart, A.-L. (1993). Floral visitors of *Vaccinium* species in the high Ardennes, Belgium. *Flora*, *188*, 263–273. https://doi.org/10.1016/S0367-2530(17)32276-4

Jacquemart, A.-L., & Thompson, J. (1996). Floral and pollination biology of three sympatric *Vaccinium* (Ericaceae) species in the Upper Ardennes, Belgium. *Canadian Journal of Botany*, *74*, 210–221. https://doi.org/10.1139/b96-025

Karban, R. (2001). Communication between sagebrush and wild tobacco in the field. *Biochemical Systematics and Ecology*, *29*, 995–1005. https://doi.org/10.1016/S0305-1978(01)00046-1

Karban, R., & Baldwin, I. (1997). *Induced resistance to herbivory*. Chicago, IL: Chicago University Press. https://doi.org/10.7208/chicago/9780226424972.001.0001

Karban, R., Baldwin, I. T., Baxter, K. J., Laue, G., & Felton, G. (2000). Communication between plants: Induced resistance in wild tobacco plants following clipping of neighboring sagebrush. *Oecologia*, *125*, 66–71. https://doi.org/10.1007/PL00008892

Karban, R., Ishizaki, S., & Shiojiri, K. (2012). Long-term demographic consequences of eavesdropping for sagebrush. *Journal of Ecology*, *100*, 932–938. https://doi.org/10.1111/j.1365-2745.2012.01974.x

Karban, R., & Maron, J. (2002). The fitness consequences of interspecific eavesdropping between plants. *Ecology*, *83*, 1209–1213. https://doi.org/10.1890/0012-9658(2002)083[1209:TFCOIE]2.0.CO;2

Karban, R., Yang, L. H., & Edwards, K. F. (2014). Volatile communication between plants that affects herbivory: A meta-analysis. *Ecology Letters*, *17*, 44–52. https://doi.org/10.1111/ele.12205

Kéry, M. (2010). *Introduction to WinBUGS for ecologists: Bayesian approach to regression, ANOVA, mixed models and related analyses*. Burlington, MA: Academic Press.

Koo, A. J., & Howe, G. A. (2009). The wound hormone jasmonate. *Phytochemistry*, *70*, 1571–1580. https://doi.org/10.1016/j.phytochem.2009.07.018

Lundberg, S., Järemo, J., & Nilsson, P. (1994). Herbivory, inducible defence and population oscillations: A preliminary theoretical analysis. *Oikos*, *71*, 537–540. https://doi.org/10.2307/3545843

Macel, M., & Vrieling, K. (2003). Pyrrolizidine alkaloids as oviposition stimulants for the cinnabar moth, Tyria jacobaeae. *Journal of Chemical Ecology*, *29*, 1435–1446. https://doi.org/10.1023/A:1024269621284

Melis, C., Buset, A., Aarrestad, P. A., Hanssen, O., Meisingset, E. L., Andersen, R., … Røskaft, E. (2006). Impact of red deer *Cervus elaphus* grazing on bilberry *Vaccinium myrtillus* and composition of ground beetle (Coleoptera, Carabidae) assemblage. *Biodiversity and Conservation*, *15*, 2049–2059. https://doi.org/10.1007/s10531-005-2005-8

Moen, A. (1999). *National atlas of Norway: Vegetation*. Hønefoss, Norway: Norwegian Mapping Authority.

Myers, J. H. (1988). Can a general hypothesis explain population cycles of forest Lepidoptera? *Advances in Ecological Research*, *18*, 179–242. https://doi.org/10.1016/S0065-2504(08)60181-6

Neuvonen, S., Haukioja, E., & Molarius, A. (1987). Delayed inducible resistance against a leaf-chewing insect in four deciduous tree species. *Oecologia*, *74*, 363–369. https://doi.org/10.1007/BF00378931

Nieminen, M., Suomi, J., Van Nouhuys, S., Sauri, P., & Riekkola, M.-L. (2003). Effect of iridoid glycoside content on oviposition host plant choice and parasitism in a specialist herbivore. *Journal of Chemical Ecology*, *29*, 823–844. https://doi.org/10.1023/A:1022923514534

Nykänen, H., & Koricheva, J. (2004). Damage-induced changes in woody plants and their effects on insect herbivore performance: A meta-analysis. *Oikos*, *104*, 247–268. https://doi.org/10.1111/j.0030-1299.2004.12768.x

Paré, P. W., & Tumlinson, J. H. (1999). Plant volatiles as a defense against insect herbivores. *Plant Physiology*, *121*, 325–332. https://doi.org/10.1104/pp.121.2.325

Pickett, J., Rasmussen, H., Woodcock, C., Matthes, M., & Napier, J. (2003). *Plant stress signalling: Understanding and exploiting plant–plant interactions*. London, UK: Portland Press Limited.

Pieterse, C. M., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, *28*, 489–521. https://doi.org/10.1146/annurev-cellbio-092910-154055

Plummer, Martyn. (2013). *rjags: Bayesian graphical models using MCMC. R package version 3-10*. URL: http://CRAN.R-project.org/package=rjags

Price, P. W., Denno, R. F., Eubanks, M. D., Finke, D. L., & Kaplan, I. (2011). *Insect ecology: Behavior, populations and communities*. Cambridge, UK: Cambridge University Press. https://doi.org/10.1017/CBO9780511975387

R Core Team (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing, 2014.

Rhoades, D. F. (1979). Evolution of plant chemical defense against herbivores. In G. A. A. J. D. H. Rosenthal (Ed.), *Herbivores: Their interaction with secondary plant metabolites* (pp. 3–54). Burlington, MA: Academic Press.

Ritchie, J. (1956). *Vaccinium myrtillus* L. *Journal of Ecology*, *44*, 291–299. https://doi.org/10.2307/2257181

Rodriguez-Saona, C. R., Polashock, J., & Malo, E. A. (2013). Jasmonate-mediated induced volatiles in the American cranberry, *Vaccinium macrocarpon*: From gene expression to organismal interactions. *Frontiers in Plant Science*, *4*, 115.

Rodriguez-Saona, C. R., Rodriguez-Saona, L. E., & Frost, C. J. (2009). Herbivore-induced volatiles in the perennial shrub, *Vaccinium corymbosum*, and their role in inter-branch signaling. *Journal of Chemical Ecology*, *35*, 163–175. https://doi.org/10.1007/s10886-008-9579-z

Ruther, J., & Kleier, S. (2005). Plant–plant signaling: Ethylene synergizes volatile emission in Zea mays induced by exposure to (Z)-3-hexen-1-ol. *Journal of Chemical Ecology*, *31*, 2217–2222. https://doi.org/10.1007/s10886-005-6413-8

Sampedro, L., Moreira, X., & Zas, R. (2011). Costs of constitutive and herbivore-induced chemical defences in pine trees emerge only under low nutrient availability. *Journal of Ecology*, *99*, 818–827. https://doi.org/10.1111/j.1365-2745.2011.01814.x

Schultz, J. C., & Baldwin, I. T. (1982). Oak leaf quality declines in response to defoliation by gypsy moth larvae. *Science*, *217*, 149–151. https://doi.org/10.1126/science.217.4555.149

Selås, V. (1997). Cyclic population fluctuations of herbivores as an effect of cyclic seed cropping of plants: The mast depression hypothesis. *Oikos*, *80*, 257–268. https://doi.org/10.2307/3546594

Selås, V. (2000). Population dynamics of capercaillie *Tetrao urogallus* in relation to bilberry *Vaccinium myrtillus* production in southern Norway. *Wildlife Biology*, *6*, 1–11. https://doi.org/10.2981/wlb.2000.032

Selås, V. (2001). Autumn population size of capercaillie *Tetrao urogallus* in relation to bilberry *Vaccinium myrtillus* production and weather: An analysis of Norwegian game reports. *Wildlife Biology*, *7*, 17–25. https://doi.org/10.2981/wlb.2001.005

Selås, V. (2006). Explaining bank vole cycles in southern Norway 1980–2004 from bilberry reports 1932–1977 and climate. *Oecologia*, *147*, 625–631. https://doi.org/10.1007/s00442-005-0326-7

Selås, V., Kobro, S., & Sonerud, G. A. (2013). Population fluctuations of moths and small rodents in relation to plant reproduction indices in southern Norway. *Ecosphere*, *4*, 1–11.

Seldal, T., Andersen, K.-J., & Högstedt, G. (1994). Grazing-induced proteinase inhibitors: A possible cause for lemming population cycles. *Oikos*, *70*, 3–11. https://doi.org/10.2307/3545692

Seldal, T., Hegland, S. J., Rydgren, K., Rodriguez-Saona, C., & Töpper, J. P. (2017). How to induce defense responses in wild plant populations? Using bilberry (*Vaccinium myrtillus*) as example. *Ecology and Evolution*, *7*, 1762–1769. https://doi.org/10.1002/ece3.2687

Spencer, K. C. (2013). *Chemical mediation of coevolution*. Amsterdam, the Netherland: Elsevier.

Staswick, P. E., & Lehman, C. C. (1999). Jasmonic acid-signaled responses in plants. In A. A. Agrawal, S. Tuzun & E. Bent (Eds.), *Inducible plant defenses against pathogens and herbivores: Biochemistry, ecology, and agriculture* (pp. 117-136). St. Paul, MN: Phytopathological Society Press.

Tolvanen, A., & Laine, K. (1997). Effects of reproduction and artificial herbivory on vegetative growth and resource levels in deciduous and evergreen dwarf shrubs. *Canadian Journal of Botany*, *75*, 656–666. https://doi.org/10.1139/b97-073

Tuomi, J., Niemelä, P., Haukioja, E., Sirén, S., & Neuvonen, S. (1984). Nutrient stress: An explanation for plant anti-herbivore responses to defoliation. *Oecologia*, *61*, 208–210. https://doi.org/10.1007/BF00396762

Underwood, N. (1999). The influence of plant and herbivore characteristics on the interaction between induced resistance and herbivore population dynamics. *The American Naturalist*, *153*, 282–294. https://doi.org/10.1086/303174

Valentine, H. T., Wallner, W. E., & Wargo, P. M. (1983). Nutritional changes in host foliage during and after defoliation, and their relation to the weight of gypsy moth pupae. *Oecologia*, *57*, 298–302. https://doi.org/10.1007/BF00377171

Wasternack, C., Miersch, O., Kramell, R., Hause, B., Ward, J., Beale, M., … Feussner, I. (1998). Jasmonic acid: Biosynthesis, signal transduction, gene expression. *European Journal of Lipid Science and Technology*, *100*, 139–146.

Welch, C. A., Keay, J., Kendall, K. C., & Robbins, C. T. (1997). Constraints on frugivory by bears. *Ecology*, *78*, 1105–1119. https://doi.org/10.1890/0012-9658(1997)078[1105:COFBB]2.0.CO;2

Zvereva, E. L., Kozlov, M. V., Niemelä, P., & Haukioja, E. (1997). Delayed induced resistance and increase in leaf fluctuating asymmetry as responses of Salix borealis to insect herbivory. *Oecologia*, *109*, 368–373. https://doi.org/10.1007/s004420050095

How to cite this article: Benevenuto RF, Hegland SJ, Töpper JP, et al. Multiannual effects of induced plant defenses: Are defended plants good or bad neighbors?. *Ecol Evol*. 2018;8:8940–8950. https://doi.org/10.1002/ece3.4365

Paper II

Benevenuto *et al. BMC Plant Biology* (2019) 19:70
https://doi.org/10.1186/s12870-019-1650-0

BMC Plant Biology

RESEARCH ARTICLE Open Access

Transcriptional profiling of methyl jasmonate-induced defense responses in bilberry (*Vaccinium myrtillus* L.)

Rafael Fonseca Benevenuto[1,2], Tarald Seldal[1], Stein Joar Hegland[1], Cesar Rodriguez-Saona[3], Joseph Kawash[4] and James Polashock[4*]

Abstract

Background: Bilberry (*Vaccinium myrtillus* L.) is one of the most abundant wild berries in the Northern European ecosystems. This species plays an important ecological role as a food source for many vertebrate and invertebrate herbivores. It is also well-recognized for its bioactive compounds, particularly substances involved in natural defenses against herbivory. These defenses are known to be initiated by leaf damage (e.g. chewing by insects) and mediated by activation of the jasmonic acid (JA) signaling pathway. This pathway can be activated by exogenous application of methyl jasmonate (MeJA), the volatile derivative of JA, which is often used to stimulate plant defense responses in studies of plant-herbivore interactions at ecological, biochemical, and molecular organismal levels. As a proxy for herbivore damage, wild *V. myrtillus* plants were treated in the field with MeJA and changes in gene expression were compared to untreated plants.

Results: The de novo transcriptome assembly consisted of 231,887 unigenes. Nearly 71% of the unigenes were annotated in at least one of the databases interrogated. Differentially expressed genes (DEGs), between MeJA-treated and untreated control bilberry plants were identified using DESeq. A total of 3590 DEGs were identified between the treated and control plants, with 2013 DEGs upregulated and 1577 downregulated. The majority of the DEGs identified were associated with primary and secondary metabolism pathways in plants. DEGs associated with growth (e.g. those encoding photosynthesis-related components) and reproduction (e.g. flowering control genes) were frequently down-regulated while those associated with defense (e.g. encoding enzymes involved in biosynthesis of flavonoids, lignin compounds, and deterrent/repellent volatile organic compounds) were up-regulated in the MeJA treated plants.

Conclusions: Ecological studies are often limited by controlled conditions to reduce the impact of environmental effects. The results from this study support the hypothesis that bilberry plants, growing in natural conditions, shift resources from growth and reproduction to defenses while in a MeJA-induced state, as when under insect attack. This study highlights the occurrence of this trade-off at the transcriptional level in a realistic field scenario and supports published field observations wherein plant growth is retarded and defenses are upregulated.

Keywords: Differential expression, Transcriptome, Herbivory, Secondary metabolism, Flowering, Signaling

---

* Correspondence: James.Polashock@ars.usda.gov
[4]Genetic Improvement of Fruits and Vegetables Lab, Philip E. Marucci Center for Blueberry and Cranberry Research, United States Department of Agriculture-Agricultural Research Service, Chatsworth, NJ, USA
Full list of author information is available at the end of the article

Background

Bilberry (*Vaccinium myrtillus* L.), also known as European blueberry, is one of the most abundant wild berries in the Northern European ecosystems. This is a long-lived deciduous clonal shrub, with evergreen stems usually 10–60 cm tall occurring mainly in the Eurasiatic boreal zone where it regularly constitutes about 40% of the ground cover [1]. Bilberry plays an important ecological role as food source for many species of vertebrate and invertebrate herbivores, pollinators, and fruit-eating birds and mammals in the boreal ecosystems [2–8]. This species is also well recognized for its bioactive properties and has attracted worldwide interest for being considered as one of the best sources of phenolic compounds, especially anthocyanins and other flavonoids [9–11]. From an ecological standpoint, such phenolic compounds and other secondary metabolites are known to play both direct and indirect roles in plant defense against biotic and abiotic stresses. For instance, some secondary metabolites are volatile organic compounds (VOCs), which can directly deter herbivores and/or provide insects with oviposition and feeding cues [12, 13]. These compounds can also act indirectly as chemical defenses by recruiting natural enemies [14, 15].

Jasmonic acid (JA), and its VOC analog methyl jasmonate (MeJA) are signaling molecules produced by plants, especially when subjected to environmental stresses such as wounding or pathogen attack. Once the plant perceives JA signals, a considerable reprogramming of gene expression occurs. Consequently, changes in the regulation of important pathways are made, including the induction of defensive genes and their associated biosynthetic pathways [16]. Inducible defense responses in plants can also be activated by exogenous application of MeJA. These responses to MeJA are similar to those induced by natural induction and include production of a range of toxic metabolites and anti-digestive proteins, such as proteinase inhibitors, which harm both specialist and generalist herbivores [17]. Studies in *Nicotiana attenuata* plants showed that trypsin proteinase inhibitor activity increased after MeJA elicitation [18]. As MeJA-induced responses are generally similar to those induced by insect herbivory [19], the application of exogenous MeJA is a useful tool to stimulate plant resistance in studies of plant-herbivore interactions at multiple organismal levels.

Recent ecological studies have reported significant changes in bilberry plants induced with MeJA treatment in their natural environments, including significant reduction in insect herbivory and plant growth [3, 20–22]. These studies have documented an apparent trade-off between growth/reproduction and defense in bilberry plants. However, little is known about the global changes in gene expression in induced plants to optimize their resource allocations from growth/reproduction to defense. Mayrose et al. [23] identified several genes, including a protein phosphatase 2C and the HD-Zip transcription factor Athb-8, whose expression are associated with trade-offs between growth and defense in common sunflower (*Helianthus annuus*). Similarly, Mitra and Baldwin [24] showed that RuBPCase activase, an abundant photosynthetic protein, mediates growth-defense trade-offs in *N. attenuata* by attenuating JA-induced defenses. However, changes in bilberry plants at the transcriptome level in response to defense induction by using MeJA have not yet been reported, though such changes must be a prerequisite to induce the synthesis of defensive metabolites and proteins related to plant defense [25].

The aim of our current work is to report, for the first time, a comprehensive transcriptome profile of MeJA-induced bilberry plants as compared to untreated control plants. Since defense- induced bilberry plants display an effective trade-off between growth/reproduction and defense [21, 22], we predict finding supporting evidence at the transcriptional level. Specifically, we expect to find down-regulation of important genes involved in growth and reproduction and up-regulation of defense-related genes. From this, we aim to provide a catalog of the primary genes, including those that are differentially expressed, involved in growth, reproduction and plant defense pathways of this ecologically important species in the boreal ecosystem.

Results

Transcriptome assembly, functional annotation, and gene expression

An average of 71,799,744 reads were generated for MeJA-treated samples after filtering, with 95.37% > Q20 and 46.1% GC content. Control samples generated 62,801,772 reads after filtering, 95.49% > Q20 and 46.3% GC content. The de novo transcriptome assembly consisted of 231,887 unigenes. Among the total assembled unigenes, 60,519 were 500 bp – 1 kbp, 51,029 were 1–2 kbp and 42,432 were ≥ 2 kbp, with a mean unigene length of 1226 bp and an N50 of 1987 bp. Of the 231,877 unigenes detected, 164,262 or 70.83%, were annotated in at least one of the databases interrogated.

For expression analysis, the de novo transcriptome filtered by Corset was used as the reference and nearly 80% of all reads mapped back to the reference. The fragments per kilobase of exon per million fragments mapped (FPKM) distribution showed that overall expression levels were similar between the MeJA plants and the untreated controls. A total of 3590 DEGs were identified between control and MeJA-treated bilberry plants with 2013 DEGs being up regulated and 1577 being down regulated (Fig. 1).

Predicted genes were analyzed with Blast2GO for Gene Ontology (GO) classification and grouped into

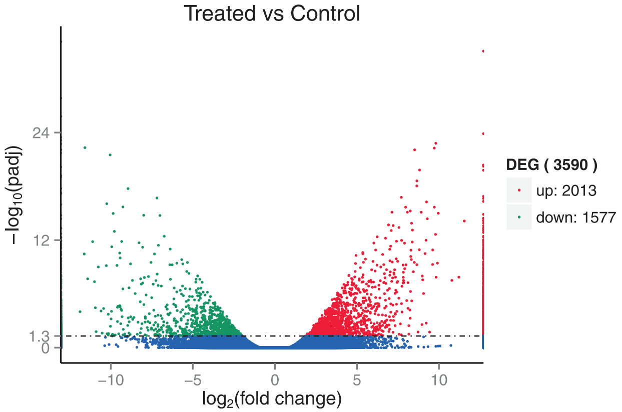


Fig. 1 Volcano plot of overall distribution of DEGs between Treated vs Control. The x-axis shows the $\log_2$fold change in gene expression between treated and control samples. The y-axis shows the -$\log_{10}$ (normalized pvalue) of the difference in expression. The further from 0 on the x-axis, the greater the change in expression and the higher on the y-axis, the greater the significance

three main GO domains: Biological Process (BP), Cellular Component (CG) and Molecular Function (MF). The seven predominant GO terms were: cellular process, metabolic process, single-organism process, cell, cell part, binding and catalytic activity (Fig. 2a). All associated GO term categories contained several differentially expressed genes, both upregulated and downregulated (Fig. 2b). The significantly enriched GO terms in DEGs were oxidation-reduction process and single-organism metabolic process, both within the GO domain of BP, as well as oxidoreductase activity in the GO domain of MF.

The annotated sequences were categorized into clusters of eukaryotic orthologous groups (KOG) classifications. In a total of 26 KOG categories, general function prediction only showed to be the largest group, followed by signal transduction mechanisms and posttranslational modification, protein turnover and chaperones (Fig. 3). Lastly, aiming to understand the biological pathways activated in MeJA-treated bilberry and its untreated control, all unigenes were mapped against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and 49,627 were annotated to 129 different KEGG pathways. The most represented pathways were translation, carbohydrate metabolism and folding, sorting and degradation. Mapping the DEGs in the KEGG database revealed that the pathways with the most significant changes in response to MeJA treatment were anthocyanin biosynthesis, nitrogen metabolism, tyrosine metabolism, and glutathione metabolism (Fig. 4).

Pathways with differentially expressed genes

Plant hormone signaling

Groups of genes involved in different plant hormone signaling pathways, such as abscisic acid (ABA), auxin (AUX), salicylic acid (SA), ethylene (ET) and brassinosteroid (BR), were identified as differentially expressed in MeJA-treated bilberry plants compared to the water/ethanol-treated control. MeJA treatment induced genes related to ABA, ET and BR signaling pathways, while genes involved in AUX and SA pathways were repressed.

In the MeJA treated plants, two important genes in the ABA signaling pathway were found to be up-regulated— those encoding the PYL ABA-receptor and ABA-responsive element binding factor (*PYL* and *ABF* genes, respectively) (Fig. 5). Two genes in the ET signaling pathway— encoding ET-insensitive protein 3 (*EIN3* gene) and ET-responsive transcription factor 1 (*ERF1* gene)—and one in the BR pathway— encoding BRI1 kinase inhibitor (*BKI1* gene) were also significantly up-regulated MeJA-treated bilberry plants. In contrast, three main groups of genes involved in the AUX signaling pathway— encoding AUX1/LAX influx carrier family (*AUX1/LAX* gene), auxin-responsive protein IAA (*IAA* gene), and Small auxin upregulated RNA protein (*SAUR* gene)— as well as two main down-regulated genes involved in the SA pathway—encoding TGA transcription factor (*TGA* gene) and *NPR1* regulatory gene — were identified as significantly down-regulated in the MeJA-treated bilberry plants (Fig. 5).

Secondary metabolites

KEGG pathway enrichment analysis of significantly up-regulated DEGs was performed to predict what secondary metabolites might be synthesized in response to MeJA treatment of bilberry plants in the wild. We found that the anthocyanin biosynthesis pathway was the most enriched up-regulated pathway, where 9 of 45 annotated

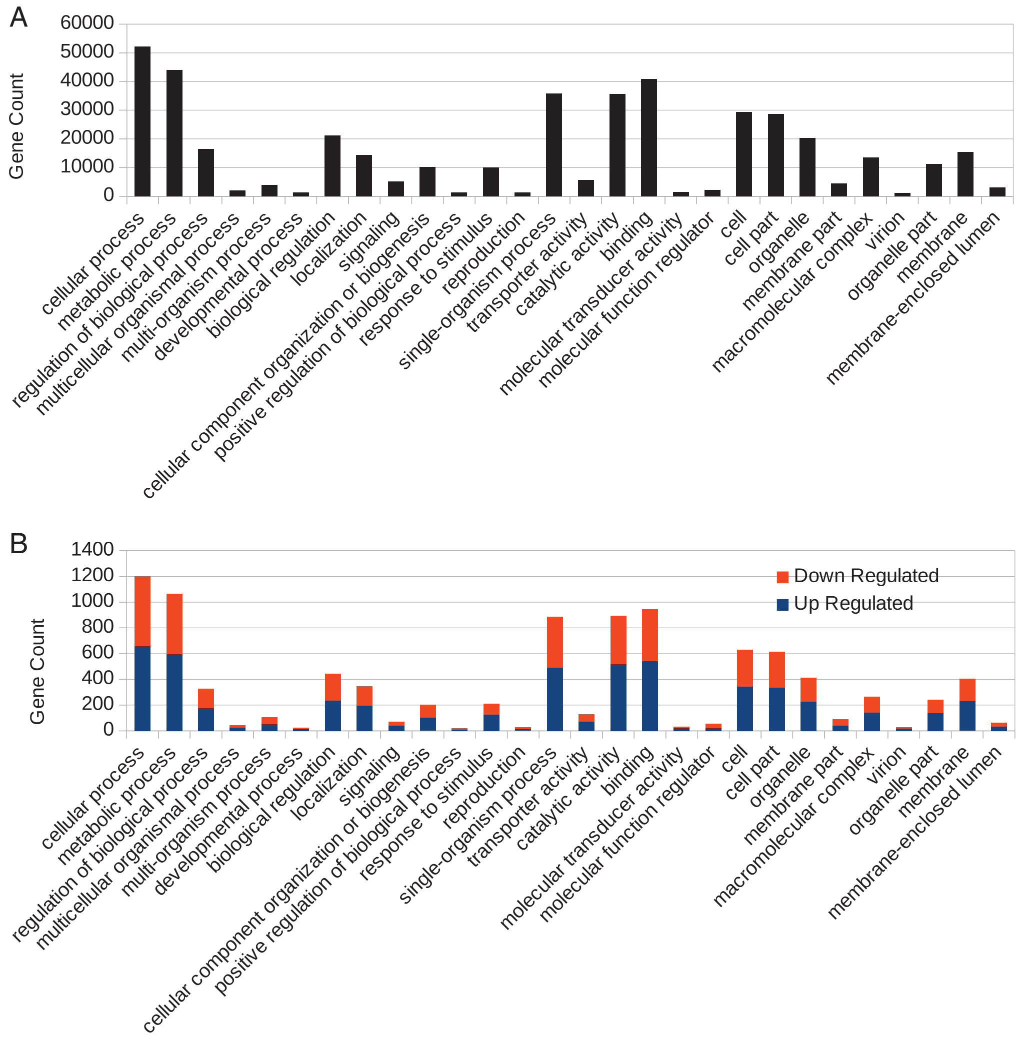


Fig. 2 GO Classification of Differentially Expressed Unigenes. Total gene counts of GO terms associated with all unigenes (**a**) and differentially expressed unigenes (**b**). Up-regulated unigenes are in blue and down-regulated unigenes are in red

genes involved in the pathway were significantly up-regulated in response to MeJA treatment (Fig. 6). We also found DEGs in the flavone and flavonol biosynthesis pathways in MeJA-treated bilberry leaves, where two key genes in the pathway were up-regulated— those encoding flavonoid 3′,5′-hydroxylase (*F3′5′H* gene) and flavonol 3-O glucosyltransferase (*F3OGT* gene) (Fig. 6). The phenylpropanoid biosynthetic pathway appeared to be up-regulated in the MeJA induced bilberry plants (Fig. 6), as evidenced by significant up-regulation of three genes in different steps of the phenylpropanoid pathway— those encoding shikimate O-hydroxycinnamoyltransferase (*HCT* gene), cinnamyl-alcohol dehydrogenase (*CAD* gene), and peroxidase (*POX* gene) (Fig. 6).

We found the tyrosine metabolic pathway was also affected by the MeJA treatment in bilberry plants. Specifically, we found many genes in the pathway significantly up-regulated—those encoding tyrosine aminotransferase (*TAT* gene), primary-amine oxidase (*AOC3*, *AOC2*, *tynA* genes), polyphenol oxidase (*PPO*

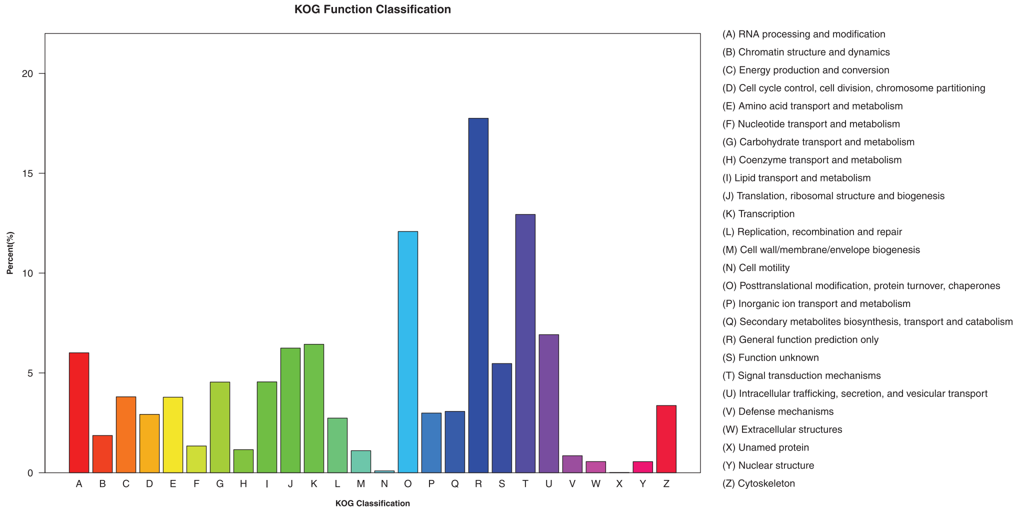


Fig. 3 KOG function classification of all unigenes

gene), alcohol dehydrogenase (*frmA*, *ADH5*, *adhC* genes), homogentisate phytyltransferase (*HPT*, *HGGT*, *ubiA* genes), and maleylacetoacetate isomerase (*maiA*, *CSTZ1* genes) (Fig. 6).

Additionally, MeJA induced genes in the glutathione metabolic pathway. The four up-regulated genes in the pathway are responsible for encoding the enzymes glutathione reductase (*GSR* gene), glutathione peroxidase (*GPX* gene), glutathione S-transferase (*GST* gene), and ornithine decarboxylase (*ODC1* gene) (Fig. 6). Finally, two terpene synthase genes that are directly involved in the synthesis of germacrene-type sesquiterpenoids were found to be up-regulated in MeJA induced plants— those encoding germacrene D synthase (*GERD* gene) and vetispiradiene synthase (*HVS* gene) (Fig. 6).

Circadian rhythm and flowering timing

MeJA treatment modified gene expression of the circadian clock in bilberry plants. A group of four important genes involved in circadian rhythm as well as flowering time had altered expression in response to MeJA induction. Genes encoding E3 ubiquitin-protein ligase COP1 (*COP1* gene) and MYB-related transcription factor late elongated hypocotyl (*LHY* gene) were significantly up-regulated in the MeJA-treated plants, while genes encoding the proteins early flowering 3 (*ELF3* gene) and flowering locus T (*FT* gene) were down-regulated (Fig. 7).

