

Sari Piippo

GRAZING TOLERANCE OF
BIENNIAL MEADOW PLANTS
IN RELATION TO RESOURCE
AVAILABILITY

FACULTY OF SCIENCE,
DEPARTMENT OF BIOLOGY,
UNIVERSITY OF OULU

A

SCIENTIAE RERUM
NATURALIUM



ACTA UNIVERSITATIS OULUENSIS
A Scientiae Rerum Naturalium 557

SARI PIIPPO

**GRAZING TOLERANCE OF BIENNIAL
MEADOW PLANTS IN RELATION TO
RESOURCE AVAILABILITY**

Academic dissertation to be presented with the assent of
the Faculty of Science of the University of Oulu for public
defence in Kuusamonsali (Auditorium YB210), Linnanmaa,
on 9 June 2010, at 12 noon

UNIVERSITY OF OULU, OULU 2010

Copyright © 2010
Acta Univ. Oul. A 557, 2010

Supervised by
Professor Juha Tuomi
Docent Annamari Markkola
Docent Pasi Rautio

Reviewed by
Doctor Mari Moora
Docent Päivi Tikka

ISBN 978-951-42-6210-4 (Paperback)
ISBN 978-951-42-6211-1 (PDF)
<http://herkules.oulu.fi/isbn9789514262111/>
ISSN 0355-3191 (Printed)
ISSN 1796-220X (Online)
<http://herkules.oulu.fi/issn03553191/>

Cover design
Raimo Ahonen

JUVENES PRINT
TAMPERE 2010

Piippo, Sari, Grazing tolerance of biennial meadow plants in relation to resource availability.

Faculty of Science, Department of Biology, University of Oulu, P.O.Box 3000, FI-90014 University of Oulu, Finland

Acta Univ. Oul. A 557, 2010

Oulu, Finland

Abstract

In this thesis I studied responses of three biennial, monocarpic plants *Erysimum strictum*, *Gentianella amarella*, and *G. campestris*, to various aspects in resource availability (*i.e.* competition, mineral nutrition, neighbor removal) and environmental stress (early frost) at adult or rosette stages and how these effects are related to grazing tolerance. I also studied how manipulations in resource availability affected arbuscular mycorrhizal colonization of the roots.

All three species were relatively tolerant to simulated grazing and in most cases plants were able to compensate quite well for minor biomass losses. According to the compensatory continuum hypothesis, tolerance is most pronounced in resource-rich conditions, but this was not always the case in the present experiments. *Erysimum strictum* compensated for defoliation at the rosette stage but the reproductive output of adult plants was reduced markedly in the next year. This reduction was strongest among fertilized plants. Moreover, apex removal at the adult stage resulted in overcompensation (*i.e.* clipped plants were more productive) but only in the absence of fertilization and in the presence of competition, which is against the compensatory continuum hypothesis. In *E. strictum* a potential cost of compensation appeared as delayed flowering and fruit maturation among clipped plants. However, in spite of early frost treatment clipped plants were still able to overcompensate. In *Gentianella amarella* and *G. campestris*, apex removal reduced growth and reproductive performance in most cases. Effects on root fungal parameters were positive or neutral. This pattern suggests that simulated above-ground herbivory tends to increase carbon limitation, and therefore regrowing shoots and the fungal symbionts may appear as alternative, competing sinks for the limited carbon reserves of the host plant.

Both shoot architecture and resource availability modify the responses of the study plants to apical damage at both rosette and adult stages. In addition, different environmental stress factors affect success in compensatory growth.

Keywords: compensatory continuum, compensatory growth, competition, herbivory, mycorrhiza, plant architecture, seminatural grasslands

To my loved ones

Acknowledgements

First of all, I would like to thank my supervisors Juha Tuomi, Annamari Markkola and Pasi Rautio. They have been very supportive and patient with me during all these years. Juha has been an encouraging supervisor who always was there for me and allowed me to take my time with this thesis. Annamari has been a valuable help with my work with mysterious AM fungi. I will never forget our joyful fieldtrips to Keminmaa, Oulanka and the Neidonkenkä-café. Pasi has helped me especially with my endless statistical and scientific problems. I want to thank other members of our research group as well. Many thanks to Ari-Pekka Huhta for sharing his knowledge about gentians and thanks to Kalle Hellström for help in the field and during my Ph.D. studies. Both of you have been great help for me.

I want to thank the personnel at the Department of Biology, at the Botanical Garden, at the Oulanka Research Station and at the Finnish Forest and Park Service. Many thanks to Tuulikki Pakonen, Satu Huttunen, Esteri Ohenoja, Annu Ruotsalainen, Tuomas Kauppila, Annikki Kestilä, Tuula Kangas, Pirkko Siikamäki and Anne Jäkäläniemi. Also, many thanks to the coauthors of my papers: Jouni Aspi, Tom Juenger, Esa Härmä and Mirja Saari. Thanks to reviewers Dr. Mari Moora and Doc. Päivi Tikka for valuable comments on my summary and on manuscripts. Thanks to Sirkka-Liisa Leinonen and Aaron Bergdahl for checking the language of my papers and summary. The study was financially supported by the Academy of Finland, the Jenny and Antti Wihuri Foundation, the Kone Foundation, the Oulanka Research Station Scholarship Foundation, the Oulu University Scholarship Foundation, Societas pro Fauna et Flora Fennica Foundation, the Oskar Öflund Foundation, Oulu University Grant for Postgraduate Education, the Faculty of Science and Natural History Society of Oulu.

I warmly thank my special friends at the Department of Biology. Huge thanks to Kati, Anu, Niina, Marika, Oili, Anna and Riikka. You have been very helpful and encouraging for me when I was not so sure of myself. If I forgot everything else about my years writing this thesis, I won't forget our times at Humus café and in several restaurants, theaters, etc., in Oulu. Special thanks to Anu for being a great roommate for many years, to Niina for sharing the painful and cheerful time of finishing this thesis and to Kati for being a supportive friend.

The most important group of people behind my thesis is my loved ones. Suuret kiitokset vanhemmilleni Tuulalle ja Sepolle. He ovat aina kannustaneet

minua opiskelemaan. Special thanks to my sisters, Virpi and Sirpa. They have always trusted me and supported me through days and nights with this project. Especially through nights via Skype... And they have shared many unbelievable moments during my free time as well! Thanks for the laughter we have shared during the last 35 years! And many thanks to the families of my sisters and of all my aunts and uncles. Special thanks to my aunt Terttu. Thanks to Jari's family: Airi, Mauri, Hanna, Markku and Juha with their families. You are very important people for me as well! I also want to thank the most important men in my life: Aapeli, Oskari and Jari. Though Jari surely has wondered at my research and writing habits and times, he always has been encouraging and supportive. Moreover, he has given countless hours of time to solve my unbelievable problems with computers. And the greatest thanks of all to my special little guys, Aapeli and Oskari. You have been a major reason and resource for me to finish this thesis. I love you guys!

Oulu, May 2010

Sari Piippo

List of original publications

This thesis is based on the following original papers, which are referred to in the text by their Roman numerals.

- I Piippo S, Huhta AP, Rautio P & Tuomi J (2005) Resource availability at the rosette stage and apical dominance in the strictly biennial *Erysimum strictum* (Brassicaceae). *Can J Bot* 83(4): 405–412.
- II Rautio P, Huhta AP, Piippo S, Tuomi J, Juenger T, Saari M & Aspi J (2005) Overcompensation and adaptive plasticity of apical dominance in *Erysimum strictum* (Brassicaceae) in response to simulated browsing and resource availability. *Oikos* 111(1): 179–191.
- III Piippo S, Hellström K, Huhta AP, Rautio P & Tuomi J (2009) Delayed flowering as a potential benefit-decreasing cost of compensatory regrowth. *Botany* 87: 837–844.
- IV Piippo S, Markkola A, Härmä E & Tuomi J (2010) Host and symbiont responses to grazing and competition in the intensively mycorrhizal meadow plant *Gentianella amarella*. Manuscript.
- V Piippo S, Huhta AP, Rautio P & Markkola A (2010) Grazing tolerance and mycorrhizal colonization: effects of resource manipulation and plant size in biennial *Gentianella campestris*. Manuscript.

Author's contribution: S. Piippo participated in establishing the experiments (I, III, IV & V), laboratory work (II, III, IV & V) and fungal studies (IV, V). She planned the experiments (I, III, IV & V) together with the research group. She performed the statistics in papers IV-V and part of the statistics in papers I & III. She was the responsible author for papers I and III-V, and participated in the writing of paper II.

Contents

Abstract	
Acknowledgements	7
List of original publications	9
Contents	11
1 Introduction	13
1.1 Tolerance to herbivory	16
1.2 Role of plant architecture and apical dominance in tolerance.....	19
1.3 Effects of resource availability on compensation.....	20
1.4 Mycorrhizal symbiosis and herbivory.....	22
1.5 Mycorrhizal symbiosis in gentians.....	23
1.6 Aims	24
2 Material and methods	29
2.1 Study species.....	29
2.2 Experimental design.....	30
2.2.1 Compensation studies with <i>Erysimum strictum</i>	31
2.2.2 Cost of compensation	31
2.2.3 Mycorrhizal and compensation studies with <i>Gentianella</i> <i>amarella</i> and <i>G. campestris</i>	31
2.3 Data analysis	32
2.3.1 Statistics	32
2.3.2 Selection gradient analysis	33
3 Results and discussion	35
3.1 Tolerance at rosette stage	35
3.2 Shoot architecture and cost of compensation at adult stage	36
3.3 Tolerance in relation to flowering time and plant size	40
3.4 Symbiont responses.....	41
3.5 Grazing tolerance in relation to resource availability	44
4 Conclusions	47
5 References	49
Original publications	57

1 Introduction

Traditional cattle grazing practices on meadows and pasture fields has almost vanished because of increased intensive farming (Pykälä 2000). As a result of ceased grazing, the number of plant species adapted to repetitive disturbance (*i.e.* loss of biomass) has decreased resulting from faster growing herbs and woody species gaining ground. Semi-natural meadows are mainly formed by traditional agricultural methods and nowadays they are listed as endangered habitats (Schulman *et al.* 2008). Lately the means of restorative ecology, such as grazing by cattle, has been increased as a management practice due to agricultural subsidies in Finland (Niemi 2009). Also mowing has been used to restore the lost biotopes and rare meadow species (Pykälä 2000, Tikka *et al.* 2001a, Hellström *et al.* 2003). In addition, road-side habitats with regular mowing as a routine management practice during last decades have also become very important refuges for several meadow plant species (Tikka *et al.* 2001b, Huhta & Rautio 2007). Yet these management practices have often been conducted without careful studies on the ecology of species and their adaptative strategies (Zobel *et al.* 2006).

Responses of meadow plants to management measures depend on two principal factors: their compensatory capacity (ability to replace the lost biomass by regrowth) and on their ability to compete with other plants (McNaughton 1979). Tall, usually perennial herbs have become more abundant in abandonment meadows because they are better competitors. When meadows are mown and/or grazed, tall herbs will suffer proportionally more than low growing plants that succeed better in these conditions (Huhta *et al.* 2000b, Hellström *et al.* 2003). High risk of biomass loss as well as competition are also factors which may have selected for very unbranched stem architecture in some meadow plant species. This feature may be associated with their regrowth capacity following damage to the shoot or apical meristem, *e.g.* because of herbivory (Paige & Whitham 1987, Crawley 1987, Vail 1992, Tuomi *et al.* 1994). Alternatively, the unbranched architecture of ungrazed plants may be associated with competition for light in dense vegetation and other selection pressures which favor fast vertical growth through apical dominance (Aarssen & Irwin 1991, Aarssen 1995). Rapid vertical growth is achieved by allocating resources to a single growing axis, rather than allocating limiting resources among multiple growing meristems. Plants will be released from this selection pressure in disturbed habitats when herbivores reduce the height and density of surrounding vegetation. Branched architecture then

again should be favored in environments of weak competition for light and nutrients (Bonser & Aarssen 1996, Duffy *et al.* 1999).

Different habitats are characterized by plants with distinct growth forms and habits. Annual plants complete their life cycles in a single year and they have only one chance to reproduce. Perennial plants live for many years and they are able to flower and produce seeds for many growing seasons. Biennials have a life cycle which lasts two years so they need to store energy and build up a strong root system in the first year, overwinter, and flower and produce seeds in their second summer (Fig. 1). After producing seeds, monocarpic (plants that produce fruit only once during their lives), biennial plants die. As herbaceous plants, annuals and biennials devote most of their energy to relatively rapid growth and production of flowers and seeds, whereas perennial plants usually delay reproduction and devote their first few years to production of energetically expensive tissue (Grime 2002). Several meadow plants are short-lived and poor competitors in resource-rich environments (Hellström *et al.* 2003).

Although the growing conditions in the second summer most strongly affect the shoot architecture of flowering biennial plants, architecture is already partially determined by their growth in the first year at the rosette stage. Herbivory at the rosette stage may affect apical dominance by reducing resources that rosette plants store in their taproots. Successful rosette establishment and growth are key factors determining the fitness of monocarpic plants since fecundity often correlates positively with rosette size (Young 1984, Simons & Johnston 1999, Buckley *et al.* 2003). Therefore, loss of photosynthetic biomass, due to herbivory, can be detrimental to the final reproductive success or fitness of monocarpic plants. Plants defoliated at the rosette stage are shown to grow more slowly and produce fewer flowers than undefoliated ones (Dhileepan *et al.* 2000), although tolerance to browsing at the flowering stage may not be directly comparable to their tolerance at the rosette stage.

