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Responses of forest insects to climate change

Herbivory and plant quality along European elevational gradients

Direttore della Scuola: Prof. Angelo Ramina

Coordinatore d'indirizzo: Prof. Francesco Favaron

Supervisore: Prof. Andrea Battisti

Dottorando: Ewelina Czwienczek

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21 December, 2012 Ewelina Czwienczek

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To my parents and my sister

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Riassunto

In questa tesi vengono presentati i risultati di uno studio svolto lungo gradienti altitudinali per verificare la risposta di insetti forestali alla temperatura. A questo scopo sono stati scelti vari siti in Europa, in modo da coprire varie situazioni climatiche e considerando in ciascuno di essi il gradiente altitudinale come analogo spaziale del cambiamento climatico, in relazione al tasso di riduzione della temperatura di 0.6°C ogni 100 m di quota. Sono stati considerati gli effetti diretti e indiretti della temperatura sulle principali categorie funzionali di insetti, considerati a livelli endemici di densità. La qualità della pianta è stata considerata mediante indicatori come la durezza delle foglie, il rapporto C/N, le caratteristiche dimensionali degli alberi. L'effetto della quota sulla fitofagia è stato positivo o indifferente in relazione alle categorie funzionali e alle piante ospiti. Insetti masticatori e minatori fogliari hanno risposto in un maggiore numero di casi rispetto a fitomizi e galligeni, mentre le conifere (abete rosso, larice, pino silvestre) hanno presentato risposte più chiare rispetto alle latifoglie (faggio). Sono stati messi in evidenza effetti positivi della quota su dimensione e durezza delle foglie (eccetto il larice) pur non trovando un legame significativo con la performance degli insetti. Non sono stati inoltre evidenziati fenomeni di competizione e facilitazione tra categorie ecologiche, con l'unica eccezione del larice.

Uno studio specifico ha riguardato l'analisi dell'abbondanza dei nidi di processionaria del pino lungo gradienti altitudinali nella Sierra Nevada in Spagna meridionale. È stato osservato che i nidi aumentano con la quota, fatto che contrasta con i modelli previsionali esistenti per questa specie. Sono stati quindi analizzati alcuni dei potenziali fattori esplicativi quali le caratteristiche del popolamento, la qualità degli aghi, la parassitizzazione delle uova e l'azione dei competitori. La temperatura invernale non spiega la distribuzione altitudinale dei nidi, che invece risponde ad alcune caratteristiche della pianta ma soprattutto alla fecondità, che aumenta con l'altitudine, e al parassitismo, che diminuisce decisamente con l'aumentare della quota.

In un ulteriore studio specialistico sono stati considerati alcuni insetti dei coni dell'abete rosso le cui tracce erano reperibili in coni maturi caduti al suolo lungo 4 gradienti altitudinali alpini, in Italia e in Francia. Gli insetti specializzati nel nutrirsi di seme (Cydia strobilella e Strobilomyia anthracina) non hanno risposto alla variazione di altitudine

mentre gli insetti generalisti consumatori dei tessuti del cono (Dioryctria abietella e Ernobius abietis) hanno risposto in modo negativo all'aumentare della quota. E' stato anche possibile mettere in evidenza una interazione positiva tra lo specialista C. strobilella e le due specie generaliste.

Complessivamente gli studi svolti nell'ambito della presente tesi hanno fornito risultati utili a comprendere gli effetti del cambiamento climatico sull'attività trofica di alcuni insetti forestali, tuttavia si ritiene che sussista ancora dell'incertezza sulle previsioni da svolgere in merito. Si auspica che per la continuazione degli studi vengano prese in maggiore considerazione le interazioni tra altitudine, pianta ospite e insetti.

Summary

This thesis describes the effect of elevation on the abundance of forest insects along steep mountain slopes, to be used as a spatial analogue of climate change. The elevational gradients in different places in Europe were used as a proxy for the temperature change, using the lapse rate of 0.6 °C every 100 m. Elevational gradients provide an optimal experimental setting to test the relationship between temperature variation and herbivory. Direct and indirect (through the host plant and by the natural enemies) effects of the temperature were checked. The tree quality was measured by leaf toughness, leaf size, C/N ratio, tree diameter and height and, for cone and seed insects, by the length of cones. Insect herbivores were taken into account because they are amongst the most important primary consumers of forest ecosystem, even when they are studied at endemic density as in the present study. The response to the changing temperature was checked for insects guilds (leaf sap feeder, chewers, gall makers, miners) in the Chapter 2, for selected insect species like pine processionary moth (*Thaumetopoea pityocampa*) in Chapter 3, and also for cone and seed insects of Norway spruce (*Cydia strobilella*, *Dioryctria abietella*, *Ernobius abietis*, *Strobilomyia anthracina*) in Chapter 4. We concentrated on four main European tree species (*Fagus sylvatica, Larix decidua, Picea abies*, *Pinus sylvestris*) because they are the most important trees regarding their abundance and economy value.

In the Chapter 1 we used a large data set where we measured leaf herbivory and leaf traits along steep elevational gradients in different biogeographical regions of Europe. We found either neutral or positive effect of increasing temperatures on herbivory while no clear effect of biotic interactions emerged. Among guilds, chewers and leaf miners responded more often than sap feeders and gall makers to temperature. The response to temperature of different host species varied strongly, with conifers more sensitive than broadleaves (*Fagus sylvatica*). There was a clear elevational pattern of toughness and leaf size (except for *Larix decidua*) but leaf traits were almost never related with changes in herbivory. There was not interaction between the herbivory guilds except for the miners and chewers of *Larix decidua*. This community approach (like dividing the insect species into the guilds) is, however, crucial to help understanding the ecosystem level response of insect herbivory to temperature.

In the Chapter 3 the abundance of the nests of pine processionary moth (*Thaumetopoea pityocampa*) along two elevational gradients in southern Spain (Sierra Nevada mountains) was considered. The number of nests increased with elevation, which is opposite to what was expected. Possible explanatory factors were searched in stand conditions (tree density, host percentage), tree quality (tree height, needle toughness, C/N ratio, needle length), egg parasitoids, and possible competitors (leaf sap feeders and chewers). Winter temperature did not explain moth abundance, which was in turn explained by tree height, leaf C/N, but mainly by a higher realized fecundity at high elevation, likely linked to better growth conditions, and by higher egg parasitism at lower elevation.

In Chapter 4 the colonization of Norway spruce mature cones by cone and seed insects was analyzed along four alpine elevational gradients (three in Italy, one in France). Specialised insects feeding on seeds (*Cydia strobilella* and *Strobilomyia antracina*) did not respond to temperature whereas generalits species feeding on cone tissues did (*Dioryctria abietella* and *Ernobius abietis*). There was also a significant interaction, as the seed feeder *Cydia strobilella* affected positively the generalists *Dioryctria abietella* and *Ernobius abietis*.

Generally, all chapters based on the elevational gradient approach provided results useful to better understand the effect of future climate change on insect herbivores, although many of the interactions among elevation, host plant, and insects are still to unveil.

Chapter 1

Introduction

Parque Natural de Massís del Montseny (Spain)

1. Climate change and insect herbivory

1.1 Climate change

Global climate change is the set of changes in the time which are slow processes happening on the Earth. “Global average surface temperature has increased by around 0.6°C during the past century, with the 1990s being the warmest decade” (Menéndez 2001) and this trend is expected to continue in the future (Solomon *et al.* 2007). By 2100 global mean air temperature will rise of 1.8 to 4.0˚C (Metz *et al.* 2007). Climate warming caused changes in some natural ecosystems because they have limited adaptive capacity (Watson *et al.* 1997; Adger *et al.* 2007).

In the last years the changes become more visible in the environments: ecological impacts of global warming of organisms (shifts in ranges, extinction of species, migration, new adaptations, changes in species interactions) (Menéndez 2001). Parmesan and Yohe (2003) noted that due to the change of climate, insect species spread at mean rate of 6.1 km per decade.

Recent studies suggest that the conditions in the upper elevation and also in higher latitude will became more suitable for organisms, because global warming will exceeded threshold of tolerance (heating and aridity) in lowlands. The uphill can became refuges for escaping organisms from lowlands (Hardy *et al.* 2010).

Netherer and Schopf (2010) reported that some insect herbivores have already changed dynamics and range expansion associated with climate change has been observed in many places (Williams & Liebhold 1995; Parmesan 2006; Hillstrom & Lindroth 2008). The examples concern changes of frequency and spatial patterns of pest outbreaks, changes in voltinism, changes in overwintering and in the host plant. Some organisms switch their host and change their elevational range, like pine processionary moth *Thaumetopoea pityocampa* (Hódar & Zamora 2004; Battisti *et al.* 2005; 2006; Krehan & Steyrer 2006; Buffo *et al.* 2007; Petrucco-Toffolo & Battisti 2008).

Most of the studies support the theory that climate change will cause negative influence on organisms and generally biodiversity (Parmesan & Yohe 2003; Franco *et al.* 2006). Laštůvka (2009) marked that the climate change can influence positively the insect pests, because they can easy adapt to new conditions, and they may disperse over landscapes maintained by man and occupy new habitats which become for them suitable.

The question asked by Menéndez (2001) is "why should we expect an effect of climate change on insects"? Bale *et al.* (2002) explained that insects can by possibly affected by climate change because of their development, reproduction and survival and also Menéndez (2001) added that they can be likely sensitive for this processes because they have short generation times and high reproductive rates, that is why the response to climate can be quicker than for example vertebrates or plants.

There are many studies about insects along the different gradients: latitudinal, elevational (altitudinal) in many sites around the world. Some of the known papers about the life along the different gradients (latitudinal, altitudinal) describe insect species (Straw *et al.* (2009) or communities (Beránek 2008), another prefer to focus on more general unit which are guilds, like leaf sap feeders, chewers, miners (Andrew & Hughes 2005; Lara *et al.* 2006; Garibaldi *et al.* 2011).

There are some studies showing different patterns of insect **abundance** along the gradients. In some of them we can observe that with increasing elevation abundance of insects is declining (in Garibaldi *et al.* 2011: Galen (1990), Kelly (1998), Suzuki (1998), Alonzo (1999)) as well as the opposite trend (Erelli *et al.* (1998), Hagen *et al.* (2007), Haynes *et al.* (2012), Bess *et al.* (1947), Houston and Valentine (1977), (Liebhold *et al.* 1994) . There are also studies about **species richness** along the altitudinal gradient like for example Röder *et al.* (2010), indicating that with increasing elevation insect species total richness decreases, while for some guilds such as the fungivores an opposite pattern has been found. Rahbek (1995) showed in his review paper that the richness of species decreases with elevation, not always monotonically. He showed some example of possible patterns of species richness (not only insects) in the literature (review on the base of 97 papers on this topic). The most common trend according to this research is that the biggest richness appears in the mid elevation compared to high and low elevations. As a difficulty in this type of studies he mentioned the area of study, as "it is difficult to find appropriate gradients with continuous natural habitat along the entire gradient". Also Wilson *et al.* (2007) marked that in the experiment along the elevational gradient humped shaped relationship between insect richness and elevation appeared.

Some of the hypotheses says that insect richness is related to plant species richness, because more diverse plant species can by colonised by more diverse insect herbivores (Southwood 1961) in Lara *et al.* (2006).

1.2 Insect herbivory

The organisms feeding on the plant tissue are commonly named herbivores. Insects also belong to this group. Guilds are the groups of insects which are exploring the same resources in similar way (like feeding or reproduction). They are not systematic groups. The guilds which we tested in our experiments are: chewers, leaf sap feeders, gall makers, leaf miners.

Chewers they have mandibulate type of mouthparts. This means that they have three pairs of appendages opposing each other (Schoonhoven *et al.* 1998). They possess mandibles which serve to cut and grind the food. They have also tooth – similar ridges to cut food and grinding surface to crush the food. Just below mandibles maxillae are located. Each maxilla has a segmented structure called maxillary palp, which is equipped with chemoreceptors called sensilla. Maxillae are helping in moving food and guiding it toward the mouth. Preoral cavity is made by labrum (upper lip) and labium (lower lip). Labium has also one pair of palps with mechanoreceptors. The ventral part of preoral cavity is called epipharynx and very often have taste sensilla (Schoonhoven *et al.* 1998).

According to Fontaine *et al.* (1991), some insects can have harder mandibles because of enrichment of cuticle in zinc and manganese. The toughness of leaves matters to insects because some of them like for example the polyphagous beet armyworm (*Spodoptera exigua*) chews three times longer on celery (*Apium graveolens*) because of tough leaves than on nettle leaf goose foot (*Chenopodium murale*) (Schoonhoven *et al.* 1998).

Sap feeders are equipped in haustellate mouthparts. This type of mouthpart is devoted piercing plant tissues and sucking the liquid food. There are different modifications of this type of mouthparts, belonging to different orders of insects like: Hemiptera and Thysanoptera. (Schoonhoven *et al.* 1998)

"The more delicate feeding strategy developed by sap – feeding insect places a restriction on larger size": chewers are generally bigger than sucking insects (Schoonhoven *et al.* 1998).

Sap – sucking insect mouthpart are very diverse: for example: aphids and scale insects often selecting phloem tissue – they have longer stylets to reach it (Hodkinson & Hughes 1982). In the contrary, some leafhoppers which feed on the mesophyll cells have short stylets with barbed apices (Pollard 1972). In the group of Thysanoptera the right mandible is reduced and in some species is absent. The left mandible is bigger and makes a narrow stylet to pierce the cells of the tissues (Childers & Achor 1989).

Gall makers

This group of phytophagus insect can appear on different part of the plant: leaf, shoot, stem, root, plant reproductive organ. Galls are formed when the normal development of tissue are disturbed by insects (Speight *et al.* 2008). The insects create the chamber in which the larvae and nymphs of them can grow. To the orders of insect which forms galls we can include Diptera, Hymenoptera and some of Hemiptera (Homoptera). Some of the species are able to change the chemistry of the plant to adapt the tissue as a food for themselves (Nyman & Julkunen-Tiitto 2000).

Leaf miners

Miners represent few orders of insects like: Coleoptera, Diptera, Hymenoptera, Lepidoptera) (Speight et al. 2008). While miners are feeding on the leaves they leave characteristic trails on leaves.

The mines often contain frass and droppings. Following the patterns of leaf miners left on the leaves – it is possible to recognize sometimes the miner to the genus or even species.