Photosynthesis and carbohydrate metabolism

Plant growth is directly tied to photosynthesis and carbohydrate metabolism. To investigate the possible trade-off between growth and defense at the transcript level, we analyzed the expression of pivotal genes involved in pathways related to photosynthesis and carbohydrate metabolism in the leaves of MeJA-treated bilberry plants. Our RNA-seq results showed significant down-regulation of three genes directly involved in photosynthesis— those encoding photosystem II CP47 chlorophyll apoprotein (*psbB* gene), photosystem II psbW (*psbW* gene), and photosystem I subunit VI (*psaH* gene) (Fig. 8). Other genes involved in carbohydrate metabolism-related pathways, such as glycolysis and the pentose phosphate pathway, were also affected by the MeJA treatment— those encoding glucose-6-phosphate isomerase (*GPI* gene), glucose-6-phosphate 1-epimerase (*GPE* gene), fructose-biphosphate aldolase (*ALDO* gene), triosephosphate isomerase (*TPI* gene), 2,3-biphosphoglycerate-independent phosphoglycerate mutase (*gpmI* gene), pyruvate decarboxylase (*pdc* gene), aldehyde dehydrogenase (*NAD+* gene), alcohol dehydrogenase (*ADH5* gene), 6-phosphoglucolactonase (*PGLS* gene), pectinesterase (*PE* gene), and UDPglucose 6-dehydrogenase (*UGDH* gene) (Fig. 8).

Nitrogen metabolism

Nitrogen metabolism is also tied to growth and development as nitrogen is an important constituent of DNA, RNA, proteins, hormones, chlorophyll, and other critical plant compounds. We found a group of important DEGs involved in the nitrogen metabolic pathway also being affected by MeJA treatment in bilberry leaves. Two genes involved in transport and reduction of nitrite were

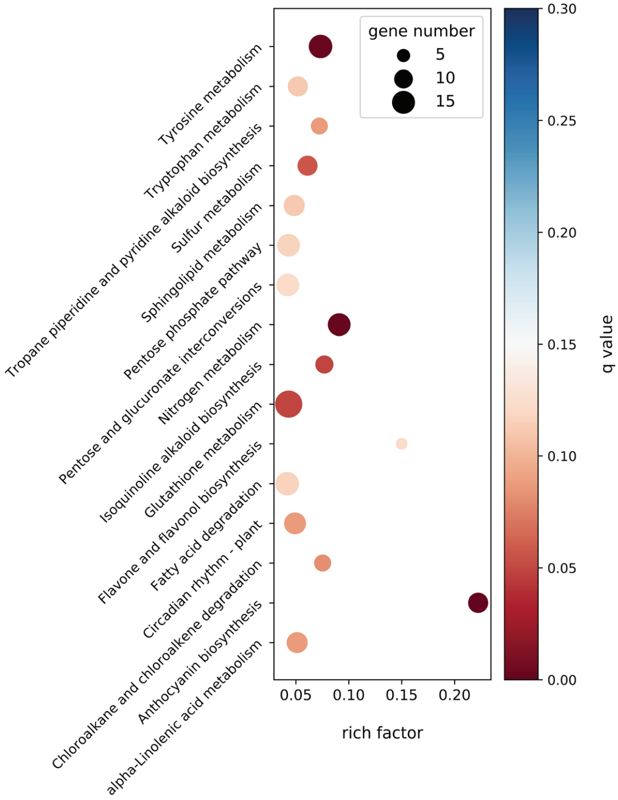


Fig. 4 KEGG Enrichment Scatter Plot of top 16 enriched pathways. KEGG enrichment scattered plot shows the DEGs enrichment analysis results in KEGG pathway. The top 16 significantly DEGs enriched pathways are displayed in the report (padj < 0.05). The degree of KEGG enrichment is measured by rich factor (x-axis), described as the ratio of the number of DEGs in the pathway compared to the total number of genes found in the pathway. The q-value is the *p*-value adjusted to sample distribution (also referred to as padj) and indicates significance of pathway enrichment. Dot size represents the number of different genes and the color indicates the q-value

up-regulated— those encoding NRT nitrate/nitrite transporter (*NRT* gene) and ferredoxin-nitrite reductase (*nirA* gene). Yet, expression levels of six other genes linked to glutamate metabolism were repressed— those encoding carbonic anhydrase (*cah* gene), chloroplastic glutamine synthetase (*glnA* gene), NADH-dependent glutamate synthase (*NADH-GOGAT* gene), ferredoxin-dependent glutamate synthase (*Fd-GOGAT* gene), and spermidine synthase (*speE*, *SRM* genes) (Fig. 9).

Discussion

Plant hormone signaling

Plant hormones operate in a complex "crosstalk" network between different pathways [26] and it is evident that MeJA treatment impacts this signaling. In the MeJA treated plants, two important genes in the ABA signaling pathway were found to be up-regulated—*PYL* and *ABF* (Fig. 5). Lackman et al. [27] showed that PYL ABA-receptor encoding genes are involved in the cross-talk between the JA and ABA signaling pathways to regulate metabolism and growth. Altered expression of PYL ABA-receptor encoding genes, *PYL4* and *PYL5*, was shown to affect JA responses, both in terms of biomass and anthocyanin production, which contributes to understanding the role of JAs in balancing the trade-off between growth and defense [27]. This trade-off has also been demonstrated in ecological studies where MeJA-treated wild bilberry plants showed consistent induction of defense responses leading to suppressed vegetative growth and reduced caterpillar herbivory, suggesting the allocation of resources from growth to defense [20, 21].

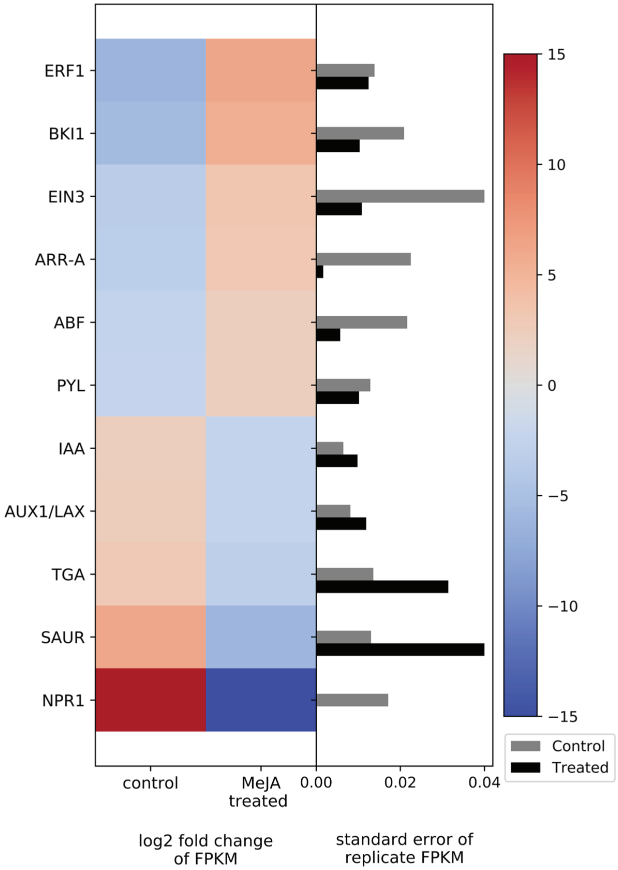


Fig. 5 Heat map of DEGs related to hormone signaling transduction. The left side of the plot is the heatmap of log2 fold change of FPKM between all treated and control groups. The right side of the plot is the standard error for all replicates divided by the average FPKM for the control and treated groups

Several studies provide evidence for positive interactions between the JA and ET signaling pathways, especially regarding the regulation of defense-related genes [28–31]. MeJA-treated bilberry plants showed two significantly up-regulated genes in the ET signaling pathway— *EIN3* and *ERF1*—and one in the BR pathway— *BKI1*. *EIN3* is well documented as a JA-responsive ethylene-signaling gene [32]. Downstream in the ET signaling pathway, *ERF1*, encoding an ET-responsive transcription factor, is known to play important roles in the regulation of defenses. Lu et al. [33] identified *ERF3* in rice (*Oryza sativa*) as a gene that positively affects expression of trypsin proteinase inhibitors and mediates resistance against caterpillars.

The involvement of AUX in plant growth and development, through induction of cell division and expansion, elongation, and cell differentiation is well characterized [34–36]. We identified three main groups of genes involved in the AUX signaling pathway as significantly down-regulated in the MeJA-treated bilberry plants— *AUX1/LAX*, *IAA*, and *SAUR* (Fig. 5). Yan et al. [37] studied the effects of MeJA treatment on root growth and found that JA signaling modulates AUX signaling pathways by regulating the expression of key genes involved in AUX transport. Among other AUX transporter-related genes, such as *PIN1*, *PIN2*, *PIN3*, they also found the *AUX1/LAX* family influx carrier gene to be down-regulated in response to MeJA treatment [26, 37].

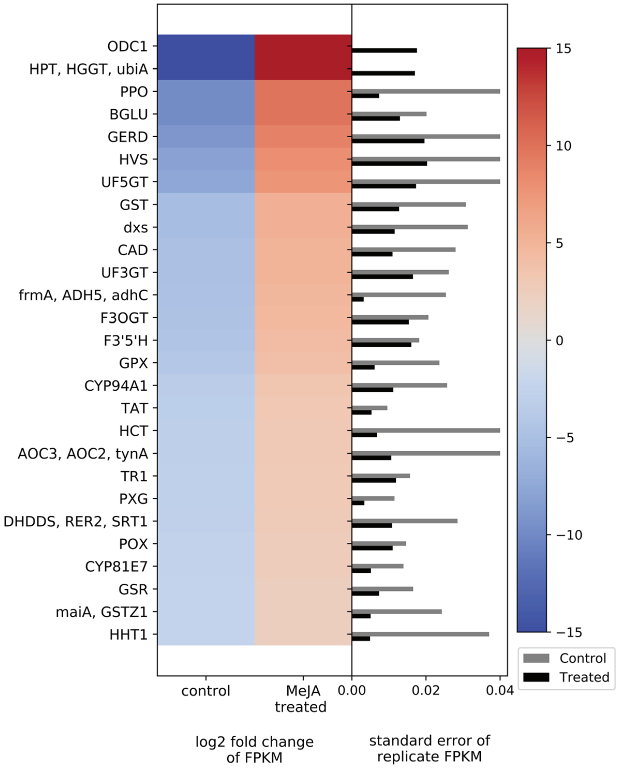


Fig. 6 Heat map of DEGs related to secondary metabolite biosynthesis. The left side of the plot is the heatmap of log2 fold change of FPKM between all treated and control groups. The right side of the plot is the standard error for all replicates divided by the average FPKM for the control and treated groups

Although the interactions between JA and SA appear to be complex, several genetic studies provided evidence for an antagonist regulatory interaction between them. As such, the SA signaling pathway was shown to be negatively modulated by MeJA treatment in bilberry plants. Specifically, we found two main down-regulated genes involved in the SA pathway—*NPR1* regulatory gene and *TGA* transcription factor. Studies in tobacco showed that endogenous and exogenous JA inhibit the expression of SA-dependent genes [38, 39]. The benefits and potential costs of cross talk between these important signaling pathways involved in defense have been extensively discussed [40–43]. Specifically, different defense signaling pathways have evolved the capacity to cross talk and modulate each other, allowing plants the flexibility to adjust defense responses according to a specific pathogen or herbivore attack [26]. In bilberry, JA signaling appears to synergize with ABA, ET, and BR, and to antagonize SA and AUX.

Secondary metabolites

MeJA treatment can trigger pathways involved in the biosynthesis of certain secondary metabolites, such as terpenoids, phenylpropanoids and alkaloids, as evidenced by several studies [16, 44–48]. We conducted KEGG pathway enrichment analysis of significantly up-regulated DEGs to determine what secondary metabolites might be synthesized in response to MeJA treatment of bilberry plants. Anthocyanin biosynthesis was the most affected secondary metabolite pathway in the MeJA-treated plants, where 9 of 45 annotated genes in the pathway were significantly up-regulated in response to the MeJA treatment (Fig. 6). Anthocyanins, a class of flavonoid, are pigments present in fruits, leaves, and flowers of several plant species which act as insect and animal attractants and have been identified as an important component in plant defense mechanisms against herbivores [49]. In addition, bilberry fruit are recognized for their high anthocyanin content as compared to those

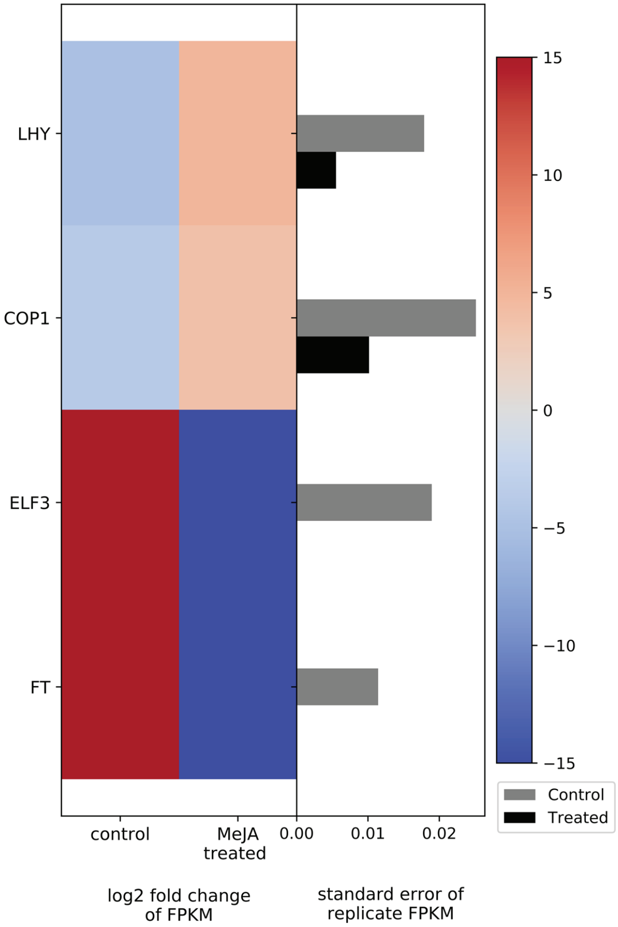


Fig. 7 Heat map of DEGs related to circadian rhythm and flowering time. The left side of the plot is the heatmap of log2 fold change of FPKM between all treated and control groups. The right side of the plot is the standard error for all replicates divided by the average FPKM for the control and treated groups

of other *Vaccinium* species. Several studies have shown the health benefits of anthocyanins, especially with respect to their antioxidant activity [50–52], but there are few studies regarding the ecological importance of this secondary metabolite in the context of plant defense in these species. Although the specific role(s) of anthocyanins in plant defense is not known, the genes up-regulated in response to MeJA treatment include— genes encoding anthocyanidin 3-O-glucosyltransferase and anthocyanidin 3-O-glucoside 5-O-glucosyltransferase, also known as UDP-glucose:flavonoid 3-O-glucosyltransferase (*UF3GT* gene) and UDP-glucose:flavonoid 5-O-glucosyltransferase (*UF5GT* gene) respectively (Fig. 6). In the anthocyanin biosynthetic pathway, these genes encode key enzymes responsible for the glucosylation of anthocyanidins (i.e. pelargonidin, cyanidin, and delphinidin) to produce stable molecules [53–55].

It is well documented that flavonoids are secondary metabolites that protect plants against pathogens and herbivores, and according to the phytochemical co-evolution theory, these are important mediators of plant-insect interactions [56]. For instance, Samac and Graham [57] found a sharp and rapid up-regulation of genes encoding enzymes in the synthesis of flavone and flavonol biosynthesis by analyzing the transcriptome profile of pathogen-infected soybean (*Glycine max*) and

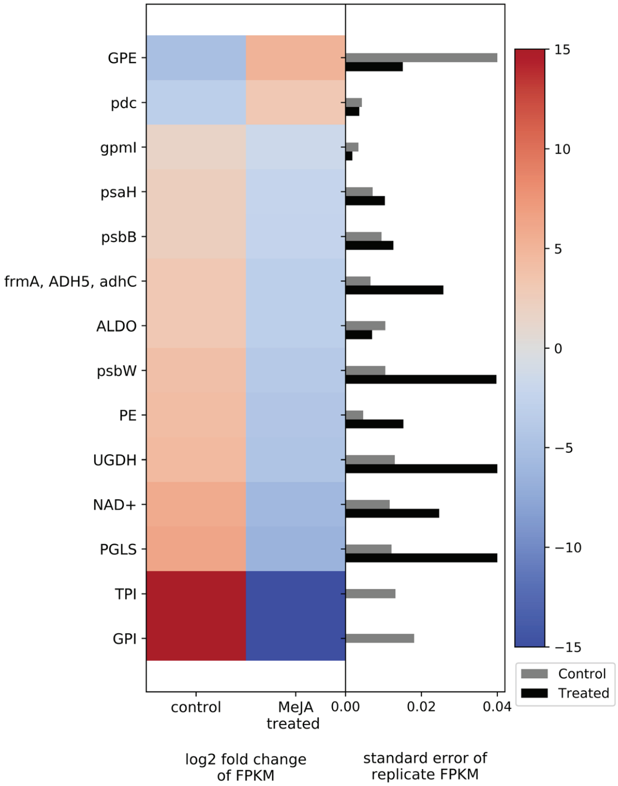


Fig. 8 Heat map of DEGs related to photosynthesis and carbohydrate metabolism. The left side of the plot is the heatmap of log2 fold change of FPKM between all treated and control groups. The right side of the plot is the standard error for all replicates divided by the average FPKM for the control and treated groups

Medicago truncatula. In this study, two key genes in the flavonoid pathway were found to be up-regulated in the treated plants— *F3'5'H* and *F3OGT* (Fig. 6). These genes encode enzymes that catalyze reactions for the synthesis of the flavones and flavonols: quercetins, kaempferols, syringetins, and luteolins.

The phenylpropanoid biosynthesis pathway was also up-regulated in the MeJA induced bilberry plants as indicated by up-regulation of three genes at different steps of the phenylpropanoid pathway—*HCT*, *CAD*, and *POX* (Fig. 6). *HCT* produces a transferase enzyme that catalyzes a reaction of p-Coumaroyl CoA to form intermediate substrates for the formation of important phenylpropanoids including caffeol-CoA. p-Coumaroyl CoA is an important precursor of different classes of secondary metabolites involved in plant defenses such as isoflavonoids, anthocyanins, stilbenes, and phenylpropanoid volatiles [58]. For example, the biosynthesis of chavicol, a phenylpropene VOC acting as defensive compound and floral attractant, was shown to occur via the phenylpropanoid pathway to p-Coumaroyl CoA [59]. The second up-regulated gene, *CAD*, encodes an enzyme which catalyzes the reduction of aldehyde compounds (e.g. p-coumaraldehyde, caffeoyl-aldehyde, sinapaldehyde, etc.) to form alcohol intermediates that are important substrates of lignin biosynthesis [60]. Finally, a peroxidase gene (*POX*) was up-regulated. Peroxidase acts in the very last step of the phenylpropanoid pathway, being responsible for the formation of different molignols such as syringyl lignin, p-hydroxyphenyl lignin, and guaiacyl lignin. Previous work suggested that the constitutive activation of the jasmonic acid (JA) signaling pathway leads to increased lignin deposition [61]. Among several important functions, the deposition of different lignins in the secondary plant cell walls acts as a physical barrier against pathogen attacks [62]. Studying the transcriptional profile of Chinese yew (*Taxus*

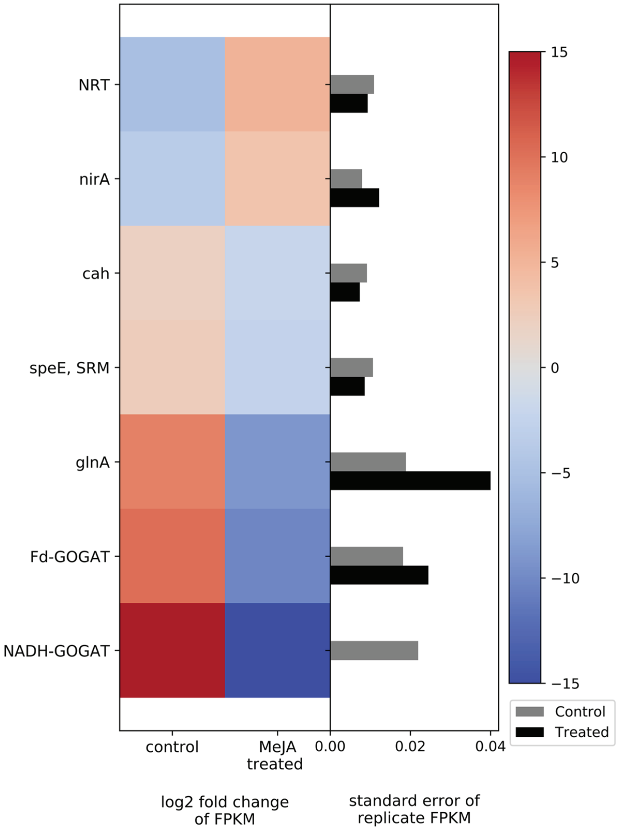


Fig. 9 Heat map of DEGs related to nitrogen metabolism. The left side of the plot is the heatmap of log2 fold change of FPKM between all treated and control groups. The right side of the plot is the standard error for all replicates divided by the average FPKM for the control and treated groups

chinensis) cells, Li et al. [63] also found that MeJA treatment up-regulated the expression of genes encoding key enzymes in the phenylpropanoid metabolic pathway.

We found the tyrosine metabolic pathway was also affected by the MeJA treatment in bilberry plants. Tyrosine is an aromatic amino acid which is considered a central molecule in a diverse array of plant metabolic processes including defenses and secondary metabolite biosynthesis [64]. We found six groups of genes in the pathway significantly up-regulated— *TAT*, *AOC3/AOC2/tynA*, *PPO*, *frmA/ADH5/adhC*, *HPT/HGGT/ubiA*, and *maiA/CSTZ1* (Fig. 6). Previous work showed that *TAT* was induced by MeJA and wounding at both the RNA and protein level in *Arabidopsis thaliana* [65, 66]. The *TAT* gene codes for the first enzyme in this biosynthetic pathway, which catalyzes the reaction from tyrosine to p-hydroxyphenylpyruvate, and is known to function as a radical scavenger, thus protecting plants under biotic and abiotic stress situations [66].

Additionally, MeJA induced genes in the glutathione metabolic pathway. Glutathione is an essential metabolite involved in multiple molecular functions in plants, such as redox turnover, metabolism, and signaling [67]. Glutathione is also involved in reactions linked to plant defense responses against pathogen and herbivore attack [67–69]. The four up-regulated genes in the pathway are responsible for encoding the enzymes glutathione reductase (*GSR*), glutathione peroxidase (*GPX*), glutathione S-transferase (*GST*), and ornithine decarboxylase (*ODC1*) (Fig. 6). The first two—*GSR* and *GPX*—are directly involved in the fundamental function of glutathione in redox signaling through thiol-disulfide interactions. This is a metabolic process wherein reduced glutathione (GSH) is continuously oxidized to a disulfide form

(GSSG) by glutathione peroxidase, which in turn is recycled to GSH by glutathione reductase. Then, GSH is used as a source of reduced S glutathione during the biosynthesis of secondary metabolites used in defense, detoxification, and signaling processes [67]. Previous studies have shown accumulation of glutathione in response to pathogen infection [70, 71] and similar changes have been reported in response to exogenous SA treatment [72–74]. *GST* encodes an enzyme associated with a range of biochemical and physiological functions, such as antioxidative and peroxidase activities [75, 76]. However, its main function in the glutathione metabolic pathway is the catalysis of GSH for several downstream reactions, including biosynthesis of secondary metabolites [67]. It should be noted that in addition to a role in SA response, glutathione also modulates mechanisms of response to pathogens and herbivores through the JA pathway. Xiang and Oliver [77] showed that JA induces the expression of genes encoding GSH and glutathione reductase, while Sasaki-Sekimoto et al. [78] showed the same effect for other genes involved in oxidative stress and antioxidant defense.

The release of volatile terpenes is associated with anti-herbivory and also plays important roles in pollinator and natural enemy (i.e. predators and parasitoids of herbivores) attraction as well as in interactions with the surrounding environment (i.e. plant-plant signaling) [79]. Specifically, it has been suggested that germacrene D itself has deterrent effects against herbivores, as well as repellent activity against aphids and ticks [80–82]. Germacrene D has also been reported as precursor of other sesquiterpenes such as cadinenes and selinenes [83, 84]. In the MeJA-treated bilberry leaves, two genes directly involved in the synthesis of germacrene-type sesquiterpenoids were found to be up-regulated in MeJA induced plants—*GERD* and *HVS* (Fig. 6). Thus, induced bilberry plants also invest in the synthesis of specific VOCs as part of their defense strategies to either interact with its neighboring environment by, for example, recruiting natural enemies, or they could act directly as deterrents and repellents against herbivore attack.

Circadian rhythm and flowering timing

The transition from vegetative growth to reproduction is a remarkable 'timed' developmental switch which can ensure the plant's reproductive success. For timing this event, plants utilize diverse environmental indicators, such as temperature, photoperiod and light intensity, to determine the ideal time of flowering [85]. A group of four important genes involved in the circadian rhythm and flowering time had altered expression in response to MeJA induction. *COP1* and *LHY* genes were significantly up-regulated in the MeJA-treated plants, while *ELF3* and *FT* were down-regulated (Fig. 7).

The *COP1* gene codes for E3 ubiquitin ligase COP1, which is a central regulator of light-dependent physiological processes including photomorphogenesis [86], as well as circadian oscillation and flowering transition [87, 88]. *ELF3* is a clock-associated gene which encodes a protein that acts as a transcriptional modulator, controlling the expression of flowering-time regulator genes [89]. Previous studies have shown that *COP1* represses flowering by promoting degradation of Constans (*CO*), a flowering inducer gene which encodes a protein that activates the *FT* gene [87, 90]. Another study showed that downstream of the flowering pathway, *COP1* mediates *ELF3* ubiquitination and degradation [91]. Moreover, altering *ELF3* expression can cause arrhythmic expression of important morning-specific clock-regulated transcription factors, such as MYB-related LHY [92, 93], encoded by the *LHY* gene, which in our study showed to be up-regulated in response to MeJA treatment. The overexpression of LHY-related transcription factors causes not only arrhythmicity in expression of clock-regulated genes, but also in leaf movement and hypocotyl elongation [94–96]. Some plants have the capacity of altering flowering timing as a strategy to avoid insect herbivory [97, 98]. Since jasmonates are plant hormones with diverse roles in biotic and abiotic stress tolerance, the link between JA and its derivates with circadian pathways and flowering timing have been explored. For instance, JA delayed flowering in *Arabidopsis* [99, 100], and exogenous MeJA application had similar effects on flowering timing in wheat plants [101].

Based on our results, MeJA appears to induce arrhythmicity in bilberry plants via the expression of clock-regulated genes related to flowering by inducing *COP1* and *LHY*, and repressing *ELF3* and *FT*. Since induced plant defenses are energetically costly, the plant's fitness is presumably increased by suppressing genes associated with reproduction and activating those related with defenses in response to MeJA, resulting in switching allocation of resources from growth and reproduction to defense [20–22, 102, 103].

Photosynthesis and carbohydrate metabolism

Previous genomic studies have shown that JA and derivatives induce the expression of genes related to defenses, oxidative stress responses, senescence, and cell wall modification, while repressing the expression of genes involved in photosynthesis and metabolism-related pathways [30, 104, 105]. We found important genes from Photosystem II, *psbB* and *psbW*, which encode a protein complex responsible for light-harvesting and chlorophyll content, being down-regulated in response to MeJA treatment in bilberry leaves. The transcript encoded by *psaH* from Photosystem I was downregulated as well. These results suggest that the inhibitory effect of MeJA on photosynthesis is effectively due to the reduction in the light-harvesting complexes and, consequently, decreases in the carbon fixation process. Down-regulation of chlorophyll-related genes and chlorophyll-protein complexes were also reported in previous studies using genomic and proteomic tools to identify molecular changes in response to JA treatment [97, 104, 106, 107]. In the presence of JA, synthesis of chloroplast proteins involved in photosynthesis is immediately decreased by negative control of translation while the transcript levels remain constant. After 12 to 24 h, the transcript levels also declined, and the corresponding proteins were degraded [108]. In our study, leaf samples were collected the day after the third of three MeJA applications. Taking this into account, our results indicate that MeJA application continues repression of these transcripts for at least 24 h. The influence of JA on the expression of this group of genes leads to typical symptoms of leaf senescence—chlorophyll destruction, protein degradation, and subsequent strong yellowing [109–111].

The regulation of carbohydrate metabolism-related genes was also affected by MeJA treatment in bilberry plants. In the glycolysis and gluconeogenesis pathways, MeJA broadly down-regulated some genes involved in the fructose biosynthesis, pentose phosphate, and the TCA cycle (e.g. *GPI*, *NAD+*, *ALDO*, etc.). Interestingly, transcripts of *pdc* and *GPE*, two genes also involved glycolysis, were up-regulated in response to MeJA. Cheng et al. [112], studying the proteome of *Arabidopsis* in response to MeJA, found similar changes in carbohydrate metabolism. Sánchez-Sampedro et al. [113] reported a reprogramming in carbohydrate metabolism, where sucrose levels decreased and glucose levels increased, in milk thistle (*Silybum marianum*) suspension cultures in response to MeJA. Thus, our results suggest that MeJA induces carbohydrate catabolism, while repressing carbohydrate anabolism. In the context of induced defenses in plants, carbohydrate catabolism has an important role providing basic carbon skeletons for the biosynthesis of some secondary metabolites, such as flavonoids [113]. The results shown here indicate that at the transcript level, MeJA application induces bilberry plants to allocate carbon resources from primary towards secondary metabolism, consistent with previous findings [112, 114, 115].

Nitrogen metabolism

As well as decreases in photosynthesis and chlorophyll content, physiological studies show that remobilization of nitrogen (N) compounds from leaves to roots and shoots is a typical senescence-like response of plants to MeJA treatment [116, 117]. We found up-regulation in the nitrite and nitrate transporter and reduction genes in the MeJA-treated bilberry leaves, which might be a preventive strategy of defense by exporting N resources out of leaves for safeguard and storing them in other tissues away from the foraging herbivores, such as shoots and roots. Gomez et al. [118] found that MeJA treatment accelerated export of N compounds from tomato leaves and described this shuttling as a strategy of defense. While nitrate transport and reduction genes were shown to be induced by MeJA-treatment, most of the genes involved in the metabolism of glutamate were down-regulated in bilberry leaves. Chloroplastic glutamine synthetase and glutamate synthase play crucial roles in amino acid synthesis and nitrogen metabolism via assimilation of ammonium obtained from the nitrate reduction and photorespiration [119]. The majority of assimilated N in plants is invested in photosynthesis, suggesting a strong positive correlation between N assimilation and photosynthetic rate [120–122]. Our observed down-regulation of genes encoding glutamine synthetase and glutamate synthase enzymes in MeJA induced bilberry leaves agrees with the idea of allocation of nitrogen-related resources from growth and development to storage as means of conserving resources while potentially under herbivore attack.

Genetic trade-off

We found complex transcriptional changes in MeJA induced bilberry and provided evidence for allocation of resources from growth, development, and reproduction, to defense related pathways. Transcripts of receptor and response-related genes in plant hormone signal transduction, such as ABA, ET, AUX, and SA, responded to MeJA treatment through cross talk and regulation of genes involved in growth/development (e.g. *ABA* and AUX) and defense (e.g. ET and SA). Genes encoding key enzymes in metabolic pathways involved in biosynthesis of flavonoids (i.e. anthocyanins, flavones/flavonols), lignin compounds (e.g. syringyl, guaiacyl and p-hydroxyphenyl), and deterrent/repellent VOCs (e.g. phenylpropenes, sesquiterpenes) were significantly up-regulated.