In this thesis, I will study how different life stages of three monocarpic biennial plant species growing in human influenced habitats are related to availability of resources and various environmental strains. Shoot architecture, size, and ecotypes of these species are assumed to be main factors in the responses of plants to disturbance and fluctuation in resource availability.

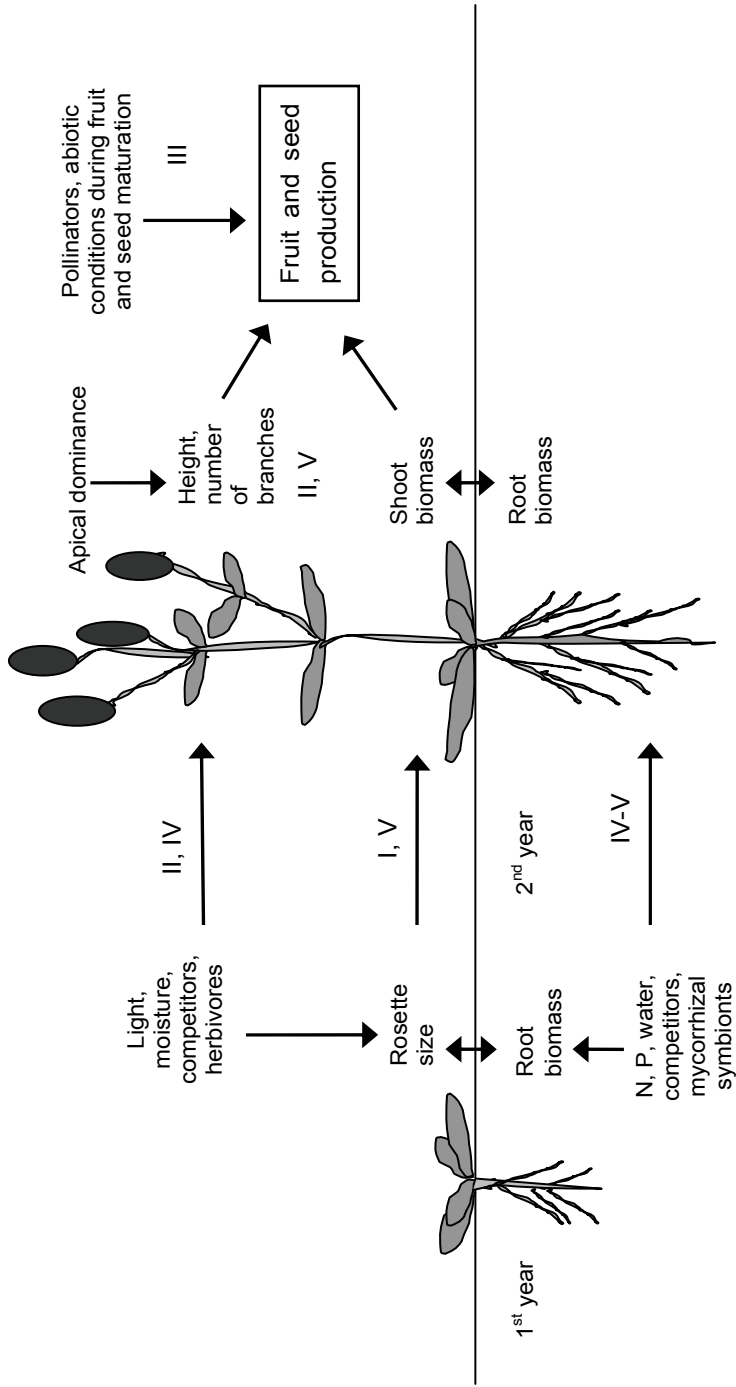


Fig. 1. Life-cycle of biennial, monocarpic plant. Roman numerals refer to papers in this thesis.

1.1 Tolerance to herbivory

Meadow plants have many kinds of biotic interactions with the surrounding environment. Usually herbivory is considered to be negative for plants but in some favorable conditions herbivory may even be beneficial to plants. Resistance and tolerance (Fig. 2) are two main antiherbivore strategies that allow plants to cope with herbivory (*cf.* Belsky *et al.* 1993, Juenger & Lennartsson 2000). Plant resistance reduces herbivore damage, while tolerance reduces the negative effects of damage (Stevens *et al.* 2007, but see Karban & Balwin 1997). Resistance traits include mechanical and chemical characters that deter herbivore feeding whereas tolerance mechanisms include increased photosynthetic activity, compensatory regrowth, utilization of stored resources, phenological changes, and mechanisms related to physiology and morphology at the time of damage (Tiffin 2002). Furthermore, there might be a trade-off between resistance and tolerance since high investment in resistance can reduce the resources available to compensate for herbivore damage (Rosenthal & Kotanen 1994, de Jong & van der Meijden 2000, Koskela *et al.* 2002). In addition, among plants with high resistance there should be weak selection for tolerance because they should experience minor biomass loss to herbivores (Fig. 2) and hence, weak fitness advantages to tolerance-related traits. On the other hand, other studies (*e.g.* Stevens *et al.* 2007) have not observed such trade-offs.

Tolerance depends on many factors, including the timing of herbivory in relation to the age and physiological stage of the plants (Bennett *et al.* 2009), the degree of damage in terms of the proportion of removed biomass, and the amount of resources available to repair the damage (Strauss & Agrawal 1999). A damaged plant alters its resource allocation, physiology and/or phenology to reduce the impacts of damage on growth and reproduction. Overcompensation is considered as a positive response to plants to injury, whereas undercompensation (no or partial compensation) can be viewed as a negative effect of herbivory on plants. When injured, overcompensating plants grow larger (vegetative overcompensation) and/or produce a greater number of mature fruits and viable seeds (reproductive overcompensation) in comparison with undamaged ones (Crawley 1987, Paige & Whitham 1987, Lennartsson *et al.* 1997, Huhta *et al.* 2000b).

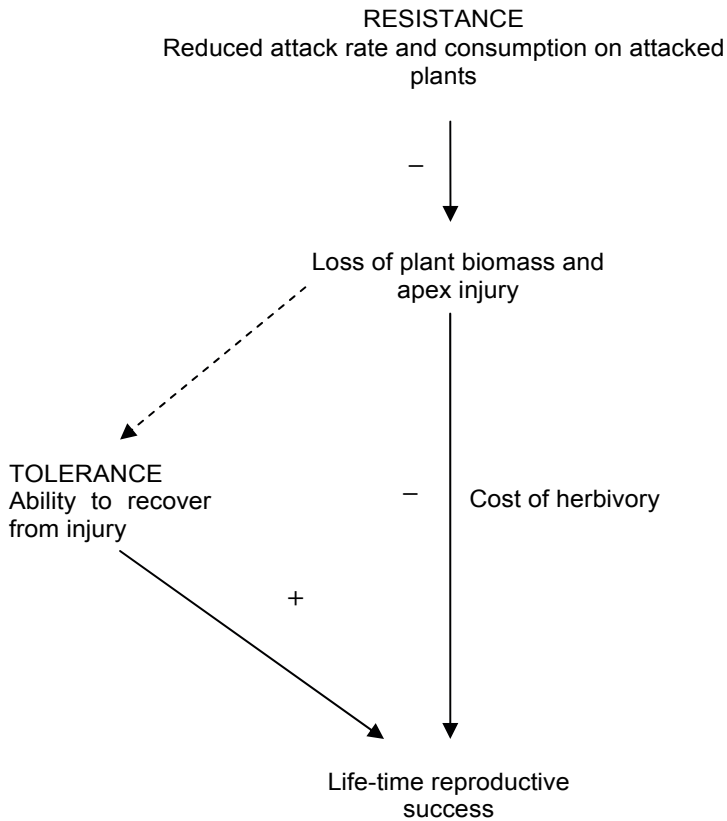


Fig. 2. Two antiherbivore strategies. Resistance traits reduce biomass loss and hence the cost of herbivory on plant life-time reproductive success. Biomass loss and /or apex injury, on the other hand, can trigger compensatory regrowth (broken line arrow) that contributes to plant tolerance, i.e. the ability to survive and reproduce in spite of grazing damage. The continuous line arrows indicate positive or negative causal relationships.

In biennial *Gentianella campestris* overcompensation has been observed in some late-flowering populations in grazed and/or mown habitats but not in the early-flowering populations when injured in the middle of the season (Lennartsson *et al.* 1997, 1998, Huhta *et al.* 2000b, Huhta & Rautio 2007). In contrast, early-flowering plants of *G. amarella* in northern Finland were able to compensate for the lost aboveground biomass and even overcompensate in terms of fruit

production (Huhta *et al.* 2003). Furthermore, in overcompensating plant populations, the responses vary greatly in relation to conditions (*e.g.* weather) during the growing season, as damaged plants may overcompensate in good years and perform poorly in bad years (Lennartsson *et al.* 1998, Levine & Paige 2004). Thus, plants are able to tolerate biomass loss if timing, degree of injury and favorable growth conditions coincide suitably (Järemo *et al.* 1999, Strauss & Agrawal 1999).

Monocarpic plants can tolerate or even overcompensate as a response to herbivory when damage takes place at the flowering stage (Paige & Whitham 1987, Paige 1994, Huhta *et al.* 2000c). Paige & Whitham (1987) studied *Ipomopsis aggregata* and suggested that there was a benefit to be eaten in terms of growth and reproduction, so herbivory can be beneficial when comparing fitness of grazed and ungrazed plants. According to the compensatory continuum hypothesis by Maschinski & Whitham (1989), overcompensation is expected to be most probable in conditions where competition is weak and the plants are growing in nutrient-rich conditions (but see Wise & Abrahamson 2005). According to this hypothesis, the probability of compensation for herbivory increases with increasing nutrient availability, since at high nutrient levels tissues can be replaced more easily and plants are able to grow faster after damage (Maschinski & Whitham 1989).

The ability to regrow might be a strategy for dealing with expected herbivory, and thus, a way to minimize the negative effects of damage (Crawley 1987, Vail 1992, Hicks & Turkington 2000). According to Huhta *et al.* (2000c) the overcompensatory response in *Erysimum strictum* might be an expression of the costs of apical dominance rather than adaptation to predictable damage because some of the populations able to overcompensate have not been regularly damaged. Lennartsson *et al.* (1997) showed that simulated grazing can positively influence the lifetime seed production of *G. campestris* and that the capacity for overcompensation appears to be an adaptive trait that has an evolutionary history related to habitats with a high and predictable risk of damage. Early flowering might be a strategy for avoiding mowing or herbivory (seeds mature before the damage), whereas late flowering plants use compensatory growth to get around herbivory-mediated damage (Lennartsson *et al.* 1997, Järemo *et al.* 1999).

Usually, damage bears costs on plant performance. Apex injury causes direct losses of the resources in the form of removed tissue, and indirect losses for the future photosynthetic capacity of the lost tissues (Benner 1988). The impact of these losses on fruit and seed production is likely to depend on the developmental

stage of a plant. The later in the season the damage occurs, the more harmful the damage often is as the loss of inflorescence at the bud stage represents a minor loss of resources compared to the loss of fully developed flowers or fruits (Ruiz *et al.* 2006).

Moreover, delayed flowering can be also understood as a cost of compensation if the phenological delay leads to adverse effects on the performance of damaged plants, for instance because of pollination failure. Furthermore, damage often delays seed maturation which may expose seeds to harsh autumnal conditions (*e.g.* early frost) (Lennartsson *et al.* 1998). In study of Huhta *et al.* (2009) with perennial *Pimpinella saxifraga* the regrowth followed by clipping caused delay in flowering, and therefore, the seed yield was reduced. Resource-rich conditions mediated the damages but delayed phenology decreased these benefits. This differs from the cost of compensation capacity on the performance of undamaged plants. For instance, a cost on undamaged plants could follow if individuals produce fewer fruits initially because they direct a higher proportion of total resources to ensure regrowth later on in the growing season (Vail 1992, Tuomi *et al.* 1994, Simons & Johnston 1999, Strauss & Agrawal 1999, but see Juenger *et al.* 2000).

I will test whether the compensatory responses of the study plants vary in accordance with the compensatory continuum hypothesis and whether there is a cost of compensation in terms of delayed flowering and, hence, higher susceptibility to early autumn frosts.

1.2 Role of plant architecture and apical dominance in tolerance

The architecture of plants is crucial when considering plant reactions to herbivory. If the architecture is strongly altered, plant reproduction can be markedly affected. Depending on the environmental conditions, as well as target and degree of herbivory, the effect can be favorable or unfavorable, and even overcompensation can sometimes occur (Escarré *et al.* 1996). These effects depend, on one hand, on how the environment affects plant height and lateral branching, and, on the other hand, how strongly plant reproduction depends on height and branch number or other features of plant architecture. Because of these relationships, resource availability, as well as damage caused by grazing, may impose selection on plasticity in plant growth architecture (Juenger *et al.* 2000).