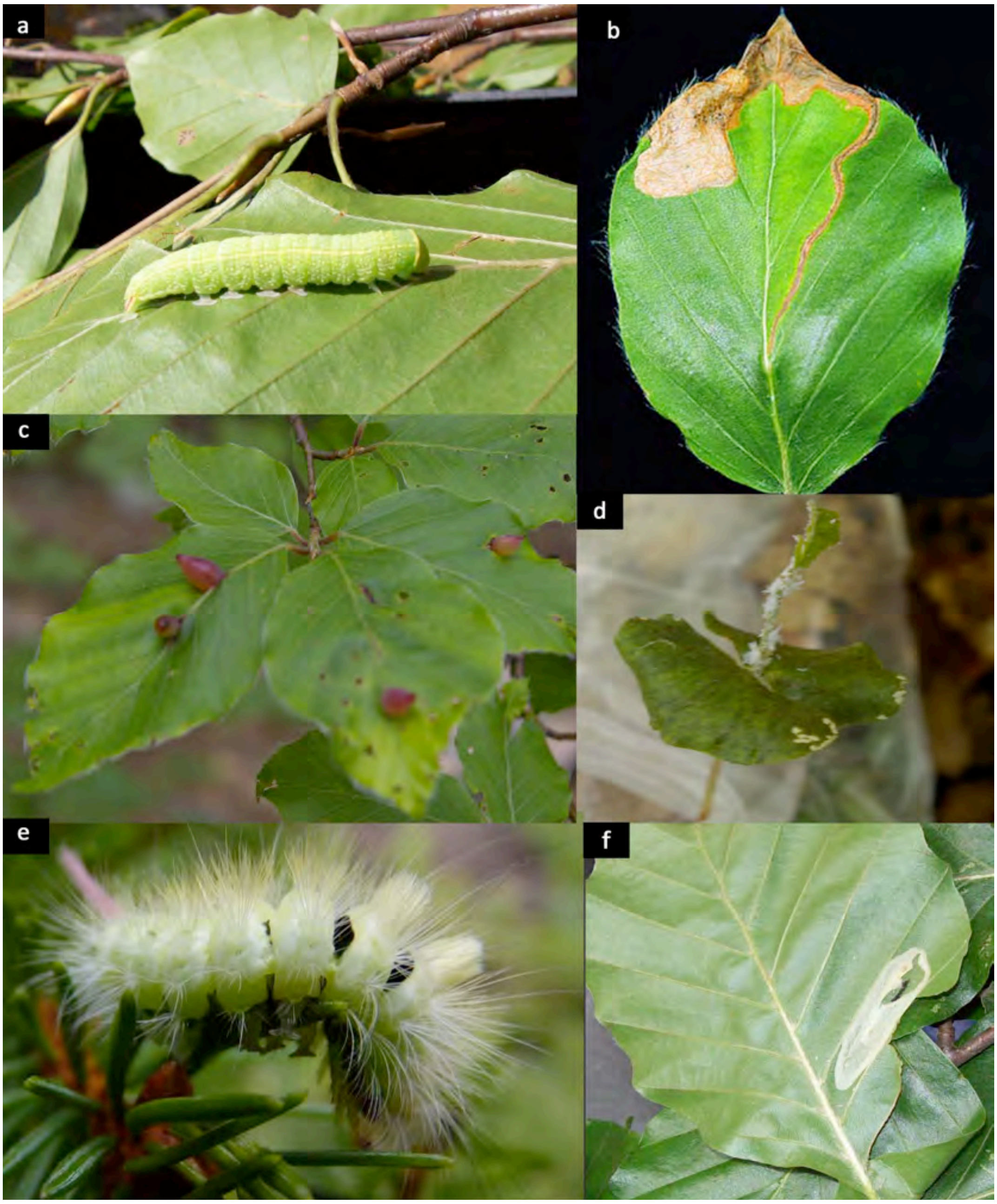

Plate 1. Examples of Insect herbivory a. Chewer caterpillar of *Pseudoips prasinana* (Poland) on beech; b. Sap feeder *Phyllaphis fagi* on beech seedling (Spain); c. Leaf chewer and also leaf miner *Rhynchaenus fagi* (photo by Gyorgy Csoka in Hungary); d. *Calliteara pudibunda* larva chewer (photo by Elena Granda in Poland); e. Gall maker *Mikiola fagi* in Spain; f. Leaf miner *Phyllonorycter* sp. Poland

2. Altitude as a proxy for the temperature and future climate change

According to the well – known Rapoport's rule about the latitudinal gradient and the species richness, ”low-latitude localities have relatively more species near the edge of their range than do high latitude sites” (Stevens 1992). Is the Rapoport's rule applicable also for altitudinal gradient? How looks the situation of abundance of the species along elevational gradient?

Hodkinson (2005) in his review is trying to show all variables of elevational gradients, which are for example: temperature, precipitation, solar radiation, partial pressure and respiratory gases, turbulence and wind speed. He mentioned that all the rules can find the exceptions because of local climate changeability. He tells also that in the last years awareness of the altitudinal gradients characterization is useful as climate change analogue. In the future, with climate warming we can assume that there will be shifts in the elevational steps – in the upper elevation the temperature will increase so the species from lowland can migrate uphill. He explains that the influence of different altitude can be direct or indirect, provided by host plant. Sometimes to understand one species appearance or guild presence it is necessary to consider all trophic food chain like: host plant, herbivore, specific parasitoid, predators, competitors and pathogens. Very important is to find the way of standardize all disturbing factors, for example abiotic factors.

The advantages of studying elevational gradients is that there is no problem with some additional disturbances like: long distance, day length (Garibaldi *et al.* 2011). But with studying elevational gradients there are other disturbing factors described below.

Climate change can influence forest pests and the size of damage caused by them: directly through their development, reproduction and distribution and indirectly by altering host

Plate 2. Examples of insect herbivory (on pine and spruce) a. *Thaumetopoea pityocampa* nest (in Italy, Val Venosta); b. *Leucaspis loewi* (in Spain, Sierra de Guadarrama); c. *Strobilomyia sp*. on spruce cone (in France in Belledonne); d. *Petrova resinella* on pine in Spain (in Spain, Sierra Nevada); e. *Gilpinia hercyniae* on spruce in Poland (Krościenko).

physiology and defence mechanisms. The climate change might have impact on the relationships between pests, their natural habitats and other elements of the natural ecological chain, like natural enemies and competitors.

2.1 Abiotic factors

The mountain gradients are characterized by all complex physical parameters which vary along steep elevational gradients (Hodkinson 2005).

Temperature lapse rate, that is mean decrease changes with increasing temperature around 5.5 and 6.5°C for each 1000 m of elevation (Anslow & Shawn 2002). With studies along the gradient it is necessary to take into account the meteorological circumstances and also which is the type of climate for example continental vs. maritime (Pepin 2001). There is also a problem of time appearance of the insects along the gradient like their phenology and seasonal differences.

The temperature is recognized as one of the most contributory factors in relation to plant – insects (Bale *et al.* 2002; Deutsch *et al.* 2008; Tylianakis *et al.* 2008; Garibaldi *et al.* 2011). "The effects of temperature (particular in the case of altitudinal gradients) cannot be considered in isolation from other factors that also change with altitude" (Bale *et al.* 2002). The good example of direct effect of temperature on insect is *Cydia strobilella* in Switzerland on *Picea abies* (Wermelinger *et al.* 1995). Abundance of this species with increasing altitude decreases. The same pattern we can find in Italian study of *Lymantria monacha* on *Picea abies* (Cescatti & Battisti 1992).

According to Caldwell *et al.* (1998) **UV short – wave radiation** has a deleterious effects on organisms.

With increasing altitude, the **pressure of gases** like oxygen or carbon dioxide is linearly decreasing (Peacock 1998). The conditions for insects will change around 11% - 1000 m a.s.l, 31% - 3000 m a.s.l. and 46% at 5000 m a.s.l. (Hodkinson 2005). The direct influence of thinner air at higher elevation can implicate "convective energy transfer for exothermic organisms" and also for the possibility of flight (Hodkinson 2005).

Precipitation is playing one of the major roles in insect distribution and diversity, because influence directly host quality (Hodkinson 2005). Orographic precipitation, like snow or rain, often rises with increasing elevation (Egger & Hoinka 1992). Precipitation is also one of the most confounding factors in altitudinal environmental gradients (Körner 2007). There is no clear and repeated trend on altitudinal precipitation and many gradients can show different patterns (Laternser & Schneebeli 2003; Weltzin *et al.* 2003). Some of the mountain forests are dry at low altitude and humid at high altitude (like for example slopes around the Intermountain Basin in the western USA) but other can show intermediate humidity peak (for example Mount Kilimanjaro) or can be humid at the lower elevation and dry on the top (for example some parts of Andes and Tenerife) (Körner 2007). It is better to consider changes in precipitation in the local range, avoiding comparison with other "precipitation gradients".

Turbulence of air and **wind** can influence in two ways: negative, because it will interrupt insects with their flight and will bring them to less suitable conditions and positive – when the wind will help to distribute and disperse insects to another parts where can increase population. Speed of wind usually increases with altitude, but there are exceptions of local differences due to the surface and topography (Hodkinson 2005). Very important case of the wind is topography of the mountains (Körner 2007). The windiest fields are for example mountains situated on the coastal areas. It is difficult to compare wind exposition between coastal and continental mountains.

Available land area is very important in gradient studies. It allows to analyze organismic diversity and evolution and include mountain geometry and history (Körner 2007). With increasing altitude this land area is becoming smaller, what is a crucial thing in habitat studies. To understand land area we should consider bed rock, degree of erosion and also steepness, which are also mentioned by Körner (2007). "As altitude increases, the associated climatic changes thus apply to a dramatically diminishing fraction of land" (Körner 2007). Rosenzweig (2003) considers the land area changes like an important and major driver of organismic diversity and evolution.

2.2 Direct effects on insects

Direct effect of altitude on insects is mainly driven by **temperature** but also there is an influence of other direct factors like: **radiation input** (including UV – B), **precipitation**,

oxygen availability and also **wind disturbances** (Hodkinson 2005). Variation in morphology like wing size polymorphism; harsh cold conditions are making very hard to fly for insects that is why the insects in the colder conditions have reduced their wings (Strathdee & Bale 1998). Differences in colour, absorbance and spectral reflectance – some of the insects species shows colour pattern polymorphism or body colour variation with respect to the elevation (Hodkinson 2005). Dark colour of the body at higher altitudes maximizes heat absorption and the lighter or reflective colouration minimizes taking heat under warmer conditions at the lower steps in the mountains (Heinrich 1996).

The body size of insects is positively correlated with altitude and latitude (Alonzo 1999; Smith *et al.* 2000; Malo & Baonza 2002) or it is not (Hawkins & Lawton 1995; Hawkins & DeVries 1996). This pattern is consistent with size – temperature hypothesis, which says that the size of the body increases with low temperatures (Atkinson 1994) but there are observed also opposite patterns (Sota 1996; Brehm & Fiedler 2004; Kubota *et al.* 2007). Very interesting example showed Guevara and Aviles (2007) on the example of social spiders and insects in Ecuador. They found that, on average, insects in the lowland rain forest are larger than insects at the higher altitude. The same findings had Janzen *et al.* (1976) and Powers and Avilés (2007). Those results are opposite to well known Bergmann's rule, which says that the animals will be bigger at altitude (latitude) where temperature is lower (Bergmann 1847). Guevara and Aviles (2007) use the converse Bergmann's rule, which says that the size of insect increases while the elevation is decreasing (or latitude) (this was summarized in Mousseau (1997), Blanckenhorn and Demont (2004), and Dillon *et al.* (2006)). This situation is explained by Guevara and Aviles (2007) by longer growing season in those areas.

The temperature affects all of the insects in the mountains. It depends on the insects how do they adapt to changes of the temperature and how are their limits to survive those changes. Temperature is the main determining factor for distribution, so the insect species must match its thermal tolerance range to the altitudinal temperature profile of its habitat (Hodkinson 2005). There is example of pine processionary moth (*Thaumetopoea pityocampa*) which reacts very clear with climate change (Battisti *et al.* 2005). In the warm summer of 2003 the pine processionary moved in the higher elevation in the Italian Alps

(Battisti *et al.* 2006). The explanation of this was unusual high temperature at night which increased nocturnal activity of females (Battisti *et al.* 2006).

Reaction for declining oxygen is very important for insect living in the mountains. It is related to increase in air intake through insect tracheal system. This in turn leads to increase of the rate of water loss in the respiratory exchange cycle (Chown 2002). If the insects is not able to compensate for decreasing oxygen it can have a problem with energy budget and growth (Hodkinson 2005). The hibernation is prolonged under the snow cover and the insects have short and rapid period of development in colder conditions (Mani 1968). The soil under the snow is not frozen, that means that under the snow, in cold conditions there can be alive faunas, even if the atmospheric temperature can drop -45°C (Mani 1968). The high altitude environment favours univoltine species, but some bivoltine species also occurs (Mani 1968). The fecundity prediction might show a decreasing pattern with increasing altitude, but this also can be related to body size of the insects, quality of the food, shorter time for oviposition (Hodkinson 2005).

2.3 Indirect effect through the host plant.

Growth and morphology of the trees are very important for the insects as a potential food. Trees differ in the upper part of the gradient to compare with the lower parts. Leaves are stronger usually and tougher up, prepared for harder temperature and solar radiation conditions. Roots of trees are deeper in the upper parts of gradient where can allow root feeders to become more abundant (Hodkinson 2005). Also probably for herbivores important is plant host genotype, which can cause problems for the insects encountering more resistant tree genotypes in higher elevation (Garibaldi *et al.* 2011). The bark beetles depend a lot on host tree quality. They choose for example the weakened trees like *Ips typographus* on *Picea abies*. *Ips typographus* is able to respond very quickly for the suitable conditions: like host abundance and weather extremes or stand general conditions (Faccoli 2009). Usually trees which are growing slower can defence themselves better (Stamp 2003; Fine *et al.* 2004). Also very important for insects are nutrients inside the host plant.

The C/N ratio hypotheses is telling that with increasing altitude C/N ratio decreases, that is mean that the foliar N content is bigger on the upper part of mountain than in the lower. The opposite pattern can be observed with gradient of the soil, because more nutrient – soils are present in the lowest parts of the mountains (Hodkinson 2005). The content of N can vary between the species of trees as well. Some hypotheses say that larger amount of N in the upper part is due to bigger space for trees, without competition (McMillin & Wagner 1998).

The water content decreases with increasing elevation which is contrary to secondary metabolites (Netherer & Schopf 2010).

Plant seasonality is different along the gradient as well, so that appearance of insects can differ. There is well known fact that those seasons become shorter when altitude is increasing. That is why it is difficult to standardize seasons in different elevations. All "seasonality" factors are complex because it depends on temperature, humidity and then local microenvironment."Ecological tests for general season – length effects in mountain biota, therefore need to account for confounding moisture effects" (Körner 2007). Sometimes cyclicity of the tree can influence life cycle of an insect.

The influence of elevational gradient on cyclicity of insects is very important but not widely explored. This type of studies were done by Haynes *et al.* (2012), who was analyzing cyclicity of gypsy moth like an example of generalists in populations along elevational gradients on the base of 31 – year records (1975 – 2005) of damage. In this paper the conclusion of the model is that density of preferred host tree species could explain patterns of gypsy moth cyclicity (Haynes *et al.* 2012).