Some variation in gene expression among replicates, for both the MeJA and water/ethanol-treated control, was evident (Figs. 5, 6, 7, 8, 9). This is to be expected since the experiment was conducted in the natural environment. The genotypes of the plants likely differ and thus their response to treatment may also differ. In addition, the specific environmental stresses to which the plants are naturally exposed over the 15d treatment period likely differ. Nonetheless, the changes in the transcription regulation associated with MeJA treatment highlighted in this paper are statistically significant and support the concept of genetic trade-off.

Under natural conditions, MeJA induced bilberry plants seem to invest more in the synthesis of quantitative rather than qualitative defense compounds, thus aiming to be equally effective against both specialists and generalist herbivores. Defense-induced bilberry plants synthesize a range of phenolic compounds, from lignin to more complex flavonoids, interfering in their digestion and palatability towards potential herbivores. Although MeJA induced responses effectively activate defense-related pathways, this appeared to be costly to the plant as evidenced by the fact that important genes involved in primary metabolism, such as those associated with photosynthesis, circadian rhythm, carbohydrate and nitrogen metabolism, were down-regulated, presumably to optimize the allocation of resources towards defense. Genes involved in carbohydrate anabolism were repressed, while the ones linked to carbohydrate catabolism were induced, as a possible means to allocate C resources from primary to secondary metabolism. Genes responsible for the remobilization of N sources were up-regulated, while key enzymes playing crucial roles in glutamate metabolism through ammonium assimilation, mostly invested in photosynthesis, were down-regulated. Circadian clock genes were also affected, especially transcription factors involved in flowering time. Previous studies have presented ecological evidence that inducible plant defense responses are energetically costly, where MeJA treatment increased resistance against caterpillar feeding and reduced growth and reproduction of bilberry plants [20–22]. This study provides the first evidence of resource allocation at the transcriptional level in induced bilberry plants under natural conditions.

Conclusions

We investigated the transcriptome of MeJA treated bilberry plants compared to water/ethanol treated controls in their natural environment. Of the 23,187 unigenes annotated, 3590 differentially expressed genes (DEG) were identified, with 2013 up-regulated, and 1577 down-regulated. Further investigation of the annotated unigenes showed a significant reprogramming at the transcriptional level, whereby MeJA induced bilberry plants generally allocated resources from primary metabolism (growth, development and reproduction) to secondary metabolism (defense). The heat-maps presented suggest changes in unifying networks of genes involved in the MeJA response. Analysis of specific genes annotated as transcription factors will help us to better understand the regulation of the underlying mechanisms of the coordinated response to MeJA as a proxy for herbivore attack. This study highlights the occurrence of genetic trade-off at the transcriptional level in a realistic field scenario and supports published field observations wherein plant growth is retarded and defenses are upregulated.

Methods

Plant material and MeJA treatment

The experiment was conducted in a boreal forest located in Kaupanger, Western Norway (61.2^0 N, 007.2^0 E). The study area consisted mainly of Scots pine (*Pinus sylvestris*) with an understory dominated by bilberry and is locally recognized as one of the most important winter ranges for red deer (*Cervus elaphus*) in the inner part of the Sognefjord area. The area selected for the experiment was a 20-year-old clear-cut replanted with pines (currently ca. 150 cm tall), dominated by bilberry and other dwarf shrubs in the understory. The trees were planted ca. 2 m apart and cast relatively little shadow. In June 2016, two groups of 15 bilberry plants each were randomly selected and exposed to two treatments: 10 mM MeJA application (treated) and water/ethanol application (control). To achieve the desired concentration of MeJA, 4.1 M MeJA stock (Bedoukian Research, Danbury, CT) was diluted 1:10 with 95% (*v*/v) ethanol, and re-diluted with water to get a final concentration of 10 mM MeJA. Ethanol (95%) was added to water at the same final concentration as that in the 10 mM MeJA solution for the control. To avoid rapid evaporation of MeJA, a cotton wad was attached to the stem close to the ground and saturated with 10 mM MeJA or with water/ethanol (control). This MeJA concentration (10 mM) was shown in our earlier ecological studies to be effective in reduction of growth and levels of herbivory in bilberry [20, 21]. The applications were repeated three times at one-week intervals (Fig. 10) to simulate attack by herbivores, following the protocol used in several studies on bilberry in the field [20–22]. One day after the last treatment application, leaves from the apical part of all plants were collected and immediately frozen in liquid nitrogen and stored at − 80 °C. Samples were then transferred to RNA*later*-ICE (Life Technologies, Carlsbad, CA) and allowed to thaw at − 20 °C before RNA isolation.

Library construction, sequencing and de novo assembly

The 15 plants in each treatment were randomly separated into five groups of three plants each. The three plants of each group were pooled and considered as one biological replicate (sample) for a total of five biological replicates per treatment for the transcriptomic analysis. Total nucleic acid was extracted from each sample using a modified CTAB procedure. Briefly, 50–70 mg of the stored leaf tissue was suspended in 800 μL cetyltrimethylammonium bromide (CTAB) buffer [123] in a 2 mL centrifuge tube with two 5 mm stainless steel beads. Samples were placed in a TissueLyserII (Qiagen, Germantown, MD) and ground for 1 min at 30 Hz. The suspension was extracted with 700 μL chloroform. After centrifugation at 11,000 *g* for 5 min, the aqueous supernatant was transferred to a new tube and total nucleic acid was precipitated by adding 0.7 volumes of isopropanol and incubating on ice for 10 min, followed by centrifugation at 13,000 *g* for 5 min. Pellets were resuspended

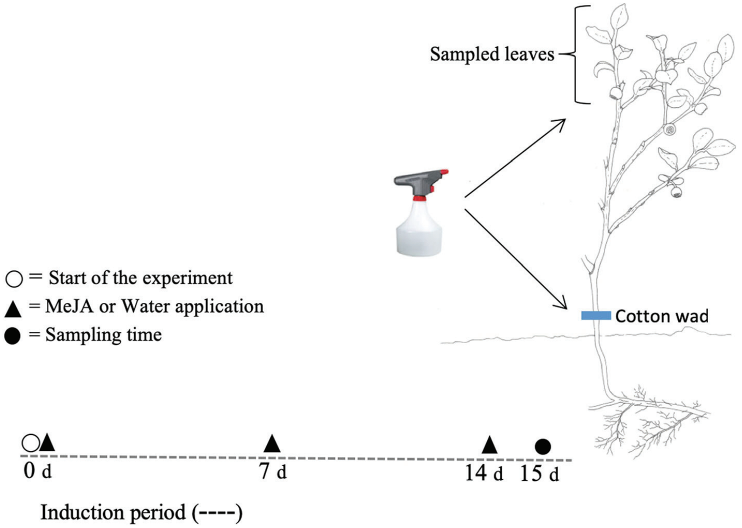


Fig. 10 Experimental design and timeline. Bilberry plants was treated with either Methyl Jasmonate (MeJA) or Water/ethanol at 0, 7, and 14 days (d). Leaf sampling was from the most apical shoots of all plants and was performed one day after the final treatment

in 400 μL of RNAse free water. Lithium chloride (100 μL of 10 M stock) was added to precipitate the total RNA and samples were incubated on ice overnight. Samples were centrifuged at 13,000 *g* for 5 min and the pellets were resuspended in 400 μL RNAse free water. The RNA was reprecipitated using ammonium acetate and ethanol. Pellets were washed with 70% ethanol and resuspended in 50 μL RNAse free water.

Libraries were constructed by Novogene Corporation (Sacramento, CA). Briefly, mRNA was enriched from total RNA using oligo (dT) beads. The mRNA was then randomly fragmented and cDNA was synthesized using random hexamers. After cDNA synthesis and library construction (terminal repair, A-tailing, ligation of sequencing adapters, size selection and PCR enrichment). The libraries were sequenced on the Illumina HiSeq platform (PE150). Raw reads were quality screened to remove adapters and those of poor quality. Clean reads were de novo assembled using Trinity [124]. The assembled transcriptome was annotated using BLAST for NR, NT, SwissProt, and KOG. For NR, NT and SwissProt databases, the evalue threshold was 1e-5 and for KOG the evalue threshold was 1e-3. PFAM, the prediction of protein structure domain: HMMER 3.0 package, hmmscan, the evalue threshold was 0.01; GO: based on the protein annotation results of NR and Pfam: Blast2GO v2.5 [125] and Novogene script, the evalue threshold was 1e-6; KEGG: KAAS, KEGG Automatic Annotation Server, the evalue threshold was 1e-10. Corset [126] was used for categorical clustering of de novo assembled contigs while individual reads were aligned with RSEM [127]. Differentially expressed genes (DEGs) between MeJA-treated and untreated control bilberry plants were identified using DESeq [128].

Abbreviations

ABA: Abscisic acid; AUX: Auxin; BP: Biological process; BR: Brassinosteroid; CG: Cellular component; CTAB: Cetyltrimethylammonium bromide; DEGs: Differentially expressed genes; ET: Ethylene; FPKM: Fragments per kilobase of exon per million fragments mapped; GO: Gene ontology; JA: Jasmonic acid; KEGG: Kyoto Encyclopedia of Genes and Genomes; KOG: Eukaryotic Orthologous Groups; MeJA: Methyl jasmonate; MF: Molecular function; mRNA: Messenger RNA; Nr: Non-redundant protein sequences; ORFs: Open reading frames; PCR: Polymerase chain reaction; SA: Salicylic acid; VOCs: Volatile organic compounds

Acknowledgements

We thank Torbjørn Stokke, Sigurd Daniel Nerhus and Mark Andrew Gillespie for their help with establishment and execution of the field experiment. We also thank Kristia Adams for all the help and assistance during the molecular analyses.

Funding

This research was financially supported by the Norwegian Research Council (project no. 204403/E40) and the Norwegian Environment Agency.

Availability of data and materials

Sequence data generated for this study are publicly available at NCBI SRA under accession number SRP153766.

Authors' contributions

RFB led the writing of the manuscript. All authors contributed to critical reading and editing of the manuscript. RFB, TS, SJH and CRS conceived the initial idea, designed and performed field work. JP led the RNA-seq portion of the project and writing of the manuscript. JK participated in the bioinformatics analysis and interpretation of the data. All authors approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

Author details
[1]Faculty of Engineering and Science, Western Norway University of Applied Sciences, Sogndal, Norway. [2]Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Ås, Norway. [3]Rutgers, Department of Entomology, Philip E. Marucci Center for Blueberry and Cranberry Research, The State University of New Jersey, Chatsworth, NJ, USA. [4]Genetic Improvement of Fruits and Vegetables Lab, Philip E. Marucci Center for Blueberry and Cranberry Research, United States Department of Agriculture-Agricultural Research Service, Chatsworth, NJ, USA.

Received: 25 June 2018 Accepted: 14 January 2019

References

1. Kuusipalo J. Factors affecting the fruiting of bilberries: an analysis of categorical data set. Plant Ecol. 1988;76:71–7.
2. Dahlgren J, Oksanen L, Sjödin M, Olofsson J. Interactions between gray-sided voles (*Clethrionomys rufucanus*) and bilberry (*Vaccinium myrtillus*), their main winter food plant. Oecologia. 2007;152:525–32.
3. Hegland SJ, Jongejans E, Rydgren K. Investigating the interaction between ungulate grazing and resource effects on *Vaccinium myrtillus* populations with integral projection models. Oecologia. 2010;163:695–706.
4. Hjälten J, Danell K, Ericson L. Hare and vole browsing preferences during winter. Acta Theriol. 2004;49:53–62.
5. Jacquemart AL. Floral visitors of *Vaccinium* species in the high Ardennes. Belgium Flora. 1993;188:263–73.
6. Selås V. Autumn population size of capercaillie *Tetrao urogallus* in relation to bilberry *Vaccinium myrtillus* production and weather: an analysis of Norwegian game reports. Wildlife Biol. 2001;7:17–25.
7. Wegge P, Olstad T, Gregersen H, Hjeljord O, Sivkov AV. Capercaillie broods in pristine boreal forest in northwestern Russia: the importance of insects and cover in habitat selection. Can J Zool. 2005;83:1547–55.
8. Welch CA, Keay J, Kendall KC, Robbins CT. Constraints on frugivory by bears. Ecology. 1997;78:1105–19.
9. Canter PH, Ernst E. Anthocyanosides of *Vaccinium myrtillus* (bilberry) for night vision-a systematic review of placebo-controlled trials. Surv Ophthalmol. 2004;49:38–50.
10. Ghosh D, Konishi T. Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. Asia Pac J Clin Nutr. 2007;16:200–8.
11. Jaakola L, Määttä K, Pirttilä AM, Törrönen R, Kärenlampi S, Hohtola A. Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. Plant Physiol. 2002;130:729–39.
12. Macel M, Vrieling K. Pyrrolizidine alkaloids as oviposition stimulants for the cinnabar moth, *Tyria jacobaeae*. J Chem Ecol. 2003;29:1435–46.
13. Nieminen M, Suomi J, Van Nouhuys S, Sauri P, Riekkola ML. Effect of iridoid glycoside content on oviposition host plant choice and parasitism in a specialist herbivore. J Chem Ecol. 2003;29:823–44.
14. Dicke M, Vet LEM. Plant-Carnivore Interactions: Evolutionary and Ecological Consequences for Plant, Herbivore and Carnivore. In: Olff H, Brown VK, Drent RH, editors. Herbivores between Plants and Predators. London: Blackwell Science; 1999. p. 483–520.
15. Paré PW, Tumlinson JH. Plant volatiles as a defense against insect herbivores. Plant Physiol. 1999;121:325–32.
16. Pauwels L, Inzé D, Goossens A. Jasmonate-inducible gene: what does it mean? Trends Plant Sci. 2009;14:87–91.
17. Baldwin IT. Inducible nicotine production in native *Nicotiana* as an example of adaptive phenotypic plasticity. J Chem Ecol. 1999;25:3–30.
18. Van Dam NM, Baldwin IT. Competition mediates costs of jasmonate-induced defences, nitrogen acquisition and transgenerational plasticity in *Nicotiana attenuata*. Funct Ecol. 2001;15:406–15.
19. Yang S, Wu H, Xie J, Rantala MJ. Depressed performance and detoxification enzyme activities of *Helicoverpa armigera* fed with conventional cotton foliage subjected to methyl jasmonate exposure. Entomol Exp Appl. 2013; 147:186–95.
20. Hegland SJ, Seldal T, Lilleeng MS, Rydgren K. Can browsing by deer in winter induce defence responses in bilberry (*Vaccinium myrtillus*)? Ecol Res. 2016;31:441–8.
21. Seldal T, Hegland SJ, Rydgren K, Rodriguez-Saona C, Töpper JP. How to induce defense responses in wild plant populations? Using bilberry (*Vaccinium myrtillus*) as example. Ecol Evol. 2017;7:1762–9.
22. Benevenuto R, Hegland SJ, Töpper JP, Rydgren K, Moe SR, Rodriguez-Saona C, Seldal T. Multiannual effects of induced plant defenses: are defended plants good or bad neighbors? Ecol Evol. 2018;8:8940–50.
23. Mayrose M, Kane NC, Mayrose I, Dlugosch KM, Rieseberg LH. Increased growth in sunflower correlates with reduced defences and altered gene expression in response to biotic and abiotic stress. Molec Ecol. 2011;20:4683–94.
24. Mitra S, Baldwin IT. RuBPCase activase (RCA) mediates growth–defense trade-offs: silencing RCA redirects jasmonic acid (JA) flux from JA-isoleucine to methyl jasmonate (MeJA) to attenuate induced defense responses in *Nicotiana attenuata*. New Phytol. 2014;201:1385–95.
25. Yang F, Zhang Y, Huang Q, Yin G, Pennerman KK, Yu J, Liu Z, Li D, Guo A. Analysis of key genes of jasmonic acid mediated signal pathway for defense against insect damages by comparative transcriptome sequencing. Sci Rep. 2015;5:16500.
26. Kunkel BN, Brooks DM. Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol. 2002;5:325–31.
27. Lackman P, González-Guzmán M, Tilleman S, Carqueijeiro I, Pérez AC, Moses T, Seo M, Kanno Y, Häkkinen ST, Van Montagu MC. Jasmonate signaling involves the abscisic acid receptor PYL4 to regulate metabolic reprogramming in *Arabidopsis* and tobacco. Proc Natl Acad Sci U S A. 2011;108:5891–6.
28. Norman-Setterblad C, Vidal S, Palva ET. Interacting signal pathways control defense gene expression in Arabidopsis in response to cell wall-degrading enzymes from *Erwinia carotovora*. Mol Plant-Microbe Interact. 2000;13:430–8.
29. Penninckx IA, Thomma BP, Buchala A, Métraux J-P, Broekaert WF. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis. Plant Cell. 1998;10:2103–13.
30. Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. Proc Natl Acad Sci U S A. 2000;97:11655–60.
31. Xu Y, Chang P-FL, Liu D, Narasimhan ML, Raghothama KG, Hasegawa PM, Bressan RA. Plant defense genes are synergistically induced by ethylene and methyl jasmonate. Plant Cell. 1994;6:1077–85.
32. Thaler JS, Humphrey PT, Whiteman NK. Evolution of jasmonate and salicylate signal crosstalk. Trends Plant Sci. 2012;17:260–70.
33. Lu J, Ju H, Zhou G, Zhu C, Erb M, Wang X, Wang P, Lou Y. An EAR-motif-containing ERF transcription factor affects herbivore-induced signaling, defense and resistance in rice. Plant J. 2011;68:583–96.
34. Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B. The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature. 2005;433(7021):39.
35. Rahman A, Bannigan A, Sulaman W, Pechter P, Blancaflor EB, Baskin TI. Auxin, actin and growth of the *Arabidopsis thaliana* primary root. Plant J. 2007;50:514–28.
36. Stepanova AN, Robertson-Hoyt J, Yun J, Benavente LM, Xie D-Y, Doležal K, Schlereth A, Jürgens G, Alonso JM. TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. Cell. 2008; 133:177–91.
37. Yan S, Zhang T, Dong S, McLamore ES, Wang N, Shan X, Shen Y, Wan Y. MeJA affects root growth by modulation of transmembrane auxin flux in the transition zone. J Plant Growth Regul. 2016;35:256–65.
38. Niki T, Mitsuhara I, Seo S, Ohtsubo N, Ohashi Y. Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. Plant Cell Physiol. 1998;39:500–7.
39. Vidal S, León IP, Denecke J, Palva ET. Salicylic acid and the plant pathogen Erwinia carotovora induce defense genes via antagonistic pathways. Plant J. 1997;11:115–23.

40. Felton G, Korth K, Bi J, Wesley S, Huhman D, Mathews M, Murphy J, Lamb C, Dixon R. Inverse relationship between systemic resistance of plants to microorganisms and to insect herbivory. Curr Biol. 1999;9:317–20.
41. Feys BJ, Parker JE. Interplay of signaling pathways in plant disease resistance. Trends Genet. 2000;16:449–55.
42. Reymond P, Farmer EE. Jasmonate and salicylate as global signals for defense gene expression. Curr Opin Plant Biol. 1998;1:404–11.
43. Thomma BP, Penninckx IA, Cammue BP, Broekaert WF. The complexity of disease signaling in Arabidopsis. Curr Opin Immunol. 2001;13:63–8.
44. Cheong JJ, Choi YD. Methyl jasmonate as a vital substance in plants. Trends Genet. 2003;19:409–13.
45. De Geyter N, Gholami A, Goormachtig S, Goossens A. Transcriptional machineries in jasmonate-elicited plant secondary metabolism. Trends Plant Sci. 2012;17:349–59.
46. Gundlach H, Müller MJ, Kutchan TM, Zenk MH. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc Natl Acad Sci U S A. 1992;89:2389–93.
47. Sun G, Yang Y, Xie F, Wen J-F, Wu J, Wilson IW, Tang Q, Liu H, Qiu D. Deep sequencing reveals transcriptome re-programming of *Taxus* x *media* cells to the elicitation with methyl jasmonate. PLoS One. 2013;8:e62865.
48. Zhao J, Davis LC, Verpoorte R. Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol Adv. 2005;23:283–333.
49. Lattanzio V, Lattanzio VM, Cardinali A. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Phytochemistry: Advances in research. 2006;661:23–67.
50. Ehlenfeldt MK, Prior RL. Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. J Agric Food Chem. 2001;49:2222–7.
51. Prior R, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, Ehlenfeldt M, Kalt W, Krewer G, et al. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. J Agric Food Chem. 1998;46:2686–93.
52. Smith MAL, Marley KA, Seigler D, Singletary KW, Meline B. Bioactive properties of wild blueberry fruits. J Food Sci. 2000;65:352–6.
53. Li Y, Baldauf S, Lim EK, Bowles DJ. Phylogenetic analysis of the UDP-glycosyltransferase multigene family of *Arabidopsis thaliana*. J Biol Chem. 2001;276:4338–43.
54. Stretch AW. Occurence and control of *Glomerella cingulata* on highbush blueberry. Plant Dis Rep. 1967;51:401–4.
55. Yamazaki M, Yamagishi E, Gong Z, Fukuchi-Mizutani M, Fukui Y, Tanaka Y, Kusumi T, Yamaguchi M, Saito K. Two flavonoid glucosyltransferases from *Petunia hybrida*: molecular cloning, biochemical properties and developmentally regulated expression. Plant Mol Biol. 2002;48:401–11.
56. Ferreyra MLF, Rius SP, Casati P. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Front Plant Sci. 2012;3:222.
57. Samac DA, Graham MA. Recent advances in legume-microbe interactions: recognition, defense response, and symbiosis from a genomic perspective. Plant Physiol. 2007;144:582–7.
58. Hoffmann L, Besseau S, Geoffroy P, Ritzenthaler C, Meyer D, Lapierre C, Pollet B, Legrand M. Silencing of hydroxycinnamoyl-coenzyme a shikimate/quinate hydroxycinnamoyltransferase affects phenylpropanoid biosynthesis. Plant Cell. 2004;16:1446–65.
59. Vassão DG, Gang DR, Koeduka T, Jackson B, Pichersky E, Davin LB, Lewis NG. Chavicol formation in sweet basil (*Ocimum basilicum*): cleavage of an esterified C9 hydroxyl group with NAD (P) H-dependent reduction. Org Biomol Chem. 2006;4:2733–44.
60. Trabucco GM, Matos DA, Lee SJ, Saathoff AJ, Priest HD, Mockler TC, Sarath G, Hazen SP. Functional characterization of cinnamyl alcohol dehydrogenase and caffeic acid *O*-methyltransferase in *Brachypodium distachyon*. BMC Biotechnol. 2013;13:61.
61. Hamann T, Bennett M, Mansfield J, Somerville C. Identification of cell-wall stress as a hexose-dependent and osmosensitive regulator of plant responses. Plant J. 2009;57:1015–26.
62. Sarkanen KV, Ludwig CH. Eds. Lignins. Occurrence, formation, structure, and reactions. New York: Wiley-Interscience; 1971.
63. Li ST, Zhang P, Zhang M, Fu C, Zhao C, Dong Y, Guo A, Yu L. Transcriptional profile of *Taxus chinensis* cells in response to methyl jasmonate. BMC Genomics. 2012;13.
64. Tzin V, Galili G. The biosynthetic pathways for shikimate and aromatic amino acids in *Arabidopsis thaliana*. Arabidopsis Book. 2010;8:e0132.
65. Lopukhina A, Dettenberg M, Weiler EW, Holländer-Czytko H. Cloning and characterization of a coronatine-regulated tyrosine aminotransferase from Arabidopsis. Plant Physiol. 2001;126:1678–87.
66. Sandorf I, Holländer-Czytko H. Jasmonate is involved in the induction of tyrosine aminotransferase and tocopherol biosynthesis in *Arabidopsis thaliana*. Planta. 2002;216:173–9.
67. Noctor G, Mhamdi A, Chaouch S, Han YI, Neukermans J, Marquez-Garcia B, Queval G, Foyer CH. Glutathione in plants: an integrated overview. Plant Cell Env. 2012;35:454–84.
68. Dron M, Clouse SD, Dixon RA, Lawton MA, Lamb CJ. Glutathione and fungal elicitor regulation of a plant defense gene promoter in electroporated protoplasts. Proc Natl Acad Sci U S A. 1988;85:6738–42.
69. Edwards R, Blount JW, Dixon RA. Glutathione and elicitation of the phytoalexin response in legume cell cultures. Planta. 1991;184:403–9.
70. Edwards MD, Helentjaris T, Wright S, Stuber CW. Molecular-marker-facilitated investigations of quantitative trait loci in maize. Theor Appl Genet. 1992;83:765–74.
71. May MJ, Hammond-Kosack KE, Jones JD. Involvement of reactive oxygen species, glutathione metabolism, and lipid peroxidation in the Cf-gene-dependent defense response of tomato cotyledons induced by race-specific elicitors of *Cladosporium fulvum*. Plant Physiol. 1996;110:1367–79.
72. Koornneef A, Leon-Reyes A, Ritsema T, Verhage A, Den Otter FC, Van Loon LC, Pieterse CM. Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. Plant Physiol. 2008;147:1358–68.
73. Mateo A, Funck D, Mühlenbock P, Kular B, Mullineaux PM, Karpinski S. Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. J Exp Bot. 2006;57:1795–807.
74. Mou Z, Fan W, Dong X. Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell. 2003;113:935–44.
75. Dixon DP, Hawkins T, Hussey PJ, Edwards R. Enzyme activities and subcellular localization of members of the Arabidopsis glutathione transferase superfamily. J Exp Bot. 2009;60:1207–18.
76. Wagner U, Edwards R, Dixon DP, Mauch F. Probing the diversity of the Arabidopsis glutathione S-transferase gene family. Plant Molec Biol. 2002;49:515–32.
77. Xiang C, Oliver DJ. Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in Arabidopsis. Plant Cell. 1998;10:1539–50.
78. Sasaki-Sekimoto Y, Taki N, Obayashi T, Aono M, Matsumoto F, Sakurai N, Suzuki H, Hirai MY, Noji M, Saito K, et al. Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerance in Arabidopsis. Plant J. 2005;44:653–68.
79. Nagegowda DA. Plant volatile terpenoid metabolism: biosynthetic genes, transcriptional regulation and subcellular compartmentation. FEBS Lett. 2010;584:2965–73.
80. Birkett MA, Al Abassi S, Kröber T, Chamberlain K, Hooper AM, Guerin PM, Pettersson J, Pickett JA, Slade R, Wadhams LJ. Antiectoparasitic activity of the gum resin, gum haggar, from the east African plant, *Commiphora holtziana*. Phytochemistry. 2008;69:1710–5.
81. Bruce TJ, Wadhams LJ, Woodcock CM. Insect host location: a volatile situation. Trends Plant Sci. 2005;10:269–74.
82. Kiran SR, Devi PS, Reddy KJ. Bioactivity of essential oils and sesquiterpenes of *Chloroxylon swietenia* DC against *Helicoverpa armigera*. Curr Sci. 2007:544–8.
83. Bülow N, König WA. The role of germacrene D as a precursor in sesquiterpene biosynthesis: investigations of acid catalyzed, photochemically and thermally induced rearrangements. Phytochemistry. 2000;55:141–68.
84. Telascrea M, de Araújo CC, Marques MO, Facanali R, de Moraes PL, Cavalheiro AJ. Essential oil from leaves of *Cryptocarya mandioccana* Meisner (Lauraceae): composition and intraspecific chemical variability. Biochem Syst Ecol. 2007;35:222–32.
85. Jang K, Lee HG, Jung S-J, Paek N-C, Seo PJ. The E3 ubiquitin ligase COP1 regulates thermosensory flowering by triggering GI degradation in Arabidopsis. Sci Rep. 2015;5:12071.
86. Deng X-W, Matsui M, Wei N, Wagner D, Chu AM, Feldmann KA, Quail PH. COP1, an Arabidopsis regulatory gene, encodes a protein with both a zinc-binding motif and a Gβ homologous domain. Cell. 1992;71:791–801.
87. Liu L-J, Zhang Y-C, Li Q-H, Sang Y, Mao J, Lian H-L, Wang L, Yang H-Q. COP1-mediated ubiquitination of CONSTANS is implicated in cryptochrome regulation of flowering in Arabidopsis. Plant Cell. 2008;20:292–306.
88. McNellis TW, Torii KU, Deng X-W. Expression of an N-terminal fragment of COP1 confers a dominant-negative effect on light-regulated seedling development in Arabidopsis. Plant Cell. 1996;8:1491–503.