Apical dominance refers to the preferential growth of a plant shoot from the apical or terminal meristem and the corresponding suppression of lateral subtending meristems and branches (Aarssen 1995). Strong apical dominance may be beneficial for plants growing in competitive environments but costly for the plants growing singly (Aarssen & Irwin 1991). The most obvious advantage of apical dominance is when plants are competing for light. Apical dominance incurs an apparent cost (*i.e.* reduced branch growth) which can transform into realized fitness costs (Aarssen 1995). The cost of apical dominance may be expressed as a higher production of viable seeds by branched than unbranched plants (Huhta *et al.* 2000a). Release from apical dominance can trigger vigorous branching which, in turn, can lead to enhanced flowering and eventually seed production, *i.e.* overcompensation (Aarssen 1995).

Unbranched forms of *Erysimum* may have a selective advantage when the gaps in the vegetation begin to close, while branched individuals do better in more open environments (Huhta *et al.* 2000c). Thus, vertical growth is favorable under a low grazing pressure with high and dense surrounding vegetation and low damage risk. On the other hand, the ability to produce compensatory branches is more important when grazing pressure is high, decreasing the intensity of competition for light and increasing the probability of more severe damage (Huhta *et al.* 2000a).

For compensatory regrowth a sufficient amount of undifferentiated meristems (for initiation of the development of branches) and sufficient resource pools (to support the initial growth of branches and the maturation of fruits and seeds) are required. To reach compensation in fruit production requires that reproductive effort should have a priority in the resource reallocation after damage, *i.e.* the developing fruits and seeds must provide sufficiently strong sinks for the limiting resources. If undamaged plants already have many branches, simulated browsing increases branching less or may even reduce the number of branches produced (Escarré *et al.* 1996, Huhta *et al.* 2000a).

As the extent of compensatory regrowth vary in different environments, I will study how plants respond to simulated grazing in both competitive environments and when neighboring plants are removed.

1.3 Effects of resource availability on compensation

Plants require basic resources (light, carbon dioxide, water, and inorganic nutrients) for growth, maintenance and reproduction (Wise & Abrahamson 2005).

Resource availability as such does not necessarily directly improve performance if the other environmental factors are not optimal. Resource availability (e.g. herbivory and fertilization) might have interactive effects on plant performance. The compensatory continuum theory by Maschinski & Whitham (1989) predicts that the more abundant the resources in an environment, the greater the compensatory potential should be. Further the compensatory continuum theory claimed that plants can tolerate herbivory best in resource-rich conditions. Supporting results have been found by studying the tolerance of *Ipomopsis arizonica* (Maschinski & Whitham 1989). Also the study of Huhta *et al.* (2000a) supported the compensatory continuum hypothesis: fertilization improved and competition reduced compensatory capacity of *Erysimum strictum*. On the other hand, plants have often been found to be less tolerant of herbivory in resource-rich environments than in resource-poor environments (Wise & Abrahamson 2005). Hawkes & Sullivan (2001) did not find strong support for the compensatory continuum hypothesis in their meta-analysis. In fact in their study dicot herbs and woody plants growing in resource-rich conditions were less tolerant of herbivory than those growing in resource-poor conditions (Hawkes & Sullivan 2001). Furthermore, in resource-poor conditions perennial plants may postpone their reproduction until the following growing season in response to grazing (Huhta *et al.* 2009) or, production of flowers and fruits may be supported by stored resources from previous years (Vallius & Salonen 2006).

Positive responses of plants to herbivory may be due to the enhanced nutrient cycling by herbivory. This positive response can occur under poor nutrient conditions and high nutrient recycling rates (Yamauchi & Yamamura 2004). Benner (1988) noticed that full compensation of *Thlaspi arvense* after apex removal occurred only when additional mineral nutrients were supplied early in growth and that the plants that received added nutrients produced more secondary branches and more fruits and seeds than unfertilized controls, whether they were apically damaged or not. According to Benner (1988) nutrient addition may have direct positive effects on branching, and it also allows plants to recover better from damage.

Plant size results largely from age and resource availability (Bonser & Aarssen 2003). Competition for mineral nutrients is often size symmetric (*i.e.* restricted resources are divided in relation to competitor size) (Schwinning & Weiner 1998). Usually allocation to active reproductive meristems and growth meristems increases with increasing plant size if larger size is due to greater resource (light and nutrient) availability. In resource-poor environments plants

may have insufficient resources to supply branching or reproduction, whereas plants allocating to growth, may have insufficient time for successful reproduction by the end of the growing season (Bonser & Aarssen 2003).

I will test how manipulation of resource availability (mineral nutrients and competition) will affect the performance of plants after simulated herbivory. Moreover, I try to find out if the responses of plants to these manipulations are different in large and small plants.

1.4 Mycorrhizal symbiosis and herbivory

Over 80 % of herb and grass species are arbuscular mycorrhizal (AM), *i.e.* their roots are colonized by Glomalean fungi forming inter- and intracellular structures (Smith & Read 2008). The plant receives nutrients and water from the fungi, and fungi get carbohydrates from the host plant. Moreover, mycorrhizae may provide protection against environmental stress, herbivores, parasites or pathogens (Bennett *et al.* 2006). From the plant's point of view fungi-plant relationship can be mutualistic, antagonistic, or neutral (Brundrett 2004, Jones & Smith 2004). In a mutualistic relationship both partners benefit, but in an antagonistic relationship the fungi reduces the yield and fitness of the host (Francis & Read 1995). Arbuscular mycorrhizal fungi are obligate symbionts and need carbon resources for several purposes: for their growth and metabolism, and for dispersal by their asexual spores. Stress and, *e.g.* herbivory, that the host plant experience can reduce the amount of carbon that the host is able to provide for fungi, which may in turn affect the amount of nutrients fungi is able to provide to the host (Bennett & Bever 2009). In addition to AM fungi, herbs and grasses commonly harbor dark septate endophytes (DSE) in their roots. These are considered as saprophytic but in some environments they may also function like mycorrhizal fungi (Jumpponen 2001).

Mycorrhizal colonization increases the carbon demand of the roots and attached fungal structures, which represent costs for the host plant (Jones & Last 1991). Costs may be 10–20 % of the current carbon assimilation (Smith & Read 2008). Basically mycorrhizal associations are beneficial (mutualistic) to plants when net costs are lower than benefits and detrimental (parasitic) when costs exceed benefits. Fertilization alleviates nutrient acquisition of plants and thus diminishes relative benefits of mycorrhizal associations and this, in turn, can switch a mutualistic association to parasitic or decrease colonization in roots.

Factors that limit photosynthate production, *i.e.* reduced irradiance and herbivory, may generate parasitic associations between fungus and plant by increasing relative costs (Johnson *et al.* 1997). The intensity of AM fungal colonization has been reported to decline in relation to natural or simulated herbivory in 21 of 35 studied plant species due to decreased carbon flow to the fungal partner (Gehring & Whitham 1994). The effect was neutral in 10 species, positive in two species and variable in two species. More recently, effects of herbivory on mycorrhizal colonization have been reported to vary from negative (Gehring & Whitham 2002), to neutral (Lugo *et al.* 2003, Pietikäinen *et al.* 2005) or positive (Kula *et al.* 2005, Wearn & Gange 2007).

Genotypes of the involved plants and fungi determine the potential function of the symbiosis. Plant taxa vary in mycorrhizal dependence and fungal taxa vary in mycorrhizal effectiveness (Johnson *et al.* 1997). For instance, mycorrhizal colonization appeared to be of great benefit to *Plantago lanceolata*, but detrimental to *Senecio jacobaea* during insect herbivory (Gange *et al.* 2002). Plant responses to herbivory depend upon the mycorrhizal fungal symbiont as well (Bennett & Bever 2007) and different mycorrhizal fungal species also result in varying allocation to plant reproduction (Klironomos *et al.* 2004, Bennett & Bever 2009).

I will test how the intensive fungal colonization in gentians responds to simulated herbivory and nutrient manipulation.

1.5 Mycorrhizal symbiosis in gentians

Arbuscular mycorrhizal colonization in plant roots have traditionally been classified into two morphological types, Arum and Paris types. The Paris type of structure is defined by the absence of the intercellular phase and presence of extensive intracellular hyphal coils. Coils are convoluted or branched hyphal structures and arbuscules are intercalary densely branched structures on the coils (Smith & Smith 1997). The Arum type is defined on the basis of an extensive intercellular phase of hyphal growth in the root cortex and development of terminal arbuscules on intracellular hyphal branches. Both types are common among plant taxa and intermediate types also occur (Dickson *et al.* 2007). Mycorrhizal colonization in Gentianaceae exhibits a Paris type of morphology (Jacquelinet-Jeanmougin & Gianinazzi-Pearson 1983). Gentians become mycorrhizal (usually hyphal colonization) 7–10 days after germination, coils and arbuscules begin to appear about two weeks after germination and vesicles later.

The possibility of carbon movement between plants linked by AM fungi is widely recognized (Hirrel & Gerdemann 1979, Francis & Read 1984, Watkins *et al.* 1996). When plant individuals are linked by a shared hyphal network, the issue of cost and benefits encountered by an individual plant may become more complicated (Francis & Read 1984, Newman *et al.* 1992). In addition, effects of mycorrhiza on host plants and competitive relationships differ depending upon the density of neighboring plants and host plant species (Hartnett *et al.* 1993). The main means of AM colonization in the field may be probably via root-to-root contacts between gentians and neighboring perennial plant species (Gay *et al.* 1982). Some genera of the Gentianaceae (*e.g.* *Centaurium*, Gay *et al.* 1982) require repeated inocula from the roots of other AM plants and this is connected with the survival of these species in closed meadow vegetation. Some achlorophyllous genera in the family (*e.g.* *Voyria* spp., Imhof 1999) are fully mycoheterotrophic and they receive their carbon and nutrients from other plants via a mycorrhizal hyphal network.

By far, partial mycoheterotrophy (or mixotrophy) has been found in photosynthesizing plant taxa obtaining a considerable part of their carbon resources from a green host via ectomycorrhizal fungal hyphae. Common partial mycoheterotrophs include, *e.g.* evergreen shrub-like pyroloids, such as genera *Pyrola* and *Orthilia* (Tedersoo *et al.* 2007). Recently, Merckx *et al.* (2009) suggested that partial mycoheterotrophy could possibly be found in AM plants, if families including taxa with total mycoheterotrophy, such as Gentianaceae, were examined. Actually, the genus *Gentianella* has already been suggested to be partially mycoheterotrophic by Karlsson (1974).

I will study if removal of neighboring plants affects fungal colonization of gentians.

1.6 Aims

In this thesis, I have studied three biennial monocarpic plants growing in human-influenced habitats and their responses to simulated grazing. The study plant *Erysimum strictum* originally grows on sandy and gravely sea- and riversides but increasingly in human-influenced habitats, such as dry meadows and railroad embankments (Ahti 1965). *Gentianella amarella* and *G. campestris* grow on semi-natural meadows and pastures and nowadays on roadsides. I tested the tolerance (I–V) and mycorrhizal colonization (IV–V) of the species in relation to leaf damage at the rosette stage (I) and simulated grazing at the flowering stage

(II–V). In addition, I was interested in how the effects of simulated grazing on plant performance interact with plant resource availability (Table 1, Fig.1).

I studied how resource availability (mineral nutrient availability, manipulated by fertilization (I, II), and competition (II)) affected rosette and shoot architecture of *Erysimum strictum* and how those effects are related to tolerance. I expected that the size and architecture of flowering plants in the second year could partially be determined by their growth and size in the first year at the rosette stage (I). Study II is based on an experiment in which plants were grown with or without supplemental fertilizer and either with or without a tall competitor. I tested whether the shoot architecture and seed production of *E. strictum* responds to changes in resource availability as expected by the compensatory continuum hypothesis.

Compensatory growth following apical damage is often related to delay of flowering. In study III I tested the potential costs of delayed flowering with *Erysimum strictum*. I simulated herbivory either before flowering or after flowering had started, after which part of the plants were subjected to early night frost. I expected that the plants with no frost treatment could compensate fully for the 25% loss of stem height in vegetative parameters. I assumed that compensatory growth would delay fruit maturation and that the frost treatment would adversely affect fruit and seed production, being more pronounced among the clipped plants, but not among intact plants. Hence, I expected to find a clipping \times frost interaction effect on fruit and seed production.

I tested the effects of simulated herbivory (IV, V), nutrient manipulation (V), and removal of neighboring plants (IV) on the vegetative and reproductive performance of the grazing-tolerant grassland biennials *Gentianella amarella* and *G. campestris* and on fungal colonization in their roots. Estimating fungal colonization is a new perspective compared to many earlier compensatory continuum studies. Furthermore, I studied how timing of clipping (IV) or size (V) and ecotype (flowering time) (IV) of plants affect plant responses to resource manipulations. In study IV, two simulated herbivory treatments were conducted: in 1998 treatment with late-flowering *G. amarella* and in 2000 separately with early- and late-flowering *G. amarella*. Additionally in 2001 repeated removal of vegetation surrounding *G. amarella* individuals was conducted. In study V, herbivory was simulated by clipping and at the same occasion the plants were fertilized. I expected that the plants could compensate for the biomass loss in vegetative parameters and that the fertilization would improve the fitness of the

plants. I assumed that both clipping and fertilization would decrease the mycorrhizal colonization.