Netherer and Schopf (2010) also mentioned about tree and insect phenology regulated by temperature and the risk of disturbance by climate change.

Climate change can modify the natural synchronization between the host plant and insects, for example causing warmer springs, which will disrupt bud burst of oak (*Quercus robur*) and egg hatching of *Operophtera brumata* feeding on it (Visser & Holleman 2001). The time of hatching is important for larvae because they should feed on fresh leaves of oak. If they emerge too early they will die of starving, if too late the food will have too high concentration of tannins (Van Dongen *et al.* 1997).

“Conspecific tree growing at high and low elevation encounter different growing conditions and may vary in their suitability as hosts for herbivorous insects. Mountain tree populations may be more resistant to herbivory if low temperature constrain growth more than they constrain photosynthesis, resulting in increased secondary metabolism (temperature hypothesis). Alternatively mountain trees may be fertilized by atmospheric nitrogen deposition and became more palatable to insects (atmospheric deposition hypothesis)” (Erelli *et al.* 1998).

Secondary compounds and defensive chemicals are also changing with altitudinal gradient (Hodkinson 2005). Plants, which are growing in more stressful conditions, like in the upper parts of the mountains – they invest less in producing defensive compounds (Erelli *et al.* 1998). Erelli *et al.* (1998) gives an example of *Betula papyrifera* – in New Hempshire. Concentrations of condensed tannins in mature trees at lower altitude were around twice than those at higher altitude.

The structure of forest can change along the altitudinal gradient. When the trees are growing beyond their normal range – they can be more palatable to insect herbivores attack, like in Japan happened with *Tsuga* spp. which grows in the not natural range, is very susceptible to the scale insect *Neuculaspis tsugae* (McClure 1985).

2.3.1 Tree features along altitudinal gradient and C/N ratio

The carbon – nitrogen hypothesis tells also that plant defences are connected to variations in the environmental nutrients. This hypothesis shows carbon/nitrogen ratio of plants determined by their secondary metabolites (Bryant *et al.* 1983). For example the plants growing on the soils poor of nitrogen will use carbon – based defences and those which grow in carbon – poor soils will produce nitrogen – based components. This theory suggests that plant can modify their defence according to available nutrients.

Reich and Oleksyn (2004) reviewed many studies about C, N, P and C/N and N/P from plant species worldwide and they tried to mark some common patterns. They included data from 5,087 observations of leaf nitrogen and phosphorus for 1,280 plant species at 452 study sites. The observations were reported for five groups of plants like: grasses, herbs, shrubs, coniferous and broadleaved trees. Results included in this review supported two hypotheses:

- The content of leaf N and P increases from the tropics to cooler latitudes because of temperate – related plant physiological stoichiometry and gradients in biogeochemistry;
- N/P ratio increases with mean temperature and toward equator because P is a major limiting nutrient in older tropical soils and N is the major limiting nutrient in younger temperate and high – latitude soils.

They also found significant relationships of leaf N, P, N/P to mean annual temperature and also to latitude for all species. They have noticed that mean annual temperature was stronger correlated with N, P and N/P than with the growing season length, growing season temperature, annual rainfall or other climate variables.

There are many papers describing the influence of temperature and latitude on carbon and nitrogen content among different groups of plants. Körner (1989) reported that deciduous trees growing in colder conditions along altitudinal and latitudinal gradients have greater leaf N, "a pattern consistent across and within species". Evergreen tree species, however, have similar N level all along the gradient (Vitousek *et al.* 1988; Körner 1989). According to Yin (1993), evergreen conifers have the biggest % N in the middle latitudes (around 45°N) and they decreased to the north.

It is possible to find among those papers field experiments and also common garden experiments, in which authors studied physiological and chemical parameters, like C/N ratio, content of C and N, and others like isotopes.

2.3.1.1. Field experiments

Trees

Cordell *et al.* (1998) checked physiological and morphological variation in *Metrosideros polymorpha* in Hawaii along an altitudinal gradient (field and common garden experiment) to be able to detect if the phenotypic variation is because of genetic differences or by phenotypic modifications due to environmental conditions. Results showed that: foliar $\delta13$ C increased with elevation (in the field experiment). Foliar $\delta13$ C remained stable (in the common garden experiment). Foliar N varied significantly with elevation in the field and also in common garden (increase with elevation).

Herbaceous plants

According to experiment of Huber *et al.* (2007) which was carried in alpine – nival ecotone in Tyrol in short altitudinal gradient of 173 m: soil pH increased with increasing altitude, soil C/N ratio, total N and total C decreased with increasing altitude, amount of plant available N decreased with increasing altitude, soil δ^{13} C - no changes along the gradient, soil δ^{15}N decreased with increasing altitude, δ^{15}N of plant tissue (leaves and roots) decreased with increasing altitude, soil δ^{15}N values were more positive than those of plant tissues.

Huber *et al.* (2007) used natural abundance of the stable isotope ^{15}N to explain the differences in N cycle. "Natural abundance of ^{15}N was used as a tool to study ecosystem processes as it acts as an integrator of the long – term ecosystem N balance (Högberg 1997)".

The soil was taken under the alpine *Carex curvula* grassland and the patchy alpine – nival plant assemblages. The plants belonged to two vegetations types: alpine grasslands (*Caricion curvulae*) and patchy nival plant assemblages (*Androsacion alpinae*).

Ferns

There were also C/N studies considering other plant species like ferns along altitudinal gradient. Wegner *et al.* (2003) found that on the level of fern community: N concentration increases with altitude, C concentration decreases with altitude, C/N ratio decline with increasing altitude.

This pattern was detected also in previous studies: (Körner & Cochrane 1985) for eucalypt; (Woodward 1986) for blueberry; (Körner 1989; 1999); (Diemer 1998a, 1998b) for Andean shrub species.

2.3.1.2. Common garden experiments

Trees

Reich *et al.* (1996) and (Oleksyn *et al.* 1998; 2002) found that population of *Pinus sylvestris* and *Picea abies* "originating from latitudinal and altitudinal gradients, differed in needle macro – and micronutrient concentrations". "These results indicated that plants from cold environments had significantly higher foliage N, P and Mg concentrations when grown in common conditions, and that this may be an adaptive feature that enhances metabolic activity and growth rates under low temperatures of their native habitats (Reich *et al.* 1996; Weih & Karlsson 2001)". Oleksyn *et al.* (2003) asked the question: "Are the resorption patterns observed in the common garden environment similar to those in situ along a temperature gradient in Europe?" They consider both latitude and altitude. They found different geographic patterns in foliage N and P for populations in the common garden vs. the population samples in situ along the latitudinal transects: Common garden situation: green foliage of *Pinus sylvestris* N and P concentrations increase with increasing latitude of seed origin (Oleksyn *et al.* 2003).

Plants sampled along the latitudinal/temperature gradient show that N and P (less visible with P) decrease with increasing site latitude (Oleksyn *et al.* 2003). The nutrient (N and P) resorption patterns depend on many factors: like partial genetic control which can determine adaptive processes (higher nutrient conservation in plants from cold habitats) (Oleksyn *et al.* 2003).

In common garden Reich *et al.* (1996) have done experiment with *Pinus sylvestris*. The results were that: needle N (%) decrease with mean annual temperature, needle N (%) increase with latitude of origin. Reich *et al.* (1996) marked that: "gradient patterns must be examined carefully to separate genotypic from environmental effects".

Reich *et al.* (1996) wanted to investigate biogeographically variation in foliar N and Rd (dark respiration rates) in "*Pinus sylvestris* populations of wide geographic origin, utilizing a common garden approach to examine genotypic differentiation among populations". They checked 14 *Pinus sylvestris* populations of wide latitudinal origin. They wanted to test hypothesis: "that population originating in colder habitats have a genetically determined tendency towards higher needle N and Rd than populations from warmer habitats, and that there is a significant association between these two leaf traits".

According to the Weih and Karlsson (2001) studies with *Betula pubescens* seedlings in the research station the "major effects of increase in air temperature were increases in shoot growth and leaf area allocation, and decreases in plant N concentration and leaf N content". They suggest that this increasing leaf N pattern with decreasing air temperature may be caused by physiological adaptation to low – temperature conditions and it is not only a consequence of weaker N dilution by reduced growth (smaller plants).

According to Jian *et al.* (2009): (research station experiment) leaf N, P and K of *Quercus liaotungensis* increased along the altitudinal gradient (1020 – 1770 m a.s.l.). N/P ratio decreased along the altitudinal gradient – P limitation for *Quercus liatungensis*. They found that leaf N and P have very weak correlation with soil N and P (out of their expectations).

Herbaceous plants

Experiments described by Morecroft *et al.* (1992) on the causes of altitudinal differences in the leaf nutrient contents, size and δ^{13} C – the carbon isotope ratio of ^{13}C:^{12}C according to international standards (Craig 1957) of *Alchemilla alpina* conclude that:

In higher altitude leaf N concentration and δ^{13} C are both higher than in lower altitude (the range of altitudes is from near sea level to about 1300 m). In field transplant experiment N concentration increased with altitude. In the field transplant experiment P concentration increased with altitude.

C/N ratio, N/P ratio increased with altitude. They carry out experiment in both field and controlled environment, with more clear results from the field.

Effects of climate warming on mountain ecosystem have already been observed through shifts in soil – plants nutrients along altitudinal gradients, like for example shift in δ^{15}N values was more pronounced in soils (5.5‰) than in plants where δ ^{15}N values only decreased by about 2‰" (Huber *et al.* 2007).

Natural abundance of ^{15}N was used as a tool to explain differences in N cycling along altitudinal gradient.

Based on this Huber *et al.* (2007) made a hypothesis that "lower temperatures reduced rates of ecosystem N turnover and N accumulation".

Changing temperature with altitude influences plant assemblages and "cover which in turn affect soil N cycling through litter quality and quantity" (Steltzer & Bowman 1998; Bowman *et al.* 2004). On those quantity and quality may have influence factors like changing climate can change composition of plant communities species which may affect soil compounds (N dynamics changes) (Hobbie 1996) and also microbes tightly connected with plants "may play a crucial role in driving the cycling of N" (Huber *et al.* 2007).

Woods *et al.* (2003) showed that: "cold exposure leads to significant increases in nutrient content and amount". They have checked groups of organisms like: plants (21 species), animals (25 species), algae (20), bacteria (8) and yeast (1). "Three of four groups contained significantly more nitrogen following cold exposure, and all groups contained more P after cold exposure. The separate groups, with the exception of bacteria, thus showed systematic effects of temperature on chemical composition. For algae and yeast, the mean increase in P – containing compounds, tough large ($78\%=10^{0.250}$), was not significantly different than zero, probably reflecting small sample size (N=6). The primary exception to the overall pattern was bacteria, which showed no evidence for increased N or protein in the cold".

Körner (1989) said that high – altitude plants have higher N content than low – altitude and that leaves generally decrease in size and area with increasing elevation.

There is also influence of light availability on foliar nitrogen concentration, nitrogen content and ^{13}C abundance for different tree species (Kranabetter *et al.* 2010). Natural abundance of ^{13}C is useful tool to establish index in ecophysiological studies and it is made on the base of discrimination against the heavier ^{13}C isotope during CO_2 gas exchange and photosynthesis (Farquahar *et al.* 1989).

According to Kranabetter *et al.* (2010) (In research forest they used 4 tree species*: Tsuga heterophylla*, *Picea glauca* x *sitchensis*, *Betula papyrifera*, *Thuja plicata*) – Foliar % N declined in shade for *T. plicata* (40%) and for *B. papyrifera* (20%) and no shading effect for foliar N % of *T. heterophylla* or *P.glauca*.

"The natural abundance of ^{13}C was strongly positively correlated with light availability for all species, with the best fit as an exponential curve for conifer species and a linear relationship for *B. papyrifera*".

2.3.2 Leaf toughness

There are different types of defenses in host plants. It could be chemical defense like releasing alkaloids, steroids, phenolics, saponins, tannins, resins or other chemicals or physical defense, which includes anatomical adaptations of the plant like leaf toughness, production of trichomes, spines, thorns and stiff hairs.

"Toughness is the ability to resist crack growth and is defined as the energy consumed in growing a crack of given area. Its units are Joules per square meter (Jm^{-2}) or a unit 1000-times larger ($kJ\ m^{-2}$). High toughness does not mean that a solid is strong, or vice versa, and, in general, high toughness is incompatible with high strength." (Lucas *et al.* 2000).

Increased in toughness interference with leaf feeding and oviposition mechanism of insects (Maxwell & Jennings 1980).

According to many authors (Maxwell & Jennings 1980; Schoonhoven *et al.* 1998) toughness of leaf tissue is very efficient and important defence mechanism. Its shows the strength and condition of the plant and can explain many situations like phenology of the tree along the elevational gradient.

Amount of damaged made by insect herbivores depends on plant fitness and general health conditions.

Leaf toughness can be due to high fiber content or by silica (Maxwell & Jennings 1980). Plant susceptibility to the insect attack is often influenced by mechanical defences (Paul *et al.* 2012). Leaf toughness can indicate plasticity of the cell wall and the avoidance of intracellular collapse upon a stress such as the leaf chewing or puncturing action of insects (Lucas *et al.* 2000).

Many tropical forest plants are less palatable to insect herbivores when they are tougher, or they have less N amount (Howlett & Davidson 2001).

The insects can have different preferences of choice the plant. It can depend on chemicals and also on the plant leaf surface. Some of them can prefer rough over smooth artificial surface like diamondback mooth *Plutella maculipennis* (Schoonhoven *et al.* 1998). It is said that plant structural characteristics like surface morphology and toughness, the same like the levels of primary and secondary compounds, can be affected by air pollutants (Hughes 1988; Heliövaara & Häisänen 1993; Brown 1995; Schoonhoven *et al.* 1998).

2.4 Indirect effect through the natural enemies

Other important factors influencing insects along the gradient are biotic factors like: competition, predation, parasitism. The studies of population dynamics are very important in altitudinal gradient, because it allows to understand insect abundance of the target species over its elevational range (Hodkinson 2005). Many studies have been done on three – trophic level, for example: in Poland there was a decrease of target species of *Coccinella septempunctata* along the gradient with observations of *Dinocampus cocinellae* which is parasite on it (Ceryngier 2000) and another example of decreasing pattern of target species

(*Dendroctonus micans*) with predator *Rhizophagus grandis* in France (Gilbert & Grégoire 2003).