89. Liu XL, Covington MF, Fankhauser C, Chory J, Wagner DR. ELF3 encodes a circadian clock–regulated nuclear protein that functions in an Arabidopsis PHYB signal transduction pathway. Plant Cell. 2001;13:1293–304.
90. An H, Roussot C, Suárez-López P, Corbesier L, Vincent C, Piñeiro M, Hepworth S, Mouradov A, Justin S, Turnbull C. CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. Development. 2004;131:3615–26.
91. Yu J-W, Rubio V, Lee N-Y, Bai S, Lee S-Y, Kim S-S, Liu L, Zhang Y, Irigoyen ML, Sullivan JA. COP1 and ELF3 control circadian function and photoperiodic flowering by regulating GI stability. Mol Cell. 2008;32:617–30.
92. Hicks KA, Albertson TM, Wagner DR. EARLY FLOWERING3 encodes a novel protein that regulates circadian clock function and flowering in Arabidopsis. Plant Cell. 2001;13:1281–92.
93. Kim W-Y, Hicks KA, Somers DE. Independent roles for EARLY FLOWERING 3 and ZEITLUPE in the control of circadian timing, hypocotyl length, and flowering time. Plant Physiol. 2005;139:1557–69.
94. Alabadi D, Yanovsky MJ, Mas P, Harmer SL, Kay SA. Critical role for CCA1 and LHY in maintaining circadian rhythmicity in Arabidopsis. Curr Biol. 2002;12:757–61.
95. Green R, Tobin E. Loss of the circadian clock-associated protein 1 in Arabidopsis results in altered clock-regulated gene expression. Proc Natl Acad Sci U S A. 1999;96:4176–9.
96. Mizoguchi T, Wheatley K, Hanzawa Y, Wright L, Mizoguchi M, Song H-R, Carré IA, Coupland G. LHY and CCA1 are partially redundant genes required to maintain circadian rhythms in Arabidopsis. Dev Cell. 2002;2:629–41.
97. Agrawal AA, Johnson MT, Hastings AP, Maron JL. A field experiment demonstrating plant life-history evolution and its eco-evolutionary feedback to seed predator populations. Am Nat. 2013;181(S1):S35–45.
98. Parachnowitsch AL, Caruso CM. Predispersal seed herbivores, not pollinators, exert selection on floral traits via female fitness. Ecology. 2008;89:1802–10.
99. Chehab EW, Yao C, Henderson Z, Kim S, Braam J. Arabidopsis touch-induced morphogenesis is jasmonate mediated and protects against pests. Curr Biol. 2012;22:701–6.
100. Song S, Qi T, Huang H, Xie D. Regulation of stamen development by coordinated actions of jasmonate, auxin, and gibberellin in Arabidopsis. Mol Plant. 2013;6:1065–73.
101. Diallo AO, Agharbaoui Z, Badawi MA, Ali-Benali MA, Moheb A, Houde M, Sarhan F. Transcriptome analysis of an mvp mutant reveals important changes in global gene expression and a role for methyl jasmonate in vernalization and flowering in wheat. J Exp Bot. 2014;65:2271–86.
102. Karban R, Yang LH, Edwards KF. Volatile communication between plants that affects herbivory: a meta-analysis. Ecol Lett. 2014;17:44–52.
103. Rodriguez-Saona CR, Polashock J, Malo EA. Jasmonate-mediated induced volatiles in the American cranberry *Vaccinium macrocarpon*: from gene expression to organismal interactions. Front Plant Sci. 2013;4:115.
104. Jung C, Lyou SH, Yeu S, Kim MA, Rhee S, Kim M, Lee JS, Do Choi Y, Cheong J-J. Microarray-based screening of jasmonate-responsive genes in *Arabidopsis thaliana*. Plant Cell Rep. 2007;26:1053–63.
105. Sasaki Y, Asamizu E, Shibata D, Nakamura Y, Kaneko T, Awai K, Amagai M, Kuwata C, Tsugane T, Masuda T. Monitoring of methyl jasmonate-responsive genes in Arabidopsis by cDNA macroarray: self-activation of jasmonic acid biosynthesis and crosstalk with other phytohormone signaling pathways. DNA Res. 2001;8:153–61.
106. Chen Q, Sun J, Zhai Q, Zhou W, Qi L, Xu L, Wang B, Chen R, Jiang H, Qi J. The basic helix-loop-helix transcription factor MYC2 directly represses PLETHORA expression during jasmonate-mediated modulation of the root stem cell niche in Arabidopsis. Plant Cell. 2011;23:3335–52.
107. Zhai Q, Li C-B, Zheng W, Wu X, Zhao J, Zhou G, Jiang H, Sun J, Lou Y, Li C. Phytochrome chromophore deficiency leads to overproduction of jasmonic acid and elevated expression of jasmonate-responsive genes in Arabidopsis. Plant Cell Physiol. 2007;48:1061–71.
108. Wierstra I, Kloppstech K. Differential effects of methyl jasmonate on the expression of the early light-inducible proteins and other light-regulated genes in barley. Plant Physiol. 2000;124:833–44.
109. Reinbothe S, Reinbothe C, Parthier B. Methyl jasmonate-regulated translation of nuclear-encoded chloroplast proteins in barley (*Hordeum vulgare* L. cv. salome). J Biol Chem. 1993;268:10606–11.
110. Reinbothe S, Reinbothe C, Parthier B. Methyl jasmonate represses translation initiation of a specific set of mRNAs in barley. Plant J. 1993;4:459–67.
111. Weidhase R, Lehmann J, Kramell H, Sembdner G, Parthier B. Degradation of ribulose-1, 5-bisphosphate carboxylase and chlorophyll in senescing barley leaf segments triggered by jasmonic acid methylester, and counteraction by cytokinin. Physiol Plant. 1987;69:161–6.
112. Cheng Z, Sun L, Qi T, Zhang B, Peng W, Liu Y, Xie D. The bHLH transcription factor MYC3 interacts with the jasmonate ZIM-domain proteins to mediate jasmonate response in Arabidopsis. Mol Plant. 2011;4:279–88.
113. Sánchez-Sampedro A, Kim HK, Choi YH, Verpoorte R, Corchete P. Metabolomic alterations in elicitor treated *Silybum marianum* suspension cultures monitored by nuclear magnetic resonance spectroscopy. J Biotechnol. 2007;130:133–42.
114. Broeckling CD, Huhman DV, Farag MA, Smith JT, May GD, Mendes P, Dixon RA, Sumner LW. Metabolic profiling of *Medicago truncatula* cell cultures reveals the effects of biotic and abiotic elicitors on metabolism. J Exp Bot. 2004;56:323–36.
115. Frenkel M, Külheim C, Jänkänpää HJ, Skogström O, Dall'Osto L, Ågren J, Bassi R, Moritz T, Moen J, Jansson S. Improper excess light energy dissipation in Arabidopsis results in a metabolic reprogramming. BMC Plant Biol. 2009;9:12.
116. Beltrano J, Ronco M, Montaldi E, Carbone A. Senescence of flag leaves and ears of wheat hastened by methyl jasmonate. J Plant Growth Regul. 1998;17:53–7.
117. Rossato L, MacDuff J, Laine P, Le Deunff E, Ourry A. Nitrogen storage and remobilization in *Brassica napus* L. during the growth cycle: effects of methyl jasmonate on nitrate uptake, senescence, growth, and VSP accumulation. J Exp Bot. 2002;53:1131–41.
118. Gómez S, Ferrieri RA, Schueller M, Orians CM. Methyl jasmonate elicits rapid changes in carbon and nitrogen dynamics in tomato. New Phytol. 2010;188:835–44.
119. Bernard SM, Habash DZ. The importance of cytosolic glutamine synthetase in nitrogen assimilation and recycling. New Phytol. 2009;182:608–20.
120. Makino A, Nakano H, Mae T, Shimada T, Yamamoto N. Photosynthesis, plant growth and N allocation in transgenic rice plants with decreased Rubisco under CO2 enrichment. J Exp Bot. 2000;51(suppl_1):383–9.
121. Makino A, Sakuma H, Sudo E, Mae T. Differences between maize and rice in N-use efficiency for photosynthesis and protein allocation. Plant Cell Physiol. 2003;44:952–6.
122. Nunes-Nesi A, Fernie AR, Stitt M. Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. Mol Plant. 2010;3:973–96.
123. Stewart CN, Via LE. A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. BioTechniques. 1993;5:748–50.
124. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnol. 2011;29:644–52.
125. Götz S, García-Gómez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, Robles M, Talón M, Dopazo J, Conesa A. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res. 2008;36:3420–35.
126. Davidson NM, Oshlack A. Corset: enabling differential gene expression analysis for de novo assembled transcriptomes. Genome Biol. 2014;15:410.
127. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics. 2011;12:323.
128. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010;11:R106.

Paper III

Molecular and ecological plant defense responses along an elevational gradient in a boreal ecosystem

Rafael Fonseca Benevenuto[1,2, *], Tarald Seldal[1], James Polashock[4], Stein R. Moe[2], Cesar Rodriguez-Saona[3], Mark A. K. Gillespie[1], and Stein Joar Hegland[1]

[1]Faculty of Engineering and Science, Western Norway University of Applied Sciences, Sogndal, Norway.

[2]Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Ås, Norway.

[3]Rutgers, The State University of New Jersey, Department of Entomology, Philip E. Marucci Center for Blueberry and Cranberry Research, Chatsworth, NJ, USA

[4] Genetic Improvement of Fruits and Vegetables Lab, Philip E. Marucci Center for Blueberry and Cranberry Research, United States Department of Agriculture-Agricultural Research Service, Chatsworth, NJ, USA

[*]Correspondence: rafaelb@hvl.no

ABSTRACT

Some plants have the capacity to adapt to environmental changes such as herbivory through molecular reprogramming, a phenomenon called phenotypic plasticity. However, little is known about how this capacity is modulated by environmental conditions. To investigate this, we used bilberry (*Vaccinium myrtillus* L.) ramets and an experimental treatment to simulate herbivory by inducing plant defenses (application of methyl jasmonate; MeJA), to observe ecological responses and gene-expression along an elevational gradient in a boreal system in Western Norway. The gradient included optimal growing conditions for bilberry in this region (ca. 500 m a.s.l.), and the plant's range limits at high (ca. 900 m a.s.l.) and low (100 m a.s.l.) altitudes. Across all altitudinal sites, MeJA-treated plants allocated more resources to herbivory resistance while reducing growth and reproduction, but this response to simulated herbivory was more pronounced at the lowest altitude. High-altitude plants growing under less herbivory pressure but more resource-limiting conditions, exhibited consistently high expression levels of defense genes in both MeJA-treated and untreated plants at all times, suggesting a constant state of 'alert.' These results suggest that plant defense responses at both molecular and ecological levels are modulated by the combination of climate and herbivory pressure, such that plants under different environmental conditions differentially direct the resources available to specific anti-herbivore strategies. Our findings may be important in understanding the complex impact of future climate changes on plant-herbivore interactions, as this is a major driver of ecosystem functioning and biodiversity.

Keywords: Climate change; constitutive and induced defenses; gene expression; trade-off; plant-herbivore interactions

INTRODUCTION

Over the next century, climate change is projected to considerably alter soil and air temperatures in seasonally snow-covered temperate forest ecosystems, where high-latitude tundra and boreal forests are particularly at risk (IPCC 2018; Serreze et al. 2000). Increases in mean annual air temperature during the growing season will consequently reduce snow depth and alter timing of snow melt in high-latitude ecosystems (Fan et al. 2014; Hayhoe et al. 2007). The combination of these climate change drivers can have direct and indirect implications for natural ecosystem functioning (Post et al. 2009). For instance, if an increase in temperature dramatically weakens the plant immune system, it could lead to the spread of plant diseases and elevated insect herbivory rates (Cheng et al. 2013; Velásquez et al. 2018). Also, less snow in high-latitude ecosystems would result in more mammalian browsing intensity (Danell et al. 1994). Alternatively, plants may adapt to rising temperatures by investing in effective defense strategies (Bidart-Bouzat and Imeh-Nathaniel 2008). The trade-offs between such opposing responses and their impacts on plant communities and ecosystem functioning remain poorly understood. In this study, we attempt to address this issue by combining molecular and ecological work across an elevational gradient, and incorporate a simulated grazing treatment to investigate changes in plant defense responses in the boreal system.

Elevational gradients serve as ‘natural experiments’ that surrogate for inferring global change-driven effects (Garibaldi et al. 2011; Körner 2007; Rasmann et al. 2014a) because they provide excellent natural laboratories to investigate species traits and interactions across gradual changes in biotic and abiotic conditions, but on small spatial scales (Pratt and Mooney 2013; Rasmann et al. 2014a; Schemske et al. 2009). However, conflicting evidence and a lack of study complexity make generalizing results difficult. For example, in a review by Moreira et al. (2018), such research was found to overly simplify the ecological complexity of plant-herbivore interactions along elevational gradients. Our understanding of boreal plant-herbivore interactions is further complicated by variable defense strategies of some species along elevational gradients, and differential responses to disturbance at molecular and ecological levels (Moreira et al. 2018). These challenges can be addressed by studies that combine molecular analysis with ecological studies of induced defenses under natural systems. Such studies can help us to better understand how abiotic conditions affect plant-herbivore interactions by exploring molecular mechanisms that underpin the ecological responses found. For instance, to cope with environmental variability, some plant species have evolved the capacity to sense these changes, quickly reprogram at the molecular level, and adapt through shifts in phenotypic characteristics (phenotypic plasticity) (Li et al. 2008; Rodriguez et al. 2009; Winning et al. 2009; Chen et al. 2006). Adaptive strategies to biotic and abiotic stress are coordinated by cellular and molecular activities aiming to minimize damage and, at the same time, conserve valuable resources for growth and reproduction (Ahuja et al. 2010). Recently, the use of 'omic' approaches has been effective in identifying molecular changes at transcript, protein and metabolite levels that confer resistance or are regulated in response to environmental stressors (Bokhari et al. 2007; Springer et al. 2008; Zeller et al. 2009; Zobayed et al. 2005). Variation in gene expression, and consequently synthesis of its functional products (i.e., proteins and metabolites), is known to play a role in the evolutionary processes of natural populations in response to environmental adaptation (Oleksiak et al. 2002; Schadt et al. 2003). Whether these effects of adaptation to a novel environment, alone or in combination, can affect ecosystem functioning, depends on how these molecular and phenotypical changes influence trophic interactions (Hegland et al. 2009).

Bilberry (*Vaccinium myrtillus* L.) is a long-lived dwarf shrub that provides an excellent model organism for combined molecular and ecological study. The plant is considered as a key food source for herbivores, pollinators, and fruit eating birds and mammals in northern

European boreal forest ecosystems (Hegland et al. 2009; Hjalten et al. 2004; Jacquemart 1993; Selas 2001), and has been found to be sensitive to environmental changes. We have also shown in previous studies that defenses induced by herbivore feeding or treatment with methyl jasmonate (MeJA; a ubiquitous defense hormone in plants released in response to stress) reduce herbivory and increase reproduction of the damaged or treated plants (Seldal et al. 2017; Hegland et al. 2016; Benevenuto et al. 2018). These studies provide evidence of potential trade-offs between growth and defense in induced bilberry plants. Additionally, MeJA-induced bilberry plants were shown to exhibit a multiannual herbivore resistance (Benevenuto et al. 2018). However, is still unclear if these trade-off responses are due to changes in plant defense system, by investing in effective defense strategies (i.e., constitutive or induced), and if these responses are also modulated by abiotic conditions.

Using wild bilberry as a model organism and MeJA application to simulate the effects of herbivory, we investigated the effects of the treatment on plant defense responses, in terms of gene expression and ecological traits, across an elevational gradient and over two consecutive years. The gradient used in this study included optimal growing conditions for bilberry at a “medium” altitude (ca. 400 m a.s.l.), and the plant’s range limits in this region at high (ca. 900 m a.s.l.) and low (ca. 100 m a.s.l.) altitudes. We expected MeJA-treated plants to allocate resources from growth and reproduction to induced defenses at the molecular (up-regulate expression of defense genes while down-regulate photosynthesis/nitrogen metabolism genes) and ecological levels (reduction of growth, reproduction and consequently herbivory). As induced defenses are presumed to be energetically costly (Benevenuto et al. 2018; Karban et al. 2014; Nabity et al. 2013; Rodriguez-Saona et al. 2013; Seldal et al. 2017) and decline with increasing elevation (Moreira et al. 2018; Pellissier et al. 2016), we predicted that this effect would be most pronounced at the lowest altitude (*Prediction I*). In addition, as bilberry is a slow-growing species adapted to limiting abiotic conditions (Flower-Ellis 1971), and the plant defense system is affected by environmental changes (Bidart-Bouzat and Imeh-Nathaniel 2008; DeLucia et al. 2012; Veteli et al. 2002b), we expected plants from the high altitude to be under constant state of 'alert.' We expected this to manifest as an investment in constitutive defenses (Moreira et al. 2014; Pellissier et al. 2016), resulting in consistently reduced seasonal growth and reproduction, and decreased herbivory rates, and higher basal expression levels of defense genes compared to plants at the optimum and low altitude (*Prediction II*).

MATERIAL & METHODS

Study system

The study was conducted within a pine bilberry forest ecosystem in Kaupanger, inner Sognefjord, western Norway (61.2°N, 007.2°E), between May/June and August/September of 2016 and 2017. The area has annual precipitation of 700–900 mm, and a mean summer temperature range of 12–16°C (Moen 1999). The inner part of Sognefjord offers marked topographical variation, representing elevational gradients of 0–2400 meters above sea level (m a.s.l.) over horizontal distances of about 2 km, which makes it a practical and convenient location for gradient-based field experiments. The most abundant vascular plant species in the field area are bilberry, Scots pine (*Pinus sylvestris* L.), birch (*Betula pendula* Roth and *B. pubescens* Ehrh.), lingonberry (*Vaccinium vitis-idaea* L.), and crowberry (*Empetrum nigrum* L.). The area has a dense winter population of red deer (*Cervus elaphus* L.) (pers. obs. S.J. Hegland), which is the most abundant wild ungulate in Norway (Norwegian Mapping Authority, 2017).

Bilberry, our study species, is a long-lived deciduous clonal dwarf shrub, with evergreen stems generally 10–60 cm high (Flower-Ellis 1971; Ritchie 1956). Although we do not have specific information regarding clone size and distribution for the study area, we have based our work on the assumption that rhizomes can reach around 200 cm in length, depending on age, and the proportion of genetic variation within population is high (Albert et al. 2003, 2004; Flower-Ellis 1971). Bilberry is considered a key species in the boreal ecosystem because of its ecological role as food source for many vertebrate and invertebrate herbivores, (Hegland et al. 2010). The main mammalian herbivores feeding on the plant in the study area are red deer and various rodent species (Hegland et al. 2016), whereas the most common insect herbivores are larvae of the moth family Geometridae (Atlegrim 1989). Bumblebees, honeybees, and syrphid flies are the main pollinators of bilberry (Jacquemart 1993; Jacquemart and Thompson 1996).

Study design

A natural elevational gradient ranging from just above sea level to the tree line was used to investigate the effects of variation in abiotic conditions (mainly temperature and timing of snow melt) on plant defense responses at molecular and ecological levels. The experiment was conducted at three different elevational sites: 'Low' elevation = ca. 100 m a.s.l. (submontane zone); 'Medium' elevation = ca. 500 m a.s.l. (mid-montane zone); and 'High' elevation = ca. 900 m a.s.l. (subalpine zone) (Fig. 1). The middle part of the range, at mid-montane zone, is considered as the optimum elevational site for bilberry growing conditions. The low-altitude at submontane zone is considered as suboptimal because of warmer temperatures and heavy grazing, while the high-altitude at subalpine zone mainly because of the cooler average temperatures and exposure. Data on average temperature and relative humidity were obtained from four data loggers (TRIX 8 LogTag, Auckland, New Zealand) placed at each site during the entire study season (June through September). Timing of snow melt was recorded as the Julian day on which each site became snow free (Fig. 1). This gradient covers a relatively large part of climatic distribution of the species, from temperate to alpine areas (Moen 1999). At all elevations, the experiment was established in sites with similar characteristics, such as south-facing slopes and open sites with little shadow aiming to reduce other environmental factors, such as irradiance. Low and Medium sites consisted of more than 15-year-old clear cuts with relatively low abundances of small pines, birches and alders that produced negligible shadow effects. The High site was a naturally open subalpine area just below the tree line. Vegetation structure was similar among the sites, with a field layer and scattered tree layer up to ca. 5 m. Unpublished data from 2016 showed that there was relatively little variation in pH (mean, 4.19) and slope (16.4 degrees) among sites, although soil organic matter (mean, 60.4%) was slightly lower at the low altitude (Knut Rydgren pers. comm. 2016).

In May/June of 2016, 20 blocks were established at each site measuring 150 m^2 (10 m x 15 m). Within each block, two ramets (between 10-25 cm height) at least 10 m apart were randomly selected (N = 120 plants), and each was exposed to one of two different treatments following the same methodology as previous studies (Benevenuto et al. 2018; Seldal et al. 2017): 1) 10 mM MeJA application (treated), and 2) water/ethanol application (control). Several studies have shown that inducible defense responses in plants can be activated by exogenous application of MeJA (Baldwin 1999; Benevenuto et al. 2018; Hegland et al. 2016; Van Dam and Baldwin 2001; Yang et al. 2013). To achieve the desired concentration of MeJA, 4.1 M MeJA stock (Bedoukian Research, Danbury, CT) was diluted 1:10 with 95% (v/v) ethanol and re-diluted with water to get a final concentration of 10 mM MeJA (Seldal et al. 2017). Ethanol was added to water at the same final concentration as that in the 10-mM MeJA solution (41:1) for the control. To avoid rapid evaporation of MeJA, a cotton wad was attached to the stem at the ground and saturated with 10 mM MeJA or water/ethanol (control) until the point of run off. Applications were repeated three times with 1-week intervals to induce the plant defense response associated with an attack by herbivores. The plants were only exposed to treatments in 2016 to evaluate possible multiannual effects on induced plant defenses.

Sampling procedure

For the ecological analyses, the sampling procedure followed a similar methodology as in a previous ecological study (Benevenuto et al. 2018). Before the start of treatments in May/June 2016, sampling time 1 (ST1), we measured the height from the ground to crown with a ruler and stem diameter at the surface using a digital caliper of the all experimental ramets (Fig 2). We also counted the number of annual shoots, flowers, leaves, browsed shoots, and chewed leaves. We recorded the same variables 6 weeks (42 days) later, at sampling time 2 (ST2). At this subsequent recording, we also counted the number of berries. The same sampling procedure was repeated in 2017. Plant height (H), stem diameter (DS), and number of annual shoots (AS) were used to calculate dry mass (DM) of each ramet as a nondestructive estimate of plant size using the formula described by Hegland et al. (2010): $\log_2(DM) = 1.41700 \times \log_2(DS) + 0.97104 \times \log_2(H) + 0.44153 \times \log_2(AS + 1) - 7.52070$.

To analyze gene expression, we collected 10 leaves from the apical part of each treatment in each block at 10:00 the day after the last treatment application, and they were immediately frozen in liquid nitrogen and stored at −80°C to avoid degradation. In June of 2017, 1 year after the last of the three treatment applications, leaf samples were collected in the same manner from all experimental plants. Samples were subsequently transferred to RNAlater-ICE (Life Technologies, Carlsbad, CA) and allowed to thaw at −20°C prior to RNA isolation according to the manufacturer's protocol, as detailed below.

Gene expression analysis

All samples collected in 2016 and 2017 were randomly separated into three groups, each containing a pool of five plants. Total RNA from each group was extracted using the Rneasy plant mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's directions. The total RNA was eluted in 100 µl sterile dH2O and quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Products; Wilmington, DE, USA). The cDNA was synthesized using 100 ng of total RNA per reaction and the Superscript VILO cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

A list of target genes for real-time PCR and the primers used in the analysis are provided in Supplementary Information S1. The selection of these eight genes involved in defense and growth-related pathways was based on our previous RNA-seq study of MeJA-treated wild bilberry plants (Benevenuto et al. 2019). Defense target genes, involved in circadian rhythm, phenylpropanoid, tyrosine, flavonoid, and anthocyanin biosynthesis pathways, are shikimate O-hydroxycinnamoyltransferase (*SHIKIMATE*), tyrosine aminotransferase (*TYR*), leucoanthocyanidin dioxygenase (*LDOX*), UDP-glycosyltransferase (*UDP*), MYB-related transcription factor LHY (*LHY*), and flavonoid 3′,5′-hydroxylase (*FLAV*). Growth-related target genes involved in photosynthesis and nitrogen metabolism are photosystem II PsbW (*PHOTO*) and glutamine synthetase chloroplastic (*GLU*).

Several genes were tested to be used for normalization, based on those commonly used in publications and our own transcriptome data (i.e., those not differentially expressed). These were β-tubulin (*TUB*), metallothionein (*MET*), an F-box gene (*Fbox*), ubiquitin-conjugating enzyme E2 28 (*Ubc28*), and glyceraldehyde-3-phosphate dehydrogenase (*GAP*). The software package NormFinder (C.L. et al. 2004) was used to select the two most stable genes (*FBOX* and *GAP*) across our samples that were used for normalization. Ubc28 was also found to be stable and was used as the interplate calibrator. The gene sequences used for primer design were selected from the RNA-seq de novo assembly of the bilberry transcriptome (Benevenuto et al. 2019), because the complete genome sequence of this species is not available. The primers were designed based on the predicted coding sequence of each target gene by using the online tool PrimerQuest (Integrated DNA Technologies Inc., Skokie, IL, USA). Real-time PCR reactions were set up using the Power SYBR green PCR master mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's directions and run on a QuantStudio 5 Real-Time PCR system (Applied Biosystems). Thermocycling conditions were 50°C for 2 min; 95°C for 10 min; and 40 cycles at 95°C for 15 s, 60°C for 1 min, with melt curve set at 95°C for 15 s, 60°C for 1 min, 95°C for 30 s, and 60°C for 15 s. There were three biological replicates (pool of five plants) of each sample; in addition, three technical replicates were run for each biological replicate. Relative expression levels were calculated, based on the average cycle threshold (Ct) of the technical replicates for each biological replicate, by the ΔΔCt method using the QuantStudio Design & Analysis Software v1.4.3 (Applied Biosystems).

Data analyses

For the ecological responses, the response variables of interest were: proportion of chewed leaves (number or chewed leaves/total number of leaves), proportion of browsed shoots (number of browsed shoots/total number of shoots), changes in biomass (through dry mass calculation), and fruit set (proportion of berries to flowers). For the variables related to herbivory and growth, each of the values from ST1 (beginning of the season; Fig. 2) were subtracted from ST2 (end of the season; Fig. 2) to obtain seasonal changes for each year. For these variables, we used the *lme4* (Bates et al. 2014) and *mixlm* (Liland and Sæbø 2014) libraries in R (Team 2016) to fit generalized linear mixed effect models with Gaussian error distribution and identity link and to perform posterior ANOVA, respectively. For all models, we entered Treatment (MeJA vs. Control), Site (Low, Medium and High), and Year (2016 and 2017), with interaction terms, as fixed effects. Block within each elevational site was fitted as a random effect in the models. To account for the possibility that plant size variation impacts the plant response to treatments, covariates were also included in the models: total number of leaves at ST1 (for insect herbivory model), total number of shoots at ST1 (for mammalian herbivory model), total biomass at ST1 (for growth model), and total number of flowers at ST2 (for model in reproduction). The treatment effects on reproduction were analyzed yearly, based on records from the last census in each year and subsequent calculation of fruit set. A visual inspection of residual plots did not reveal any obvious deviation from homoscedasticity or normality for any models. To determine the presence of an interaction effect, we performed likelihood ratio tests of the full model against the model without the effect and/or interaction in question. Considering the significant interaction terms in all models, we assessed the statistical significance (p-values) of single factors and its respective interactions by using the ANOVA tables for each model (Supplementary Information S2). In order to easily visualize MeJA treatment effects, seasonal effect sizes representing the mean difference between Control and MeJA-treated plants for each response variable in each site and year are presented. When effect size was significant (ANOVA $p < 0.05$) for more than one site, differences and mean separations between elevational sites within each year were analyzed by Tukey *post hoc* comparisons ($p < 0.05$).

We also analyzed the effect of elevational gradient on the mean values of response variables taken at ST2 across the two years to find effects that we could not see in models of seasonal changes, such as herbivory pressure/intensity, plant size, and total number of flowers and berries across the elevational gradient. The exception to this was total flowers, which we calculated as the mean of the total number of flowers over the two sampling sessions. Linear mixed models were fitted for each of these response variables by using Treatment and Site as fixed predictor variables, and Block within each elevational site as random effect. After models were fitted, they were submitted to ANOVA followed by posterior mean separation by Tukey post hoc comparisons ($p < 0.05$).

For gene expression analyses, normalized relative expression values were log transformed to satisfy the assumptions of linear modeling (homogeneity of variance and normality in the residual distributions). Linear models were fitted with the *lme4* package (Bates et al. 2014) in

R (Team 2016) to each target gene. Log-transformed relative expression levels of each gene were used as the response variable, while Treatment (MeJA or Control), Site (Low, Medium, High), and Year (2016, 2017) were the explanatory variables. A factorial ANOVA was conducted with the *mixlm* package (Liland and Sæbø 2014) to determine if there was an interaction effect between the three predictor variables (factors) (Supplementary Information S2). Differences and means within each target gene were analyzed by Tukey *post hoc* comparisons ($p < 0.05$). We presented fold change values as a measure of describing how much the expression of specific gene changes from control to MeJA-treated plants. It is defined as the ratio of MeJA-treated expression value in relation to control expression value. Fold changes are presented in logarithm base 2 (Log2FC) for comparisons of MeJA-treated and control plants in the same site and year. Log2FC is the most widely used alternative transformation of the ratio, because it has the advantage of producing a continuous spectrum of values and representing up- and down-regulated gene expression values in a reader friendly fashion (Quackenbush 2002). A positive Log2FC value indicates that the respective gene was significantly up-regulated for the MeJA-treated plant. A negative Log2FC value means that the respective gene was significantly down-regulated for the MeJA-treated plant.

RESULTS

Ecological responses

Total insect herbivory at the Low site (0.18 ± 0.03 [SE] proportion of chewed leaves) was 60% higher than at the High (0.11 ± 0.01) site but not significantly different from the Medium site (0.15 ± 0.02) (Fig. 3A). Mammalian herbivory was 40% greater at the Low (0.39 ± 0.03 proportion of browsed shoots) and Medium (0.39 ± 0.02) sites than the High site (0.27 ± 0.03) (Fig. 3B). Plant size was ~50% larger in Low (0.74 ± 0.14 dry mass $\log_2$) and Medium

(0.78 ± 0.04) than at the High-altitude site (0.50 ± 0.02) (Fig. 3C). Bilberry plants from the Medium site produced 6 ± 0.4 flowers, which is 40% more than plants from the Low site (4.1 ± 0.3) and twice as much as plants at the High site (3 ± 0.1) (Fig. 3D). Bilberry plants at the Low (1.9 ± 0.5 berries) and Medium (2.4 ± 0.5) sites produced about twice as many berries compared with plants from the High site (0.9 ± 0.2) (Fig. 3E).

Seasonal changes in the proportion of chewed leaves (insect herbivory) (Treat*Site*Year effect: F = 12.09; p = 0.01), proportion of browsed shoots (mammalian herbivory; Treat*Site*Year effect: F = 10.58; p = 0.03), dry mass (growth; Treat*Site*Year effect: F = 14.19; p = 0.006), and fruit set (Treat*Site*Year effect: F = 18.59; p = 0.0009) showed significant interaction effects among all three factors involved (S2). In the year of treatment (2016), MeJA-treated plants had significantly lower proportions of insect and mammalian herbivory than control plants across all elevational sites, although to varying degrees. Bilberry plants from Low and Medium sites had ~80% higher levels of insect herbivory than plants growing at High site (Table 1; Fig. 4A). Mammalian herbivory did not significantly differ between elevational sites (Table 1; Fig. 4B). MeJA-treated plants from Low and High sites grew significantly less than the control in 2016, whereas no significant effect was found for plant growth at the Medium site (Table 1; Fig. 5). Effect size of growth was ~80% greater at the Low site than the High site (Table 1; Fig. 5A). Fruit set in 2016 was reduced by the MeJA treatment in all sites, although the effect size was significantly stronger at the Low site, followed by the Medium and the High site (Table 1; Fig. 5B).

One year after treatment (2017), insect herbivory remained lower in MeJA-treated plants than the control at Low and Medium sites. Effect sizes were not significantly different between sites (Table 1; Fig. 4A). Mammalian herbivory was also significantly lower in 2017 for MeJA-treated plants, but only for those located at the Low site (Table 1; Fig. 4B). Negative effects of MeJA treatment on dry mass were maintained for plants growing at the Low and High sites in 2017; however, in contrast to 2016, MeJA-treated plants from the Medium site also exhibited lower growth rates than the control plants in 2017. Effect sizes of seasonal growth did not differ between sites 1 year after treatment (Table 1; Fig. 5A). MeJA treated plants maintained a significant reduction in fruit set at the Low site in 2017 (Table 1; Fig. 5B).

Gene expression

Considering single-factor effects (without interaction), all defense-related genes tested in our study were up-regulated in response to the MeJA treatment, while photosynthesis- and nitrogen metabolism-related genes were down-regulated (Supplementary Information S3; Treatment effect). The single effect for ‘Site’ occurred because all bilberry plants located at the High site had significantly higher levels of expression for *FLAV*, *LHY*, *SHIKIMATE*, and *LDOX* than plants from the Low site (Supplementary Information S3; Site effect). Regarding ‘Year’ as a factor, all target genes tested were more highly expressed in the year of treatment (2016) than a year later (2017) (Supplementary Information S3; Year effect).

All defense-related target genes showed a significant ($p < 0.05$) or marginally significant ($p < 0.1$) interaction effect between the two or three factors involved in our study, which means that their expression level depends on the interaction between specific treatment, elevational site, and time after induction. *FLAV* (Treat*Site*Year effect: $F = 5.81$; $p = 0.009$), *TYR* (Treat*Site*Year effect: $F = 4.66$; $p = 0.02$), *LHY* (Treat*Year effect: $F = 4.98$; $p = 0.03$), *SHIKIMATE* (Treat*Site*Year effect: $F = 3.11$; $p = 0.06$), and *LDOX* (Treat*Year effect: $F =17.33$; $p= 0.0003$; and Treat*Site effect: $F = 6.43$; $p = 0.005$) were similarly affected: MeJA-treated plants showed higher expression levels than control plants at all sites to varying degrees and mainly for 2016 (Supplementary Information S3; Table 2; Fig. 6A–E). In the same year, the MeJA treatment also induced the up-regulation of *UDP* (Treat*Site*Year effect: $F = 4.33$; $p = 0.02$) in bilberry plants located only at Low and Medium sites (Table 2; Fig. 6F). *GLU*

(Treat*Site*Year effect: F = 3.57; p = 0.04) and *PHOTO* (Treat*Site*Year effect: F = 6.79; p = 0.004) expression levels also depended on the interaction between the three factors; in 2016, MeJA treatment down-regulated the expression of *GLU* only in plants at the Low site (Table 2; Fig. 7A). MeJA-treated plants from Low and Medium sites showed reduced expression levels of *PHOTO* compared with the controls at each site (Table 2; Fig. 7B).