As a whole, in this thesis I wanted to clarify how both above-ground parts and fungal colonization of monocarpic biennial plants react to fluctuation in availability of resources and various environmental stress factors in different stages of their life cycle. In addition I tried to determine how the original architecture, size, and ecotypes of study plants affect their responses to these manipulations.

Table 1. Experimental design of my studies I-V. I have both common garden and field experiments at three different study sites. I examined how the manipulations of resource availability (clipping, fertilization, competition, neighbor removal) or stress factor (early frost) affect the vegetative and reproductive performance of the study plants and mycorrhizal colonization of gentians. Furthermore, I studied how time of clipping or size and ecotype of plants affect plant responses to these manipulations.

Study	I	II	III	IV	V
Study site	Oulu	Oulu	Oulu	Kuusamo	Keminmaa
Type of experiment	Common garden	Common garden	Common garden	Field (road-side)	Field (meadow)
Clipping	50 % of rosette leaves	25 % of adult height	25 % of adult height	50 % of adult height	25 % of adult height
Other treatments or grouping factors	Fertilization	Competition and fertilization	Frost treatment	Timing of damage, neighbor removal, ecotype of plant	Fertilization, plant size
Response variables	Vegetative and reproductive performance	Vegetative and reproductive performance	Vegetative and reproductive performance	Vegetative and reproductive performance, mycorrhizal colonization	Vegetative and reproductive performance, mycorrhizal colonization

2 Material and methods

2.1 Study species

All three study species are monocarpic, strictly biennial herbs. *Erysimum strictum* is generally considered to be a quite common, non-mycorrhizal plant in Finland, whereas gentian species, *Gentianella amarella* and *G. campestris*, are rare and threatened, intensively mycorrhizal meadow plants found only in less than 50 restricted populations in Finland.

Tall wormseed mustard, *Erysimum strictum* P. Gaertn., B. Mey. and Scherb. (Brassicaceae) (syn. *E. hieracifolium* auct., *E. virgatum* Roth), is a 50–100-cm-tall, biennial herb. Seeds germinate in spring and develop into a rosette during the first summer, and the plant flowers during the second summer. The second-year shoot usually has one unbranched stalk, but plants sometimes branch vigorously (Huhta *et al.* 2000a,c). *Erysimum* species are pollinated by bumblebees, beetles, bees, sirphids, and flies (Gómez 2003). The flowering time is from mid-June until the end of July, and after reproduction the plant dies. Because *E. strictum* is a monocarpic species, seed production in the year of flowering is a good estimate of lifetime reproductive success. In Finland, *E. strictum* grows on sandy and gravelly sea- and riversides and, increasingly, in human-influenced habitats, such as dry meadows and railroad embankments (Ahti 1965). Plants may be subject to minor insect herbivory, or to mammalian herbivory by hare, reindeer, or moose, especially in their respective primary habitats. *E. strictum* studies were carried out in the common garden of the Botanical Gardens of the University of Oulu.

The autumn gentian, *Gentianella amarella* (L.), Börner, and the field gentian *Gentianella campestris* (L.), Börner, are biennial meadow plants (Hultén & Fries 1986, Lennartsson *et al.* 1997, Huhta *et al.* 2003) occurring in temperate and cool regions. As meadow habitats have decreased during the past few decades, populations of *G. amarella* and *G. campestris* have declined (Lennartsson & Oostermeijer 2001, Huhta *et al.* 2000b, 2003) and both species are now endangered. In both species seeds germinate in spring and the plants form a vegetative rosette during the first summer. The taproot is the overwintering form and the plant flowers in the second summer. Both gentians include early- and late-flowering ecotypes, which flower either in June-July (early-flowering type, *G. amarella* var. *lingulata*; *G. campestris* var. *suecica*) or August-September (late-flowering type, *G. amarella* var. *amarella*; *G. campestris* var. *campestris*)

(Hämet-Ahti *et al.* 1998). Gentians are pollinated by bumblebees (*pers. obs.*) although at least field gentian is highly selfing and can produce about 80–95% seed set without pollinators (Lennartsson *et al.* 2000). Both gentian species usually are 10–30 centimeters tall when flowering and they have 3–8 internodes (Hämet-Ahti *et al.* 1998). Branches normally grow from the second to fourth nodes and can produce 10–20 flowers. *Gentianella* spp are known to have bitter-tasting defensive chemical compounds against herbivory (Janković *et al.* 2005). In spite of this, gentian individuals will occasionally become either grazed (13–40 % of *G. campestris* individuals) or mown (over 80% of individuals being injured) in traditionally managed meadows (Lennartsson *et al.* 1997). Especially the late-flowering ecotypes are able to recover from certain damages, for instance mowing and grazing (Lennartsson *et al.* 1998, but see Huhta *et al.* 2003). The habitat preference is semi-natural meadows and pastures (from dry to mesic) and nowadays roadsides. The study population of autumn gentian is located in Kuusamo, Liikasenvaara and that of field gentian in Keminmaa, Finland.

2.2 Experimental design

In this thesis, I examined how the manipulations of resource availability and stress factors affect the vegetative and reproductive performance of the study plants and mycorrhizal colonization of gentians (Table 1). As a resource manipulation I used clipping (I–V) of rosette leaves or shoot, fertilization (I, III, V), competition (III), and removal of neighboring plants (IV). Early frost was used as a stress factor in study III. Furthermore, I studied how time of clipping (IV) or size (V) and ecotype (IV) of plants affect plant responses to these manipulations.

In all the studies I measured both vegetative and reproductive parameters of plants after harvesting. I measured height, biomass (I–IV), number of branches, fruits and seeds, biomass of roots (I–III) and seeds (I–IV) (Table 1). In study III, I measured also germinability of seeds.

I measured fungal parameters in the gentian studies (IV–V, Table 1). I estimated root colonization by arbuscular mycorrhizal (AM) structures (hyphae, arbuscules, coils, vesicles) as well as dark septate endophyte (DSE) colonization. In the *G. amarella* study I measured the fluctuation of fungal colonization during the second growing season as well (IV).

2.2.1 Compensation studies with *Erysimum strictum*

In the first study *E. strictum* rosettes were defoliated twice and fertilized three times. Newly emerged rosette-stage plants of similar size were transplanted from a gravel roadside into a common garden in mid-June 2001. In the defoliation treatment, half of the rosette leaves were removed with scissors immediately after planting and a second defoliation was performed in early August 2001. Fertilizer was applied three times immediately after the defoliations and once in mid-July.

The second study with *E. strictum* in a common garden was conducted by removing 25% of shoot height, adding supplemental nutrients, and growing plants in competition with *Anthriscus sylvestris*. One-year-old rosette-stage plants were transplanted from a natural habitat into a common garden in mid-June 2001. In half of every planting bed, seedlings of *A. sylvestris* were transplanted around every *E. strictum* individual and half of the plants were fertilized. The presence of *A. sylvestris* resulted in intensive competition for light and nutrients. The remaining half of each planted bed was weeded weekly to keep the conditions competition free.

2.2.2 Cost of compensation

The potential costs of delayed flowering were tested with adult plants of *E. strictum* (III). The plants were collected in early June 2001 in their second growing season and transplanted into the common garden. Herbivory was simulated by removing 25% of the shoot height with scissors either before flowering or after flowering had started. Later on, in August-September, some of the intact and damaged plants were subjected to artificial night frost in climate chamber 3 weeks earlier than normal.

2.2.3 Mycorrhizal and compensation studies with *Gentianella amarella* and *G. campestris*

The effects of simulated herbivory and removal of neighboring plants on the AM fungal colonization of *G. amarella* were tested (IV). In autumn 1998, gentian individuals from the late-flowering population were selected pairwise for clipping and control treatments. The clipping was performed in early August 1998 by removing *ca.* 50% of the shoot biomass. The effects on plant performance and root colonization by fungi were studied on two occasions: 20 days after clipping

and 50 days after clipping. In the 2000 experiment autumn gentian individuals were clipped and collected from early-flowering and late-flowering populations separately. To obtain the data regarding how colonization varies during the growing season the colonization percentages of intact plants were measured three times per growing season. In 2001, a neighbor removal experiment in the same late-flowering population was conducted. In mid-June the surrounding vegetation of 15 gentians was removed and 15 gentians served as controls. The removal treatment was repeated three times during the growing season, and the plants were harvested in late August.

Effects of host size, simulated herbivory and fertilization on the vegetative and reproductive performance of *Gentianella campestris* and on its arbuscular mycorrhizal colonization were studied (V). Herbivory was simulated by removing 25% of the shoot of small and large plants with scissors in mid-June before flowering had started. Fertilizer was applied directly to the base of each plant after clipping.

In both the experiments relating to mycorrhiza (IV–V), plants were collected using a shovel so that *ca.* 1–1.5 liter of compact soil cake was taken with each plant, and the whole plants with soil were stored in a cold room 1–7 days before washing. Roots were gently washed and collected carefully under dissection microscope, after which they were stored in 50% ethanol. Roots were stained with trypan blue (Phillips & Hayman 1970), and their fungal colonization percentages were determined with magnified intersect method under a light microscope (McGonigle *et al.* 1990, magnification of 100–400). Total fungal colonization percentage as well as colonization of arbuscular mycorrhizal (AM) structures, *i.e.* hyphae, arbuscules, coils, and vesicles as well as dark septate (DSE) endophytes, were determined

2.3 Data analysis

2.3.1 Statistics

In study I, the data was analyzed by means of factorial ANOVA with defoliation, fertilization, and the defoliation \times fertilization interaction as fixed factors. Variables were log-transformed if the assumptions of normality or homogeneity of variances were not met. The relationships between plant height, total plant biomass, total branch number, and seed number per plant in the second year

(dependent variables) with rosette diameter in the first year (explanatory variable) were analyzed with linear regressions.

In study II, the response variables from the study were analyzed using a split-plot ANOVA approach. Planting bed was considered as a blocking factor. Competition (within each block) was considered as the whole plot term, and each whole plot was further divided into subplot treatments (fertilization, clipping and their combination).

In study III, the effects of clipping and frost treatment on vegetative and reproductive parameters were tested by two-way factorial ANOVA with clipping, frost treatment and their interaction as fixed factors. Differences in the frequency of intact and early- or late-clipped plants at different phenological stages were analyzed by means of a *G*-test. Mortality in different clipping treatments was tested with the *G*-test separately for observations done before mid-July and for those done after that.

In study IV, the results were analyzed using the clipping treatment and the time of collection as fixed factors in a two-way ANOVA or in the case of the neighbor removal experiment, using the paired t-test. In study V, the results were statistically analyzed using the clipping treatment, fertilization and size group and their interactions as factors in a three-way ANOVA. Variables were log-transformed if the assumptions of normality or homogeneity of variances were not met.

Statistical analyses were performed with different versions of SPSS for Windows.

2.3.2 Selection gradient analysis

In study II, the strength and pattern of selection was explored on plant architectural traits using standard phenotypic selection analyses. Directional selection on the measured plant characters was examined with multiple linear regression. Partial regression coefficients β_H and β_B indicate the strength of directional selection on plant height and branch number, respectively. Stabilizing, disruptive and correlational selection was evaluated by multiple quadratic regression (Phillips & Arnold 1989, Brodie *et al.* 1995). As fitness measures related to Fig. 3 (see below), total seed number per plant and total seed mass per plant (II, Table 3) were used.

3 Results and discussion

Below, I will discuss tolerance of *Erysimum strictum* in relation to leaf damage at the rosette stage and simulated grazing at the flowering stage. Moreover I will consider tolerance and mycorrhizal colonization in relation to flowering time and plant size of *Gentianella amarella* and *G. campestris*. Finally, I will consider how the effects of simulated grazing on plant performance interact with plant resource availability.

3.1 Tolerance at rosette stage

The effects of resource manipulation on the reproductive output of *E. strictum* were different at rosette and adult stages. First, apical damage of mature adult plants often stimulates lateral branching and, hence, increases fruit and seed production (II–III, Huhta *et al.* 2000a), whereas rosette defoliation reduced branch production and, consequently, fruit production (I, see also Suwa *et al.* 2010). Second, rosette fertilization increased both basal and upper branchiness (I), but in adult plants fertilization usually increases and competition decreases the number of branches in the middle and top parts of the stem (Huhta *et al.* 2000a). Moreover, fertilization at the rosette stage stimulated upper branches only among undefoliated plants. Branch number and reproductive output in mature plants therefore correlate with resource availability and growth conditions at both the rosette and adult stage. This gives support to the results of Dhileepan *et al.* (2000) that final adult-stage architecture and reproductive output of a plant is partially already affected by herbivory at the rosette stage.

The size of rosette is important when considering the lifetime fitness of a plant since resources stored in taproots during the rosette stage affect apical dominance at the adult stage (Dhileepan *et al.* 2000). Usually, lifetime fecundity and fitness of monocarpic species correlate positively with rosette size (Young 1984, Simons & Johnston 1999, Buckley *et al.* 2003). In study I, the performance of adult, flowering plants showed positive dependence on rosette diameter in most cases. Rosette defoliation reduced reproductive output more strongly in large plants and fertilization intensified these effects. Presumably, rosette defoliation caused a higher cost on fertilized plants because defoliation reduces the ability of plant to utilize additional nutrients or because defoliation may have suppressed the development of axillary meristems. Apical dominance suppresses meristems

in the rosette leaf axils but nutrient surplus may break this suppression and cause basal branching.