Some of studies showed that with increasing elevation damage made by insects is lower (Galen 1990; Kelly 1998; Suzuki 1998) and sometimes some entomocenosis observations showed that with increasing elevation there are less insects (Beránek 2008; Röder *et al.* 2010) which in consequence can cause less damage in the highest altitude. There are also some opposite patterns: with increasing altitude – damage made by insects also increases (Erelli *et al.* 1998). In some papers we can observe not very clear pattern with altitude like for example the case of *Elatobium abietinum* in Straw *et al.* (2009) studies where the highest abundance of this insects appears in the mid altitude, however natural enemies (invertebrates) of *Elatobium abietinum* like coccinellid and cantharid beetles, syrphid larvae, brown lacewings, spiders and harvestman were the most abundant in the lowest elevation. In this paper they consider the influence of the natural enemies and also the temperature on *Elatobium abietinum*.

Many species of insects live in the mountains conditions along all elevational gradients. They adapt to different environments in the lowest and highest altitudes. Insect respond to the gradient directly and through its host plant (Hodkinson 2005).

The presence and abundance of insects along the slope (gradients) can be regulated by their predators, competitors and parasitoids, like in the case of spatial distribution of pine sawfly, *Neodiprion autumnalis* (McMillin & Wagner 1998). There are main significant drivers of oviposition, which are tree density and elevation. *Neodiprion sertifer* (*Hymenoptera*) on *Pinus sylvestris* increases with increasing altitude in Finland (Niemelä *et al.* 1987) and possibly relate to absence of natural enemies.

To get a full picture of appearance of insects and their dynamics along the gradient, it is necessary to see tritrophic interaction between host plant – herbivorous insect and its parasitoids and predators.

Randall (1982) studied population dynamics along the altitudinal gradient (15 – 610 m a.s.l) of *Juncus squarrosus* seed feeders – *Coleophora alticolella* in England in between 1977 – 1979 and he found that: there is parabolic altitudinal pattern of abundance in all years studied, there was the highest density of *Coleophora alticolella* eggs and larvae in the middle altitude (with respect to the food supply), mortality associated with parasitoids declines with rising altitude and host plant producing suitable seed capsules increases.

Virtanen and Neuvonen (1999) studied *Epirrita autumnata* larvae feeding on *Betula pubescens* in Finland in the mountain (80 – 320 m a.s.l) in between 1993 – 1996. They found that:

differences in performance of *E. autumnata* were related to temperature conditions that is mean that in the lower altitude where the temperature was higher, survival and the egg production index were lower and parasitism higher than in the higher altitude, secondly that for *E. autumnata* the most important factor in the life cycle was egg mortality related to minimum winter temperature, followed by parasitism and also variation in food quality and the last that the potential area and also frequency of *E. autumnata* outbreaks will be bigger in the future with climate change. The summer temperature can increase parasitoid and predator activity that is why in summer the abundance can decrease due to this process. In the contrary – in the cooler summers, the outbreaks may be more frequent. So according to this conclusions, they hypothesis is that with climate change and global warming the outbreaks of *E. autumnata* will decrease.

3. Objectives of the study

3.1 Insect herbivory and climate change

We wanted to test if the difference in temperature between altitudinal (elevational) steps, will change also appearance of the insect herbivory in respective the host plants. The aims of the insect studies along the altitudinal gradients is also to distinguish abundance pattern of insect guilds like leaf chewers, leaf sap feeders, leaf miners, gall makers on pine, spruce, beech and larch and also abundance and richness of some single species.

3.2 C/N ratio study

The aim of the studies with C/N is to build a hypothesis about how variation in carbon and nutrients content of plants (especially trees) along temperature gradients may affect the performance of herbivorous insects. We know that the use of altitudinal and latitudinal gradients as surrogates of temperature can be misleading because of several confounding factors (e.g. different biogeochemistry, water availability, solar radiation, nutrient deposition). In our experimental setup we tried to keep such factors at the lowest impact as possible by choosing short and steep altitudinal gradient with very similar type of stand composition and structure. We also added a generic measure of plant suitability to insect herbivores consisting of leaf toughness. We tested our hypothesis with measurement of insect performance on – site and in field and laboratory bioassays. By testing this hypothesis, we may obtain information allowing predicting the effects of the climate warming on the performance of insect herbivores of forest ecosystems. Our climate – warming related predictions would be trivial if the adaptive response of the organisms involved would be the same (everything is shifting) but we know that herbivores are highly mobile whereas plants (and especially trees) are much slower in moving along gradients. So we predict that the gap in the response of plants and herbivores to climate warming may disrupt the equilibrium reached in a long time of coexistence in the forest ecosystems. To quantify the direction and the magnitude of such a change is the challenge of our project.

3.3 Toughness study

We would like to see relative importance of direct vs. indirect effect of temperature on herbivory via modified host tree quality like toughness.

We assumed that with increasing temperature – the tree leaves are less tough. This is very general assumption because as we know from the literature, there is combine effect of many factors influencing the insect appearance. We would like to know if all of the factors are the same important for the insects or there are some of them which are particularly important and the rest are less.

References

Adger N., Aggarwal P., Agrawala S., Alcamo J., Allali A., Anisimov O., Arnell N., Boko M., Canziani O., Carter T., Casassa G., Confalonieri U., Cruz R.V. & e a.a. (2007). IPCC 2007 climate change 2007: impacts, adaptation and vulnerability. Working Group II Contribution to the Intergovermental Panel on Climate Change Fourth Assessment Report. Cambridge University press., P 23.

Alonzo C. (1999). Variation in herbivory by Yponomeuta mahalebella on its only hostplant Prunus mahaleb along an elevational gradient. *Ecological Entomology*, 24, 371 - 379.

Andrew N.R. & Hughes L. (2005). Herbivore damage along a latitudinal gradient: relative impacts of different feeding guilds. *Oikos*, 108, 176 - 182.

Anslow F.S. & Shawn M.J. (2002). An investigation of local alpine terrestrial lapse rates in the Canadian Rockies. pp.1. Proceedings of the 32nd Annual Artic Workshop, INSTAAR, University of Colorado, Boulder.

Atkinson D. (1994). Temperature and Organism Size - A Biological Law for Ectotherms? In: *Advances in Ecological Research* (eds. Begon M & Fitter AH). Academic Press, pp. 1 - 58.

Bale J.S., Masters G.J., Hodkinson I.D., Awmack C., Bezemer T.M., Brown V.K., Butterfield J., Buse A., Coulson J.C., Farrar J., Good J.E.G., Harrington R., Hartley S., Jones T.H., Lindroth R.L., Press M.C., Symrnioudis I., Watt A.D. & Whittaker J.B. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, 8, 1 - 16.

Battisti A., Stastny M., Buffo E. & Larsson S. (2006). A rapid altitudinal range expansion in the pine processionary moth produced by the 2003 climatic anomaly. *Global Change Biology*, 12, 662 - 671.

Battisti A., Stastny M., Netherer S., Robinet C., Schopf A., Rocques A. & Larsson S. (2005). Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. *Ecological Applications* 15, 2084 - 2096.

Beránek J. (2008). The response of weevil communities (Coleoptera:Curculionoidea) to the altitudinal zones of beech stands. *Journal of Forest Science - UZPI*, 54, 262-272.

Bergmann C. (1847). Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grosse. . *Gottinger Studien* 1, 595 – 708

Bess H.A.S., Spurr H. & Littlefield E.W. (1947). Forest site conditions and the gypsy moth. *Harvard forest bulletin* no 22. Harvard University press, Cambridge.

Blanckenhorn W.U. & Demont M. (2004). Bergmann and Converse Bergmann Latitudinal Clines in Arthropods: Two Ends of a Continuum? *Integrative and Comparative Biology*, 44, 413 - 424.

Bowman W.D., Steltzer H., Rosenstiel T.N., Cleveland C.C. & Meier C.L. (2004). Litter effect of two co – occurring alpine species on plant growth, microbial activity and immobilization of nitrogen. *Oikos*, 104, 336 – 344.

Brehm G. & Fiedler K. (2004). Bergmann's rule does not apply to geometrid moths along an elevational gradient in an Andean montane rain forest. *Global Ecology and Biogeography*, 13, 7 - 14.

Brown V.C. (1995). Insect herbivores and gaseous air pollutants - current knowledge and predictions, in Insects in a Changing Enviroment, (eds R. Harrington and N.E Stork), Academic Press, London,. 219 - 249.

Bryant J.P., Chapin I.F.S. & Klein D.R. (1983). Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory". *Oikos* (Blackwell Publishing), 40(3), 357 – 368.

Buffo E., Battisti A., Stastny M. & Larsson S. (2007). Temperature as a predictor of survival of the pine processionary moth in the Italian Alps. *Agricultural and Forest Entomology*, 9, 65 - 72.

Caldwell M.M., Björn L.O., Bornman J.F., Flint S.D., Kulandaivelu G., Teramura A.H. & Tevini M. (1998). Effects of increased solar ultraviolet radiation on terrestrial ecosystems. *Journal of Photochemistry and Photobiology B: Biology*, 46, 40 - 52.

Ceryngier P. (2000). Overwintering of Coccinella septempunctata (Coleoptera: Coccinellidae) at different altitudes in the Karkonosze Mts. *SW Poland European Journal of Entomology* 97, 323 - 328.

Cescatti A. & Battisti A. (1992). Distribution and ecology of Lymantria monacha L. and Cephalcia spp. in non - outbreak areas of Trentina (N-Italy). *Anzeiger für Schädlingskunde Pflanzenschutz Umweltschutz* 65, 92 - 99.

Childers C.C. & Achor D.S. (1989). Structure of the mouthparts of Frankliniella bispinosa (Morgan) (Thysanoptera: Thripidae). In B. L. Parker, M. Skinner and T. Lewis [eds.], Towards Understanding Thysanoptera. Proceedings of the International Conference on Thrips. USDA Technical Report NE-147, Radnor, PA.

Chown S.L. (2002). Respiratory water loss in insecta. *Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology*, 133, 791 - 804.

Cordell S., Goldstein G., Mueller - Dombois D., Webb D. & Vitousek P.M. (1998). Physiological and morphological variation in Metrosideros polymorpha, a dominant Hawaiian tree species, along an altitudinal gradient: the role of phenotypic plasticity. *Oecologia*, 113, 188-196.

Craig H. (1957). Isotopic standards for carbon and oxygen correlation factors for mass spectrometric analysis of carbon dioxide. *Geochimica & Cosmochimica Acta* 12, 133 - 149.

Deutsch C.A., Tewksbury J.J., Huey R.B., Sheldon K.S., Ghalambor C.K., Haak D.C. & Martin P.R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, 105, 6668 - 6672.

Diemer M. (1998a). Leaf lifespans of high – elevation, aseasonal Andean shrub species in relation to leaf traits and leaf habit. . *Global Ecol. Biogeogr. Lett.* , 7, 457 – 465.

Diemer M. (1998b). Life span and dynamics of leaves of herbaceous perennials in high – elevation environments: "news from the elephant's leg" . *Funct. Ecol.* , 12, 413 - 425.

Dillon M.E., Frazier M.R. & Dudley R. (2006). Into thin air: Physiology and evolution of alpine insects. *Integrative and Comparative Biology*, 46, 49 - 61.

Egger J. & Hoinka K.P. (1992). Fronts and orography. *Meteorology and Atmospheric Physics*, 48, 3 - 36.

Erelli M.C., Ayres M.P. & Eaton G.K. (1998). Altitudinal patterns in host suitability for forest insects. *Oecologia*, 117, 133 - 142.

Faccoli M. (2009). Effect of Weather on Ips typographus (Coleoptera Curculionidae) Phenology, Voltinism, and Associated Spruce Mortality in the Southeastern Alps. *Environmental Entomology*, 38, 307 - 316.

Farquahar G.D., Ehleringer J.R. & Hubick K.T. (1989). Carbon isotope discrimination and photosynthesis. *Ann Rev Plant Physi Plant Mol Biol*, 40, 503 - 537.

Fine P.V.A., Mesones I. & Coley P.D. (2004). Herbivores promote habitat specialization by trees in Amazonian forests. *Science*, 305.

Fontaine A.R., Olsen N., Ring R.A. & Singla C.L. (1991). Cuticular metal hardening of mouthparts and claws of some forest insects of British Columbia. *Journal - Entomological Society of British Columbia.*, 88, 45 - 55.

Franco A.M.A., Hill J.K., Kitschke C., Collingham Y.C., Roy D.B., Fox R., Huntley B. & Thomas C.D. (2006). Impacts of climate warming and habitat loss on extinctions at species low-latitude range boundaries. *Global Change Biology*, 12, 1545 - 1553.

Galen C. (1990). Limits to the distributions of alpine tundra plants: herbivores and the alpine skypilot, Polemonium viscosum. *Oikos*, 59.

Garibaldi L., Kitzberger T. & Chaneton E. (2011). Environmental and genetic control of insect abundance and herbivory along a forest elevational gradient. *Oecologia*, 167, 117 - 129.

Gilbert M. & Grégoire J.-C. (2003). Site condition and predation influence a bark beetle's success: a spatially realistic approach. *Agricultural and Forest Entomology*, 5, 87 - 96.

Guevara J. & Aviles L. (2007). Multiple techniques confirm lavational differences in insect size that may influence spider sociality. *Ecology*, 88(8), 2015 – 2023

Hagen S.B., Jepsen J.U., Ims R.A. & Yoccoz N.G. (2007). Shifting altitudinal distribution of outbreak zones of winter moth Operophtera brumata in sub - arctic birch forest: a response to recent climate warming? *Ecography*, 30, 299 - 307.

Hardy P., Kinder P., Sparks T. & Dennis R. (2010). Elevation and habitats: the potential of sites at different altitudes to provide refuges for phytophagous insects during climatic fluctuations. *Journal of Insect Conservation*, 14, 297 - 303.

Hawkins B.A. & DeVries P.J. (1996). Altitudinal gradients in the body sizes of Costa Rican butterflies. v. 17, no. 3, p. 185 - 194.

Hawkins B.A. & Lawton J.H. (1995). Latitudinal gradients in butterfly body sizes: is there a general pattern? *Oecologia*, 102, 31 - 36.

Haynes K., Liebhold A. & Johnson D. (2012). Elevational gradient in the cyclicity of a forest - defoliating insect. *Population Ecology*, 54, 239 - 250.

Heinrich B. (1996). The Thermal Warriors: Strategies of Insect Survival. Harvard University Press, London.

Heliövaara K. & Häisänen R. (1993). Insects and pollution, CRC Press, Boca raton, FL.

Hillstrom M.L. & Lindroth R.L. (2008). Elevated atmospheric carbon dioxide and ozone alter forest insect abundance and community composition. *Insect Conservation and Diversity*, 1, 233 - 241.

Hobbie S.E. (1996). Temperature and plant species control over litter decomposition in Alaskan tundra. *Ecol. Monogr.,*, 66, 503 – 522.

Hodkinson I.D. (2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews*, 80, 489 - 513.