One year after induction (2017), up-regulation of *FLAV*, *TYR*, *SHIKIMATE*, and *LDOX* defense genes was maintained for the MeJA-treated plants in Low and/or Medium sites. In the High site, no significant changes were found for any defense genes tested (Table 2; Fig. 6A–E). *GLU* maintained the same response from 2016, with reduced expression in MeJA-treated plants at the Low site (Table 2; Fig. 7A). MeJA-treated plants at the Medium site had significantly higher levels of *PHOTO* expression than control plants 1 year after treatment (2017) (Table 2; Fig. 7B).

DISCUSSION

The plant defense system in bilberry varied over the elevational gradient. Induced defenses were strongly activated for all plants growing at the optimum (Medium site) and suboptimal lower altitude (Low site), suggesting that these plants have more resources available to invest in defense against herbivore attack, compared with high-altitude plants growing under colder and resource-limiting conditions. The MeJA activation of induced defenses were strongest at the Low site and this was supported by the molecular mechanisms found: low-altitude plants effectively reduced seasonal growth, fruit set, and herbivory rates, while significantly down-regulated growth-related genes and up-regulated defense-related genes in response to MeJA treatment. In summary, these combined responses are highly suggestive of resource allocation from growth and reproduction to induced defenses (trade-off at ecological and molecular levels), especially for plants growing at the warmest low-altitude site (*Prediction I*). Moreover, we showed that these effects persisted for at least one year after treatment. On the other hand, bilberry plants growing at the suboptimal and coldest upper elevation (High site) invested more in constitutive than induced defenses, indicating that they are under constant 'alert.' This was supported by the small effect sizes of changes in growth, fruit set, and herbivory resistance, and the consistently high expression levels of defense-related genes in both MeJA-treated and untreated plants at high-altitude (*Prediction II*).

Herbivory and defense

Ecological responses related to defense showed that, in the year of treatment (2016), plants growing under optimal (Medium site) and suboptimal lower elevation (Low site) had the largest effect sizes (differences between control and MeJA) in seasonal changes of herbivory. Although MeJA treatment also appeared to increase herbivore resistance in bilberry plants from the suboptimal upper elevation (High site) in the year of treatment, this effect was considerably lower when compared to plants from lower altitudes (Low and Medium sites). A year after treatment (2017), significant herbivore resistance effects persisted in MeJA-treated plants from Low and Medium sites, but the same was not found at the highest altitude. This finding corroborates classical expectations that plant defenses decline with increasing elevation (Rasmann et al. 2014a; Rodríguez-Castañeda et al. 2010).

We found molecular responses at gene expression level that could help explain the ecological findings. Expression of important defense-related genes were up-regulated by MeJA (positive log2FC) across all sites in 2016. This was particularly shown in the genes involved in the phenylpropanoid, flavonoid, and anthocyanin biosynthesis pathways (i.e., *FLAV*, *TYR*, SHIKIMATE, and *LDOX*), which are directly involved in the synthesis of secondary metabolites involved in plant defense. For example, bilberry plants are particularly known for a naturally high content of anthocyanins, pigments present in fruits, leaves, and flowers, which, in addition to providing color to plant tissues, are considered an important component in defenses against herbivore attack (Lattanzio et al. 2006). The *SHIKIMATE* gene acts early in the phenylpropanoid biosynthesis pathway, producing a transferase enzyme that catalyzes a reaction of p-Coumaroyl CoA to form important precursors of different classes of secondary metabolites, including anthocyanins (Hoffmann et al. 2004). *FLAV* and *LDOX* act later in the flavonoid biosynthesis pathways, encoding enzymes to catalyze precursors, such as p-Coumaroyl CoA, for the synthesis of flavones and flavonols. These genes also participate in the formation of anthocyanidin, which is used as a substrate by enzymes encoded by *UDP* to produce stable anthocyanin compounds (Li et al. 2001). Besides secondary metabolites, MeJA also induced *LHY,* an important morning-specific transcription factor involved in the circadian rhythm and flowering time, in 2016, especially in low-altitude bilberry plants. Changes in expression of LHY-related transcription factors cause arrhythmicity in the expression of other clock-regulated genes and can also alter leaf movement and flowering (Alabadí et al. 2002; Green and Tobin 1999; Mizoguchi et al. 2002). Previous studies have shown that plants have the capacity to alter flowering time to avoid insect herbivory of flower tissues (Agrawal et al. 2013; Parachnowitsch and Caruso 2008).

Our results indicate that bilberry plants rapidly invest in the synthesis of these metabolites, in response to herbivory (as simulated by MeJA induction), especially at the optimal medium and warmer low-altitude site. Although significant effects of defense induction at gene level were found along all elevational gradients, as shown in the ecological results, plants growing at the low and medium-altitudes showed stronger up-regulation of defense genes (higher log2FC; Table 1) than high-altitude plants. In 2017, up-regulation of most defense-related genes tested persisted in MeJA-treated plants from the low and medium altitudes, while no differences were found in plants located in the highest altitude. Thus, such molecular results together with ecological findings of reduced herbivory at the optimal medium and warmer low-altitude sites can partly be interpreted as allocation of resources in response to environmental pressure.

Growth and reproduction

Ecological results related to growth and reproduction showed that, in the year of treatment (2016) and one year later (2017), bilberry plants growing at the suboptimal lower elevation (Low site) presented the largest effect sizes in biomass and fruit set measurements, compared to plants located at the medium and high-altitudes. As found in measurements related to defense, even though MeJA treatment affected seasonal growth and reproduction in plants growing across all elevational gradient, evidence of resource allocation to defense was less apparent in MeJA-treated plants from Medium and High sites than for plants at the lower altitude.

As for results in defense, our molecular findings of genes related to growth and development support ecological responses in relation to our first prediction. GLU, an important gene associated with nitrogen metabolism, had its expression significantly down-regulated in MeJA-treated plants growing at the low-altitude in the year of treatment (2016), and one year later (2017). GLU plays a crucial role in nitrogen metabolism via assimilation of ammonium obtained from photorespiration (Bernard and Habash 2009). Most of the assimilated nitrogen in plants is invested in photosynthesis; hence, nitrogen assimilation and photosynthetic capacity are strongly correlated (Makino et al. 2000; Makino et al. 2003; Nunes-Nesi et al. 2010). MeJA-treated plants growing at the Low site reduced the expression of GLU, probably to allocate nitrogen-related resources from growth and development to storage in case of herbivore attack. PHOTO, an important gene from photosystem II, was also down-regulated by MeJA in bilberry plants from Low and Medium sites in the year of treatment. However, the same effect did not persist until the next season. PHOTO is directly linked to growth and development, since it encodes a protein complex responsible for light-harvesting and chlorophyll content (Shi et al. 2012). The down-regulation of PHOTO gene, as well as substantial decreases in seasonal growth found in our study, suggests that the inhibitory effect of MeJA on photosynthesis is effective due to the reduction in the light-harvesting complexes, consequently decreasing carbon fixation. Our findings corroborate with a previous transcriptomic study, where PHOTO and other important chlorophyll-related genes from the Photosystem II complex were down-regulated in response to MeJA treatment in wild bilberries (Benevenuto et al. 2019). Such gene expression results underpin molecular mechanisms underlying ecological responses found related to reductions in growth and reproduction of MeJA-treated bilberry plants.

Trade-offs along the elevational gradient

Our findings suggest an effective trade-off between growth, reproduction, and defense in plants under simulated herbivory pressure from warmer low-altitude environments in the year of treatment, suggesting an investment strategy of protecting against future or persistent herbivore attacks. In a previous study exploring the transcriptional profiling of MeJA-induced defense responses in bilberry, we proposed the term ‘genetic trade-off,’ which is the up-regulation of genes involved in important defense-related pathways and the corresponding down-regulation of genes related to growth and nitrogen metabolism pathways (Benevenuto et al. 2019). Trade-offs persisted one year after MeJA induction only at the lowest altitude site. In 2017, down-regulation of *GLU* correlated with the up-regulation of *SHIKIMATE* and *LDOX* defense genes for plants located at the Low site, indicating that genetic trade-offs between defense and growth-related genes can be multiannual in warmer suboptimal conditions. Ecological responses also showed trade-offs between growth/reproduction and herbivory resistance one year after induction in low-altitude bilberry plants of the boreal system. In general, the effects of MeJA disappeared in plants growing at the colder and resource-limiting high altitude one year after treatment.

Previous studies have shown that high temperature can directly affect the synthesis of secondary metabolites involved in defense (Estiarte et al. 1999; Gouinguene and Turlings 2002; Guo et al. 2012; Mosolov and Valueva 2011; Sun et al. 2011; Veteli et al. 2002a). Indeed, when induced and according to environmental conditions, bilberry plants in the boreal system appear to invest in the up-regulation of defense-related pathways (i.e., synthesis of secondary metabolites), while down-regulating growth-related genes, as well as consequently reducing seasonal growth and reproduction to increase herbivory resistance. However, although still significant in low-altitude plants, these effects seem to be relaxed one year after induction. Induced defense responses can persist from hours and days to years, depending on the plant species, previous herbivory pressure, and life history of the plant (Haukioja et al. 1985; Karban and Baldwin 1997). Besides, as abiotic factors can profoundly influence plant development through changes in metabolic rates (O'Connor 2009), and phenotypic plasticity is a developmental phenomenon, spatial or temporal variation in temperature can also affect speed, magnitude, and costs (allocation of resources from growth /reproduction to defense) associated with induced defenses (Trussell and Smith 2000). Our results suggest that bilberry plants are more responsive to induced defenses in the suboptimal lower elevation, where the average temperature is higher and the timing of snow melt is earlier, by allocating resources from growth and reproduction to effective herbivory defense for at least one year after induction. This result corroborates with our previous ecological study showing that the induced defense system in bilberry plants functions in a multiannual manner (Benevenuto et al. 2018).

As predicted, high-altitude bilberry plants invested more in constitutive than induced defenses. Small effect sizes for some of the ecological response variables tested (i.e., insect herbivory and fruit set) indicate that high-altitude plants may invest limited resources to defense at all times. At the molecular level, all bilberry plants (MeJA-treated and control) growing at the high-altitude showed high basal levels of expression for most defense genes tested (i.e., *FLAV*, *LHY*, *SHIKIMATE*, and *LDOX*). Furthermore, we observed that general herbivory pressure was less intense (i.e., lower total proportion of chewed leaves) at the high-altitude than the low-altitude site of the elevational gradient, consistent with recent studies (Pellissier et al. 2012; Rasmann et al. 2014b). This suggests that bilberry plants growing in suboptimal upper elevations are under a constant state of 'alert' (constitutive defenses), possibly because of the resource-limiting and stressful environmental conditions (i.e., low nutrients, cool average temperatures, late timing of snow melt, and consequent short-growing season); as well as lower herbivory pressure in subalpine habitats of the boreal system (Moreira et al. 2018). The combination of these conditions likely affects the type of defense strategy expressed in bilberry plants from a reliance on constitutive defenses instead of inducible defenses. Anti-herbivore defense strategies frequently trade-off along an elevational gradient, within and among plant species (Koricheva et al. 2004). Moreira et al. (2014); Pellissier et al. (2016) found similar ecological responses among pine species, where constitutive and induced levels of defensive chemicals traded off along elevation. Bilberry plants from higher elevations may have developed consistent levels of plant defenses as an adaptation process to severe climatic conditions, which indirectly confer increased resistance to herbivores. Therefore, high-altitude plants may be not adapted, but ecologically fitted to future herbivore pressure due to possible rapid shifts in herbivore range under climate change (Rasmann et al. 2014b).

CONCLUSION

We showed that bilberry defense responses are modulated by the combination of climate and herbivory pressure at both ecological and molecular levels in the boreal system. High-altitude plants invested more in constitutive defenses, whereas low-altitude plants relied strongly on induced defenses. Although herbivory pressure is lower at high altitudes of the boreal system, the cooler average temperatures and limiting resource availability were associated with constantly ‘alert’ bilberry plants, whether treated with MeJA or not. The plants located at low altitudes, where herbivory rates and seasonal temperature are higher, appeared to be more responsive to the simulated herbivore attack by effectively investing resources away from growth and reproduction to induced anti-herbivory defenses. This result suggests that under increasingly warmer conditions and higher herbivory pressure, bilberry plants may respond by altering their defense strategy.

Our results agree with previous studies showing that plant defensive traits are influenced by, and can adapt to, a combination of both biotic (i.e., herbivory pressure and biodiversity) and abiotic (i.e., temperature and resource availability) factors along an elevational gradient in the forest (Moreira et al. 2014; Pearse and Hipp 2012). Under the current scenario of global warming (IPCC 2018; Serreze et al. 2000), how would we expect the defense system of key plant species in the boreal system to change? And what is the possible consequences of it? In the near future, suboptimal upper elevation likely to become the new optimal growing environment for most species in the boreal system. If so, the defense system of low and medium-altitude plants that migrate to higher elevations might encounter novel abiotic (e.g. harsher environment and less resource availability) and biotic (e.g. less diversity and herbivory pressure) conditions to which they need to adapt. On the other hand, rapid shifts of herbivore range to higher elevation due to increasing temperatures may result in limited damage to plants not adapted to such herbivory pressure. These combinations of events in response to climate change can consequently catalyze new ecological and co-evolutionary dynamics through modulating plant–herbivore interactions in the subalpine zones of the boreal system. Plant-herbivore interactions are one of the major drivers of ecosystem functioning and diversity, thus the evolution of plant defense system has been suggested to sculpt such patterns (Ehrlich and Raven 1964). The ecological and molecular work in this study has provided useful insights into the underlying of plant defense strategies along abiotic gradients. These approaches could be used to monitor migration and analyze changes in plant-herbivore relationships, which can help us to better predict possible modifications on the boreal ecosystem functioning during climate change.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

All data generated for this study are publicly available at NCBI SRA under BioProject ID number PRJNA481170.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was financially supported by the Norwegian Research Council (project no. 204403/E40), the Norwegian Environment Agency, and a hatch project No. NJ08140 to CR-S.

Authors' Contributions

RFB led the bioinformatics and statistical analysis, interpretation of the data and writing of the manuscript. All authors contributed to critical reading and editing of the manuscript. RFB, TS, SJH, MAG, SRM and CR-S conceived the initial idea, designed and performed field work. RFB and JP led the RT-qPCR portion of the project. All authors approved the final manuscript.

Acknowledgements

We thank Torbjørn Stokke and Sigurd Daniel Nerhus for their help with establishment and execution of the field experiment. We also thank Kristia Adams for all the help and assistance during the molecular analyses.

TABLES

Table 1. Effect sizes of seasonal changes in herbivory, growth, and reproduction of wild bilberry plants (*Vaccinium myrtillus* L.) across the season in response to MeJA treatment and elevational gradient for two consecutive years.

Changes in proportion of insect chewed leaves (no. chewed leaves/total number of leaves); proportion of deer browsed shoots (no. browsed shoots/total number of shoots), and growth (dry mass) were calculated as difference between value at the end of the season (Aug-Sep) minus value at the beginning of the season (May-June) each year. Reproduction (fruit set) was calculated as the proportion between total number of berries/total number of flowers in each year. Effect sizes represent the mean difference (with standard error) between Control and MeJA seasonal effect for each response variable within each site and year. ‘NS’ indicates no significant difference (ANOVA $p < 0.05$) in effect size for the specific comparison within each site and year. For significant effect sizes, different letters represent mean separation between elevational sites within each year (Tukey $p < 0.05$).

Effect sizes (Control – MeJA)	**2016**			**2017**		
	L	**M**	**H**	**L**	**M**	**H**
Insect chewed leaves (proportion)	0.11 ± 0.02 a	0.11 ± 0.02 a	0.06 ± 0.01 b	0.05 ± 0.01 a	0.05 ± 0.02 a	NS
Deer browsed shoots (proportion)	0.13 ± 0.03 a	0.15 ± 0.04 a	0.16 ± 0.04 a	0.12 ± 0.03	NS	NS
Growth ($\log_2$ g dry mass)	0.37 ± 0.1 a	NS	0.2 ± 0.04 b	0.12 ± 0.05 a	0.2 ± 0.04 a	0.13 ± 0.02 a
Reproduction (fruit set)	0.6 ± 0.06 a	0.4 ± 0.03 b	0.21± 0.05 c	0.5 ± 0.05	NS	NS

Table 2. Differentially expressed genes ($\log_2$ fold change) in wild bilberry plants (*Vaccinium myrtillus* L.) in response to MeJA treatment and elevational gradient for two consecutive years.

A positive value indicates that the respective gene was significantly up-regulated for the MeJA-treated plant. A negative value means that the respective gene was significantly down-regulated for the MeJA-treated plant. ‘ns’ indicates that no significant difference (ANOVA $P < 0.05$) between MeJA-treated and control plant was found for the relative expression of the respective gene on that specific comparison.

	Log_2 fold change of gene expression (log2FC)					
	2016			**2017**		
	Low	**Medium**	**High**	**Low**	**Medium**	**High**
Defense-related genes						
FLAV	2.84	2.00	1.18	NS	0.91	NS
TYR	3.75	1.86	1.5	NS	0.72	NS
LHY	1.49	0.92	0.89	NS	NS	NS
SHIKIMATE	1.28	2.33	1.48	0.94	1.6	NS
LDOX	3.74	4.06	1.87	1.32	2.27	NS
UDP	3.36	2.00	NS	NS	NS	NS
Growth-related genes						
GLU	-2.83	NS	NS	-1.12	NS	NS
PHOTO	-1.51	-1.88	NS	NS	0.85	NS

FIGURES

Figure 1. Average timing of snow melt, temperature and relative humidity across the elevational gradient. Snow free = Julian Day Number (JDN) for complete snow melt; T = air temperature; RH = relative air humidity.

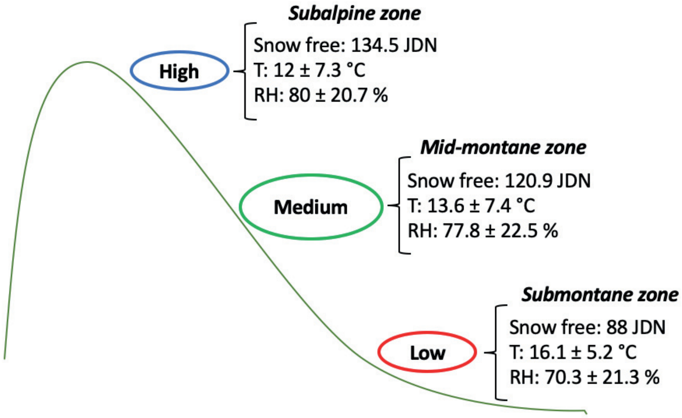


Figure 2. A bilberry ramet including size recordings, and timeline for the induction and response period when the measurements were recorded. ds: days**.**

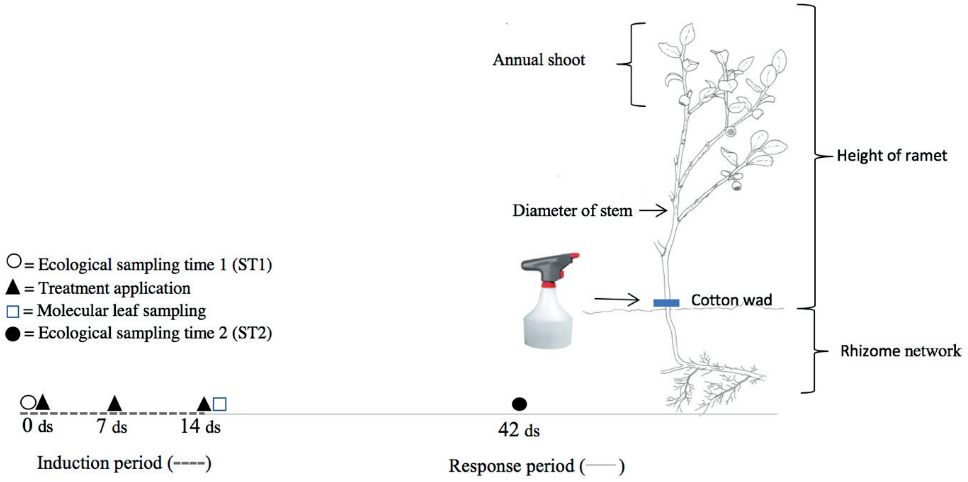


Figure 3. Mean of total herbivory, plant size, number of flowers and berries across an elevation gradient over two years (2016 and 2017). Means of each response variable were calculated from the values collected in the end of the season (Aug-Sep; sampling time 2) in both years of study. Error bars represent standard error of the mean. Different letters represent mean separation between sites (Tukey $p < 0.05$).

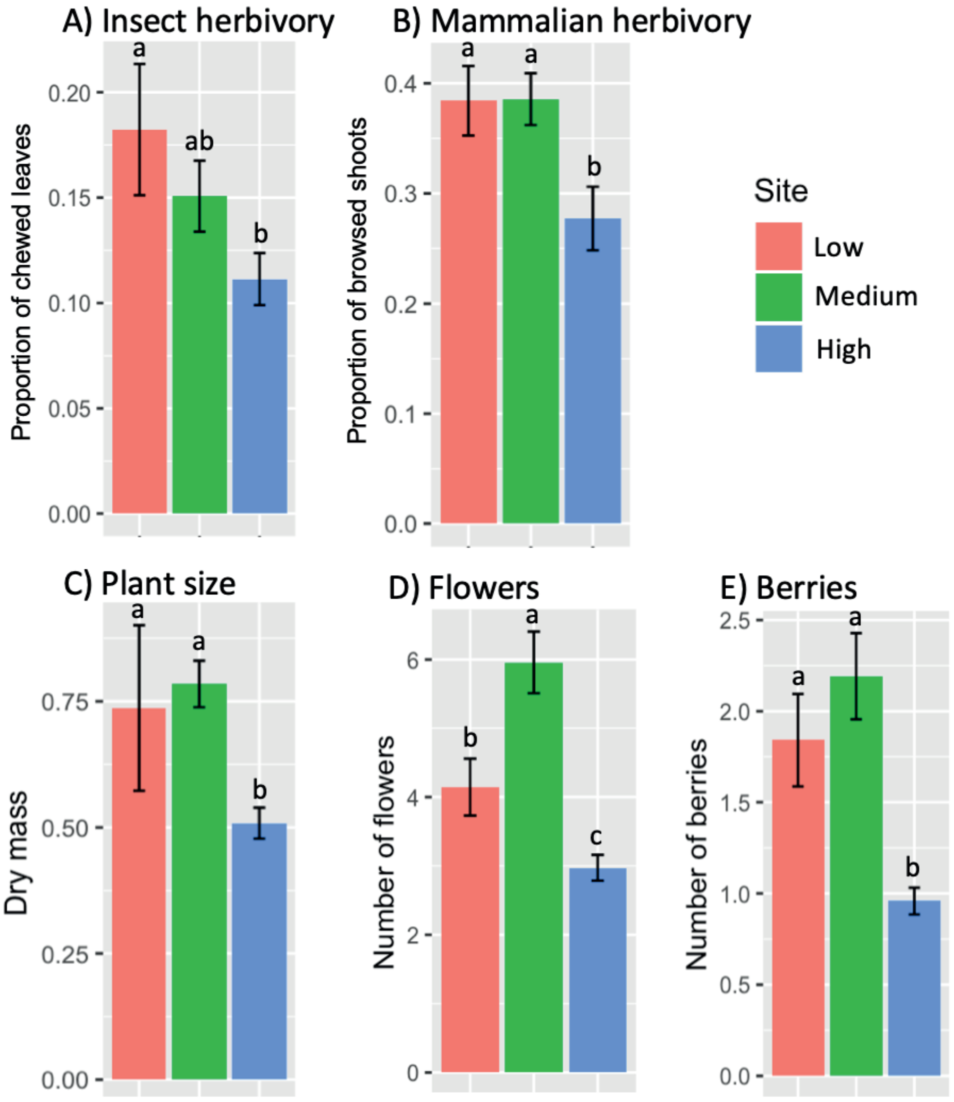


Figure 4. Effect sizes of seasonal changes in insect (A) and mammalian (B) herbivory of wild bilberry plants (*Vaccinium myrtillus* L.) in response to MeJA treatment across a elevational gradient (Low, Medium, High) for two consecutive years (2016 and 2017). Seasonal changes in proportion of insect chewed leaves (no. chewed leaves/total number of leaves) and proportion of deer browsed shoots (no. browsed shoots/total number of shoots), were calculated as difference between value at the end of the season (Aug-Sep; sampling time 2) minus value at the beginning of the season (May-June; sampling time 1) in each year. Effect sizes represent the mean difference between treatments (Control vs. MeJA) within each site and year. NS' indicates that effect size is not significantly different (ANOVA $p < 0.05$). Different letters represent mean separation of significant effect sizes between sites within each year (Tukey $p < 0.05$). Error bars represent standard error of the mean.

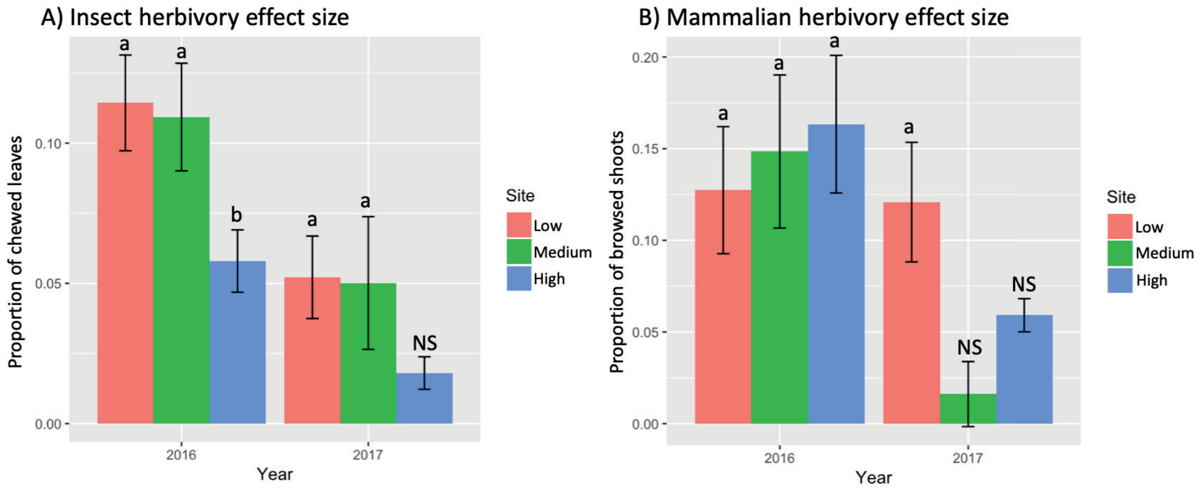


Figure 5. Effect sizes of seasonal changes in growth (A) and reproduction (B) of wild bilberry plants (*Vaccinium myrtillus* L.) in response to MeJA treatment across a elevational gradient (Low, Medium, High) for two consecutive years (2016 and 2017). Seasonal growth in dry mass was calculated as difference between value at the end of the season (Aug-Sep; sampling time 2) minus value at the beginning of the season (May-June; sampling time 1) in each year. Reproduction is the proportion of total number of berries/total number of flowers in each year. Effect sizes represent the mean difference between treatments (Control vs. MeJA) within each site and year. NS' indicates that effect size is not significantly different (ANOVA $p < 0.05$). Different letters represent mean separation of significant effect sizes between sites within each year (Tukey $p < 0.05$). Error bars represent standard error of the mean.

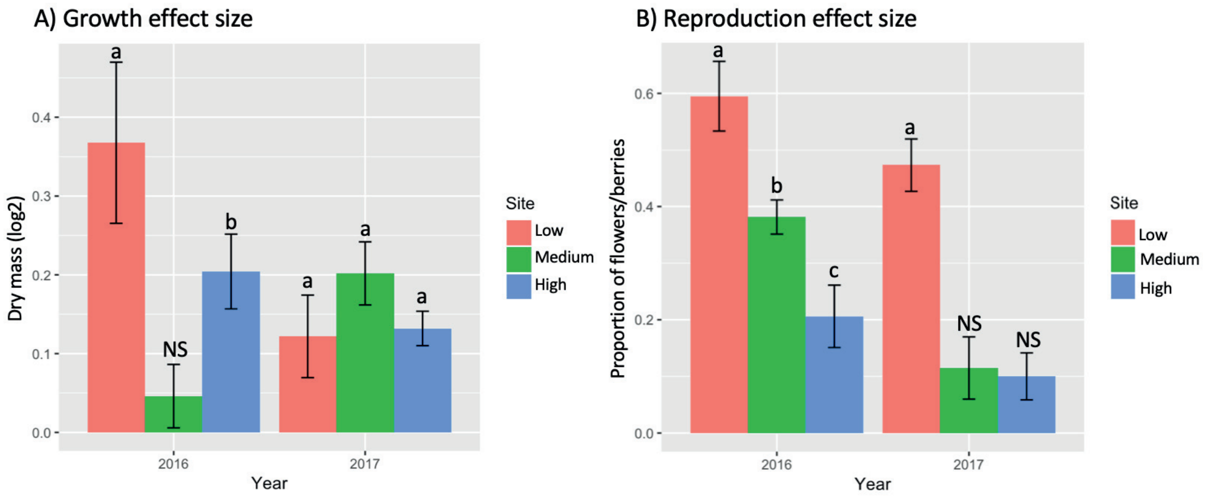


Figure 6. Relative expression levels of six (A-F) defense-related target genes in bilberry plants (*Vaccinium myrtillus* L.) in response to MeJA treatment and elevational gradient (Low, Medium, High) for two consecutive years (2016 and 2017). The log of relative expression shown is the mean for each treatment in each elevational site and in each year; error bars represent confidence interval at 95%. Full names of target genes are presented in Supplementary Information S1.