Small and large plants may respond differently to nutrient surplus (*e.g.* when growing in a nutrient-rich patch). After defoliation, large plants are expected to increase branch production, whereas small plants are expected to respond by increased height to reach the height of the competing sward. Large plants should rather invest their additional resources to improve branch and flower production. Large size and abundant flower production are impending, since they have positive effects on plants due to increased pollinator attraction (Vallius & Salonen 2006). Furthermore, competition for mineral nutrients is usually size symmetric (*i.e.* resources are divided in relation to plant size) whereas light competition is size asymmetric (*i.e.* a large plant obtains a higher proportion of light resource related to its size) (Schwinning & Weiner 1998). However, large size may also imply some costs if, *e.g.*, the risk of grazing increases with increased size (Ehrlén 1997, Gómez 2003).

In conclusion, branch number in mature *E. strictum* plants is a response to resource availability and growth conditions at both the rosette and adult stage. The leading stalk suppresses axillary meristems at the rosette base but nutrient-rich conditions during the rosette stage can break this suppression. Large plants are expected to increase branch production, whereas small plants suffering from competition for light are expected to increase height growth after defoliation.

3.2 Shoot architecture and cost of compensation at adult stage

In study II, *Erysimum strictum* plants were able to compensate or even overcompensate for shoot apex injury at the adult stage in most cases. Clipping and fertilization positively affected most growth and reproductive parameters whereas competition had a negative effect on the number of branches and stem biomass. The greatest fitness overcompensation was found in the absence of fertilization and in the presence of competition, contrary to the compensatory continuum hypothesis (Maschinski & Whitham 1989) but in accordance with Hawkes & Sullivan (2001).

Because competition reduced and fertilization increased the number of branches, it is likely that resource deficiency suppressed lateral branching. However, as overcompensation was associated with resource-poor environments, the compensation to simulated herbivory was primarily not determined by nutrient resources, but rather by meristem limitation as in *Gentianella* plants in

the study of Juenger *et al.* (2000). Therefore compensatory response was more dependent on number of available meristems than amount of nutrient resources. Resource availability thus affected compensation ability more strongly through phenotypic plasticity. The result is consistent with the corresponding changes in shoot architecture, as suggested by Escarré *et al.* (1996).

The responses of plants to herbivory are not only related to the architecture of the plants but the timing of damage and the phenology of the plant as well (Escarré *et al.* 1996). Simulated grazing is known to increase lateral branching in growth conditions where undamaged plants have few branches (Huhta *et al.* 2000a). In study II we predicted selection for increased vertical growth among plants grown in competition and for increased branching among plants grown without competition. Indeed, when fitness was measured as seed number per plant, results partially followed this prediction. There was stronger directional selection for increased height than branching among clipped plants grown in competition (Fig. 3a). However, among intact plants selection for increased branching was always stronger or equally strong as compared to selection on plant height (Fig. 3 b). As intact and apically damaged plants have made their investments in vertical growth and lateral branching at different phenological states, the shading effect by *Anthriscus* may have been stronger during the regrowth period of apically damaged plants. These patterns were somewhat similar but less clear when total seed mass was used as the fitness measure (Fig. 3c & 3d, II: Table 3). The difference between the fitness parameters could be due to a decrease in individual seed weight among short plants in some treatment combinations. The actual importance of this difference is not, however, clear because lighter seed weight does not necessarily mean decreased seedling success (Reader 1993).

It is known that herbivory affects the balance between vegetative and reproductive meristems (McNaughton 1979). For compensatory regrowth plants needs a sufficient amount of undifferentiated meristems and sufficient resource pools for growth and reproduction. After damage, the reproductive organs should be the most important sinks in resource allocation in monocarpic herbs (Huhta *et al.* 2000a). It is noteworthy that typically the increase in plant size increases the absolute number of meristems. According to Bonser & Aarssen (2001) meristem allocation is size-dependent: allocation to active reproductive and growth meristems is greater in larger plants than in smaller plants.

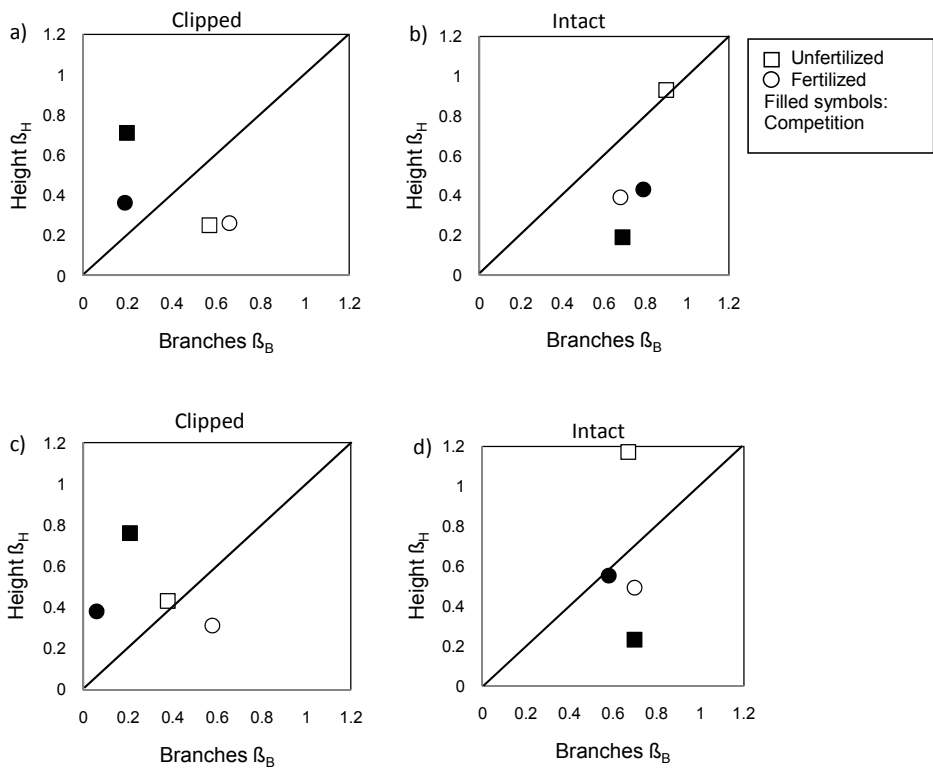


Fig. 3. Directional selection gradients (β) in *Erysimum strictum*. Predictions: there is selection for increased vertical growth among plants grown in competition ($\beta_H > \beta_B$) and for increased branching without competition ($\beta_H < \beta_B$). Total seed number per plant (a-b) and total seed mass per plant (c-d) were used as fitness measures. Open symbols: plants grown without competition. Filled symbols: plants grown in competition. Square: unfertilized plants. Circle: fertilized plants. Results: a) There was stronger directional selection for increased height than branching among clipped plants grown in competition. b) Among intact plants the selection gradient on number of branches was always larger than or equally large as the selection gradient on height. c) Among clipped plants in competition, the selection gradient on height was always larger than the selection gradient on increased branching. d) There was stronger directional selection for increased branching than height among intact plants grown in competition.

Removal of the shoot apex of adult *E. strictum* improved most of growth and reproductive parameters (III). In other words, plants were able to overcompensate for apical damage as clipped plants produced more than twice the number of germinable seeds compared to intact plants. However, the potential cost of compensation appeared as delayed flowering and fruit maturation among clipped plants. Frost treatment reduced final total biomass but did not significantly weaken their compensatory growth or germinability of seeds. Apical damage itself slightly decreased seed weight and germinability, but these effects were weak in comparison to improved fruit set that largely determines the final seed set and, hence, the number of germinated mature seeds per plant.

Early in the growing season there might be selection for fast vertical growth and unbranched shoot architecture, especially in dense vegetation as with *E. strictum* in natural populations (Huhta *et al.* 2000c). According to Benner (1988), by clipping plants early, buds developing into branches are removed, whereas with late apex removal the apical tissue with only a few branch buds is removed. Moreover, delayed fruit maturation may lead to a higher proportion of immature fruits at the end of the growing season (Lennartsson *et al.* 1998). Delayed flowering is a quite common consequence of apical damage (III, Bergelson & Crawley 1992, Lennartsson *et al.* 1998, Martínková *et al.* 2008, Ramula 2008, but see Marshall *et al.* 2008). For instance, in the perennial *Pimpinella saxifraga*, compensatory growth after clipping caused a delay in flowering, and hence, the seed yield was reduced (Huhta *et al.* 2009). On the other hand, according to Huhta *et al.* (2000c) minor damage (10% clipping) on *E. strictum* resulted in only a slight delay in flowering but a marked decrease in seed viability in the clipped plants, possibly because of increased self-pollination.

The most important results of these studies (II–III) are that the greatest overcompensation in *E. strictum* was found in the competitive nutrient poor environment, contrary to the compensatory continuum hypothesis. Therefore the compensation for simulated herbivory was not primarily determined by nutrient resources, but rather by meristem limitation. In addition, the cost of compensation in *E. strictum* appeared as delayed flowering and fruit maturation among clipped plants. Despite this delay, frost treatment did not significantly weaken their compensatory growth or germinability of seeds. Thus, early night frosts are a potential risk to monocarpic herbs recovering from damage, but other sub-optimal environmental conditions may be a greater threat for early-flowering plants recovering from grazing.

3.3 Tolerance in relation to flowering time and plant size

In *Gentianella amarella* apex injury reduced plant performance in most cases (IV). However, early-flowering gentians completely compensated for the biomass loss in 2000. In 1998 apex injury strongly reduced vegetative and reproductive performance of late-flowering plants.

In 2000 both apex removal and flowering time of a *G. amarella* population had a remarkable effect on vegetative and reproductive performance of plants. Firstly, almost all the growth and reproductive parameters of intact plants were higher in late-flowering populations compared to early-flowering ones. Secondly, the early-flowering population compensated for clipping but the late-flowering population did not. In earlier studies with *G. campestris* it has been found that the damage can induce vigorous branching in gentians (Lennartsson *et al.* 1997, 1998) and that *G. campestris* compensated well for minor damages (Huhta *et al.* 2000b). In Huhta *et al.* (2003), *G. amarella* could compensate both in southern and northern populations in terms of above-ground biomass but overcompensate in number of fruits only in northern populations. According to Lennartsson *et al.* (1998), overcompensation should not be expected to be found at high altitudes or latitudes with a short growing season. Variation in growth and compensatory responses may reflect differences in the amount of resources for growth and regrowth. Early-flowering plants have a shorter time period for growth before damage than late-flowering plants (Huhta *et al.* 2000c), whereas late-flowering plants have less time to recover from damage. Another possible explanation for the overcompensation capacity of gentians is grazing history if grazing or mowing has favored overcompensating genotypes in gentians. In my study populations, there may not have been a history of constant grazing or mowing and therefore no need for adaptation to predictable damage (Huhta *et al.* 2003).

In the *Gentianella campestris* experiment (V) with two size groups, plant performance (vegetative and reproductive parameters) was highest in large plants, as was expected. Moreover the responses to clipping and fertilization were not always the same in different size groups. Apical damage caused an allocation shift from height growth to branches in all the groups except in large and fertilized plants, a finding which is against the compensatory continuum hypothesis. Clipping did not have the strongest effect on small plants because they were able to compensate for the lost biomass in terms of number of branches and the number of seeds per plant. A reason for that may have been that large plants may have already used most of their meristems before clipping and fertilization (II,

Escarré *et al.* 1996, Huhta *et al.* 2000a). Alternatively, damaged plants cannot utilize fertilization right after damage (I) but then again, for some reason, small plants were able to compensate in a comparable condition. In large plants resources stored in the taproot enhance the growth right from the beginning of the growing season (Lennartsson *et al.* 1997) and, hence, the nutrient addition possibly came too late for large plants, since the number of meristems could have already been determined before the addition. Small plants usually live in more resource-poor patches (Lennartsson *et al.* 1997). In dense vegetation, on the other hand, they suffer from light and nutrient limitation due to their weaker competitive ability in relation to taller neighbors. (Irwin & Aarssen 1996).

Gentianella plants are known to experience some level of meristem limitation (Juenger *et al.* 2000) so that at the beginning of the growing season the compensation capacity may be constrained by the amount of resources available for regrowth whereas the late limit of compensation may be affected mainly by the availability of undifferentiated meristems (Lennartsson *et al.* 1998). Increased branch production in *G. campestris* (V) was not reflected directly in seed production but in the treatment combinations where there was a tendency for increased branch production, damaged plants were able to reach the level of seed production of intact plants.

In summary, different results in compensatory responses in large and small plants may be due to the number of inactive meristems or in the amount of resources (light, nutrients) for growth and regrowth. The ecotype of plants affects regrowth as well because there may not be enough time or resources to recover.