Hodkinson I.D. & Hughes M.K. (1982). Insect herbivory.Chapman & Hall, London.

Houston D.R. & Valentine H.T. (1977). Comparing and predicting forest stand susceptibility to gypsy moth. *Can J For Res*, 7, 447 - 461.

Howlett B.E. & Davidson D.W. (2001). Herbivory on planted dipterocarp seedlings in secondary logged forests and primary forests of Sabah, Malaysia. *Journal of Tropical Ecology*, 17, 285 - 302.

Hódar J. & Zamora R. (2004). Herbivory and climatic warming: a Mediterranean outbreaking caterpillar attacks a relict, boreal pine species. *Biodiversity and Conservation*, 13, 493 - 500.

Huber E., Wanek W., Gottfried M., Pauli H., Schweiger P., Arndt S., Reiter K. & Richter A. (2007). Shift in soil – plant nitrogen dynamics of an alpine – nival ecotone. *Plant and Soil*, 301, 65 - 76.

Hughes P.R. (1988). Insect populations on host plants subjected to air pollution. *Insect Interactions,* (ed E.A. Heinrichs), John Wiley, New York, 111 - 137.

Högberg P. (1997). Tansley Review No. 95 15N natural abundance in soil - plant systems. *New Phytologist*, 137, 179 - 203.

Janzen D.H., Ataroff M., Farinas M., Reyes S., Rincon N., Soler A., Soriano P. & Vera M. (1976). Changes in the arthropod community along an elevational transect in the Venezuelan Andes. *Biotropica*, 8, 193 – 203.

Jian Q., Keming M. & Yuxin Z. (2009). Leaf - trait relationships of Quercus liaotungensis along an altitudinal gradient in Dongling Mountain, Beijing. *Ecological Research*, 24, 1243 - 1250.

Kelly C.A. (1998). Effects of variable life history and insect herbivores on reproduction in Solidago macrophylla (Asteraceae) on an elevational gradient. *Am Midl Nat*, 139, 243 – 254.

Körner C. (1989). The nutritional status of plants from high altitudes. A worldwide comparison. *Oecologia*, 81, 379 – 391.

Körner C. (1999). Alpine plant life: Functional plant ecology of high mountain ecosystems. Springer, Berlin, Germany.

Körner C. & Cochrane P.M. (1985). Stomatal responses and water relations of Eucalyptus pauciflora in summer along elevational gradient. *Oecologia*, 66, 433 - 455.

Kranabetter J., Simard S., Guy R. & Coates K. (2010). Species patterns in foliar nitrogen concentration, nitrogen content and ^{13}C abundance for understory saplings across light gradients. *Plant and Soil*, 327, 389 - 401.

Krehan H. & Steyrer G. (2006). Klimaveränderung – Schadorganismen bedrohen unsere Wälder. *BFW - Praxisinformation*, 10, 15 - 17.

Kubota U., Loyola R.D., Almeida A.M., Carvalho D.A. & Lewinsohn T.M. (2007). Body size and host range co - determine the altitudinal distribution of Neotropical tephritid flies. *Global Ecology and Biogeography*, 16, 632 - 639.

Körner C. (2007). The use of 'altitude' in ecological research. *Trends Ecol Evol* 22, 569 - 574.

Lara A.C.F., Fernandes G.W. & Goncalves-Alvim S.J. (2006). Tests of hypotheses on patterns of gall distribution along an altitudinal gradient.*Tropical Zoology,* North America 15

Laštůvka Z. (2009). Climate change and its possible influence on the occurrence and importance of insect pests. *Plant Protect. Sci.*, 45.

Laternser M. & Schneebeli M. (2003). Long - term snow climate trends of the Swiss Alps (1931 – 99). *International Journal of Climatology*, 23, 733 - 750.

Liebhold A.M., Elmes G.A., Halverson J.A. & Quimby J. (1994). Landscape characterization of forest susceptability to gypsy-moth defoliation. *For Sci*, 40, 18 - 29.

Lucas P.W., Turner I.M., Dominy N.J. & Yamashita N. (2000). Mechanical Defences to Herbivory. *Annals of Botany*, 86, 913 - 920.

Malo J.E. & Baonza J. (2002). Are there predictable clines in plant – pollinator interactions along altitudinal gradients? The example of Cytisus scoparius (L.) Link in the Sierra de Guadarrama (Central Spain). *Diversity and Distributions*, 8, 365 - 371.

Mani M.S. (1968). Ecology and Biogeography of High Altitude Insects. Junk, the Hauge.

Maxwell G.G. & Jennings P.R. (1980). Strategies of race - specific phytoparasitism and its control by plant breeding. *Genetika*, 9, 237 - 255.

McClure M.S. (1985). Patterns of abundance, survivorship and fecundity of Nuculaspis tsugae (Homoptera: Diaspididae) on Tsuga species in Japan in relation to elevation. *Environmental Entomology* 14, 413 – 415.

McMillin J.D. & Wagner M.R. (1998). Influence of host plant vs. natural enemies on the spatial distribution of a pine sawfly, Neodiprion autumnalis. *Ecological entomology*, 23, 397 - 408.

Menéndez R. (2001). How are insects responding to global warming? *Tijdschrift voor Entomologie*, 150, 355 - 365.

Metz B., Davidson O.R., Bosh P.R., Dave R. & Meyer L.A. (2007). IPCC, 2007. Climate change 2007: mitigation. Contribution of Working group III to the Fourth Assessment Report of the Intergovermental Panel of Climate Change. Cambridge University press, Cambridge, UK, New York, p 3.

Morecroft M.D., Woodward F.I. & Marrs R.H. (1992). Altitudinal trends in leaf nutrient contents, leaf size and delta ^{13}C of Alchemilla alpina. *Funct. Ecol.*, 6, 730 - 740.

Mousseau T.A. (1997). Ectotherms Follow the Converse to Bergmann's Rule. *Evolution*, 51, 630 - 632.

Netherer S. & Schopf A. (2010). Potential effects of climate change on insect herbivores in European forests - General aspects and the pine processionary moth as specific example. *Forest Ecology and Management*, 259, 831 - 838.

Niemelä P., Rousi M. & Saarenmaa H. (1987). Topographical delimitation of Neodiprion sertifer (Hym., Diprionidae) outbreaks on Scots pine in relation to needle quality. *Journal of Applied Entomology*, 103, 84 - 91.

Nyman T. & Julkunen-Tiitto R. (2000). Manipulation of the phenolic chemistry of willows by gall-inducing sawflies. *Proceedings of the National Academy of Sciences*, 97, 13184 - 13187.

Oleksyn J., Modrzyński J., Tjoelker M.G., Żytkowiak R., Reich P.B. & Karolewski P. (1998). Growth and physiology of Picea abies populations from elevational transects: common garden evidence for altitudinal ecotypes and adaptation to cold environment. *Funct Ecol* 12, 573 - 590

Oleksyn J., Reich P.B., Zytkowiak R., Karolewski P. & Tjoelker M.G. (2002). Needle nutrients in geographycally diverse Pinus sylvestris populations. *Ann For Sci* 59, 1 - 18.

Oleksyn J., Reich P.B., Zytkowiak R., Karolewski P. & Tjoelker M.G. (2003). Nutrient conservation increases with latitude of origin in European Pinus sylvestris populations. *Oecologia*, 136, 220 - 235.

Parmesan C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* 37, 637 - 669.

Parmesan C. & Yohe G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421, 37 - 42.

Paul G., Montagnini F., Berlyn G., Craven D., Breugel M. & Hall J. (2012). Foliar herbivory and leaf traits of five native tree species in a young plantation of Central Panama. *New Forests*, 43, 69 - 87.

Peacock A.J. (1998). ABC of oxygen - Oxygen at high altitude. *British Medical Journal*, 317, 1063 - 1066.

Pepin N. (2001). Lapse rate changes in northern England. *Theoretical and Applied Climatology*, 68, 1 - 16.

Petrucco-Toffolo E. & Battisti A. (2008). Performances of an expanding insect under elevated CO_2 and snow cover in the Alps. *iForest - Biogeosciences and Forestry*, 1, 126 - 131.

Pollard D.G. (1972). The stylet structure of leafhopper (Eupteryx melissae Curtis: Homoptera, Cicadellidae) *J. Nat. Hist.*, 6, 261 – 271.

Powers K.S. & Avilés L. (2007). The role of prey size and abundance in the geographic distribution of spider sociality. *Journal of Animal Ecology* 76, 995 – 1003

Rahbek C. (1995). The elevational gradient of species richness: a uniform pattern? *Ecography*, 18, 200 - 205.

Randall M.G.M. (1982). The dynamics of an insect population throughout its altitudinal distribution – Coleophora alticolella (Lepidoptera) in northern England. *Journal of Animal Ecology*, 51, 993 - 1016.

Reich P.B., Oleksyn J. & Tjoelker M.G. (1996). Needle respiration and nitrogen concentration in Scots pine populations from a broad latitudinal range: a common garden test with field – grown trees. *Funct Ecol* 10, 768 – 776.

Reich P.B. & Oleksyn J. (2004). Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 11001 - 11006.

Röder J., Bässler C., Brandl R., Dvořak L., Floren A., Goßner M.M., Gruppe A., Jarzabek-Müller A., Vojtech O., Wagner C. & Müller J. (2010). Arthropod species richness in the Norway Spruce (Picea abies (L.) Karst.) canopy along an elevation gradient. *Forest Ecology and Management*, 259, 1513 - 1521.

Rosenzweig M.L. (2003). How to reject the area hypothesis of latitudinal gradients. In *Macroecology: Concepts and Consequences* Blackburn, T.M and Gaston, K.J., eds Blackwell, 87 - 106.

Schoonhoven L.M., Jermy T. & van Loon J.J.A. (1998). Insect-Plant Biology. From Physiology to Evolution. Chapman & Hall 1st edition, London, *XI + 409 pp.*

Smith R.J., Hines A., Richmond S., Merrick M., Drew A. & Fargo R. (2000). Altitudinal variation in body size and population density of Nicrophorus investigator (Coleoptera: Silphidae).*Environmental Entomology*, 29, 290 - 298.

Solomon S., Qin D., Manning M., Alley R.B., Berntsen T., Bindoff N.L., Chen Z., Chidthaisong A., Gregory J.M., Hegerl G.C., Heimann M., Hewitson B., Hoskins B.J., Joos F., Jouzel J., Kattsov V., Lohmann U., Matsuno T., Molina M., Nicholls N., Overpeck J., Raga G., Ramaswamy V., Ren J., Rusticucci M., Somerville R., Stocker T.F., Whetton P., A W.R. & D W. (2007). Technical Summary. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Sota T. (1996). Altitudinal variation in life cycles of carabid beetles: life - cycle strategy and colonization in alpine zones. *Arctic and Alpine Research* 28, 441 - 447.

Southwood T.R.E. (1961). The number of species of insect associated with various trees. *Journal of Animal Ecology*, 30, 1 - 8.

Speight M.R., Hunter M.D. & Watt A.D. (2008). Ecology of Insects: Concepts and Applications, 2nd Edition. Wiley-Blackwell.

Stamp N. (2003). Out of the quagmire of plant defense hypotheses. *The quarterly review of biology* 78.

Steltzer H. & Bowman W.D. (1998). Differential influence of plant species on soil nitrogen transformations within moist meadow Alpine tundra. *Ecosystems*, 1, 464 - 474.

Stevens G.C. (1992). The elevational gradient in altitudinal range: An extension of Rapoport's latitudinal rule to altitude. *The American Naturalist*, 140.

Strathdee A.T. & Bale J.S. (1998). Life on the edge: insect ecology in arctic environments. *Annual Review of Entomology* 43, 85 - 106.

Straw N.A., Timms J.E.L. & Leather S.R. (2009). Variation in the abundance of invertebrate predators of the green spruce aphid Elatobium abietinum (Walker) (Homoptera: Aphididae) along an altitudinal transect. *Forest Ecology and Management*, 258, 1 - 10.

Suzuki S. (1998). Leaf phenology, seasonal changes in leaf quality and herbivory pattern of Sanguisorba tenuifolia at different altitudes. *Oecologia*, 117, 169–176.

Tylianakis J.M., Didham R.K., Bascompte J. & Wardle D.A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, 11, 1351 - 1363.

Van Dongen S., Backelja T., Matthysen E. & Dhondt A.A. (1997). Synchronization of hatching date with budburst of individual host tree (Quercus robur) in the winter moth (Operophtera brumata) and its fitness consequences. *Journal of Animal Ecology*, 66, 113 - 121.

Virtanen T. & Neuvonen S. (1999). Performance of moth larvae on birch in relation to altitude, climate, host quality and parasitoids. *Oecologia*, 120, 92 - 101.

Visser M.E. & Holleman L.M. (2001). Warmer springs disrupt the synchrony of Oak and Winter Moth phenology. *Proc. R. Soc. Lond. B.* , 268, 289 - 294.

Vitousek P.M., Matson.P.A. & Turner D. (1988). Elevational and age gradients in Hawaiian montane rain forest: foliar and soil nutriets. *Oecologia*, 77, 565 – 570.

Watson R.T., Zinyowera M.C., Moss R.H. & Dokken D.J. (1997). IPCC special report. The regional impacts on climate change: an assessment of vulnerability. Cambridge University Press, Cambridge, United Kingdom 527.

Wegner C., Wunderlich M. & Kessler M. (2003). Foliar C:N Ratio along an Andean Elevational Gradient. *Biotropica*, 35, 486 - 490.

Weih M. & Karlsson P.S. (2001). Growth response of Mountain birch to air and soil temperature: is increasing leaf-nitrogen content an acclimation to lower air temperature? *New Phytologist*, 150, 147 - 155.

Weltzin J.F., Loik M.E., Schwinning S., Williams D.G., Fay P.A., Haddad B.M., Harte J., Huxman T.E., Knapp A.K., Lin G., Pockman W.T., Shaw M.R., Small E.E., Smith M.D., Smith S.D., Tissue D.T. & Zak J.C. (2003). Assessing the Response of Terrestrial Ecosystems to Potential Changes in Precipitation. *BioScience*, 53, 941 - 952.

Wermelinger B., Hirschheydt J.v. & Fecker B. (1995). Abundance and emergence of spruce cone insects in different parts of Switzerland. *Journal of Applied Entomology*, 119, 9 - 15.

Williams D.W. & Liebhold A.M. (1995). Herbivorous insects and global change: potential changes in the spatial distribution of forest defoliator outbreaks. *Journal of Biogeography*, 22, 665 - 671.

Wilson R.J., Gutiérrez D., Gutiérrez J. & Monserrat V.J. (2007). An elevational shift in butterfly species richness and composition accompanying recent climate change. *Global Change Biology*, 13, 1873 - 1887.