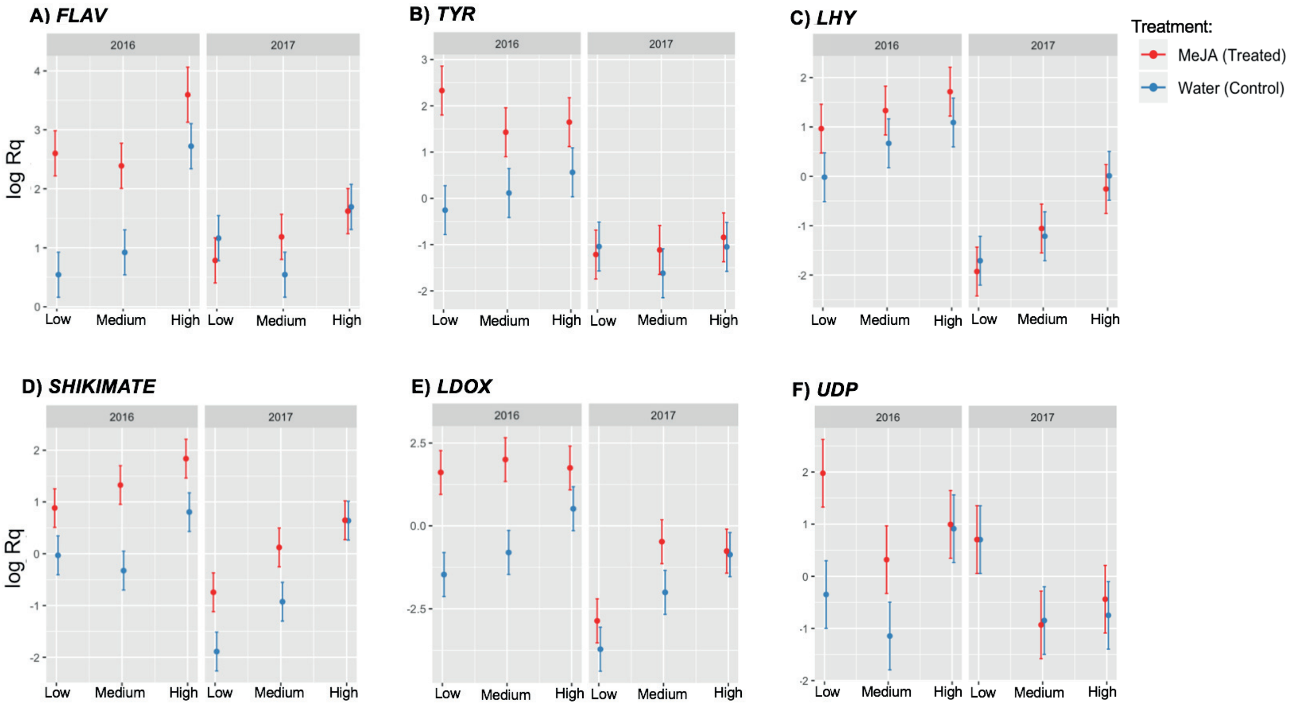
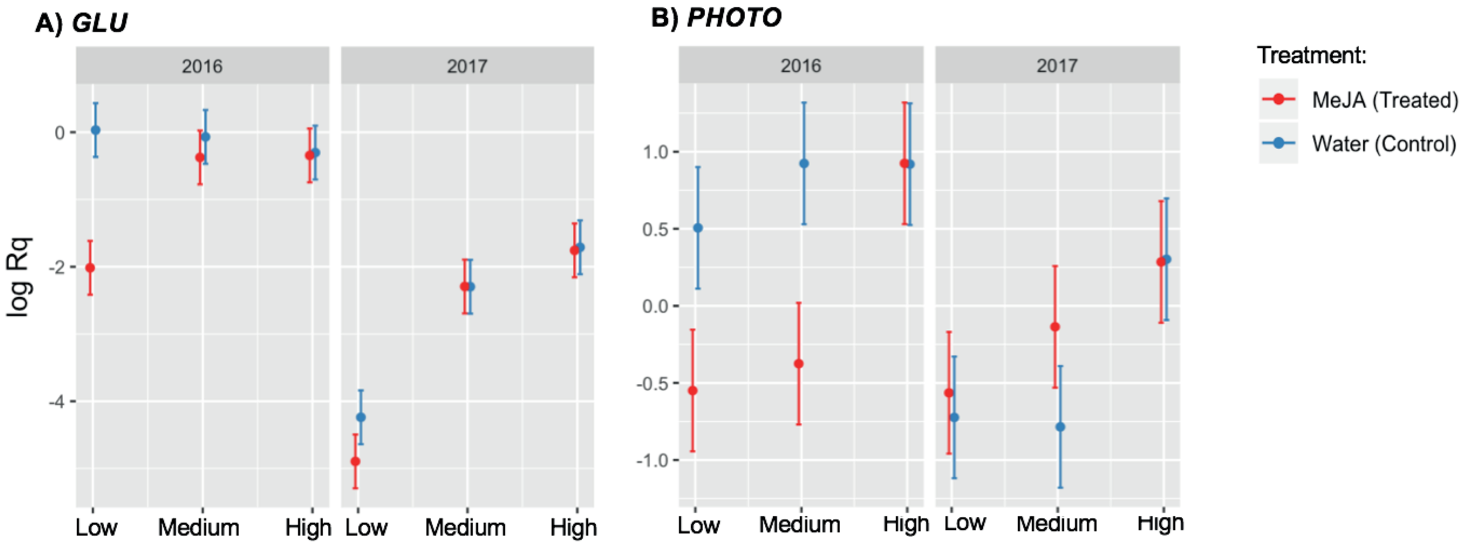


Figure 7. Relative expression level of two (A-B) growth-related target genes in bilberry plants (*Vaccinium myrtillus* L.) in response to MeJA treatment and elevational gradient (Low, Medium, High) for two consecutive years (2016 and 2017). The log of relative expression shown is the mean for each treatment in each elevational site and in each year; error bars represent confidence interval at 95%. Full names of target genes are presented in Supplementary Information S1.



Supplementary Information

S1. Target genes and primer sequences used for real-time qPCR.

Gene (*abbreviation*)	**F Primer**	**R Primer**
Shikimate O-hydroxycinnamoyltransferase (*SHIKIMATE*)	TCTTCACCGGCACATCATTAG	CGTCGTCCATTCGGGATAAA
Tyrosine aminotransferase (*TYR*)	GCCCATTCCGAGAGAAATCA	CCGAACACCATCACAATCAAAG
Leucoanthocyanidin dioxygenase (*LDOX*);	TGTAGAAGAAGGCAAGCGATAC	CGGCGATCAAATTCAGGTACTA
UDP-glycosyltransferase (*UDP*);	CAACCGCAGTTGCCATAAAC	GTGGTGGTGGTGGTGATATT
MYB-related transcription factor LHY (*LHY*);	GTTCGCCAGTCTACCACTAAA	CGAGGAGAGAGATGGAGGATTA
Flavonoid 3',5'-hydroxylase (*FLAV*);	GACCTCGGCTGGACTATTTAAC	CATACGCTTCATCCCACTCTC
Photosystem II PsbW (*PHOTO*);	CAAGGGAGATCCACAGCATATT	CAAGGCAAACCCATGGAAATAC
Glutamine synthetase chloroplastic (*GLU*).	AGCCACCATCCTCTCTCATA	GTGTTGTACTCACTCTCGATCC
Fbox protein (*FBOX*)	AATTCATAATCCCAGCCCCTC	TCCGTCCACAAAAGAGTCATC
Glyceraldehyde 3-phosphate dehydrogenase (*GAP*).	GAAGGATTGGAGAGGTGGAAG	CCACAGTAGGAACACGGAAC
Ubiquitin-conjugating enzyme E2 28 (*UBC28*)	GATGAACCTCACCAAAATACCTG	ACCCCGCTCTCAATCATAAC

S2. ANOVA supplementary table with Chi-square and P-value for seasonal changes in insect and mammalian herbivory, growth (dry mass) and reproduction (fruit set). Significance codes: ‘***’ < 0; ‘**’ < 0.001; ‘*’ < 0.05; ‘°’ < 0.1.

Response variables	***Insect herbivory***		***Mammalian herbivory***		***Growth (dry mass)***		***Reproduction (fruit set)***	
Factors	***Chisq***	***P-value***	***Chisq***	***P-value***	***Chisq***	***P-value***	***Chisq***	***P-value***
Treat	*42.22*	*1.75e-11****	*10.14*	*0.001***	*7.76*	*0.005***	*91.25*	*2.2e-16****
Site	0.51	0.77	7.05	0.03*	12.26	0.002**	9.63	0.008**
Year	*0.25*	*0.61*	*0.01*	*0.93*	*8.16*	*0.004***	*8.63*	*0.003***
Covariates								
Number of leaves	*26.14*	*3.16e-7****	-	-	-	-	-	-
Number of shoots	-	-	8.43	0.003**	-	-	-	-
Dry mass	-	-	-	-	13.81	0.0002***	-	-
Flowers	-	-	-	-	-	-	1.31	0.25
Interactions								
Treat:Site	7.14	0.02*	0.16	0.92	2.51	0.28	30.85	1.99e-7***
Treat:Year	8.53	0.003**	0.10	0.75	0.01	0.93	11.09	0.0008***
Treat:Site:Year	12.04	0.01**	9.21	0.05*	13.54	0.008**	16.21	0.002***

S3. ANOVA supplementary table with F and P-values for gene expression levels of defense and growth-related target genes. Significance codes: ‘***’ < 0; ‘**’ < 0.001; ‘*’ < 0.05; ‘°’ < 0.1.

Genes	***FLAV***		***TYR***		***LHY***		***SHIKIMATE***		***LDOX***		***UDP***		***GLU***		***PHOTO***	
Factors	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**
Treatment	49.33	3.7e-7***	38.79	1.9e-6***	3.76	0.06°	85.49	2.2e-9***	74.65	7.8e-9***	14.2	0.0009***	21.26	0.0001***	5.54	0.02*
Site	47.99	6.2e-9***	2.25	0.12	20.68	6e-6***	63.99	2.4e-10***	32.5	1.4e-7***	20.4	6.7e-6***	96.5	3.3e-12***	26.17	9.3e-7***
Year	73.3	1.3e-8***	205.5	2.8e-13***	141.6	1.5e-11***	112.25	1.6e-10***	165.6	2.9e-12***	15.4	0.0006***	442.68	2.2e-16***	35.98	3.4e-6***
Interactions																
Treat:Site	2.78	0.08°	1.2	0.31	0.19	0.82	5.33	0.01*	6.43	0.005**	2.37	0.11	14.05	9.1e-5***	1.42	0.26
Treat:Year	42.67	1.15e-6***	25.16	3.9e-5***	6.75	0.01*	4.98	0.03*	17.33	0.0003***	11.23	0.002**	6.4	0.01*	22.5	7.9e-5***
Site:Year	6.57	0.005**	0.05	0.94	1.97	0.16	9.55	0.0008***	7.02	0.004**	5.66	0.009**	32.84	1.3e-7***	0.11	0.89
Treat:Site:Year	5.81	0.009**	4.66	0.02*	0.36	0.69	3.11	0.06°	0.86	0.43	4.33	0.02*	3.57	0.04*	6.79	0.004**

REFERENCES

Agrawal AA, Johnson MT, Hastings AP, Maron JL (2013) A field experiment demonstrating plant life-history evolution and its eco-evolutionary feedback to seed predator populations. Am Nat 181 Suppl 1 (S1):S35-45. doi:10.1086/666727

Ahuja I, de Vos RC, Bones AM, Hall RD (2010) Plant molecular stress responses face climate change. Trends Plant Sci 15 (12):664-674. doi:10.1016/j.tplants.2010.08.002

Alabadí D, Yanovsky MJ, Más P, Harmer SL, Kay SA (2002) Critical role for CCA1 and LHY in maintaining circadian rhythmicity in Arabidopsis. Current Biology 12 (9):757-761

Albert T, Raspe O, Jacquemart AL (2003) Clonal structure in Vaccinium myrtillus L. revealed by RAPD and AFLP markers. Int J Plant Sci 164 (4):649-655. doi:Doi 10.1086/375373

Albert T, Raspe O, Jacquemart AL (2004) Clonal diversity and genetic structure in Vaccinium myrtillus populations from different habitats. Belgian Journal of Botany 137 (2):155-162

Atlegrim O (1989) Exclusion of birds from bilberry stands: impact on insect larval density and damage to the bilberry. Oecologia 79 (1):136-139. doi:10.1007/BF00378251

Baldwin IT (1999) Inducible nicotine production in native Nicotiana as an example of adaptive phenotypic plasticity. Journal of Chemical Ecology 25 (1):3-30. doi:Doi 10.1023/A:1020880931488

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R package version 1 (7):1-23

Beier C, Beierkuhnlein C, Wohlgemuth T, Penuelas J, Emmett B, Körner C, de Boeck H, Christensen JH, Leuzinger S, Janssens IA (2012) Precipitation manipulation experiments–challenges and recommendations for the future. Ecology letters 15 (8):899-911

Benevenuto RF, Hegland SJ, Topper JP, Rydgren K, Moe SR, Rodriguez-Saona C, Seldal T (2018) Multiannual effects of induced plant defenses: Are defended plants good or bad neighbors? Ecol Evol 8 (17):8940-8950. doi:10.1002/ece3.4365

Benevenuto RF, Seldal T, Hegland SJ, Rodriguez-Saona C, Kawash J, Polashock J (2019) Transcriptional profiling of methyl jasmonate-induced defense responses in bilberry (Vaccinium myrtillus L.). BMC Plant Biol 19 (1):70. doi:10.1186/s12870-019-1650-0

Bernard SM, Habash DZ (2009) The importance of cytosolic glutamine synthetase in nitrogen assimilation and recycling. New Phytol 182 (3):608-620. doi:10.1111/j.1469-8137.2009.02823.x

Bidart-Bouzat MG, Imeh-Nathaniel A (2008) Global change effects on plant chemical defenses against insect herbivores. J Integr Plant Biol 50 (11):1339-1354. doi:10.1111/j.1744-7909.2008.00751.x

Bokhari SA, Wan XY, Yang YW, Zhou L, Tang WL, Liu JY (2007) Proteomic response of rice seedling leaves to elevated CO2 levels. J Proteome Res 6 (12):4624-4633. doi:10.1021/pr070524z

C.L. A, J. L-J, T. Ø (2004) Normalization of real-time quantitative RT-PCR data: a model based variance estimation approach to identify genes suited for normalization - applied to bladder- and colon-cancer data-sets. Cancer Res 64:5245-5250

Chen J, Burke JJ, Velten J, Xin Z (2006) FtsH11 protease plays a critical role in Arabidopsis thermotolerance. Plant J 48 (1):73-84. doi:10.1111/j.1365-313X.2006.02855.x

Cheng C, Gao X, Feng B, Sheen J, Shan L, He P (2013) Plant immune response to pathogens differs with changing temperatures. Nat Commun 4:2530. doi:10.1038/ncomms3530

Danell K, Bergstrom R, Iedenius L (1994) Effects of Large Mammalian Browsers on Architecture, Biomass, and Nutrients of Woody-Plants. Journal of Mammalogy 75 (4):833-844. doi:Doi 10.2307/1382465

DeLucia EH, Nabity PD, Zavala JA, Berenbaum MR (2012) Climate change: resetting plant-insect interactions. Plant Physiol 160 (4):1677-1685. doi:10.1104/pp.112.204750

Ehrlich PR, Raven PH (1964) Butterflies and Plants - a Study in Coevolution. Evolution 18 (4):586-608. doi:Doi 10.2307/2406212

Estiarte M, Penuelas J, Kimball BA, Hendrix DL, Pinter Jr PJ, Wall GW, LaMorte RL, Hunsaker DJ (1999) Free-air CO2 enrichment of wheat: leaf flavonoid concentration throughout the growth cycle. Physiologia Plantarum 105 (3):423-433

Fan FX, Bradley RS, Rawlins MA (2014) Climate change in the northeastern US: regional climate model validation and climate change projections. Climate Dynamics 43 (1-2):145-161. doi:10.1007/s00382-014-2198-1

Flower-Ellis J (1971) Age structure and dynamics in stands of bilberry (Vaccinium myrtillus L.).

Garibaldi LA, Kitzberger T, Chaneton EJ (2011) Environmental and genetic control of insect abundance and herbivory along a forest elevational gradient. Oecologia 167 (1):117-129. doi:10.1007/s00442-011-1978-0

Gouinguene SP, Turlings TCJ (2002) The effects of abiotic factors on induced volatile emissions in corn plants. Plant Physiology 129 (3):1296-1307. doi:10.1104/pp.001941

Green RM, Tobin EM (1999) Loss of the circadian clock-associated protein 1 in Arabidopsis results in altered clock-regulated gene expression. Proc Natl Acad Sci U S A 96 (7):4176-4179

Guo H, Sun Y, Ren Q, Zhu-Salzman K, Kang L, Wang C, Li C, Ge F (2012) Elevated CO2 reduces the resistance and tolerance of tomato plants to Helicoverpa armigera by suppressing the JA signaling pathway. PLoS One 7 (7):e41426. doi:10.1371/journal.pone.0041426

Haukioja E, Suomela J, Neuvonen S (1985) Long-term inducible resistance in birch foliage: triggering cues and efficacy on a defoliator. Oecologia 65 (3):363-369. doi:10.1007/BF00378910

Hayhoe K, Wake CP, Huntington TG, Luo LF, Schwartz MD, Sheffield J, Wood E, Anderson B, Bradbury J, DeGaetano A, Troy TJ, Wolfe D (2007) Past and future changes in climate and hydrological indicators in the US Northeast. Climate Dynamics 28 (4):381-407. doi:10.1007/s00382-006-0187-8

Hegland SJ, Jongejans E, Rydgren K (2010) Investigating the interaction between ungulate grazing and resource effects on Vaccinium myrtillus populations with integral projection models. Oecologia 163 (3):695-706. doi:10.1007/s00442-010-1616-2

Hegland SJ, Nielsen A, Lázaro A, Bjerknes AL, Totland Ø (2009) How does climate warming affect plant-pollinator interactions? Ecology letters 12 (2):184-195

Hegland SJ, Seldal T, Lilleeng MS, Rydgren K (2016) Can browsing by deer in winter induce defence responses in bilberry (Vaccinium myrtillus)? Ecological Research 31 (3):441-448. doi:10.1007/s11284-016-1351-1

Hjalten J, Danell K, Ericson L (2004) Hare and vole browsing preferences during winter. Acta Theriologica 49 (1):53-62. doi:Doi 10.1007/Bf03192508

Hoffmann L, Besseau S, Geoffroy P, Ritzenthaler C, Meyer D, Lapierre C, Pollet B, Legrand M (2004) Silencing of hydroxycinnamoyl-coenzyme A shikimate/quinate hydroxycinnamoyltransferase affects phenylpropanoid biosynthesis. Plant Cell 16 (6):1446-1465. doi:10.1105/tpc.020297

IPCC (2018) IPCC report 2018: Global warming of 1.5 °C: an IPCC special report on the impacts of global warming of 1.5 °C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. IPCC report.

Jacquemart AL (1993) Floral Visitors of Vaccinium Species in the High Ardennes, Belgium. Flora 188 (3):263-273

Jacquemart AL, Thompson JD (1996) Floral and pollination biology of three sympatric Vaccinium (Ericaceae) species in the Upper Ardennes, Belgium. Canadian Journal of Botany-Revue Canadienne De Botanique 74 (2):210-221. doi:DOI 10.1139/b96-025

Karban R, Baldwin I (1997) Induced Responses to Herbivory University of Chicago Press Chicago. IL Google Scholar

Karban R, Yang LH, Edwards KF (2014) Volatile communication between plants that affects herbivory: A meta-analysis. Ecology letters 17 (1):44-52

Koricheva J, Nykänen H, Gianoli E (2004) Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? The American Naturalist 163 (4):E64-E75

Körner C (2007) The use of 'altitude'in ecological research. Trends in ecology & evolution 22 (11):569-574

Lattanzio V, Lattanzio VM, Cardinali A (2006) Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Phytochemistry: Advances in research 661:23-67

Li P, Ainsworth EA, Leakey AD, Ulanov A, Lozovaya V, Ort DR, Bohnert HJ (2008) Arabidopsis transcript and metabolite profiles: ecotype-specific responses to open-air elevated [CO2]. Plant Cell Environ 31 (11):1673-1687. doi:10.1111/j.1365-3040.2008.01874.x

Li Y, Baldauf S, Lim EK, Bowles DJ (2001) Phylogenetic analysis of the UDP-glycosyltransferase multigene family of Arabidopsis thaliana. J Biol Chem 276 (6):4338-4343. doi:10.1074/jbc.M007447200

Liland KH, Sæbø S (2014) mixlm: Mixed Model ANOVA and Statistics for Education. R package version 1 (7)

Makino A, Nakano H, Mae T, Shimada T, Yamamoto N (2000) Photosynthesis, plant growth and N allocation in transgenic rice plants with decreased Rubisco under CO2 enrichment. J Exp Bot 51 Spec No (suppl_1):383-389

Makino A, Sakuma H, Sudo E, Mae T (2003) Differences between maize and rice in N-use efficiency for photosynthesis and protein allocation. Plant and Cell Physiology 44 (9):952-956. doi:DOI 10.1093/pcp/pcg113

Mizoguchi T, Wheatley K, Hanzawa Y, Wright L, Mizoguchi M, Song HR, Carre IA, Coupland G (2002) LHY and CCA1 are partially redundant genes required to maintain circadian rhythms in Arabidopsis. Dev Cell 2 (5):629-641

Moen A (1999) National atlas of Norway: vegetation.

Moreira X, Mooney KA, Rasmann S, Petry WK, Carrillo-Gavilán A, Zas R, Sampedro L (2014) Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. Ecology Letters 17 (5):537-546

Moreira X, Petry WK, Mooney KA, Rasmann S, Abdala-Roberts L (2018) Elevational gradients in plant defences and insect herbivory: recent advances in the field and prospects for future research. Ecography 41 (9):1485-1496. doi:10.1111/ecog.03184

Mosolov VV, Valueva TA (2011) [Inhibitors of proteolytic enzymes under abiotic stresses in plants (review)]. Prikl Biokhim Mikrobiol 47 (5):501-507

Nabity PD, Zavala JA, DeLucia EH (2013) Herbivore induction of jasmonic acid and chemical defences reduce photosynthesis in Nicotiana attenuata. J Exp Bot 64 (2):685-694. doi:10.1093/jxb/ers364

Nunes-Nesi A, Fernie AR, Stitt M (2010) Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. Mol Plant 3 (6):973-996. doi:10.1093/mp/ssq049

O'Connor MI (2009) Warming strengthens an herbivore–plant interaction. Ecology 90 (2):388-398

Oleksiak MF, Churchill GA, Crawford DL (2002) Variation in gene expression within and among natural populations. Nature Genetics 32 (2):261-266. doi:10.1038/ng983

Parachnowitsch AL, Caruso CM (2008) Predispersal seed herbivores, not pollinators, exert selection on floral traits via female fitness. Ecology 89 (7):1802-1810

Pearse IS, Hipp AL (2012) Global patterns of leaf defenses in oak species. Evolution 66 (7):2272-2286. doi:10.1111/j.1558-5646.2012.01591.x

Pellissier L, Fiedler K, Ndribe C, Dubuis A, Pradervand JN, Guisan A, Rasmann S (2012) Shifts in species richness, herbivore specialization, and plant resistance along elevation gradients. Ecol Evol 2 (8):1818-1825. doi:10.1002/ece3.296

Pellissier L, Moreira X, Danner H, Serrano M, Salamin N, van Dam NM, Rasmann S (2016) The simultaneous inducibility of phytochemicals related to plant direct and indirect defences against herbivores is stronger at low elevation. Journal of Ecology 104 (4):1116-1125. doi:10.1111/1365-2745.12580

Post E, Forchhammer MC, Bret-Harte MS, Callaghan TV, Christensen TR, Elberling B, Fox AD, Gilg O, Hik DS, Hoye TT, Ims RA, Jeppesen E, Klein DR, Madsen J, McGuire AD, Rysgaard S, Schindler DE, Stirling I, Tamstorf MP, Tyler NJ, van der Wal R, Welker J, Wookey PA, Schmidt NM, Aastrup P (2009) Ecological dynamics across the Arctic associated with recent climate change. Science 325 (5946):1355-1358. doi:10.1126/science.1173113

Pratt JD, Mooney KA (2013) Clinal adaptation and adaptive plasticity in Artemisia californica: implications for the response of a foundation species to predicted climate change. Glob Chang Biol 19 (8):2454-2466. doi:10.1111/gcb.12199

Quackenbush J (2002) Microarray data normalization and transformation. Nat Genet 32 Suppl:496-501. doi:10.1038/ng1032

Rasmann S, Alvarez N, Pellissier L (2014a) The altitudinal niche-breadth hypothesis in insect-plant interactions. Annual plant reviews 47:339-359

Rasmann S, Pellissier L, Defossez E, Jactel H, Kunstler GJFE (2014b) Climate-driven change in plant–insect interactions along elevation gradients. 28 (1):46-54

Ritchie JC (1956) Vaccinium-Myrtillus L. Journal of Ecology 44 (1):291-299. doi:Doi 10.2307/2257181

Rodriguez-Saona C, Polashock J, Malo E (2013) Jasmonate-mediated induced volatiles in the American cranberry, Vaccinium macrocarpon: from gene expression to organismal interactions. Frontiers in plant science 4:115

Rodríguez-Castañeda G, Dyer LA, Brehm G, Connahs H, Forkner RE, Walla TR (2010) Tropical forests are not flat: how mountains affect herbivore diversity. Ecology letters 13 (11):1348-1357

Rodriguez AA, Maiale SJ, Menendez AB, Ruiz OA (2009) Polyamine oxidase activity contributes to sustain maize leaf elongation under saline stress. Journal of Experimental Botany 60 (15):4249-4262. doi:10.1093/jxb/erp256

Schadt EE, Monks SA, Drake TA, Lusis AJ, Che N, Colinayo V, Ruff TG, Milligan SB, Lamb JR, Cavet G, Linsley PS, Mao M, Stoughton RB, Friend SH (2003) Genetics of gene expression surveyed in maize, mouse and man. Nature 422 (6929):297-302. doi:10.1038/nature01434

Schemske DW, Mittelbach GG, Cornell HV, Sobel JM, Roy K (2009) Is there a latitudinal gradient in the importance of biotic interactions? Annu Rev Ecol Evol Syst 40:245-269

Selas V (2001) Autumn population size of capercaillie Tetrao urogallus in relation to bilberry Vaccinium myrtillus production and weather: an analysis of Norwegian game reports. Wildlife Biology 7 (1):17-25

Seldal T, Hegland SJ, Rydgren K, Rodriguez-Saona C, Töpper JP (2017) How to induce defense responses in wild plant populations? Using bilberry (Vaccinium myrtillus) as example. Ecology and Evolution 7 (6):1762-1769

Serreze MC, Walsh JE, Chapin FS, Osterkamp T, Dyurgerov M, Romanovsky V, Oechel WC, Morison J, Zhang T, Barry RG (2000) Observational evidence of recent change in the northern high-latitude environment. Climatic Change 46 (1-2):159-207. doi:Doi 10.1023/A:1005504031923

Shi LX, Hall M, Funk C, Schroder WP (2012) Photosystem II, a growing complex: updates on newly discovered components and low molecular mass proteins. Biochim Biophys Acta 1817 (1):13-25. doi:10.1016/j.bbabio.2011.08.008

Springer CJ, Orozco RA, Kelly JK, Ward JK (2008) Elevated CO2 influences the expression of floral-initiation genes in Arabidopsis thaliana. New Phytol 178 (1):63-67. doi:10.1111/j.1469-8137.2008.02387.x

Sun Y, Yin J, Cao H, Li C, Kang L, Ge F (2011) Elevated CO2 influences nematode-induced defense responses of tomato genotypes differing in the JA pathway. PLoS One 6 (5):e19751. doi:10.1371/journal.pone.0019751

Team RC (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2014.

Trussell GC, Smith LD (2000) Induced defenses in response to an invading crab predator: an explanation of historical and geographic phenotypic change. Proc Natl Acad Sci U S A 97 (5):2123-2127. doi:10.1073/pnas.040423397

Van Dam N, Baldwin I (2001) Competition mediates costs of jasmonate-induced defences, nitrogen acquisition and transgenerational plasticity in Nicotiana attenuata. Functional Ecology 15 (3):406-415

Velásquez AC, Castroverde CDM, He SY (2018) Plant–Pathogen Warfare under Changing Climate Conditions. Current Biology 28 (10):R619-R634

Veteli T, Kuokkanen K, JULKUNEN-TIITTO R, Roininen H, Tahvanainen J (2002a) Effects of elevated CO2 and temperature on plant growth and herbivore defensive chemistry. Global Change Biology 8 (12):1240-1252

Veteli TO, Kuokkanen K, Julkunen-Tiitto R, Roininen H, Tahvanainen J (2002b) Effects of elevated CO2 and temperature on plant growth and herbivore defensive chemistry. Global Change Biology 8 (12):1240-1252. doi:DOI 10.1046/j.1365-2486.2002.00553.x

Winning H, Viereck N, Wollenweber B, Larsen FH, Jacobsen S, Sondergaard I, Engelsen SB (2009) Exploring abiotic stress on asynchronous protein metabolism in single kernels of wheat studied by NMR spectroscopy and chemometrics. J Exp Bot 60 (1):291-300. doi:10.1093/jxb/ern293

Yang SY, Wu HH, Xie JC, Rantala MJ (2013) Depressed performance and detoxification enzyme activities of Helicoverpa armigera fed with conventional cotton foliage subjected to methyl jasmonate exposure. Entomologia Experimentalis Et Applicata 147 (2):186-195. doi:10.1111/eea.12060

Zeller G, Henz SR, Widmer CK, Sachsenberg T, Rätsch G, Weigel D, Laubinger S (2009) Stress-induced changes in the Arabidopsis thaliana transcriptome analyzed using whole-genome tiling arrays. The Plant Journal 58 (6):1068-1082

Zobayed SMA, Afreen F, Kozai T (2005) Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. Plant Physiology and Biochemistry 43 (10-11):977-984. doi:10.1016/j.plaphy.2005.07.013

Paper IV

Neighborhood effects of herbivore-induced plant resistance vary along an elevational gradient

Rafael Fonseca Benevenuto[1,2*], Tarald Seldal[1], Stein Ragnar Moe[2], Cesar Rodriguez-Saona[3], Stein Joar Hegland[1]

[1] Faculty of Engineering and Science, Western Norway University of Applied Sciences, Sogndal, Norway.

[2] Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Ås, Norway.

[3] Rutgers, The State University of New Jersey, Department of Entomology, Philip E. Marucci Center for Blueberry and Cranberry Research, Chatsworth, NJ, USA.

*Correspondence: Rafaelb@hvl.no

Author Contributions: TS, SJH, SRM, and CR-S conceived the initial idea and designed the field work. RFB carried out the field experiments, collected, analyzed and interpreted the data, and wrote the manuscript. All authors contributed to critically reading and editing manuscript, and they approved its final form.

ABSTRACT

The activation of plant defense systems in response to herbivory or experimentally applied methyl jasmonate (MeJA) involves the production of chemical defense substances functioning as warning signals to repel herbivores and protect against pathogens. They also serve as signals detectable by undamaged neighboring plants, a phenomenon called plant-plant communication. We studied how altitudinal variation in temperature and timing of snowmelt affected herbivore resistance, growth and reproduction of untreated bilberry (*Vaccinium myrtillus* L.) 20–500 cm from MeJA-treated ramets. Across two years, responses of MeJA-treated and untreated bilberry ramets were recorded twice per season along an elevational gradient in a boreal system. At low and medium altitudes, untreated bilberry showed increased herbivore resistance and reduced growth and reproduction up to 500 cm from MeJA-treated ramets. In the warmer sites at these altitudes, the effects persisted for two years for ramets up to 100 cm from the treated ramets. At high altitudes, however, only untreated ramets growing 20–100 cm from the treated ramets showed increased resistance to insect herbivores and reduced reproduction, but these effects did not persist into the second year. Altitudinal variation in climate affected trade-offs between plant defense, growth, and reproduction. Our findings indicate that plant-plant communication is also influenced by the combination of changes in climate and time after induction. Adaptations of plants growing under increasing temperature in high-latitude environments can profoundly impact ecosystem functioning, especially where bilberry, the key plant species in the boreal system, interacts with its herbivores.