3.4 Symbiont responses

In *G. amarella* (IV), effects of clipping on root fungal parameters were positive or neutral. In 1998 clipping mainly have had a positive effect on mycorrhizal colonization, whereas in 2000 clipping did not cause noticeable effects. In *G. campestris* (V) a significant interaction was found between size, clipping, and fertilization in the total fungal colonization and colonization by coils. The general trend was that simulated grazing increased colonization in large plants growing in a nutrient-rich environment whereas in small plants this effect was seen in a nutrient-poor environment. On the contrary, simulated grazing decreased colonization in small plants grown in a nutrient-rich environment, but in large plants in a nutrient-poor environment. Increase in mycorrhizal colonization may

be due to host plant's needs for additional nutrients directly after damage, which mycorrhizal symbionts provide (Eom *et al.* 2001, Kula *et al.* 2005). In a study by Pietikäinen *et al.* (2009), clipping increased AM fungal colonization in unfertilized conditions but not in fertilized, whereas fertilization decreased mycorrhizal colonization in both clipped and intact plants. Foliage removal by herbivory may reduce the function of mycorrhiza and one possible explanation for no increase in mycorrhizal colonization (IV, V) may be carbon limitation (Gange *et al.* 2002).

In both *G. amarella* (IV) and *G. campestris* (V) clipping decreased the colonization by dark septate endophyte. According to Jumpponen (2001), DSE usually are considered as saprophytic but in some environments they can enhance host growth and nutrient uptake and function like mycorrhizal fungi. Co-occurrence of high colonizations of AM fungi and DSE in gentian roots may indicate that they have complementary roles in nutrient uptake. On the other hand, a decrease in the amount of DSE among clipped plants; *i.e.* under limited carbon conditions, suggests that competition for resources may control the abundance of these two groups in host roots (Medina-Roldán *et al.* 2008).

AM fungal colonization generally does not change root morphology. However, a characteristic feature in intensively colonized gentian roots is distinct and heavily colonized patches which are slightly tuberous and often their color is slightly yellowish-pinkish (Fig. 4). Maintaining intensive fungal colonization (IV, V: 69–97%) is expensive to plants (Gange *et al.* 2002, Walling & Zabinski 2006) and in the case of gentians, high overall colonization and morphological changes in roots imply exceptionally high structural costs. However, I have no estimates for metabolic, *e.g.*, respiration costs.

In study IV in 2000 arbuscular and hyphal colonizations were higher among late-flowering plants compared to early-flowering ones and the mycorrhizal colonization in *G. amarella* increased during the growing season. According to Gay *et al.* (1982), two peaks are assumed to occur during the life cycle in the density of mycorrhizal colonization in biennial species. The first peak is in the first autumn of growth, and the second peak is in the second summer of growth during the period of maximum growth. Seasonality of mycorrhizal colonization might be due to high metabolic activity and soil moisture during the summer. On the other hand, in the perennial *Plantago lanceolata* arbuscular colonization was found to be highest in winter and spring (Gange *et al.* 2002). These earlier results are not supported by my results with autumn gentians (IV) since fungal colonization was at the highest level in late summer.

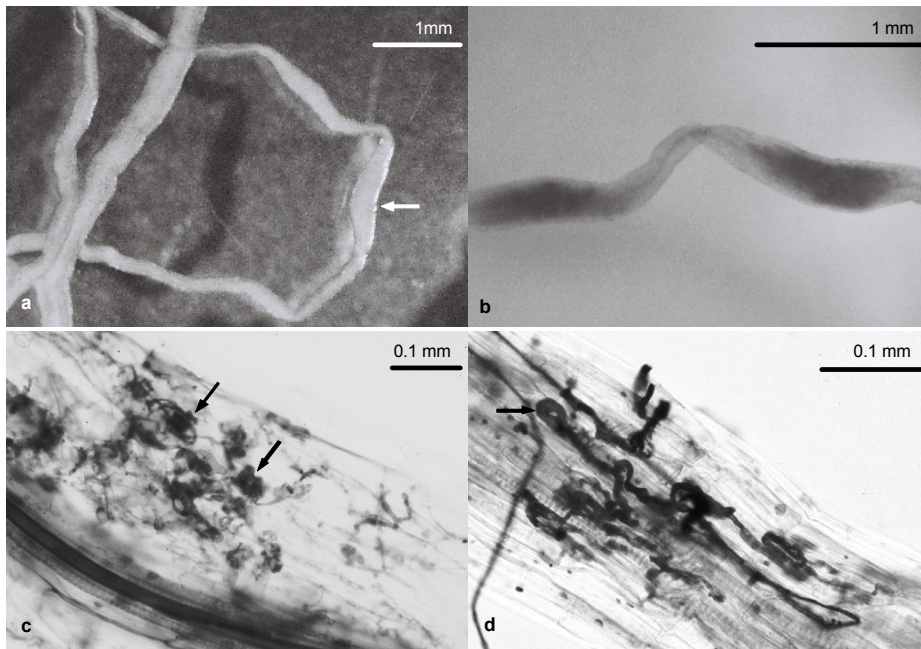


Fig. 4. Unstained (a-b) and stained roots (c-d) of *Gentianella amarella*. a-b) Roots with tuberos parts (arrow) containing intensive mycorrhizal colonization. Fungal structures: c) arbuscules (arrows) and d) coils (arrow) in intensively colonized areas. Scales presented in figures.

Increased fungal colonization (IV) that takes place in late summer may compete too strongly with the sinks of late-flowering hosts. In contrast, the early-flowering hosts may benefit from avoiding costly late-season colonization. Especially the late-flowering host is at risk not to succeed in reproduction. Therefore, grazing late in the season and, at the same time, a high demand for carbon by the fungal symbiont, may result in a decreased capacity for compensation growth (Wamberg *et al.* 2003). Three clipping experiments with *G. amarella* (IV) supported the hypothesis that root symbionts compete with the above-ground sinks. Moreover, in *G. campestris* (V), in large damaged plants grown in nutrient-rich conditions, total colonization increased but above-ground parts suffered (*e.g.* number of branches and seeds per plant declined). On the contrary, in similarly treated small plants total colonization decreased markedly, but plants compensated (in terms of seeds per plant) or even overcompensated (in terms of branch number) for the loss of above-ground biomass. Thus, it seems likely, that large clipped plants

allocate extra resources to roots and fungal partners at the expense of aboveground parts.

Some of the experiments with *G. amarella* (IV) supported the assumption that neighbor removal improves host growth and reproduction, while a greater carbon source capacity benefits mycorrhizal colonization. In other words mycorrhizal colonization increased probably due to release from light competition and enhancement of photosynthesis and, hence, increased flow of carbon from the shoot to the fungi (IV–V). Generally, positive mycorrhizal growth responses may decrease as the density of plants increases because the roots appear to compete with each other in a way that the growth of all the individual plants is reduced. Increased light competition (shading), can cause mycorrhizal costs to exceed benefits because the benefits of a mycorrhizal association (increased supply of nutrients) may remain constant while relative costs (increased carbon demand) mount up (V, Johnson *et al.* 1997, Gange *et al.* 2002, Walling & Zabinski 2006). In the neighbor removal experiment (IV), I did not find evidence supporting mycoheterotrophic interactions in *G. amarella* and neighboring plants. If these interactions have existed in this experiment, they may have been masked by the obvious release from light competition.

The main results from gentian studies are that simulated above-ground herbivory tends to increase carbon limitation. Therefore, as a novel interpretation, responses of shoot and mycorrhizal parameters were opposite to each other as regrowing shoots and the fungal symbionts may appear as alternative, competing sinks for the limited carbon reserves. The sink strength is increased by the high intensity of the mycorrhizal colonization found in both biennial monocarpic *Gentianella* species.

3.5 Grazing tolerance in relation to resource availability

Defoliation by herbivores may have a major effect on the reproductive success of plants (Crawley 1987, Paige & Whitham 1987, Vallius & Salonen 2006). If the architecture of a shoot is strongly altered, as in my thesis (I–V), the reproduction of the plants may be markedly affected. Depending on environmental conditions, the effect can be favorable or unfavorable (Escarré *et al.* 1996). According to the compensatory continuum hypothesis, plants are assumed to best tolerate herbivory in resource-rich conditions (Maschinski & Whitham 1989). On the other hand, many studies have shown that plants are less tolerant to herbivory in resource-rich environments than in resource-poor environments (I–II and V,

Hawkes & Sullivan 2001, Wise & Abrahamson 2005, Banta *et al.* 2010). Furthermore, the compensatory continuum hypothesis does not in fact explain why the plants should be more tolerant in good growth conditions (for discussion, see Huhta *et al.* 2000a).

The relationship between resource levels and tolerance may be more complex than assumed by the compensatory continuum hypothesis. When considering compensatory growth, one needs to take into account more specific, resource-related aspects as well. For instance: which resource is limiting plant fitness for the most part, which resources are affected by damage, and is resource acquisition affected by the damage? Plant tolerance depends not only on the overall level of resources, but on the specific resource that limits plant fitness the most and, moreover, which tissues are damaged. In a low nitrogen environment the fitness of an undamaged plant is nitrogen limited rather than carbon limited. Therefore plants growing in a low nitrogen environment should be rather tolerant of herbivory. On the other hand, if nitrogen fertilizer is added, undamaged plants have enough nitrogen to reproduce at their maximum level and, hence, their fitness could be carbon limited. Thus, because of carbon limitation, herbivory most likely decreases plant fitness in nutrient-rich conditions (Wise & Abrahamson 2005).

It is evident that growth conditions at the time of damage, and after it, are essential in response to herbivory (Escarré *et al.* 1996). In nutrient-rich environments apical dominance may be weak causing increased branching, whereas in nutrient-poor environments there are not enough resources to compensate for damage (Irwin & Aarssen 1996). Plants typically show higher apical dominance under competitive environments (Aarssen & Irwin 1991) as found in studies II and V. It may be that suppressed lateral branching at low availability of light and nutrients (*e.g.* under competition) is an expression of plasticity induced by restricted availability of resources. Therefore both shoot architecture and resource availability together may modify the responses of *E. strictum* to apical damage, the former effects being much stronger (I–II). At the rosette stage simulated herbivory decreases lateral branching and height in the following growing season, whereas simulated herbivory at the adult stage tends to increase lateral branching. Thus, in different life stages different resources and availability of meristems limit plant growth.

Responses of different plant species to resource availability vary and depending on species trade-offs may occur in allocation of resources among growth, tolerance, and defense. Ability of plants to allocate more resources to one

of these three strategies may mean that resources cannot be allocated to other strategies (Bennett *et al.* 2006). Allocation to active branch growth should be high in resource-rich environments, whereas allocation to inactive quiescent meristems should be high in resource-poor environments (Bonser & Aarssen 2003). In the early growing season meristem differentiation may be limited due to the low amount of resources available for regrowth and at the end of growing season mainly by the availability of undifferentiated meristems (II and V, Lennartsson *et al.* 1998). If undamaged plants have already produced several branches before damage, grazing increases branching less or may even reduce the number of branches (Huhta *et al.* 2000a). *E. strictum* may have been selected for fast vertical growth at the beginning of the second growing season (Huhta *et al.* 2000a). Hence, apical damage in adult plants causes branching in the upper parts of the stalk (II–III), whereas rosette defoliation in the first growing season decreases both basal and distal branching in the adult phase (I).

Enhanced nutrient uptake of damaged plants (Paige & Whitham 1987), possibly because of increased mycorrhizal colonization, might enhance compensatory growth without a decrease in productivity (Maschinski & Whitham 1989). However, because of a greater drain on photosynthates, reduced photosynthetic area due to herbivory may cause a greater burden on the carbon budget of mycorrhizal, rather than on non-mycorrhizal plants. In this case, mycorrhizal plants may be less tolerant to herbivory. In addition, the fungus and herbivore both consume photosynthates, so there may be competition between them (IV–V, Gehring & Whitham 1994, Borowicz 1997, Koricheva *et al.* 2009). Thus, herbivore damage may decrease the benefit of mycorrhizal colonization on host fitness (Gange *et al.* 2002, Garrido *et al.* 2010).

4 Conclusions

Grazed meadows are challenging habitats for plants as in some places there is a high risk of being grazed, whereas in some other places competition for space and light is high. The growth and flowering time of biennial herbs are adapted to this environment to compromise seed yield in both favorable and unfavorable conditions. Unbranched stem architecture with strong apical dominance is associated with regrowth capacity following damage to the shoot or apical meristem. Moreover, mycorrhizal symbiosis of meadow plants may affect the outcome of a plant in dense vegetation and especially when plants are exposed to herbivory.

Compensation capacity is strongly dependent on the timing of damage during the growing season and in relation to the developmental stage of a plant. The size of an adult, biennial plant correlates positively with its size at the rosette stage, and the size of the rosette in turn is determined largely by environmental factors (*i.e.* resources and stress). For instance, defoliation of a rosette can reduce the reproductive output of an adult even though the plant would to some extent be able to compensate for the damage at the rosette stage. Moreover, while apical dominance by the leading stalk at the adult stage restrains the axillary meristems at the rosette base, nutrient surplus at the rosette stage can break this suppression. At the adult stage all three study species were relatively tolerant to simulated grazing and in most cases plants were able to compensate for minor biomass loss. The compensatory continuum hypothesis assumes that tolerance is better in resource-rich conditions but this was not always the case in the experiments in this thesis.