Woods H.A., Makino W., Cotner J.B., Hobbie S.E., Harrison J.F., Acharya K. & Elser J.J. (2003). Temperature and the chemical composition of poikilothermic organisms. *Functional Ecology*, 17, 237 - 245.

Woodward F.I. (1986). Ecophysiogical studies on the shrub Vaccinium myrtillus L. taken from the wide altitudinal range. *Oecologia*, 70, 580 - 586.

Yin X. (1993). Variation in foliar nitrogen concentration by forest type and climatic gradients in North America. *Canadian Journal of Forest Research*, 23, 1587 - 1602.

Chapter 2

Elevational patterns of insect herbivory across European forests

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Abstract

Insect herbivores are amongst the most important primary consumers of forest ecosystems. Potential effects of temperature warming on insect herbivores can be direct, through impacts on their physiology and behaviour, or indirect, where the insects respond to climate – induced changes in host plant quality or top – down interactions. In this context, elevational gradients provide an optimal experimental setting to test the relationship between temperature variation and herbivory. We therefore used a large data set where we measured leaf herbivory and leaf traits along steep elevational gradients across several biogeographical regions. We quantified endemic herbivory on four major European tree species (i.e. Beech, Norway spruce, Scots pine, and European larch) by four feeding guilds (sap feeders, chewers, miners and gall makers) under non – outbreak conditions.

The aim was to test direct and indirect effects of temperature via modified plant quality on insect herbivory, testing also the potential role of inter – guild interactions. We found either neutral or positive effect of increasing temperatures on herbivory while no clear effect of biotic interactions emerged. Although we found relatively high variability in the response to temperature of different guilds and host tree species, herbivory on conifers exhibited a stronger sensitivity to temperature variations than herbivory on European beech. Except for larch, we found clear elevational patterns of variation in leaf toughness and size. Leaf traits were, however, almost never associated with changes in herbivory. As we considered feeding guilds as a whole, the observed mixed responses are probably the result of the simultaneous activities of several species that may idiosyncratically respond to both temperature and leaf quality. Considering the potential increased susceptibility of Norway spruce and larch to insect herbivory due to temperature warming, forest managers and local stakeholders should seek for effective measures to mitigate potential climate change effects on forest ecosystems.

Introduction

Global temperature is currently increasing at an unprecedented rate with potentially profound effects on forest ecosystems (Seppälä et al. 2009; Littell et al. 2010). Effects of global warming on insect herbivores can be direct, through impacts on their physiology and behaviour, or indirect, where the herbivores respond to climate – induced changes in the host plants or in biotic interactions (Bale et al. 2002). The quantification of the potential net effect of insect herbivory on forests due to climate variation is, however, largely unknown. From the herbivore perspective, harsh and cold environments are usually associated with low population density, lower growth rates, increased development time, reduced survival and consumption rates, but higher body size (Whittaker & Tribe, 1996; Williams 1999; Zuo et al. 2011). The expected outcome of these individual and population effects is a reduced herbivory at high elevations (Logan et al 2003). Temperature warming is therefore expected to expose temperate forests to greater herbivory (Klapwijk et al. 2012), in particular in areas where low temperatures currently constrain herbivore distribution and activity (Battisti et al. 2005; Jepsen et al. 2008; Adams & Zhang 2009; Garibaldi et al. 2010; Garibaldi et al. 2011). Existing literature has mostly focused on single species known to be highly sensitive to temperature (Battisti et al. 2005, Marini et al. 2012), while a more general approach looking at the whole herbivore community has been rarely used (but see Garibaldi et al. 2010, 2011; Jactel et al. 2012; De Sassi et al. 2012). This community approach is, however, crucial to help understanding the ecosystem level response of insect herbivory to temperature.

Biotic interactions can also be altered by temperature variations. In particular competition is considered a central factor in the community ecology of herbivore insects and both intra – and inter – guild competition is expected to be important in determining the final herbivory (Denno et al. 1995; Kaplan & Denno, 2007). For instance, there are several examples of sap – feeder attacks imposing adverse effects on the subsequent activity of external chewers such as caterpillars (e.g. Mattson et al. 1980) and vice versa (e.g. Van Zandt and Agrawal 2004). Moving from high to low elevation forests, herbivore communities are expected to be more diverse and to ingest more energy per unit mass, causing resource density to decline faster and the strength of competitive interactions to increase (Vasseur & McCann 2005). Ecological theory suggests that these biotic interactions should be more important at warmer than at colder temperatures (Jiang & Morin 2004; Lang et al. 2012). Similarly, studies on the consequences of climate change on pest herbivory rarely include indirect effect through changes in natural enemies' density and behaviour.

From the host tree perspective, low temperatures and a shorter growing season at high elevations would result in increased leaf nitrogen and phosphorous concentrations (Körner 1989), potentially favouring the performance of some herbivore species (Suzuki 1998). However, increasing elevation should be also associated with anatomical adaptations to cold temperatures and high radiation such as toughness and reduced leaf size that ultimately may serve as mechanical defences against herbivores (Brown et al. 1991). Although reduced leaf size and increased leaf toughness are expected to decrease herbivory (Agrawal 2007; Garibaldi et al. 2010), the effects of such plant defences can be sometimes more complex to predict (Coley and Barone 1996).

Herbivory response to both host quality and temperature can be highly tree species – specific (Heil 2008). Both bottom – up and top – down control of insect herbivores may therefore vary with the system studied being highly dependent on the local conditions. The relationships can therefore vary between geographical regions, making generalizations difficult to derive from single – region studies. Similarly, individual species responses to climate variations are expected to be largely idiosyncratic depending on complex interactions between top – down and bottom – up processes. However, insect feeding guild has often been shown to help explaining this response variability (Huberty & Denno 2004; Jactel et al. 2012). Amongst insect folivores four main feeding guilds can be identified: chewers, sap – feeders, leaf miners and gall makers. While the positive direct effect of temperature is expected to be similar among the four feeding guilds, the indirect effect via modified plant quality is more complex (Awmack & Leather 2002). For instance chewers and leaf miners should be more sensitive to increased leaf toughness compared to other guilds due to the complete or partial ingestion of the leaf tissue (Huberty & Denno 2004). Gall makers such as sawflies and cecidomyiid flies require vigorous, expanding plant tissue for successful gall formation (Price 1980) and should be more related to leaf size. Finally sap – feeders are expected to be more related to leaf water and nutrient content than to external anatomical leaf traits (Awmack & Leather 2002). The identification of guild – specific responses to both temperature and leaf quality may improve our ability to generalize the potential effect of climate change on insect herbivory.

In this context, elevational gradients can provide a quasi experimental setting to test the relationship between temperature, host plant quality and herbivory. Compared to latitudinal gradients, which have been more intensively studied (e.g. Coley & Barone 1996; Andrew & Hughes 2005; Adams et al. 2009), elevational gradients have the great advantage to present a steep temperature variation over short geographical distances, thus reducing the confounding effects of different local environmental conditions such as general climate, forest management and biogeographical differences in herbivore communities (Hodkinson 2005; Körner 2007). However, only a few studies have quantified the association between temperature, plant traits and insect herbivory along elevation gradients (but see McDonald et al. 1999; Holton et al. 2003; Bidart – Bouzat et al. 2008), and to our knowledge, only one (Garibaldi et al. 2011) has tried to simultaneously assess the relative importance of both direct and indirect effects of temperature variation in determining insect herbivory.

We therefore used a large data set where we measured leaf herbivory of different feeding guilds and leaf traits along elevational gradients of four major forest tree species across Europe. The general aim was to test direct and indirect effect of temperature via modified plant quality on insect herbivory, testing also the potential role of inter – guild competition. We tested the general hypothesis that herbivory decreases with increasing elevation. Specifically, we tested three underlying mechanisms explaining the expected negative herbivory − elevation relationship: i) direct negative effect of low temperature on insect herbivores, ii) indirect positive effect of low temperatures due to lower inter – guild competition, iii) indirect negative effect of low temperatures due to higher leaf defences at high elevation. By testing different feeding guilds we further aimed to identify functional response groups to temperature. Considering our elevation – related temperature gradient as a proxy for temperature warming, our large scale empirical study will help gaining insights into the potential consequences of increased temperatures on herbivory pressure on key – stone European tree species.

Materials and methods

Sampling design

The study included 21 elevational gradients across 7 European countries (Fig. 1). In all sites, an elevational gradient was selected to cover the local elevational range distribution of one or more focal tree species. The focal tree species included in the study were Norway spruce (*Picea abies* Karst.), Scots pine (*Pinus sylvestris* L.), European larch (*Larix decidua* Mill.), and European beech (*Fagus sylvatica* L.). The species were selected to include some of the most important broadleaf and conifer species dominating temperate European forests and occurring along elevation gradients. All the sampled forests were mature (i.e. producing seeds), had relatively homogenous tree composition and density along the elevation gradients, and did not show symptoms of pest and disease outbreaks, i.e. the study aimed to analyze endemic herbivory under non – outbreak condition. Within each gradient, we selected between four and seven steps depending on the elevation range of the tree species and the homogeneity of local conditions. At each step, we randomly selected 10 mature trees per focal species on which we carried out the herbivory assessment and leaf traits' measurements (see below). As for the same tree species the position of the elevation range strongly differed among the regions due to different latitude and general climate, for further analyses we transformed the elevation of each gradient into temperature values. We derived the mean annual temperature for year 2010 of the closest meteorological station to each gradient (source: http://www.geodata.us) and we transformed the elevation in temperature values using a lapse rate of 0.60°C per 100 m. With this transformation most of the temperature gradient of each tree species overlapped indicating a relatively consistent thermal niche breadth.

Herbivory estimation

On each tree, three 1 – m long branches were randomly selected from the sunny side of the lower canopy and cut down using a long pruner. Branch sun exposure was kept as homogenous as possible along each gradient to avoid confounding effects of sun radiation on both leaf traits and herbivore activity. Herbivory estimation was performed for four feeding guilds, namely leaf sap feeders, leaf chewers, leaf miners, and gall makers. Not all the guilds were present for the four tree species at each elevation. On each branch, the proportion of leaf area removed or altered by leaf chewers, leaf miners, and sap feeders was visually estimated. For gall makers, the proportion of plant organs affected (leaves, buds, shoots) was assessed at the branch level. Any further alteration of plant organs not clearly imputable to the four guilds was ignored, including signs of microorganism colonization.

Leaf size and toughness

In a subset of gradients (n= 14, Table S1), we further quantified traits that are expected to affect herbivore performance: leaf size and leaf toughness. All leaf measurements were taken from 30 randomly selected leaves for each tree (3 leaves from each of 10 shoots, three measures per leaf). Size referred in all cases to leaf length. Leaf toughness measurements were performed on fresh leaves by using a penetrometer Dial Tension Gauge (Mitutoyo). For beech (n= 3 gradients), spruce (n= 6 gradients) and Scots pine (n= 4 gradients), we further measured C/N ratio on a pooled sample obtained from the 10 trees per step (i.e. one C/N ratio value per step). To measure C/N ratio, leaves were dried at 60˚C for 24 hours and then milled and crushed using liquid nitrogen for conifer and an electric grinder (coffee grinder Braun) for beech. Dried leaves were ground to the fine powder and after weighted 50 mg into tin capsules with addition of 50 mg of WO_3 and analyzed by Vario Macro Elementar Analyzer, version CNS. As C/N ratio was not measured at the tree level it was not included in the analyses presented in the study. However, we preliminary tested if the C/N ratio was associated with temperature and found no temperature effect across the tree species investigated, i.e. C/N ratio varied in a unpredictable way along the elevation gradients for all the tree species.

Data analysis

First, considering all of the 21 gradients we tested the direct effect of temperature on total leaf herbivory (sum of the herbivory of all the feeding guilds), by using linear mixed models (LMMs) (Fig. 2). Temperature was included as a linear term after checking the plots visually for linearity. Although some regions presented slightly curvilinear relationships between temperature and herbivory, we did not include a quadratic term due to the low number of steps within each gradient and the consequent large sensitivity of the analysis to single step values. To account for the spatial nestedness in the sampling, the models included elevational step within region as random factors. The factor temperature was therefore tested using the number of steps as true replicates.

Second, using the same data as above, we tested the direct effect of both temperature and inter – guild interactions on leaf herbivory for the four feeding guilds, separately (Fig. 2). We used LMMs with the same random structure as described above. Temperature was included as a linear term and the inter – guild effect was tested by summing the cumulative herbivory of all the guilds except the one tested as response in the current analysis. We hypothesized that herbivory caused by a guild can negatively affect that caused by the others due to induced plant defence and mechanical removal or alteration of the available plant tissues.

Third, for the gradients where we measured both herbivory and leaf traits (n= 14), we further evaluated the relationship between temperature, leaf size and toughness, and herbivory using the same mixed model approach. First, the direct effect of temperature on leaf size and toughness was tested using two separate mixed models with temperature as fixed effect, gradient as random effect and either leaf size or toughness as response variable. Second, the indirect effect of temperature on herbivory via modified leaf traits was evaluated using a mixed model with both temperature and leaf traits as fixed effects and step within elevation gradient as random. We preliminary tested also the interaction between temperature and leaf traits, which however were never associated with herbivory. We therefore did not present the interaction in the present study. A significant partial coefficient of toughness or leaf size in a model including temperature would indicate an additional effect of leaf traits on herbivory. These models were performed separately for each guild. All of the LMMs were estimated using the lme(nlme) function in R, version 2.12.1 (R Development Core Team, 2010) with the restricted maximum likelihood (REML) estimation method. All explanatory variables were standardized to mean 0 and standard deviation 1. The use of centred reduced variables allows using coefficients as true measures of the direction and magnitude of the effects.

The nested nature of our sampling across different spatial scale and the cyclic nature of our multivariate causal hypothesis (Fig. 2) did not allow us using a path analysis approach to test hypotheses about direct and indirect effect of temperature on different guild herbivory. As far as we know the only analytical framework available has been developed for acyclic multivariate causal hypothesis (Shipley 2009).

Results

The average level of insect herbivory was generally below 10% with a few regions exceeding this value. Within each tree species and feeding guilds there was relatively high variability in the level of herbivory between the various regions (Fig. 3).

The total herbivory obtained by summing the four guilds' contribution decreased moving from low to high elevation for Norway spruce and larch, while pine, and beech presented a neutral relationship (Fig. 4).

The simultaneous test of temperature and inter – guild interaction on herbivory demonstrated a differential response depending on the host tree and the guild considered (Fig. 5). For beech, we found no relationship with temperature for any of the four guilds. For spruce, we found positive temperature effect on herbivory for miners, while the other guilds did not respond significantly. For Scots pine, we found a positive temperature effect only for sap – feeders. For larch, we found a contrasting response between miners and chewers, i.e. the former responded positively while the latter negatively to temperature. We found no negative effect of inter – guild interaction in the four tree species, while for larch we found a positive effect in two cases, i.e. herbivory by chewers and miners on larch was positively associated with the total herbivory caused by the other guilds.