Keywords induced defenses · methyl jasmonate · neighborhood effects · plant-plant communication · *Vaccinium myrtillus*

Introduction

Global warming is currently occurring at an unprecedented rate (Brooker 2006). Mean global temperature is projected to increase by 1.5–2.0°C in coming decades, causing longer drought periods in some areas and more precipitation in others (IPCC 2018). Climate change is likely to be a major threat to biodiversity in many regions worldwide (Sala et al. 2000; Thomas et al. 2004). High latitude tundra and boreal areas are particularly vulnerable to climate change-induced degradation and loss, with woody shrubs encroaching onto the tundra (IPCC 2018). At northern latitudes, milder winters and warmer summers will prolong the growing season, alter plant growth patterns, and eventually cause a mismatch between, for example, the time of pollinator emergence and host plants flowering (Bale et al. 2002; Stenseth and Mysterud 2002; Visser and Both 2005). Climate change is also expected to influence plant phenology (differences in growth and maturation), physiology (e.g., stomatal functioning in plants and the physicochemical properties of volatiles compounds), and resource allocation from growth or reproduction, or both, to defense when under herbivore attack. These can have direct and indirect implications for natural ecosystem functioning (Post et al. 2009; Peñuelas and Staudt 2010).

Plant coexistence can influence interactions with other organisms such as herbivores, predators, pollinators, and microorganisms. Indeed, a plant's association with its neighbors can take several forms, with competitive and facilitative interactions playing fundamental roles in shaping natural communities. Plant-plant competition has long been a key component of many classic ecological theories (Keddy 1989). In contrast, the importance of positive plant-plant interactions, such as facilitation, has only more recently been recognized as being equally important in regulating community composition and ecosystem functioning (Brooker 2006; Callaway 2007; Brooker et al. 2008). Neighboring plants can facilitate each other by promoting their growth (Plath et al. 2011), for example, through fixing nitrogen (Joëlle et al. 2010), or helping each other in defense against herbivores either directly, through reducing damage by diverting herbivores from their host plant (Morley et al. 2005), or indirectly, by attracting the herbivores' natural enemies (Landis et al. 2000). These negative and positive plant-plant interactions can be mediated by biotic (i.e., herbivore attack) and abiotic factors (i.e., environmental drivers). However, little is known about the effects of climate in modulating these interactions.

Plants under attack by herbivores and pathogens activate inducible defense mechanisms such as the emission of volatile organic compounds (VOCs) from aboveground and belowground tissues (Knudsen et al. 1993; Dudareva et al. 2004; Steeghs et al. 2004). Once VOCs are emitted, however, they convey 'public' information that can be perceived by surrounding plants and organisms, a phenomenon referred to as plant-plant communication. For instance, herbivore-induced VOCs can attract natural enemies of herbivores (Sabelis 1999), provide protection against pathogens (Shiojiri et al. 2006), and repel herbivores (Heil 2004), potentially defending plants against their antagonists (Kessler and Baldwin 2001). Moreover, undamaged neighboring plants can recognize VOCs emitted from attacked plants and use them as a cue to mobilize their own defense systems (Kobayashi and Yamamura 2003). Plant communication can be influenced by climate because VOC production, volatility and, consequently, VOC movement at the leaf surface, are controlled by temperature (Niinemets et al. 2004). Recent studies highlight possible effects of climate change on the biosynthesis and abundance of VOCs (Rosenstiel et al. 2003; Calfapietra et al. 2007), which could mitigate their ecological functions (Pinto et al. 2007). Higher VOC production and volatility under elevated temperatures (Helmig et al. 2007) are expected to change plant-herbivore interactions by altering the ability of specialist herbivores to locate their hosts, thereby increasing antiherbivore defense responses and modifying plant-plant interactions (Yuan et al. 2009). But we still do not fully understand how inter-plant communication mechanisms vary under different climate regimes, how these could be affected by changing environmental conditions, and what the consequences could be for ecological interactions and ecosystem functioning.

Plants incur costs if limiting resources, such as nitrogen and carbon, are invested in defense rather than production (Redman et al. 2001). Studies have shown that the activation of plant defense systems are energetically costly and reduce plant growth and reproduction (Accamando and Cronin 2012; Nabity et al. 2013; Seldal et al. 2017; Benevenuto et al. 2018). For example, in bilberry (*Vaccinium myrtillus* L.), a key food source for many herbivore species in boreal forest, treatment with methyl jasmonate (MeJA), a widely occurring volatile organic compound used in plant defense, increases resistance to herbivores and reduces plant growth and reproduction for several years (i.e., prolonged multiannual defense) (Benevenuto et al. 2018). These trade-offs between herbivore resistance and both growth and reproduction have previously been observed on MeJA-treated bilberry plants as well as on untreated neighbors (Benevenuto et al. 2018). Given that ecosystem composition and functioning can affect the ecosystem services on which human society depends (Peterson et al. 1998; Hooper et al. 2005), there is a strong practical need to understand those processes that regulate biodiversity, particularly in the light of potential impacts of climate change. It is therefore timely to consider how variations in climate might modulate plant-plant interactions and their effects, specially in high-latitude ecosystems.

In this study, we investigate how altitudinal variation in temperature and the time of snowmelt affect growth, reproduction and the induction of defenses in MeJA-treated and untreated neighboring bilberry ramets. Because VOCs can induce defenses in neighboring plants some distance away (Heil and Karban 2010; Hare 2011; Benevenuto et al. 2018), we predict similar patterns of defense in MeJA-induced and untreated neighboring bilberry ramets at different distances from the induced plant, for example, reduced growth, reproduction, and levels of herbivory. Such a distance effect would indicate plant-plant communication. Furthermore, because plant-induced defenses appear to decline with increasing elevation (Moreira et al. 2018) and elevated temperature is known to increase biogenic VOC emissions (Guenther et al. 1995; Helmig et al. 2007), we expect to find stronger plant-plant communication effects in the warmer, lower altitude environments.

Materials and methods

Study system

We conducted this study along an elevational gradient in the boreal pine-bilberry forest ecosystems of Kaupanger, the inner Sognefjord, western Norway (61.2°N, 007.2°E) in 2016 and 2017. Annual precipitation there is 700–900 mm with a mean summer temperature range of 12–16° C (Moen et al. 1999). The most abundant tree is pine (*Pinus sylvestris* L.), with dwarf shrubs, such as bilberry, lingonberry (*Vaccinium vitis-idaea* L.), and crowberry (*Empetrum nigrum* L.) dominating the field layer. Bilberry is a long-lived deciduous clonal dwarf shrub with evergreen stems usually 10–60 cm high (Ritchie 1956; Flower-Ellis 1971). Although we do not have specific information on clone size and distribution in the study area, we have based our work and study design on the assumptions that rhizomes can reach approximately 200 cm in length, depending on age, and that there is considerable genetic variation within the population (Flower-Ellis 1971; Albert et al. 2003; Albert et al. 2004). Bilberry is considered a key plant in boreal forests because it is a food source for many vertebrate and invertebrate herbivores (Hegland et al. 2010). The main mammalian herbivores feeding on bilberry in this area are red deer (*Cervus elaphus* L.) and various rodent species, whereas the commonest insect herbivores are Geometridae larvae (S.J. Hegland, pers. obs.). Bumblebees and solitary bees, as well as ants, are the main visitors to bilberry flowers at our sites (S.J. Hegland, unpublished data).

Gradient design

Inner Sognefjord is topographically diverse with marked altitudinal gradients occurring within a relatively small area. These are ideal for climate research, making it practical and convenient to carrying out ecological experiments over short geographical distances. We used a natural altitudinal gradient ranging from just above sea level to the subalpine zone to investigate the effects of variation in climate, mainly temperature and the timing of snowmelt, on plant-herbivore interactions and plant-plant communication. Anorthosite bedrock and moraine material dominate the whole gradient (NGU 2019) and there are no latitudinal or longitudinal influences. As such this gradient design reduces the possibility of confounding effects that can often be a challenge in climate-gradient studies.

We conducted an experiment at three different elevations representing an altitudinal gradient along which temperature, timing of snowmelt and length of the growth season varied (Fig. 1): ’Low’ elevation, ca. 100 m a.s.l. (submontane zone); ‘Medium’ elevation, ca. 500 m a.s.l. (mid-montane zone); and ‘High’ elevation, ca. 900 m a.s.l. (subalpine zone). We placed four data loggers (TRIX 8 LogTag, Auckland, New Zealand) at each site to record data on average temperature and relative humidity throughout the study season in both years. We recorded the timing of snowmelt as the Julian day on which each site became largely snow free. All experimental sites faced south west, thereby reducing incidental environmental variation, such as light conditions. Low and medium sites consisted of more than 10-year-old clear-cuts with small pines, birches and alders that produced negligible shadow. The high site was a naturally open subalpine area just below the tree line. Unpublished data from 2016 showed that there was relatively little variation in pH (mean, 4.19) and slope (16.4 degrees) among sites, although soil organic matter (mean, 60.4%) was slightly lower at the low altitude (Knut Rydgren pers. comm. 2016). Vegetation structure was similar among the sites, with a field layer and scattered tree layer up to ca. 5 m.

Experimental design and data collection

In May and June 2016, at each site (Low, Medium, and High), we established 20 150 m^2 (10 m × 15 m) blocks, leaving a minimum of 10 m between each to avoid interference from neighboring blocks. Within each block, two transects were established at least 10 m apart with five individually marked bilberry ramets in each, ranging in height from 10 to 25 cm. We located the five ramets at 10–30 cm (called dist. 20 hereafter), 40–60 cm (dist. 50), 80–120 cm (dist. 100), and 400–530 cm (dist. 500) from the first ramet (dist. 0) in each transect (Fig. 2). Transects were randomly assigned within each block. The first ramets (dist. 0) were subjected to one of two treatments: 10 mM MeJA application, termed the MeJA-treated or focal ramet, or a water : ethanol application, designated the control. To achieve the desired concentration of MeJA, 4.1 M MeJA stock solution (Bedoukian Research, Danbury, CT) was diluted 1:10 with 95% (v/v) ethanol and diluted further with water to get a final concentration of 10 mM MeJA (Seldal et al. 2017). Ethanol was added to water at the same final concentration as that in the 10 mM MeJA solution (41:1) for the control. To avoid rapid evaporation of MeJA, a cotton wad was attached to the stem at the ground and saturated with 10 mM MeJA (treatment) or water-ethanol (control) to the point of run-off. This was repeated three times at 1-week intervals after the initial application to simulate attack by herbivores. The ramets were not exposed to further treatments in 2017, to reveal any possible multiannual effects. To evaluate possible plant-plant communication between MeJA-treated and untreated neighboring ramets, only the first ramet (dist. 0) in the transect was treated (Benevenuto et al. 2018). The other ramets along the transects served as test subjects.

We used a similar sampling procedure to that adopted in a previous study (Benevenuto et al. 2018). In May/June 2016 (sampling time 1 [ST1]), before the start of treatment at all sites, we measured ramet height from the ground to crown with a ruler, and stem diameter at ground level with a digital caliper, for all the marked plants on the transects. We also counted the number of annual shoots, flowers, leaves, browsed shoots and insect-chewed leaves. We measured the same variables again at the end of the season (sampling time 2 [ST2]), 6 weeks (42 days) after the start of treatment. On this latter occasion, we also counted the number of berries. The same procedure was repeated in 2017 except that the focal plants and controls were not treated then. Plant height (H), stem diameter (DS) and the number of shoots (AS) were used to calculate the dry mass (DM) of each ramet, applying the formula proposed by Hegland et al. (2010): $\log2(DM) = 1.41700 \times \log2(DS) + 0.97104 \times \log2(H) + 0.44153 \times \log2(AS + 1) - 7.52070$. This provided a nondestructive estimate of plant size.

Data analyses

We assessed if MeJA induced anti-herbivore defenses in wild bilberry ramets by analyzing the patterns of herbivory (proportion of chewed leaves by insect herbivores and proportion of mammalian-browsed shoots), growth (changes in biomass, through dry mass calculation) and fruit set (proportion of berries to flowers) of MeJA-treated and untreated neighboring ramets at different distances, across an elevational gradient in two consecutive seasons. The analysis involved comparing control ramets with the corresponding ramets in the experimental MeJA-treated transects (dist. 0), and for untreated neighboring ramets at increasing distance from the control and experimental ramets (dist. 20–500), at each site along the elevational gradient in 2016 and 2017. The response variables were the changes in the proportion of chewed leaves, proportion of browsed shoots, and biomass, calculated by subtracting the values at ST1 (beginning of the season) from those at ST2 (end of the season). These provided measures of seasonal change for each year. Effects on reproduction were analyzed yearly, based on measurements of the number of flowers present in May–June and the number of berries in Aug–Sept in each year, from which fruit set was calculated as the proportion of berries to flowers.

For each response variable, we used the *lme4* (Bates et al. 2014) and *mixlm* (Liland and Sæbø 2014) libraries in R (RDevelopment 2012) to fit generalized linear mixed effect models (GLMM) with Gaussian error distribution with identity link and performed posterior analysis of variance (ANOVA). For all models, in each year (2016 and 2017), we entered treatment (control and MeJA dist. 0–500) and site (Low, Medium and High), with interaction terms as fixed effects. Blocks within each elevational site were fitted as random effects. To account for variation in plant size, the following covariates were included: total number of leaves at ST1 (for insect herbivory model), total number of shoots at ST1 (for mammalian herbivory model), total biomass at ST1 (for growth model), and total number of flowers at ST2 (for the fruit-set model). The treatment effects on reproduction were analyzed yearly, based on estimates of fruit set as defined above. Visual inspection of residual plots did not reveal any obvious deviation from homoscedasticity or normality for any model. To determine the presence of an interaction effect, we performed likelihood ratio tests of the full model against the model without the effect or interaction in question. We assessed the statistical significance (p-values) of single factors and their respective interactions by performing ANOVA for each model. Differences and mean separations between control and MeJA (dist. 0–500) ramets, within each elevational site, were analyzed by Tukey *post hoc* comparisons ($\alpha = 0.05$) (Supplementary Tables S1, S2).

Results

Insect herbivory, measured as the change in the proportion of chewed leaves, was significantly reduced in MeJA-treated and untreated neighboring bilberry ramets at all distances (dist. 20–

500) at the Low and Medium sites, and up to dist. 100 at the High site in 2016, compared with that of control ramets. This reduction in insect herbivory was maintained in 2017, but only in MeJA-treated ramets and in untreated neighbors up to dist. 50 at the Low and Medium sites, and not at all at the High site (Fig. 3; Supplementary Table S1).

Mammalian herbivory, measured as the change in the proportion of browsed shoots, was similarly significantly reduced in both years in MeJA-treated and neighboring ramets at all distances at both Low and Medium sites, compared with that in control plants. At the High site, however, there was no statistically significant reduction in browsing pressure other than in MeJA-treated ramets (dist. 0), and then only in 2016 (Fig. 4; Supplementary Table S1).

In 2016, seasonal growth (change in biomass) was reduced in MeJA-treated and nearby neighboring ramets (dist. 20–50) at both Low and Medium sites compared with the control plants, but no significant difference was observed at the High site. A year after treatment (2017), biomass production continued to be significantly depressed relative to control plants at the Low site at distances up to ca. 50 cm, and at all distances at the Medium site. At the High site, however, which had displayed no significant response in 2016, significant reductions in growth were recorded in 2017 in the 2016 MeJA-treated experimental ramets and their closest neighbors (dist. 20), relative to the control (Fig. 5; Supplementary Table S2).

Fruit set, measured as the proportion of berries to flowers, was also affected. In 2016, fruit set was significant reduced in MeJA-treated and untreated neighboring ramets at distances from ca. 20 to 100 cm at both Low and High sites. The effect was more marked at the Medium site, however, where reduced fruit set was recorded in untreated neighbors up to ca. 500 cm away (Fig. 6; Supplementary Table S2). Similar statistically significant reductions in fruit set were recorded for MeJA-treated and untreated neighboring ramets in 2017 at the Low and Medium sites at distances up to ca. 100 cm, but not in any of the plants at the High site at any distance (Fig. 6; Supplementary Table S2).

Discussion

Our results show that treated plants and untreated neighbors up to 500 cm away appear to have activated their defense systems in response to treatment with MeJA, as shown by reductions in both insect and mammalian herbivory, relative to control plants in the same area. This in turn resulted in reduced growth and reproduction in these MeJA-treated plants and their untreated neighbors, presumably due to the reallocation of resources from growth and reproduction to defense. This effect was observed at all elevations, but especially at Low and Medium altitude sites, where the effect persisted into a second year, in line with our predictions. At these sites the effects were more strongly induced and persistent, including among untreated conspecific ramets at intermediate to long distances (ca. 100-500 cm). Conversely, at the High site, where temperatures are lower and the growing season is shorter, only MeJA-treated and untreated neighboring ramets at short to intermediate distances (ca. 20–100 cm) showed reduced herbivory and fruit set, and this effect did not persist into the next year. The single instance of reduced growth recorded in 2017 at the High site in the 2016 MeJA-treated experimental plants and close neighbors (dist. 20) may have been coincidental and could have arisen if the plants were responding to an instance of natural herbivory and the release of volatile defense compounds.

We found clear neighborhood/distance effects of MeJA-treatment on bilberry growth, reproduction, and herbivory resistance across all elevations in both MeJA-treated and untreated neighboring ramets. These effects were largely as predicted across elevation and time, and support the idea of plant-plant communication, with both MeJA-treated and untreated neighboring ramets up to 5 m away evidently activating their defense systems, at least in 2016 when the plants were treated. These results are consistent with other studies showing that above and belowground signals emitted by induced plants can activate defense system of uninduced neighbors (Dicke and Bruin 2001; Baldwin et al. 2002; Pickett et al. 2003). Moreover, these effects were more pronounced and consistent in the sub-montane and mid-montane climatic zones (Low and Medium sites) than in the subalpine zone (High sites), suggesting that plant-plant communication in bilberry, either through airborne or belowground signaling from MeJA-treated ramets, is apparently modulated by climatic conditions.

The reduction in growth, fruit set, and levels of insect and mammalian herbivory, in untreated bilberry ramets growing within about 5 m of MeJA-treated ramets suggests that there is a trade-off between growth and defense. This trade-off apparently persisted, albeit less markedly, for at least the next year in untreated neighboring plants up to ca. 100 cm from the treated ramets at the Low and Medium sites, suggesting that defense activation of induced plants and their neighbors growing in sub-montane to mid-montane zones in the boreal forest is multiannual. In a previous study, in the same ecosystem and area, at ca. 350 m a.s.l., we also found evidence of multiannual plant-plant communication effects between MeJA-treated and untreated neighboring ramets (Benevenuto et al. 2018).

Although MeJA-treated and untreated bilberry ramets up to ca. 100 cm away showed reduced insect herbivory and fruit set initially at the High site, neither growth nor mammalian herbivory, other than on the treated ramets, were significantly reduced. Furthermore, in contrast to plants growing at lower elevations, these effects did not persist through to the next growing season, further evidence that climate impinges on the effectiveness of plant-plant communication in an induced defense system. The elevational gradient used in our study parallels a natural climatic gradient. As such, average temperatures at the Low and Medium sites are notably higher than at the High site, in what is effectively the subalpine zone. Plants growing at this latter zone experience a more stressful environment with lower average temperatures, later snowmelt, and, consequently, a shorter growing season than plants at lower altitudes in this boreal ecosystem. Overall, climate can be an important abiotic factor in determining the intensity and efficacy of induced plant defenses, for example, by affecting the release of plant volatiles (Gouinguené and Turlings 2002). Such effects can alter plant-herbivore and plant-plant interactions and can result in important changes in natural ecosystem functioning, such as cyclic fluctuations in herbivore populations (Haukioja and Hanhimäki 1985).

The relatively warmer environments at the Low and Medium elevation sites, together with their longer growth seasons, boosted the performance of the induced defense system in bilberry and produced more effective plant-plant communication. Although we have not determined the type of signaling, it is probable that both aboveground (airborne VOCs) and belowground (VOCs or ramet contact) processes were involved in activating the defense systems of untreated neighboring ramets. The emission rates of VOCs, one of the main means of plant-plant communication, are determined mainly by their synthesis rates and physicochemical characteristics (i.e., solubility, volatility, and diffusivity). They are therefore greatly affected by internal and external factors (Kesselmeier and Staudt 1999; Peñuelas and Llusià 2001, 2003; Niinemets et al. 2004). For instance, elevated temperature increases biogenic VOC emission by enhancing enzyme activity during synthesis, raising vapor pressure, and decreasing the resistance to diffusion (Tigney 1991; Guenther et al. 1993; Gouinguené and Turlings 2002). Altered VOC emission in a warmer environment can affect signaling efficacy directly in various ecological interactions in natural ecosystems. For example, higher VOC production at elevated temperatures (Helmig et al. 2007) could enhance plant-pollinator interactions by increasing flower visitation rates. They could also alter the ability of specialist insect and mammalian herbivores to locate their hosts, increase direct and indirect antiherbivore defenses, and consequently alter plant-plant interactions (Yuan et al. 2009). Moreover, at higher temperatures, insect herbivores, predators, and parasitoids in general have higher metabolic activity and could be more receptive to plant infochemicals (Valkama et al. 2007). This can enhance the defense role of induced VOCs in warmer climates. Conversely, moderately elevated temperatures enhance feeding by certain herbivores (Bale et al. 2002; Valkama et al. 2007), thereby increasing inducible VOC emissions (Gouinguené and Turlings 2002; Himanen et al. 2009). Besides temperature and other main environmental factors of climate change, VOC emissions from plants could also be altered by other abiotic factors, such as nutrient and resource availability, interacting with these main one (Gouinguené and Turlings 2002), as well as altering phenology such as plant seasonality and leaf duration (Constable et al. 1999). Our high-altitude site, located in the subalpine zone of the boreal forest biome, experienced cooler average temperatures, limited resource availability, and had a shorter growing season. This may explain the apparently reduced response by bilberry to inducible VOCs, perhaps revealing reduced plant-plant communication capacity compared with that of bilberry ramets growing at lower altitudes.

The more pronounced plant-plant communication found in bilberry ramets growing in the submontane (Low site) and mid-montane (Medium site) zones reflects the antiherbivore defense strategy of plants growing under milder environmental conditions. In separate study, our molecular and ecological data showed that high-altitude bilberry in the boreal forest invested more in constitutive defenses, whereas low-altitude plants relied more on induced defenses (Unpublished Benevenuto et al. 2019). Bilberry plants growing at lower altitudes, where herbivore pressure and seasonal temperatures are higher, appeared more responsive to simulated herbivore attack by diverting resources from growth and reproduction to induced antiherbivore defenses in both treated and untreated neighboring ramets. This suggests that bilberry plants, growing under warmer conditions and higher levels of herbivory, are adapted to be more flexible in their responses compared with conspecifics growing in subalpine zones. This flexibility has consequences both for natural ecosystem functioning, by modulating plant-herbivore interactions (e.g., outbreaks of herbivore populations), and for enhanced plant-plant interactions (e.g., detection and response to VOC emissions by adjacent plants).

Emissions of VOCs by plants contribute substantially to the hydrocarbon load on the atmosphere, emitting an equivalent to the sum of biogenic and anthropogenic methane emissions (Guenther et al. 1995). In the longer term, therefore, increased levels of VOCs in the atmosphere, resulting from bilberry growing under warmer environmental conditions in this boreal ecosystem, could contribute to climate change through aerosol formation and enhanced greenhouse effects (Peñuelas and Llusià 2003; Rosenstiel et al. 2003; Calfapietra et al. 2007), which in turn could feedback to affect their own ecological functioning. Moreover, VOCs can relieve abiotic stresses, such as higher temperature and levels of reactive oxygen species (Sharkey et al. 2001; Gouinguené and Turlings 2002; Peñuelas et al. 2005; Pinto et al. 2007), and this could be magnified in the future by higher average temperatures. Given the function of some VOCs, such as terpenoids, in plant-plant communication, changes in volatile abundance as a result of climate change could therefore affect plant-herbivore relationships by either weakening or strengthening signaling between plants (Yuan et al. 2009).

In summary, our findings provide evidence for climate modulation of plant-plant communication as part of the induced plant defense system in bilberry. Low (submontane zone) to Medium altitude (mid-montane zone) environments in the boreal system, with higher average temperatures and longer growth seasons, favored induced defenses in the ramets of neighboring conspecifics, at least within ca. 5 m of a source plant. The greater prominence of this inducible plant defense system at these lower altitudes may presage the kinds of change expected in plant-plant communication systems and associated VOC emissions in a warmer world, which will likely feedback to further influence ecological functioning. Although challenging to do, simulations of climate-change effects on plant-herbivore and plant-plant interactions in natural systems could help us predict the possible ecological consequences of a changing climate on vulnerable ecosystems, especially high-latitude systems in which there are marked dependences among species, such as bilberry and most insect and mammalian herbivores in boreal forest.

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was financially supported by the Norwegian Research Council (project no. 204403/E40), the Norwegian Environment Agency.

Authors' Contributions

TS, SJH, SRM, and CR-S conceived the initial idea and designed field work. RFB carried out the field experiments, collected, analyzed and interpreted the data, and wrote the manuscript. All authors contributed to critically reading and editing the manuscript, and they approved its final form.

Acknowledgements

We thank Torbjørn Stokke, Sigurd Daniel Nerhus, and Mark Andrew Gillespie for their help with establishment and execution of the field experiment.

References

Accamando AK, Cronin JT (2012) Costs and benefits of jasmonic acid induced responses in soybean. Environ Entomol 41 (3):551–561 doi:10.1603/EN11277

Albert T, Raspé O, Jacquemart A-L (2003) Clonal structure in *Vaccinium myrtillus* L. revealed by RAPD and AFLP markers. Int J Plant Sci 164 (4):649–655

Albert T, Raspé O, Jacquemart A-L (2004) Clonal diversity and genetic structure in *Vaccinium myrtillus* populations from different habitats. Belg J Bot 137:155–162

Baldwin IT, Kessler A, Halitschke R (2002) Volatile signaling in plant–plant–herbivore interactions: what is real? Curr Opin Plant Biol 5 (4):351–354

Bale JS, Masters GJ, Hodkinson ID, Awmack C, Bezemer TM, Brown VK, Butterfield J, Buse A, Coulson JC, Farrar J, Good JEG, Harrington R, Hartley S, Jones TH, Lindroth RL, Press MC, Symrnioudis I, Watt AD, Whittaker JB (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Glob Chang Biol 8 (1):1–16 doi: 10.1046/j.1365-2486.2002.00451.x

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R package version 1 (7) R Foundation for Statistical Computing, Vienna, Austria

Benevenuto RF, Hegland SJ, Töpper JP, Rydgren K, Moe SR, Rodriguez-Saona C, Seldal T (2018) Multiannual effects of induced plant defenses: Are defended plants good or bad neighbors? Ecol Evol 8:8940–8950 doi: 10.1002/ece3.4365

Brooker RW (2006) Plant–plant interactions and environmental change. New Phytol 171 (2):271–284

Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G, Liancourt P, Tielborger K, Travis JMJ, Anthelme F, Armas C, Coll L, Corcket E, Delzon S, Forey E, Kikvidze Z, Olofsson J, Pugnaire FI, Quiroz CL, Saccone P, Schiffers K, Seifan M, Touzard B, Michalet R (2008) Facilitation in plant communities: the past, the present, and the future. J Ecol 96 (1):18–34 doi:10.1111/j.1365-2745.2007.01295.x

Calfapietra C, Wiberley AE, Falbel TG, Linskey AR, Mugnozza GS, Karnosky DF, Loreto F, Sharkey TD (2007) Isoprene synthase expression and protein levels are reduced under elevated O3 but not under elevated CO2 (FACE) in field-grown aspen trees. Plant Cell Environ 30 (5):654–661

Callaway RM (2007) Positive interactions and interdependence in plant communities. Springer, Dordrecht, The Netherlands

Constable JVH, Litvak ME, Greenberg JP, Monson RK (1999) Monoterpene emission from coniferous trees in response to elevated CO2 concentration and climate warming. Glob Chang Biol 5 (3):255–267

Dicke M, Bruin J (2001) Chemical information transfer between plants: back to the future. Biochem Syst Ecol 29 (10):981–994

Dudareva N, Pichersky E, Gershenzon J (2004) Biochemistry of plant volatiles. Plant Physiol 135 (4):1893–1902 doi: 10.1104/pp.104.049981

Flower-Ellis J (1971) Age, structure and dynamics in stands of bilberry (*Vaccinium myrtillus* L.). Department of Forest Ecology and Forest Soils. Research Note 9. Royal College of Forestry, Stockholm,

Gouinguené SP, Turlings TC (2002) The effects of abiotic factors on induced volatile emissions in corn plants. Plant Physiol 129 (3):1296–1307

Guenther A, Hewitt CN, Erickson D, Fall R, Geron C, Graedel T, Harley P, Klinger L, Lerdau M, Mckay WA, Pierce T, Scholes B, Steinbrecher R, Tallamraju R, Taylor J, Zimmerman P (1995) A global-model of natural volatile organic-compound emissions. J Geophys Res Atmos 100 (D5):8873–8892 doi: 10.1029/94jd02950

Guenther AB, Zimmerman PR, Harley PC, Monson RK, Fall R (1993) Isoprene and monoterpene emission rate variability – model evaluations and sensitivity analyses. J Geophys Res Atmos 98 (D7):12609–12617 doi: 10.1029/93jd00527

Hare JD (2011) Ecological role of volatiles produced by plants in response to damage by herbivorous insects. Annu Rev Entomol 56:161–180

Haukioja E, Hanhimäki S (1985) Rapid wound-induced resistance in white birch (*Betula pubescens*) foliage to the geometrid *Epirrita autumnata*: a comparison of trees and moths within and outside the outbreak range of the moth. Oecologia 65 (2):223–228

Hegland SJ, Jongejans E, Rydgren K (2010) Investigating the interaction between ungulate grazing and resource effects on *Vaccinium myrtillus* populations with integral projection models. Oecologia 163 (3):695–706

Heil M (2004) Direct defense or ecological costs: responses of herbivorous beetles to volatiles released by wild Lima bean (*Phaseolus lunatus*). J Chem Ecol 30 (6):1289–1295

Heil M, Karban R (2010) Explaining evolution of plant communication by airborne signals. Trends Ecol Evol 25 (3):137–144

Helmig D, Ortega J, Duhl T, Tanner D, Guenther A, Harley P, Wiedinmyer C, Milford J, Sakulyanontvittaya T (2007) Sesquiterpene emissions from pine trees – identifications, emission rates and flux estimates for the contiguous United States. Environ Sci Technol 41 (5):1545–1553

Himanen SJ, Nerg AM, Nissinen A, Pinto DM, Stewart CN, Jr., Poppy GM, Holopainen JK (2009) Effects of elevated carbon dioxide and ozone on volatile terpenoid emissions and multitrophic communication of transgenic insecticidal oilseed rape (*Brassica napus*). New Phytol 181 (1):174–186 doi: 10.1111/j.1469-8137.2008.02646.x

Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setala H, Symstad AJ, Vandermeer J, Wardle DA (2005)

Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecol Monogr 75 (1):3–35 doi: 10.1890/04-0922

IPCC (2018) Global warming of 1.5 °C. An IPCC special report on the impacts of global warming of 1.5 °C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. Intergovernmental Panel on Climate Change, Geneva, Switzerland

Joëlle F, Fabien L, Stéphanie M, Jean-Bernard C (2010) Nitrogen rhizodeposition of legumes. A review. Agron Sustain Dev 30:57–66