Differences in compensatory capacity of the meadow plants in response to grazing lies in the original architecture of the adult plant (*e.g.* number of branches and height) and differences in habitat resource levels. The effect of grazing is partially determined by the amount of meristems available for growth and reproduction but also by resource allocation between these meristems. When considering management practices for different meadows and meadow species, it is important to clarify what are the most important resources restricting both the initial plant growth but also regrowth after damage. For instance, in the low productive environment the plant is nutrient rather than carbon limited. Thus, plants growing in the low nutrient environment are quite tolerant of herbivory. If plants happen to grow in a nutrient-rich patch, plant fitness could be carbon limited even without damage. So the timing and level of damage have to be

appropriate for each particular plant population. However, after mowing/grazing, plants may also be subjected to uncontrollable environmental stress factors (early frost, drought) which may affect their ability to recover and regrow.

Relationships between meadow plants, herbivores and fungal symbionts are complicated. The effect of resource manipulation on mycorrhizal colonization of meadow plants obviously depends on environmental factors. Usually shoot damage and nutrient surplus decrease mycorrhizal colonization because if nutrients are not limiting there is no clear advantage to invest carbon into mycorrhizal symbionts. This situation changes if plants are living in resource-poor environments, are small-sized or living in intensive competition with other plants. Above-ground herbivory tends to increase carbon limitation and, hence, regrowing shoots and the fungal symbionts may appear as alternative, competing sinks for the limited carbon reserves of the host.

Different plant ecotypes may be in different stages of development during shoot damage and, hence, they respond to the same damage dissimilarly. If environmental conditions are harsh (*e.g.* short growing season) the plant may neither be able to compensate for the damage nor gain benefit from the fungal partner. The fungal partner may help the host plant recover from damage by enhancing the nutrient supply. On the other hand, the mycorrhizal symbiont always bears a cost in terms of carbon resources for the host plant and in some cases costs may exceed benefits.

References

- Aarssen LW (1995) Hypothesis for the evolution of apical dominance in plants: implications for the interpretation of overcompensation. *Oikos* 74: 149–156.
- Aarssen LW & Irwin DL (1991) What selection: herbivory or competition? *Oikos* 60: 261–262.
- Ahti T (1965) *Erysimum hieraciifolium* L. In Jalas J (ed) *Suuri kasvikirja II*. Helsinki Finland, Otava: 567–569.
- Banta JA, Stevens MHH & Pigliucci (2010) A comprehensive test of the ‘limiting resources’ framework applied to plant tolerance to apical meristem damage. *Oikos* 119: 359–369.
- Belsky AJ, Carson WP, Ensen JCL & Fox GA (1993) Overcompensation by plants: herbivore optimisation or red herring? *Evol Ecol* 7: 109–121.
- Benner BL (1988) Effects of apex removal and nutrient supplementation on branching and seed production in *Thlaspi arvense* (Brassicaceae). *Am J Bot* 75: 645–651.
- Bennett AE, Alers-Garcia J & Bever JD (2006) Three-way interactions among mutualistic mycorrhizal fungi, plants, and plant enemies: hypothesis and synthesis. *Am Nat* 167: 141–152.
- Bennett AE & Bever, JD (2007) Mycorrhizal species differentially alter plant growth and response to herbivory. *Ecology* 88: 210–218.
- Bennett AE & Bever JD (2009) Trade-offs between arbuscular mycorrhizal fungal competitive ability and host growth promotion in *Plantago lanceolata*. *Oecologia* 169: 807–816.
- Bennett AE, Bever JD & Bovers MD (2009) Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory. *Oecologia* 160: 771–779.
- Bergelson, J & Crawley MJ (1992) The effects of grazers on the performance of individuals and populations of scarlet gilia, *Ipomopsis aggregata*. *Oecologia* 90: 435–444.
- Bonsler SP & Aarssen LW (1996) Meristem allocation: a new classification theory for adaptive strategies in herbaceous plants. *Oikos* 77: 347–352.
- Bonsler SP & Aarssen LW (2001) Allometry and plasticity of meristem allocation throughout development in *Arabidopsis thaliana*. *J Ecol* 89: 72–79.
- Bonsler SP & Aarssen LW (2003) Allometry and development in herbaceous plants: Functional responses of meristem allocation to light and nutrient availability. *Am J Bot* 90: 404–412.
- Borowicz VA (1997) A fungal root symbiont modifies plant resistance to an insect herbivore. *Oecologia* 112: 534–542.
- Brodie EDIII, Moore AJ & Janzen FJ (1995) Visualizing and quantifying natural selection. *Trends Ecol Evol* 10: 313–318.
- Brundrett M (2004) Diversity and classification of mycorrhizal associations. *Biol Rev* 79: 473–495.

- Buckley YM, Briese DT & Rees M (2003) Demography and management of the invasive plant species *Hypericum perforatum*. I. Using multi-level mixed-effects models for characterizing growth, survival and fecundity in a long-term data set. *J Appl Ecol* 40: 481–493.
- Crawley MJ (1987) Benevolent herbivores? *Trends Ecol Evol* 2: 167–168.
- de Jong TJ & van der Meijden E (2000) On the correlation between allocation to defense and regrowth in plants. *Oikos* 88: 503–508.
- Dhileepan K., Setter SD & McFadyen RE (2000) Response of the weed *Parthenium hysterophorus* (Asteraceae) to defoliation by the introduced biocontrol agent *Zygotogramma bicolorata* (Coleoptera: Chrysomelidae). *Biol Contr* 19: 9–16.
- Dickson S, Smith FA & Smith SE (2007) Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after Gallaud, where next? *Mycorrhiza* 17: 375–393.
- Duffy NM, Bonser SP & Aarssen LW (1999) Patterns of variation in meristem allocation across genotypes and species in monocarpic Brassicaceae. *Oikos* 84: 284–292.
- Ehrlén J (1997) Risk of grazing and flower number in a perennial plant. *Oikos* 80: 428–434.
- Eom A-H, Wilson GW & Hartnett DC (2001) Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. *Mycologia* 93: 233–242.
- Escarré J, Lepart J & Sentuc JJ (1996) Effects of simulated herbivory in three old field Compositae with different inflorescence architectures. *Oecologia* 105: 501–508.
- Francis R & Read DJ (1984) Direct transfer of carbon between plants connected by vesicular-arbuscular mycorrhizal mycelium. *Nature* 307: 53–56.
- Francis R & Read DJ (1995) Mutualism and antagonism in the mycorrhizal symbiosis, with special reference to impacts on plant community structure. *Can J Bot* 73: 1301–1309.
- Gange AC, Bower E & Brown VK (2002) Differential effects of insect herbivory on arbuscular mycorrhizal colonization. *Oecologia* 131: 103–112.
- Garrido E, Bennett AE, Fornoni J & Strauss SY (2010) Variation in arbuscular mycorrhizal fungi colonization modifies the expression of tolerance to above-ground defoliation. *J Ecol* 98: 43–49.
- Gay PE, Grubb PJ & Hudson HJ (1982) Seasonal changes in the concentrations of nitrogen, phosphorus and potassium, and in the density of mycorrhiza, in biennial and matrix-forming perennial species of closed chalkland turf. *J Ecol* 70: 571–593.
- Gehring CA & Whitham TG (1994) Interactions between aboveground herbivores and the mycorrhizal mutualists of plants. *Trends Ecol Evol* 9: 251–255.
- Gehring CA & Whitham TG (2002) Mycorrhizae-herbivore interactions: population and community consequences. In van der Heijden MGA & Sanders I (eds) *Mycorrhizal ecology*. Springer-Verlag: 295–320.
- Gómez JM (2003) Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant specialization. *Am Nat* 162: 242–256.

- Grime P (2002) Plant strategies, vegetation processes, and ecosystem properties. West Sussex England, John Wiley & Sons Ltd.
- Hartnett DC, Hetrick BAD, Wilson GWT & Gibson DJ (1993) Mycorrhizal influence on intra- and interspecific neighbour interactions among co-occurring prairie grasses. *J Ecol* 81: 787–795.
- Hawkes CV & Sullivan JJ (2001) The impact of herbivory on plants in different resource conditions: a meta-analysis. *Ecology* 82: 2045–2058.
- Hellström K, Huhta A-P, Rautio P, Tuomi J, Oksanen J & Laine K (2003) Use of sheep grazing in the restoration of semi-natural meadows in northern Finland. *Appl Veg Sci* 6: 45–52.
- Hicks S & Turkington R (2000) Compensatory growth of three herbaceous perennial species: the effects of clipping and nutrient availability. *Can J Bot* 78: 759–767.
- Hirrel MC & Gerdemann JW (1979) Enhanced carbon transfer between onions infected with a vesicular-arbuscular mycorrhizal fungus. *New Phytol* 83: 731–738.
- Huhta A-P, Hellström K, Rautio P, & Tuomi J (2000a) A test of the compensatory continuum: fertilisation increases and below-ground competition decreases tolerance of tall wormseed mustard (*Erysimum strictum*). *Evol Ecol* 14: 353–372.
- Huhta -P, Lennartsson T, Tuomi J, Rautio P & Laine K (2000b) Tolerance of *Gentianella campestris* in relation to damage intensity: an interplay between apical dominance and herbivory. *Evol Ecol* 14: 373–392.
- Huhta A-P, Tuomi J & Rautio P (2000c) Cost of apical dominance in two monocarpic herbs, *Erysimum strictum* and *Rhinanthus minor*. *Can J Bot* 78: 591–599.
- Huhta A-P, Hellström K, Rautio P, & Tuomi J (2003) Grazing tolerance of *Gentianella amarella* and other monocarpic herbs: why is tolerance highest at low damage levels? *Plant Ecol* 166: 49–61.
- Huhta A-P & Rautio P (2007) A case with blue gentian blues: roadside-cutters creating neograsslands as refugia for endangered *Gentianella campestris*. *Nord J Bot* 25: 372–379.
- Huhta A-P, Rautio P, Hellström K, Saari M, & Tuomi J (2009) Tolerance of a perennial herb, *Pimpinella saxifraga*, to simulated flower herbivory and grazing: immediate repair of injury or postponed reproduction? *Plant Ecol* 201: 599–609.
- Hultén E & Fries M (1986) Atlas of North European Vascular Plants, North of the Tropic of Cancer. Königstein, Koeltz Scientific Books.
- Hämet-Ahti L, Suominen J, Ulvinen T, Uotila P & Vuokko S (1998) Retkeilykasvio (A field guide to the flora of Finland). Helsinki, Suomen Luonnonsuojelun Tuki Oy.
- Imhof S (1999) Root morphology, anatomy and mycotrophy of the achlorophyllous *Voyria aphylla* (Jacq.) Pers. (Gentianaceae). *Mycorrhiza* 9: 33–39.
- Irwin DL & Aarssen LW (1996) Effects of nutrient level on cost and benefit of apical dominance in *Epilobium ciliatum*. *Am Midl Nat* 136: 14–28.
- Janković T, Krstić D, Aljančić I, Šavikin-Fodulović K, Menković N, Vajs V & Milosavljević (2005) Xanthones and C-glucosides from the aerial parts of four species of *Gentianella* from Serbia and Montenegro. *Biochem System Ecol* 33: 729–735.

- Jacquelinet-Jeanmougin S & Gianinazzi-Pearson V (1983) Endomycorrhizas in the Gentianaceae. I. The fungus associated with *Gentiana lutea* L. *New Phytol* 95: 663–666.
- Johnson NC, Graham JH & Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytol* 135: 575–585.
- Jones CG & Last FT (1991) Ectomycorrhizae and trees: implications for aboveground herbivory. In Barbosa P, Krischik VA, & Jones CG (eds) *Microbial mediation of plant-herbivore interactions*. New York, John Wiley & Sons: 65–103.
- Jones MD & Smith SE (2004) Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms? *Botany* 82: 1089–1109.
- Juenger T & Lennartsson T (eds) 2000. Plant tolerance to herbivory. *Evol Ecol* (special issue) 14: 283–570.
- Juenger T, Lennartsson T & Tuomi J (2000) The evolution of tolerance to damage in *Gentianella campestris*: natural selection and the quantitative genetics of tolerance. *Evol Ecol* 14: 393–419.
- Jumpponen A (2001) Dark septate endophytes – are they mycorrhizal? *Mycorrhiza* 11: 207–211.
- Järemo J, Ripa J & Nilsson P (1999) Flee or fight uncertainty: plant strategies in relation to anticipated damage. *Ecol letters* 2: 361–366.
- Karban R & Balwin IT (1997) *Induced Responses to Herbivory*. The University of Chicago Press.
- Karlsson T (1974) Recurrent ecotypic variation in Rhinanthae and Gentianaceae in relation to hemiparasitism and mycotrophy. *Bot Notiser* 127: 527–539.
- Klironomos JN, McCune J & Moutoglis P (2004) Species of arbuscular mycorrhizal fungi affect mycorrhizal responses to simulated herbivory. *Appl Soil Ecol* 26: 133–141.
- Koricheva J, Gange AC & Jones T (2009) Effects of mycorrhizal fungi on insect herbivores: a meta-analysis. *Ecology* 90: 2088–2097.
- Koskela T, Puustinen S, Salonen V, & Mutikainen P (2002) Resistance and tolerance in a host plant - holoparasitic plant interaction: genetic variation and costs. *Evolution* 56: 899–908.
- Kula AAR, Hartnett DC & Wilson GWT (2005) Effects of mycorrhizal symbiosis on tallgrass prairie plant-herbivore interactions. *Ecol Letters* 8: 61–69.
- Lennartsson T, Tuomi J, & Nilsson P (1997) Evidence for an evolutionary history of overcompensation in the grassland biennial, *Gentianella campestris* (Gentianaceae). *Am Nat* 149: 1147–1155.
- Lennartsson T, Nilsson P & Tuomi J (1998) Induction of overcompensation in the field gentian, *Gentianella campestris*. *Ecology* 79: 1061–1073.
- Lennartsson T, Oostermeijer JGB, van Dijk J & den Nijs HCM (2000) Ecological significance and heritability of floral reproductive traits in *Gentianella campestris* (Gentianaceae). *Basic Appl Ecology* 1: 69–81.
- Lennartsson T & Oostermeijer JGB (2001) Demographic variation and population viability in *Gentianella campestris*: effects of grassland management and environmental stochasticity. *J Ecol* 89: 451–463.