On a subset of gradients (n= 14 gradients), we first tested the effect of temperature on leaf traits using LMMs. Leaf size significantly increased with increasing temperature for beech and spruce, while it significantly declined for Scots pine (Table 1). Leaf size was not measured in larch. Leaf toughness significantly decreased with increasing temperature for, spruce and Scots pine, while it did not vary for the all deciduous species (beech and larch).

The simultaneous test of temperature, leaf traits and inter – guild interaction on herbivory indicated that leaf size was never associated with the magnitude of herbivory, while in only one case leaf toughness was positively associated with general herbivory in spruce (Table 2).

Discussion

Our study investigated the elevational pattern of herbivory in four key – stone Eupean tree species across several biogeographical regions. We found that both host tree identity and feeding guild modified herbivory response to temperature. Herbivory on conifers (i.e. Norway spruce, Scots pine, and European larch) exhibited a stronger sensitivity to warming temperature variations than herbivory on beech. However, we found in a large number of cases a neutral effect of warmer temperatures and in one case a negative response. Although we found clear elevational patterns of variation in leaf toughness and size for all the tree species except for larch, leaf traits did not affect significantly herbivory variation. Similarly, we did not find important effect of inter – guild interactions.

Against the general prediction of a generalized positive effect of warmer temperature on insect herbivory (Bale et al. 2002) more than half of the cases exhibited a neutral relationship. Our results only partly conform with those of several regional studies on single tree species that indicated a dominance of a thermal limitation to insect herbivory at high elevations (Suzuki 1998; Alonso 1999; Battisti et al. 2005; Garibaldi et al. 2011), while they are consistent with the observed mixed patterns found along latitudinal gradients (Andrew & Hughes 2005; Adams et al. 2009; Adams & Zhang, 2009; Garibaldi et al. 2010). Differential guild responses are likely to be caused by the combination of different thermal physiology of the single species, and these responses could be further complicated by shifts in top – down interactions (Huey et al. 2009) and by modifications of plant – insect interactions (Bale et al. 2002; De Sassi et al. 2012). The simultaneous analysis of several host tree species across different biogeographical regions has demonstrated that the response to temperature was highly variable and no simple generalization can be made on the response of insect herbivory to temperature.

While herbivory on beech always exhibited a neutral response to temperature, herbivory of several guilds feeding on conifers responded positively to increasing temperatures. As Norway spruce has been widely planted outside its natural climatic range, the positive effect of temperature was probably related to situations where spruce grows on the warm side of its natural climatic range. Hence, forests propagated at elevations that are too warm relative to the natural species distribution will be those most affected by current and future climate change (Marini et al. 2012). For Scots pine, only sap – feeders responded positively to temperature while for larch, we had two herbivore guilds clearly responding to temperature: miners and chewers but with a contrasting response. Based on these idiosyncratic results we cannot generalize a positive response to warmer temperature of any of the feeding guilds considered, as their response was highly tree species – dependent. It is important to stress that our results refer to endemic levels of herbivory and should not be confounded with the potential higher outbreak propensity of single species associated with global warming (see Klapwijk et al. 2012). Although large outbreaks are expected to have more severe consequences for forest ecosystems than endemic herbivory (Kurz et al. 2008), several works indicate that even relatively low levels of defoliation can have a considerable impact on tree performance. For instance, it has been demonstrated that herbivory levels of 10 – 15%, comparable with those observed in our study, are clearly associated with reduction of volume increments (Armour et al. 2003). Hence, temperature warming may negatively affect conifer forest productivity via increased herbivory, although the magnitude of this effects appeared to be low compared to the direct effect of temperature on trees.

For those guilds that responded to temperature positively, we can assume that herbivory declined with elevation mainly due to the expected decrease in population density towards higher elevation sites (Garibaldi et al. 2011). A review of existing literature on population density response to elevation of several herbivore insects across different orders and with various feeding behaviours has shown that in the large majority of the cases density declined with increasing elevation (Hodkinson et al. 2005). Colder temperatures may reduce individual growth rates and prolong developmental time, thus decreasing insect survival with negative consequences on population dynamics (Williams 1999).

Plants often mediate negative interactions between insect herbivores (competition via induced resistance or altered risk of natural enemy attack), but at the same time they can also promote positive interactions between herbivores (induced susceptibility) such as feeding facilitation, allelochemistry, or protective housing (Denno et al. 1995; Denno & Kaplan, 2007). Accordingly, we found that potential biotic interactions between different feeding guilds showed no clear effect on herbivory. Facilitation or competition between feeding guilds are complex and the expected variations among species in their demographic and physiological responses to temperature will likely affect the outcome of these interactions in an unpredictable way (Wilmers et al. 2006).

In the case of insect herbivores, it is expected that temperature may change plant quality that in turn should alter herbivore performance and host plant use. Accordingly, we found that in almost all cases (except for larch) leaf mechanical defences tended to increase along the elevation gradient. Although several empirical studies have demonstrated that leaf size strongly influence insect performance where smaller leaves are usually associated with reduced herbivory compared to larger leaves (Brown et al. 1991; Coley and Barone 1996; Feath 1991; Ribeiro et al. 1994; Low et al. 2009), we found no relationship with leaf herbivory. Along with size, leaf toughness was also expected to be one of the main non – chemical deterrents to herbivory, as tougher leaves may represent a serious constrain to insect development especially in early instars (Zovi et al. 2008). Although we found several clear elevation patterns of leaf size and toughness we did not find any association between these leaf traits and herbivory (see also Andrew & Hughes, 2005). It is possible that single species may respond to plant quality more than the whole guild and that idiosyncratic response of different species within the same guild may have obscured these relationships. Plant − insect interactions are known to be highly species – specific being linked to long evolutionary processes (Strauss & Agrawal 1999). These interactions are also often characterized by complex temporal dynamics where herbivore activity affects plant quality and *vice versa.* These effects at the population level cannot, however, be captured by a correlative analysis within one year, probably explaining the lack of relationship between leaf quality and guild herbivory.

We cannot exclude that the lack of low temperature effect on several herbivore guilds at higher elevations may be obscured by positive effects derived from reduced natural enemy pressure (Koptur 1985; Hodkinson 2005). This hypothesis has been put forward also to explain the absence of a negative latitudinal pattern in herbivory (Bjorkman et al. 2010). More studies are, however, needed to elucidate the role of top – down control of insect herbivory along elevational gradients (Berggren et al. 2009). Moreover, as we considered feeding guilds instead of single species the observed herbivory responses are the result of the simultaneous activities of several species that may interact in a complex way. As shown above for plant quality, it is likely that the potential contrasting response to temperature of different species belonging to the same feeding guild may have obscured some of the relationships. One another potential shortcoming of our approach that may have masked some relationship is that the tree and insect species included were probably not always studied over their entire elevational range (Hodkinson et al. 2005). This is mainly the result of forest management that has removed less economically valuable tree species (e.g. beech) in favour of others that instead have been propagated outside their natural range (e.g. Norway spruce).

Although based on observational data, our study provides the first large scale test of the potential consequences of temperature variations on insect herbivory on four main European tree species. The mixed effect of temperature and the lack of leaf quality and biotic interaction effect indicated that direct effect of temperature on insect herbivory is more complex than expected from simple autoecological considerations (Berggren et al. 2009; Adams & Zhang 2009), and that more attention should be given to how direct effects of temperature interact with other factors such as natural enemies (Björkman et al. 2010). Although our results indicated relatively high variability in the response of herbivory to temperature warming, it seems clear for several herbivore guilds feeding on conifers that increasing temperatures will be associated with larger herbivory, and perhaps with outbreak propensity (Klapwijk et al. 2012). Considering the potential increased susceptibility of conifer forests to insect herbivory, forest managers and local stakeholders should seek for effective measures to mitigate potential climate change effects.

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References

Adams, J. M. & Zhang, Y. (2009) Is there more insect folivory in warmer temperate climates? A latitudinal comparison of insect folivory in eastern North America. *Journal of Ecology*, 97, 933 – 940.

Adams, J.M., Rehill, B., Zhang, Y. & Gower, J. (2009) A test of the latitudinal defense hypothesis: herbivory, tannins and total phenolics in four North American tree species. *Ecological Research*, 24, 697 – 704.

Agrawal, A.A.(2007) Macro Evolution of plant defense strategies. *Trends in Ecology & Evolution* 22:103-109.

Alonso C (1999) Variation in herbivory by Yponomeuta mahalebella on its host plant Prunus mahaleb along an elevational gradient. *Ecol Entomol* 24:371 – 379.

Andrew, N.R. & Hughes, L. (2005) Herbivore damage along a latitudinal gradient: relative impacts of different feeding guilds. *Oikos*, 108, 176 – 182.

Armour H., Straw N., Day K. (2003) Interactions between growth, herbivory and long – term foliar dynamics of Scots pine. *Trees* 17:70 – 80.

Awmack C.S., Leather S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annu. Rev. Entomol.* 47:817 – 44.

Bale J.S., Masters G.J., Hodkinson I.D., Awmack C., Bezemer T.M., Brown V.K., Butterfield J., Buse A., Coulson J.C., Farrar J., Good J.E.G., Harrington R., Hartley S., Jones T.H., Lindroth R.L., Press MC, Symrnioudis I, Watt AD, Whittaker JB (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Glob Change* Biol 8:1 – 16.

Battisti A., Stastny M., Netherer S., Robinet C., Schopf A., Roques A., Larsson S. (2005): Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. *Ecol. Applic.,* 15: 2084 – 2096.

Berggren Å, Björkman C., Bylund H, and Ayres MP. (2009). The distribution and abundance of animal populations in a climate of uncertainty. *Oikos,* 118: 1121 – 1126.

Bidart – Bouzat M.G. and Imeh – Nathaniel A. (2008) Global change effects on plant chemical defenses against insect herbivores. *Journal of Integrative Plant Biology*, 50: 1339 – 1354.

Björkman, C., Berggren, Å. & Bylund, H. 2011. Causes behind insect folivory patterns in latitudinal gradients. Journal of Ecology 99: 367 – 369.

Brown VK, Lawton JH, Grubb PJ (1991) Herbivory and the evolution of leaf size and shape [and discussion]. *Philos Trans Biol Sci* 333:265 – 272.

Coley P.D., Barone J.A. (1996) Herbivory and plant defenses in tropical forests. *Annu Rev Ecol Syst* 27:305 – 335.

De Sassi C., Lewis O., and Tylianakis J. M. (2012) Plant – mediated and nonadditive effects of two global change drivers on an insect herbivore community. *Ecology* 93, 1892 – 1901.

Denno, R.F., McClure, M.S. & Ott, J.R. (1995). Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Ann. Rev. Entomol.*, 40, 297 – 331.

Denno, R.F. & Kaplan, I. (2007). Plant – mediated interactions in herbivorous insects: mechanisms, symmetry, and challenging the paradigms of competition past. In: Ecological Communities: Plant Mediation in Indirect Interaction Webs (eds Ohgushi, T., Craig, T.P. & Price, P.W.). Cambridge University Press, London, pp. 19 – 50.

Faeth, S.H. (1991). Effect of oak leaf size on abundance, dispersion, and survival of the leafminer Cameraria sp. (Lepidoptera: Gracillariidae). *Environmental Entomology* 20: 196 – 204.

Garibaldi, L.A., Kitzberger, T., Chaneton, E.J. (2011) Environmental and genetic control of insect abundance and herbivory along a forest elevational gradient. *Oecologia* 167: 117 – 129.

Garibaldi, L.A., Kitzberger, T., Ruggiero A. (2010) Latitudinal decrease in folivory within Nothofagus pumilio forests: dual effect of climate on insect density and leaf traits? *Global Ecology and Biogeography* 20:609 – 619.

Heil, M. (2008) Indirect defence via tritrophic interactions. *New Phytologist* 178: 41 – 61.

Holton M.K., Lindroth R.L., Nordheim E.V. (2003) Foliar quality influences tree – herbivore – parasitoid interactions: effects of elevated CO_2, O_3, and plant genotype. *Oecologia* 137: 233 – 244.

Hodkinson I.D. (2005) Terrestrial insects along elevation gradients: species and community responses to altitude. *Biol Rev Camb Philos Soc* 80:489 – 513.

Huberty, A.F. and R.F. Denno. (2004). Plant water stress and its consequences for herbivorous insects: A new synthesis. *Ecology* 85: 1383 – 1398.

Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Perez, H. J.A. and Garland, T. (2009). Why tropical forest lizards are vulnerable to climate warming. *Proc. R. Soc. B Biol. Sci.* 276, 1939 – 1948.

Jactel H., Petit J., Desprez – Loustau M.-L., Delzon S., Piou D., Battisti A., Koricheva J. (2012). Drought effects on damage by forest insects and pathogens: a meta – analysis. *Global Change Biol* 18:267 – 276.

Jepsen, J.U., Hagen, S.B., Ims, R.A. & Yoccoz, N.G. (2008). Climate change and outbreaks of the geometrids Operophtera brumata and Epirrita autumnata in subarctic birch forest: evidence of a recent outbreak range expansion. *Journal of Animal Ecology*, 77, 257 – 264.

Jiang L. and Morin P.J. (2004) temperature – dependent interactions explain unexpected responses to environmental warming in communities of competitors. *Journal of Animal Ecology*, 73, 569 – 576.

Kaplan I., Denno R.F. (2007) Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters*, 10, 977 – 994.

Klapwijk, M.J.; Ayres, M.P.; Battisti, A.; Larsson, S. (2012). Assessing the impact of climate change on outbreak potential. In: Barbosa, P.; Letourneau, D.L.; Agrawal, A.A., eds. Insect outbreaks revisited. [New York]: Wiley-Blackwell. 300 p.

Koptur S. (1985) Alternative defenses against herbivores in Inga (Fabaceae: Mimosoideae) over an elevational gradient. *Ecology* 66:1639 – 1650.

Körner C (1989) The nutritional status of plants from high altitudes. *Oecologia* 81:379 – 391.

Körner C. (2007) The use of 'altitude' in ecological research. *Trends in Ecology and Evolution* 22, 569 – 574.

Kurz, W.A, C.C. Dymond, G. Stinson, G. J. Rampley, E.T. Neilson, A. L. Carroll, T. Ebata, and L. Safranyik (2008) Mountain pine beetle and forest carbon feedback to climate change, *Nature* 452:987 – 990.

Lang B., Rall B.C. and Brose U. (2012) Warming effects on consumption and intraspecific interference competition depend on predator metabolism. *Journal of Animal Ecology* 81, 516 – 523.

Littell, J.S., E.E. Oneil, D. McKenzie, J.A. Hicke, J.A. Lutz, R.A. Norheim, and M.M. Elsner. (2010). Forest ecosystems, disturbance, and climatic change in Washington State, USA. *Climatic Change* 102(1-2): 129 – 158.