Keddy PA (1989) Effects of competition from shrubs on herbaceous wetland plants – a 4-year field experiment. Can J Bot 67 (3):708–716 doi: 10.1139/b89-094

Kesselmeier J, Staudt M (1999) Biogenic volatile organic compounds (VOC): An overview on emission, physiology and ecology. J Atmos Chem 33 (1):23–88 doi: 10.1023/A:1006127516791

Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Science 291 (5511):2141–2144 doi: 10.1126/science.291.5511.2141

Knudsen JT, Tollsten L, Bergström LG (1993) Floral scents—a checklist of volatile compounds isolated by head-space techniques. Phytochemistry 33 (2):253–280

Kobayashi Y, Yamamura N (2003) Evolution of signal emission by non-infested plants growing near infested plants to avoid future risk. J Theor Biol 223 (4):489-503

Landis DA, Wratten SD, Gurr GM (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu Rev Entomol 45 (1):175–201 doi:10.1146/annurev.ento.45.1.175

Liland KH, Sæbø S (2014) mixlm: Mixed Model ANOVA and Statistics for Education. R package version 1 (7). R Foundation for Statistical Computing, Vienna, Austria

Moen A, Lillethun A, Odland A (1999) Vegetation. National atlas of Norway. Norwegian Mapping Authority, Hønefoss, Norway

Moreira X, Petry WK, Mooney KA, Rasmann S, Abdala-Roberts L (2017) Elevational gradients in plant defences and insect herbivory: recent advances in the field and prospects for future research. Ecography 41:1485–1496 doi: 10.1111/ecog.03184

Morley K, Finch S, Collier RH (2005) Companion planting—behaviour of the cabbage root fly on host plants and non-host plants. Entomol Exper Appl 117 (1):15–25

Nabity PD, Zavala JA, DeLucia EH (2013) Herbivore induction of jasmonic acid and chemical defences reduce photosynthesis in *Nicotiana attenuata*. J Exp Bot 64 (2):685–694

NGU (2019) Norges Geologiske Undersøkelse. http://geo.ngu.no/kart/minkommune/?kommunenr=1420. Accessed 08/01/2019 2019

Niinemets U, Loreto F, Reichstein M (2004) Physiological and physicochemical controls on foliar volatile organic compound emissions. Trends Plant Sci 9 (4):180–186 doi: 10.1016/j.tplants.2004.02.006

Peñuelas J, Llusià J (2001) The complexity of factors driving volatile organic compound emissions by plants. Biol Plant 44 (4):481–487 doi: 10.1023/A:1013797129428

Peñuelas J, Llusià J (2003) BVOCs: plant defense against climate warming? Trends Plant Sci 8 (3):105–109

Peñuelas J, Llusià J, Asensio D, Munne-Bosch S (2005) Linking isoprene with plant thermotolerance, antioxidants and monoterpene emissions. Plant Cell Environ 28 (3):278–286 doi: 10.1111/j.1365-3040.2004.01250.x

Peñuelas J, Staudt M (2010) BVOCs and global change. Trends Plant Sci 15 (3):133–144 doi:10.1016/j.tplants.2009.12.005

Peterson G, Allen CR, Holling CS (1998) Ecological resilience, biodiversity, and scale. Ecosystems 1 (1):6–18 doi: 10.1007/s100219900002

Pickett J, Rasmussen H, Woodcock C, Matthes M, Napier J (2003) Plant stress signalling: understanding and exploiting plant–plant interactions. Biochem Soc Trans 31:123–127

Pinto DM, Blande JD, Nykänen R, Dong W-X, Nerg A-M, Holopainen JK (2007) Ozone degrades common herbivore-induced plant volatiles: does this affect herbivore prey location by predators and parasitoids? J Chem Ecol 33 (4):683–694

Plath M, Mody K, Potvin C, Dorn SJAS (2011) Do multipurpose companion trees affect high value timber trees in a silvopastoral plantation system? Agrofor Syst 81 (1):79–92 doi: 10.1007/s10457-010-9308-9

Post E, Forchhammer MC, Bret-Harte MS, Callaghan TV, Christensen TR, Elberling B, Fox AD, Gilg O, Hik DS, Høye TT (2009) Ecological dynamics across the Arctic associated with recent climate change. Science 325 (5946):1355–1358

R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Redman AM, Cipollini DF, Jr., Schultz JC (2001) Fitness costs of jasmonic acid-induced defense in tomato, *Lycopersicon esculentum*. Oecologia 126 (3):380–385 doi:10.1007/s004420000522

Ritchie J (1956) *Vaccinium myrtillus* L. J Ecol 44 (1):291–299

Rosenstiel TN, Potosnak MJ, Griffin KL, Fall R, Monson RK (2003) Increased CO_2 uncouples growth from isoprene emission in an agriforest ecosystem. Nature 421 (6920):256–259

Sabelis M, Janssen A, Pallini A, Venzon M, Bruin, J, Drukker B, Scutareanu P (1999) Behavioral responses of predatory and herbivorous arthropods to induced plant volatiles: from evolutionary ecology to agricultural applications. In: Agrawal AA, Tuzun S, Bent E (eds) Induced plant defenses against pathogens and herbivores. American Phytopathological Society Press, St Paul, MN, pp 269–296

Sala OE, Chapin FS, 3rd, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. Science 287 (5459):1770–1774

Seldal T, Hegland SJ, Rydgren K, Rodriguez-Saona C, Töpper JP (2017) How to induce defense responses in wild plant populations? Using bilberry (*Vaccinium myrtillus*) as example. Ecol Evol 7 (6):1762–1769

Sharkey TD, Chen X, Yeh S (2001) Isoprene increases thermotolerance of fosmidomycin-fed leaves. Plant Physiol 125 (4):2001–2006

Shiojiri K, Kishimoto K, Ozawa R, Kugimiya S, Urashimo S, Arimura G, Horiuchi J, Nishioka T, Matsui K, Takabayashi J (2006) Changing green leaf volatile biosynthesis in plants: An approach for improving plant resistance against both herbivores and pathogens. Proc Natl Acad Sci USA 103 (45):16672–16676 doi:10.1073/pnas.0607780103

Steeghs M, Bais HP, de Gouw J, Goldan P, Kuster W, Northway M, Fall R, Vivanco JM (2004) Proton-transfer-reaction mass spectrometry as a new tool for real time analysis of root-secreted volatile organic compounds in arabidopsis. Plant Physiol 135 (1):47–58 doi: 10.1104/pp.104.038703

Stenseth NC, Mysterud A (2002) Climate, changing phenology, and other life history traits: nonlinearity and match–mismatch to the environment. Proc Natl Acad Sci USA 99 (21):13379–13381

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BF, De Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, Van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Peterson AT, Phillips OL, Williams SE (2004) Extinction risk from climate change. Nature 427 (6970):145–148 doi: 10.1038/nature02121

Tigney DT, Turner DP, Weber JA (1991) Factors controlling the emissions of monoterpenes and other volatile organic compounds. In: Sharkey TD, Holland EA, Mooney HA (eds) Trace gas emission by plants. Elsevier, Amsterdam, The Netherlands, pp 93–119

Valkama E, Koricheva J, Oksanen E (2007) Effects of elevated O3, alone and in combination with elevated CO2, on tree leaf chemistry and insect herbivore performance: a meta-analysis. Glob Chang Biol 13 (1):184–201

Visser ME, Both C (2005) Shifts in phenology due to global climate change: the need for a yardstick. Proc R Soc Lond B-Biol Sci 272 (1581):2561–2569 doi: 10.1098/rspb.2005.3356

Yuan JS, Himanen SJ, Holopainen JK, Chen F, Stewart CN, Jr. (2009) Smelling global climate change: mitigation of function for plant volatile organic compounds. Trends Ecol Evol 24 (6):323–331 doi:10.1016/j.tree.2009.01.012

Captions to Figures

Fig. 1 Average timing of snowmelt, temperature, and relative humidity across the elevational gradient. Snow free = Julian Day Number (JDN) for complete snowmelt; T = air temperature; RH = relative air humidity.

Fig. 2 Transect design showing the distances between MeJA-treated or Control bilberry ramets and their untreated neighbors. C = control transect; T = treated transect; MeJA = methyl jasmonate.

Fig. 3 Seasonal changes in insect herbivory (proportion of chewed leaves) of MeJA-treated and untreated neighboring (dist. 20–500) bilberry plants (*Vaccinium myrtillus* L.) across an elevational gradient (Low, Medium, and High) for two consecutive years (2016 and 2017). The seasonal change in the proportion of insect chewed leaves (no. chewed leaves/total number of leaves) was calculated as the difference each year between the value at the end of the growing season (Aug/Sep) and that at the beginning (May/June). Error bars are ±1 SE, * represents significant differences between MeJA-treated and untreated neighbors (dist. 20–500) vs. Control ramets (Tukey $p < 0.05$).

Fig. 4 Seasonal changes in mammalian herbivory (proportion of browsed shoots) of MeJA-treated and untreated neighboring (dist. 20–500) bilberry plants (*Vaccinium myrtillus* L.) across an elevational gradient (Low, Medium, and High) for two consecutive years (2016 and 2017). The seasonal change in the proportion of deer-browsed shoots (no. browsed shoots/total number of shoots) was calculated as difference each year between the value at the end of the growing season (Aug/Sep) and that at the beginning (May/June). Error bars are ±1 SE, * represents significant differences between MeJA-treated and untreated neighbors (dist. 20–500) vs. Control ramets (Tukey $p < 0.05$).

Fig. 5 Seasonal changes in growth (biomass) of MeJA-treated and untreated neighboring (dist. 20–500) bilberry plants (*Vaccinium myrtillus* L.) across an elevational gradient (Low, Medium, and High) for two consecutive years (2016 and 2017). The seasonal change in growth (biomass) was calculated as the difference each year between the value at the end of the growing season (Aug/Sep) and that at the beginning (May/June). Error bars are ±1 SE, * represents significant differences between MeJA-treated and untreated neighbors (dist. 20–500) vs. Control ramets (Tukey $p < 0.05$).

Fig. 6 Fruit set (proportion of berries to flowers) of MeJA-treated and untreated neighboring (dist. 20–500) bilberry plants (*Vaccinium myrtillus* L.) across an elevational gradient (Low, Medium, and High) for two consecutive years (2016 and 2017). Fruit set is the ratio between the total number of berries divided by the total number of flowers in each year. Error bars are ±1 SE, * represents significant differences between MeJA-treated and untreated neighbors (dist. 20–500) vs. Control ramets (Tukey $p < 0.05$).

Fig. 1

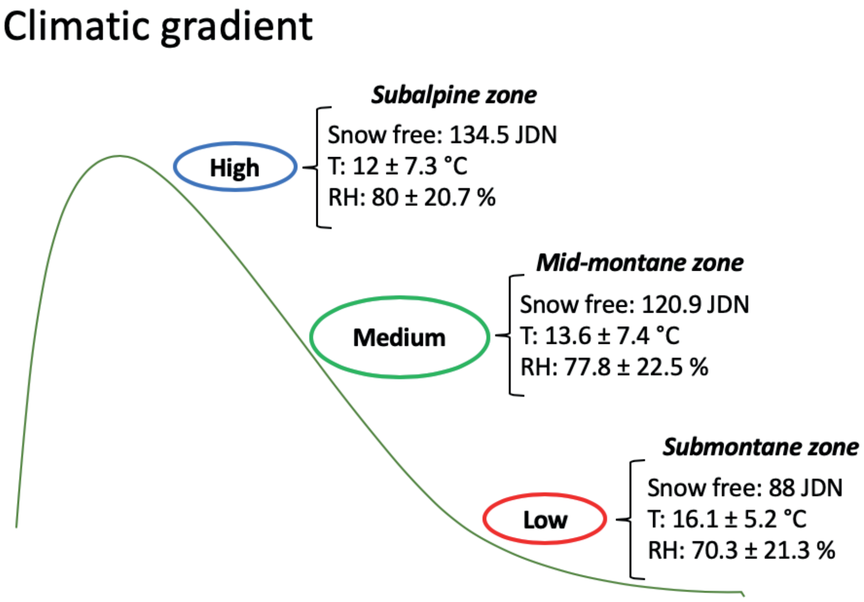


Fig. 2

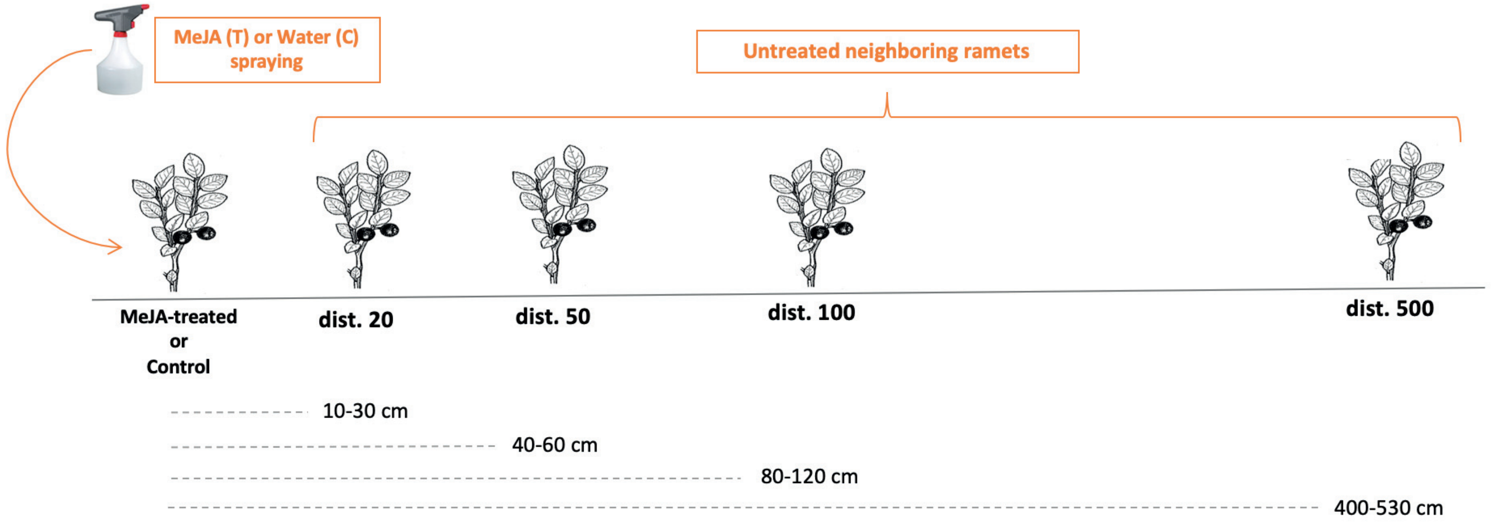

MeJA (T) or Water (C) spraying
Untreated neighboring ramets
MeJA-treated or Control
dist. 20
dist. 50
dist. 100
dist. 500
10-30 cm
40-60 cm
80-120 cm
400-530 cm

Fig. 3

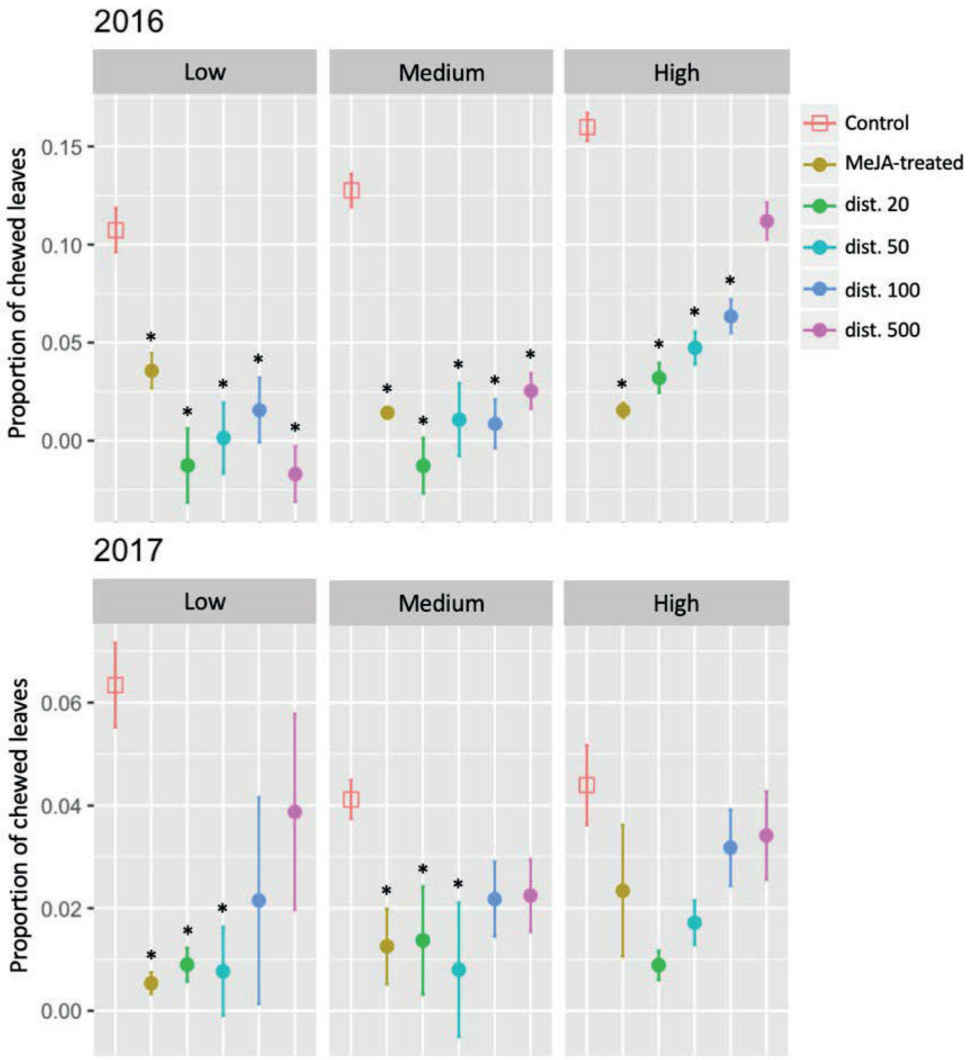

2016
Low
Medium
High
Proportion of chewed leaves
0.15
0.10
0.05
0.00
*
Control
MeJA-treated
dist. 20
dist. 50
dist. 100
dist. 500
2017
Low
Medium
High
Proportion of chewed leaves
0.06
0.04
0.02
0.00

Fig. 4

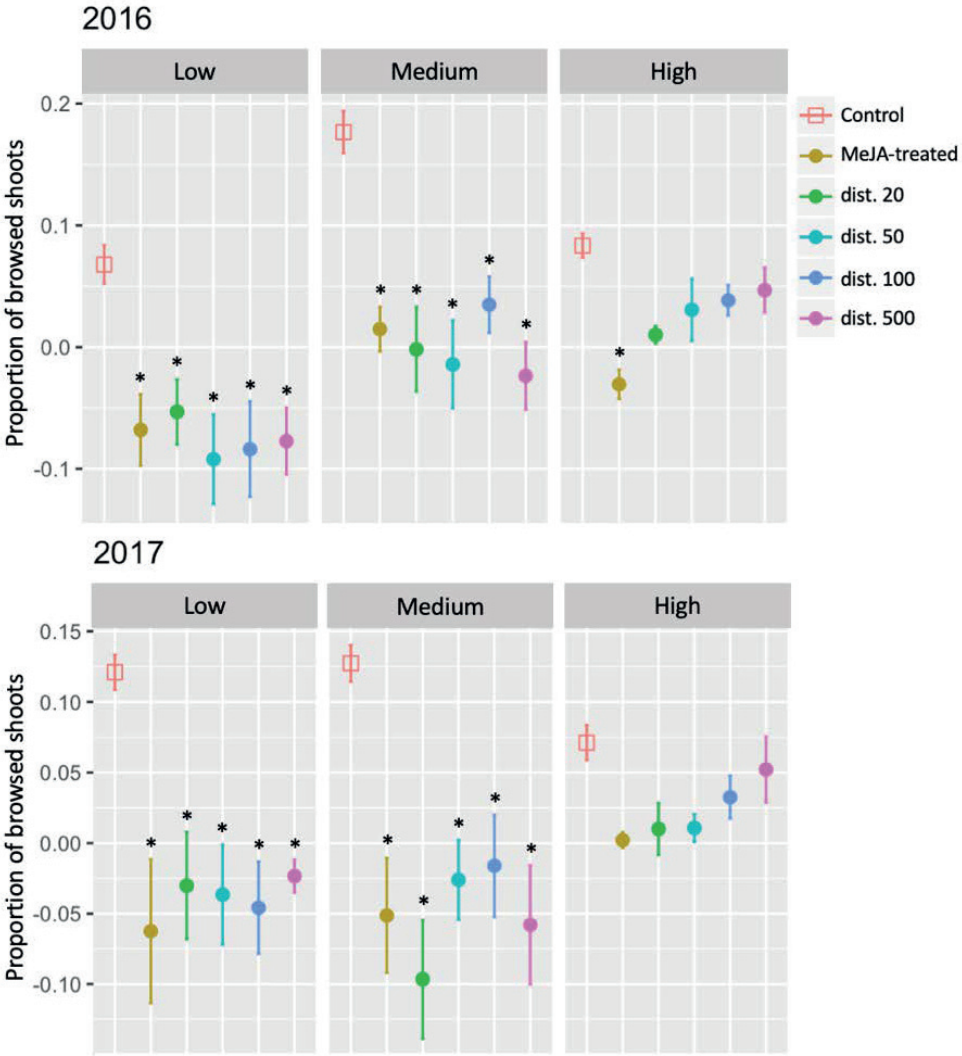

2016
Low
Medium
High
Proportion of browsed shoots
0.2
0.1
0.0
-0.1
*
Control
MeJA-treated
dist. 20
dist. 50
dist. 100
dist. 500
2017
Low
Medium
High
0.15
0.10
0.05
0.00
-0.05
-0.10

Fig. 5

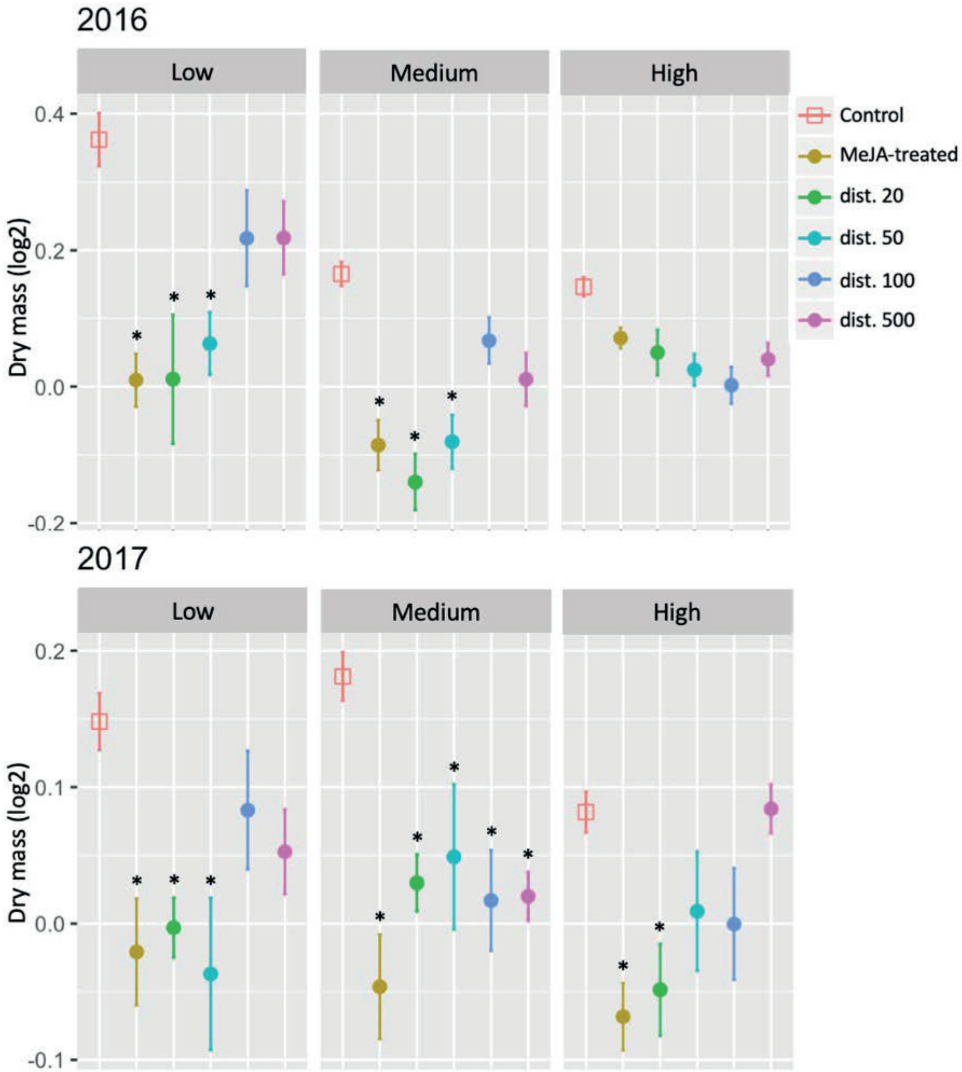
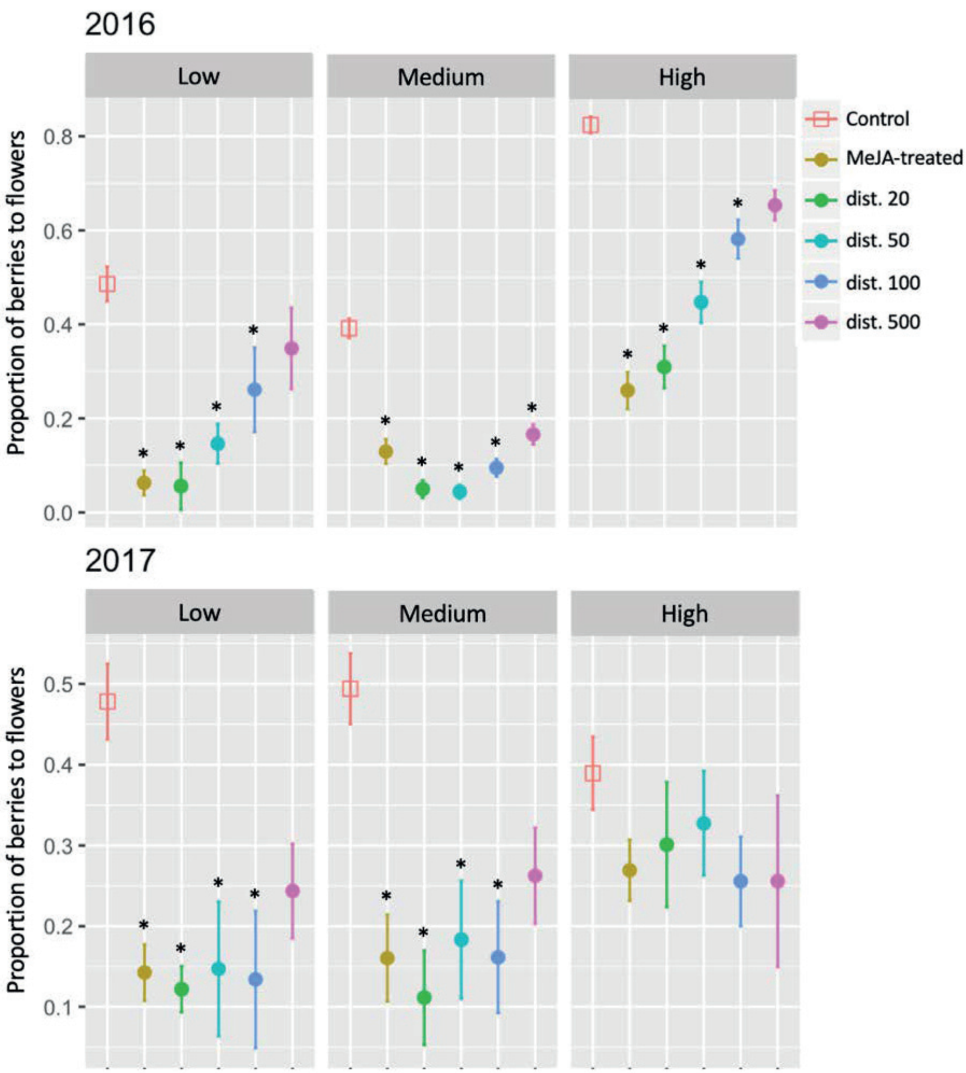

2016
Low
Medium
High
Dry mass (log2)
0.4
0.2
0.0
-0.2
*
Control
MeJA-treated
dist. 20
dist. 50
dist. 100
dist. 500
2017
Low
Medium
High
Dry mass (log2)
0.2
0.1
0.0
-0.1

Fig. 6



2016
2017
Low
Medium
High
Proportion of berries to flowers
0.0
0.2
0.4
0.6
0.8
0.1
0.3
0.5
*
Control
MeJA-treated
dist. 20
dist. 50
dist. 100
dist. 500

Supplementary information

Table S 1 ANOVA table with F and P-values for insect (proportion of chewed leaves) and mammalian (proportion of browsed shoots) herbivory in MeJA-treated and untreated neighboring (dist. 20–500) bilberry plants (*Vaccinium myrtillus* L.) across an elevational gradient (Low, Medium, and High) in Kaupanger, Western Norway, in two consecutive years (2016 and 2017)

	Insect herbivory				**Mammalian herbivory**			
	2016		**2017**		**2016**		**2017**	
Single factors	**Chisq**	**p-value**	**Chisq**	**p-value**	**Chisq**	**p-value**	**Chisq**	**p-value**
Treatment	344.67	< 0.0001	60.05	< 0.0001	148.42	< 0.0001	144.05	< 0.0001
Site	34.35	< 0.0001	0.93	NS	44.08	< 0.0001	10.62	0.005
Covariates								
Number of leaves	22.82	< 0.0001	22.14	< 0.0001	-	-	-	-
Number of shoots	-	-	-	-	18.94	< 0.0001	0.01	NS
Interaction								
Treatment x Site	22.82	0.011	7.36	NS	22.31	0.014	32.18	0.0003

Table S 2. ANOVA table with F and P-values for seasonal growth (biomass) and fruit set (proportion of berries to flowers) in MeJA-treated and untreated neighboring (dist. 20–500) bilberry plants (*Vaccinium myrtillus* L.) across an elevational gradient (Low, Medium, and High) in Kaupanger, Western Norway, in two consecutive years (2016 and 2017)

	Growth				**Fruit set**			
	2016		**2017**		**2016**		**2017**	
Single factors	**Chisq**	**p-value**	**Chisq**	**p-value**	**Chisq**	**p-value**	**Chisq**	**p-value**
Treatment	130.34	< 0.0001	105.52	< 0.0001	361.92	< 0.0001	83.91	< 0.0001
Site	17.31	0.0002	7.33	0.02	146.75	< 0.0001	5.56	0.06
Covariates								
Plant size	27.76	< 0.0001	17.32	< 0.0001	-	-	-	-
Number of flowers	-	-	-	-	0.91	NS	54.65	<0.0001
Interaction								
Treatment x Site	28.18	0.002	14.19	NS	22.53	0.01	11.59	NS

ISBN: 978-82-575-1588-1
ISSN: 1894-6402

Western Norway
University of
Applied Sciences

P.O.Box 7030
NO-5020 Bergen, Norway
+47 55 58 58 00
https://www.hvl.no

Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no

102736 / ANDVORDGRAFISK.NO