- Levine MT & Paige KN (2004) Direct and indirect effects of drought on compensation following herbivory in scarlet gilia. *Ecology* 8: 3185–3191.
- Lugo MA, González Maza ME & Cabello MN (2003) Arbuscular mycorrhizal fungi in a mountain grassland II: Seasonal variation of colonization studied, along with its relation to grazing and metabolic host type. *Mycologia* 95: 407–415.
- Marshall CB, Germán AS & Reekie EG (2008) Effects of nutrient and CO₂ availability on tolerance to herbivory in *Brassica rapa*. *Plant Ecol* 196: 1–13.
- Martínková J, Klimešová J & Milhulka S (2008) Compensation of seed production after severe injury in the short-lived herb *Barbarea vulgaris*. *Basic Appl Ecol* 9: 44–54.
- Maschinski J & Whitham TG (1989) The continuum of plant responses to herbivory: the influence of plant association, nutrient availability and timing. *Am Nat* 134: 1–19.
- McGonigle TP, Miller MH, Evans DG, Fairchild GL & Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol* 115: 495–501.
- McNaughton SJ (1979) Grazing as an optimization process: grass-ungulate relationships in the Serengeti. *Am Nat* 113: 691–703.
- Medina-Roldán E, Arredondo, JT, Huber-Sannwald E, Chapa-Vargas L & Olalde-Portugal V (2008) Grazing effects on fungal root symbionts and carbon and nitrogen storage in a shortgrass steppe in Central Mexico. *J Arid Environ* 72: 546–556.
- Merckx, V, Bidartondo, MI & Hynson, NA (2009) Myco-heterotrophy: when fungi host plants. *Ann Bot* 104: 1255 – 1261.
- Moora M & Zobel M (1996) Effect of arbuscular mycorrhiza on inter- and intraspecific competition of two grassland species. *Oecologia* 108: 79–84.
- Newman EI, Eason WR, Eissenstat DM & Ramos MIRF (1992) Interactions between plants: the role of mycorrhizae. *Mycorrhiza* 1: 47–53.
- Niemelä M (2009) Biotic interactions and vegetation management on coastal meadows. PhD thesis, Univ Oulu, Dept Biol.
- Paige KN (1994) Herbivory and *Ipomopsis aggregata*: differences in response, differences in experimental protocol: a reply to Bergelson and Crawley. *Am Nat* 143: 739–749.
- Paige KN & Whitham, TG (1987) Overcompensation in response to mammalian herbivory: the advantage of being eaten. *Am Nat* 129: 407–416.
- Phillips JM & Hayman DS (1970) Improved procedures for clearing and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc* 55: 158–160.
- Phillips PC & Arnold SJ (1989) Visualizing multivariate selection. *Evolution* 43: 1209–1222
- Pietikäinen A, Kytöviita M-M & Vuoti U (2005) Mycorrhiza and seedling establishment in a subarctic meadow: Effects of fertilization and defoliation. *J Veg Sci* 16: 175–182.
- Pietikäinen A, Mikola J, Vestberg M & Setälä H (2009) Defoliation effects on *Plantago lanceolata* resource allocation and soil decomposers in relation to AM symbiosis and fertilization. *Soil Biol Biochem* 41: 2328–2335.
- Pykälä J (2000) Mitigating human effects on European biodiversity through traditional animal husbandry. *Conserv Biol* 14: 705–712.

- Ramula S (2008) Responses to the timing of damage in an annual herb: Fitness components versus population performance. *Basic Appl Ecol* 9: 233–242.
- Reader RJ (1993) Control of seedling emergence by ground cover and seed predation in relation to seed size for some old-field species. *J Ecol* 81: 169–175.
- Rosenthal JP & Kotanen PM (1994) Terrestrial plant tolerance to herbivory. *Trends Ecol Evol* 9: 145–148.
- Ruiz N, Saltz D & Ward D (2006) The effects of herbivory and resource variability on the production of a second inflorescence by the desert lily, *Pancreatium sickenbergeri*. *Plant Ecol* 186: 47–55.
- Schulman A, Alanen A, Hægström CA, Huhta AP, Jantunen J, Kekäläinen H, Lehtomaa L, Pykälä J & Vainio M (2008) Perinnebiotoopit. In Raunio A, Schulman A & Kontula T (eds) Suomen luontotyypin uhanalaisuus – Osa II: Luontotyypin kuvaukset. Suomen ympäristö 8: 397–466.
- Schwinning S & Weiner J (1998) Mechanisms determining the degree of size asymmetry in competition among plants. *Oecologia* 113: 447–455.
- Simons AM & Johnston MO (1999) The cost of compensation. *Am Nat* 153: 683–687.
- Smith FA & Smith SE (1997) Tansley Review No. 96 Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. *New Phytol* 137: 373–388.
- Smith SE & Read DJ (2008) *Mycorrhizal Symbiosis*. Third edition. San Diego, Academic.
- Stevens MT, Waller, DM & Lindroth L (2007) Resistance and tolerance in *Populus tremuloides*: genetic variation, costs, and environmental dependency. *Evol Ecol* 21: 829–847.
- Strauss SY & Agrawal AA (1999) The ecology and evolution of plant tolerance to herbivory. *Trends Ecol Evol* 14: 179–185.
- Suwa T, Louda SM & Russell FL (2010) No interaction between competition and herbivory in limiting introduced *Cirsium vulgare* rosette growth and reproduction. *Oecologia* 162: 91–102.
- Tedersoo L, Pellet P, Kõljalg U & Selosse MA (2007) Parallel evolutionary paths to mycoheterotrophy in understorey Ericaceae and Orchidaceae: ecological evidence for mixotrophy in Pyrolae. *Oecologia* 151: 206–217.
- Tiffin P (2002) Competition and time of damage affect the pattern of selection acting on plant defense against herbivores. *Ecology* 83: 1981–1990.
- Tikka PM, Heikkilä T, Heiskanen M & Kuitunen M (2001a) The role of competition and rarity in the restoration of a dry grassland in Finland. *Appl Veg Sci* 4: 139–146.
- Tikka PM, Högmander H & Koski PS (2001b) Road and railway verges serve as dispersal corridors for grassland plants. *Landscape Ecol* 16: 659–666.
- Tuomi J, Nilsson P & Åström M (1994) Plant compensatory responses: bud dormancy as an adaptation to herbivory. *Ecology* 75: 1429–1436.
- Vail SP (1992) Selection for overcompensatory plant responses to herbivory: a mechanism for the evolution of plant–herbivore mutualism. *Am Nat* 139: 1–8.
- Vallius E & Salonen V (2006) Allocation to reproduction following experimental defoliation in *Platanthera bifolia* (Orchidaceae). *Plant Ecol* 183: 291–304.

- Walling SZ & Zabinski CA (2006) Defoliation effects on arbuscular mycorrhizae and plant growth of two native bunchgrasses and an invasive forb. *Appl Soil Ecol* 32: 111–117.
- Wamberg C, Christensen S & Jakobsen I (2003) Interaction between foliar-feeding insects, mycorrhizal fungi and rhizosphere protozoa on pea plants. *Pedobiologia* 47: 281–287.
- Watkins NK, Fitter AH, Graves JD & Robinson D (1996) Carbon transfer between C₃ and C₄ plants linked by a common mycorrhizal network, quantified using stable carbon isotopes. *Soil Biol Biochem* 28: 471–477.
- Wearn JA & Gange AC (2007) Above-ground herbivory causes rapid and sustained changes in mycorrhizal colonization of grasses. *Oecologia* 153: 959–971.
- Wise MJ & Abrahamson WG (2005) Beyond the compensatory continuum: environmental resource levels and plant tolerance of herbivory. *Oikos* 109: 417–428.
- Yamauchi A & Yamamura N (2004) Herbivory promotes plant production and reproduction in nutrient-poor conditions: effects of plant adaptive phenology. *Am Nat* 163: 138–153.
- Young TP (1984) The comparative demography of semelparous *Lobelia telekii* and iteroparous *Lobelia keniensis* on Mount Kenya. *J Ecol* 72: 637–650.
- Zobel M, Öpik M, Moora M & Pärtel M (2006). Biodiversity and ecosystem functioning: It is time for dispersal experiments. *J Veg Sci* 17: 543–547.

Original publications

- I Piippo S, Huhta AP, Rautio P & Tuomi J (2005) Resource availability at the rosette stage and apical dominance in the strictly biennial *Erysimum strictum* (Brassicaceae). *Can J Bot* 83(4): 405–412.
- II Rautio P, Huhta AP, Piippo S, Tuomi J, Juenger T, Saari M & Aspi J (2005) Overcompensation and adaptive plasticity of apical dominance in *Erysimum strictum* (Brassicaceae) in response to simulated browsing and resource availability. *Oikos* 111(1): 179–191.
- III Piippo S, Hellström K, Huhta AP, Rautio P & Tuomi J (2009) Delayed flowering as a potential benefit-decreasing cost of compensatory regrowth. *Botany* 87: 837–844.
- IV Piippo S, Markkola A, Härmä E & Tuomi J (2010) Host and symbiont responses to grazing and competition in the intensively mycorrhizal meadow plant *Gentianella amarella*. Manuscript.
- V Piippo S, Huhta AP, Rautio P & Markkola A (2010) Grazing tolerance and mycorrhizal colonization: effects of resource manipulation and plant size in biennial *Gentianella campestris*. Manuscript.

Reprinted with kind permission from NRC Research Press (I, III) and Wiley-Blackwell (II).

Original publications are not included in the electronic version of the dissertation.

ACTA UNIVERSITATIS OULUENSIS
SERIES A SCIENTIAE RERUM NATURALIUM

541. Eskelinen, Anu (2009) Plant community dynamics in tundra: propagule availability, biotic and environmental control
542. Still, Kaisa (2009) Mobile technology for interest-based communities: concept design with a knowledge-based approach
543. Tarvainen, Oili (2009) Scots pine and its ectomycorrhizal symbionts under chronic low-level urban pollution—responses and restoration
544. Saari, Eija (2009) Towards minimizing measurement uncertainty in total petroleum hydrocarbon determination by GC-FID
545. Pihlajaniemi, Henna (2009) Success of micropropagated woody landscape plants under northern growing conditions and changing environment
546. Pajunen, Anu (2010) Willow-characterised shrub vegetation in tundra and its relation to abiotic, biotic and anthropogenic factors
547. Salin, Mikko (2010) Protein crystallographic studies of A-TIM—structure based development of new enzymes
548. Kemppainen, Jukka (2010) Behaviour of the boundary potentials and boundary integral solution of the time fractional diffusion equation
549. Varga, Sandra (2010) Significance of plant gender and mycorrhizal symbiosis in plant life history traits
550. Räisänen, Teppo (2010) All for one, one for all. Organizational knowledge creation and utilization using a new generation of IT tools
551. Luimula, Mika (2010) Development and evaluation of the location-aware platform. Main characteristics in adaptable location-aware systems
552. Bisi, Jukka (2010) Suomalaisen susikonfliktin anatomia
553. Poutanen, Hillka (2010) Developing the role of human resource information systems for the activities of good leadership
554. Saukkoriipi, Jaakko (2010) Theoretical study of the hydrolysis of aluminum complexes
555. Sirviö, Anu (2010) The role of factors promoting genetic diversity within social insect colonies
556. Tahkokorpi, Marjaana (2010) Anthocyanins under drought and drought-related stresses in bilberry (*Vaccinium myrtillus* L.)

Book orders:
Granum: Virtual book store
<http://granum.uta.fi/granum/>

S E R I E S E D I T O R S

A
SCIENTIAE RERUM NATURALIUM

Professor Mikko Siponen

B
HUMANIORA

University Lecturer Elise Kärkkäinen

C
TECHNICA

Professor Pentti Karjalainen

D
MEDICA

Professor Helvi Kyngäs

E
SCIENTIAE RERUM SOCIALIUM

Senior Researcher Eila Estola

F
SCRIPTA ACADEMICA

Information officer Tiina Pistokoski

G
OECONOMICA

University Lecturer Seppo Eriksson

EDITOR IN CHIEF

University Lecturer Seppo Eriksson

PUBLICATIONS EDITOR

Publications Editor Kirsti Nurkkala

ISBN 978-951-42-6210-4 (Paperback)

ISBN 978-951-42-6211-1 (PDF)

ISSN 0355-3191 (Print)

ISSN 1796-220X (Online)