Logan, J.A., Régnière, J. & Powell, J.A. (2003). Assessing the impact of global warming on forest pest dynamics. *Front. Ecol. Environ.*, 1: 130 – 137.

Low C, Wood SN, Nisbet RG (2009) The effects of group size, leaf size, and density on the performance of a leaf – mining moth. *J Anim Ecol* 78:152 – 160.

Marini L., Ayres M.P., Battisti A., Faccoli M. (2012) Climate affects severity and altitudinal distribution of outbreaks in an eruptive bark beetle. *Climatic Change* 115, 327-341.

Mattson, Jr. W.J. (1980) Herbivory in relation to plant defence. *Annual Review of Ecology and Systematics* 11: 119 – 161.

McDonald E.P., Agrell J., Lindroth R.L. (1999). CO_2 and light effects on deciduous trees: growth, foliar chemistry, and insect performance. *Oecologia* 119, 389 – 399.

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. & Weis, A.E. (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, 11, 41 – 65.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria ISBN 3-900051-07-0, URL http://wwwR-projectorg

Ribeiro S.P., Pimenta H.R. and Fernandes G.W. (1994) Herbivory by chewing and sucking insects on Tabebuia ochracea. *Biotropica*, 26, 302 – 307.

Seppälä R, Buck A, Katila P (2009) Adaptation of forests and people to climate change: A global assessment report. International Union of Forest Research Organizations, Helsinki

Shipley B. (2009) Confirmatory path analysis in a generalized multilevel context. *Ecology* 90, 363 – 368.

Strauss, S.Y., Agrawal, A.A. (1999). The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution*, 14: 179 – 185.

Suzuki S (1998) Leaf phenology, seasonal changes in leaf quality and herbivory pattern of Sanguisorba tenuifolia at different altitudes. *Oecologia* 117:169 – 176

Vasseur, D.A. & McCann, K.S. (2005) A mechanistic approach for modeling temperature – dependent consumer – resource dynamics. *The American Naturalist*, 166, 184 – 198.

.

Van Zandt, P.A. & Agrawal, A.A. (2004b) Community – wide impacts of herbivore – induced plant responses in milkweed (Asclepias syriaca). *Ecology*, 85, 2616 – 2629.

Williams,I. S. 1999. Slow – growth, high – mortality — a general hypothesis, or is it? *Ecological Entomology* 24:490 – 495.

Wilmers C.C., Post E., Peterson R.O., Vucetich J.A. (2006) Predator disease outbreak modulates top – down, bottom – up and climatic effects on herbivore population dynamics. *Ecol Lett*. 2006 April; 9(4): 383 – 389.

Whittaker J.B. and Tribe N.P. 1996. An altitudinal transect as an indicator of responses of a spittlebug (Auchenorrhyncha: Cercopidae) to climate change. *European Journal of Entomology* 93: 319 – 324.

Zovi D, Stastny M, Battisti A, Larsson S (2008) Ecological costs on local adaptation of an insect herbivore imposed by host plants and natural enemies. *Ecology* 89: 1388 – 1398.

Zuo, W., Moses M.E., West G.B., Hou C., and Brown J.H. (2012). A general model for effects of temperature on ectotherm ontogenetic growth and development. *Proc. R. Soc. B* 279:1840 – 184.

TABLES AND FIGURES

Table 1 Effect of temperature on leaf size and toughness (n gradients=14) from a mixed model including temperature as fixed effect and step within region as random effect. Both leaf toughness and size were standardized to mean 0 and standard deviation 1. Each row represents a separate model.

	Temperature effect		
	Estimate	**SE**	**P**
(a) Fagus sylvatica (n gradients=3)			
Toughness	0.020	0.234	0.94
Leaf size	**0.293**	**0.075**	**<0.01**
(b) Picea abies (n gradients=6)			
Toughness	**-0.724**	**0.127**	**<0.01**
Leaf size	0.382	0.200	0.07
(c) Pinus sylvestris (n gradients=4)			
Toughness	**-0.476**	**0.112**	**<0.01**
Leaf size	**-0.327**	**0.113**	**0.01**
(d) Larix decidua (n gradients=3)			
Toughness	0.007	0.101	0.94
Leaf size	-		-

Table 2 Effect of toughness and leaf size on herbivory after removing the effect of temperature and inter-guild interaction (n gradients=14). Results comes from one linear mixed model for each host tree including temperature, inter-guild interaction, toughness and leaf size as fixed effect and step within region as random factors.

	Total herbivory	**P**	**Sap feeders**	**P**	**Chewers**	**P**	**Miners**	**P**	**Gall makers**	**P**
(a) Fagus sylvatica (n gradients=3)										
Toughness	-0.007	0.94	-0.036	0.74	0.002	0.97	0.012	0.89	-0.066	0.54
Leaf size	-0.007	0.94	-0.022	0.84	0.046	0.65	-0.115	0.23	-0.050	0.69
(b) Picea abies (n gradients=6)										
Toughness	**0.200**	**0.03**	0.103	0.19	0.100	0.23	0.144	0.14	0.072	0.44
Leaf size	-0.091	0.23	-0.050	0.45	0.059	0.43	-0.014	0.87	-0.138	0.08
(c) Pinus sylvestris (n gradients=4)										
Toughness	-0.112	0.20	-0.156	0.11	-0.027	0.76	-	-	0.087	0.40
Leaf size	0.103	0.14	0.006	0.94	0.120	0.09	-	-	-0.073	0.42
(d) Larix decidua (n gradients=3)										
Toughness	-0.074	0.41	-0.023	0.81	-0.071	0.48	-0.039	0.68	-	-
Leaf size	-	-	-	-	-	-	-	-	-	-

Fig. 1 Description of the nested sampling design of the study with (a) the distribution of the 13 regions where the 21 gradients were sampled. The dots are less than the number of gradients due to the low resolution of the map.

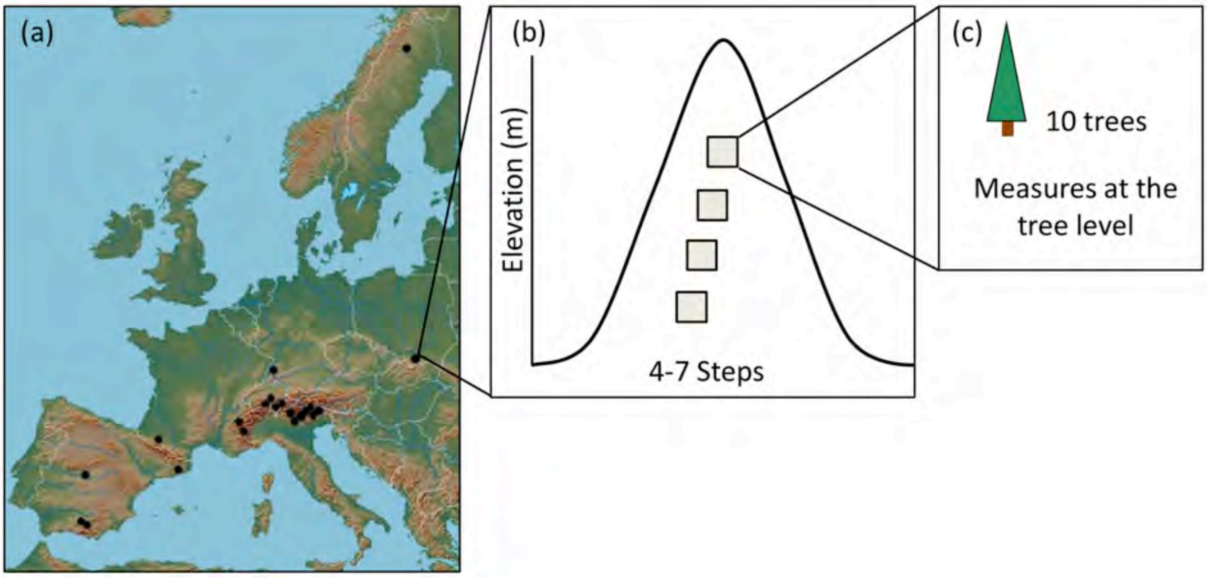


Fig. 2 Conceptual scheme of the expected relationship between temperature, host tree quality, and insect herbivory. Tritrophic interactions are not depicted as they were not measured in the present study. The sign indicates the expected relationship from literature.

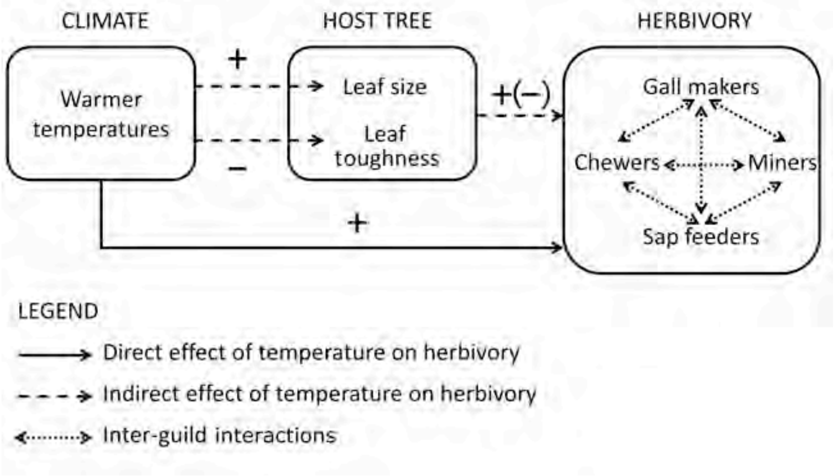


Fig. 3 Average level of herbivory (%) in the different regions across Europe separately for feeding guilds for (a) Beech, (b) Norway spruce, (c) Scots pine, and (d) Larch. For sap feeders and chewers herbivory corresponds to the proportion of damaged leaf area while for leaf miners and gall makers it indicated the proportion of leaves (or for larch the proportion of brachyblasts) with mines and galls, respectively.

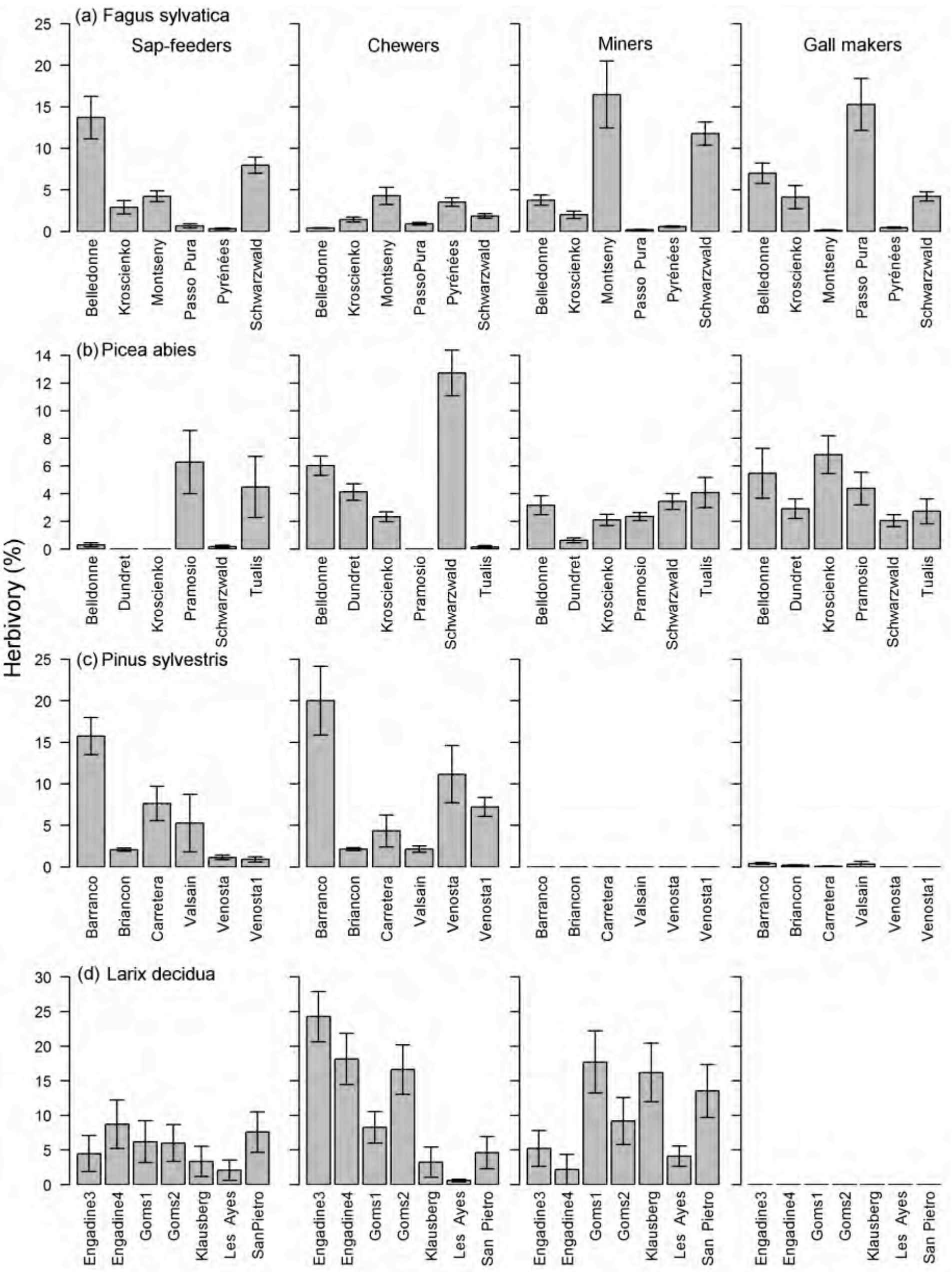


Fig. 4 Temperature effect on total herbivory (±confidence intervals at 95%) from a mixed model including elevational step within region as random effect. * Indicates significant effect at P<0.05.

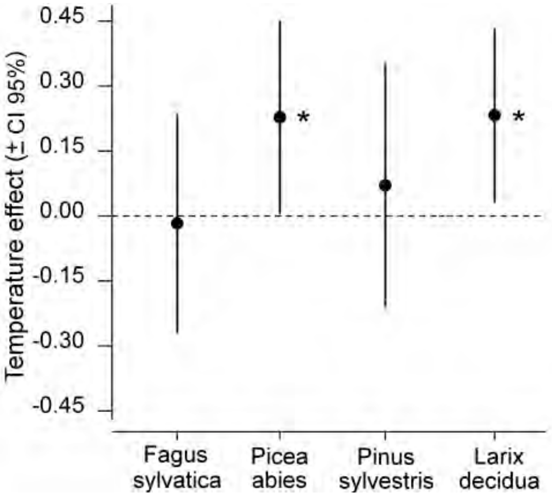
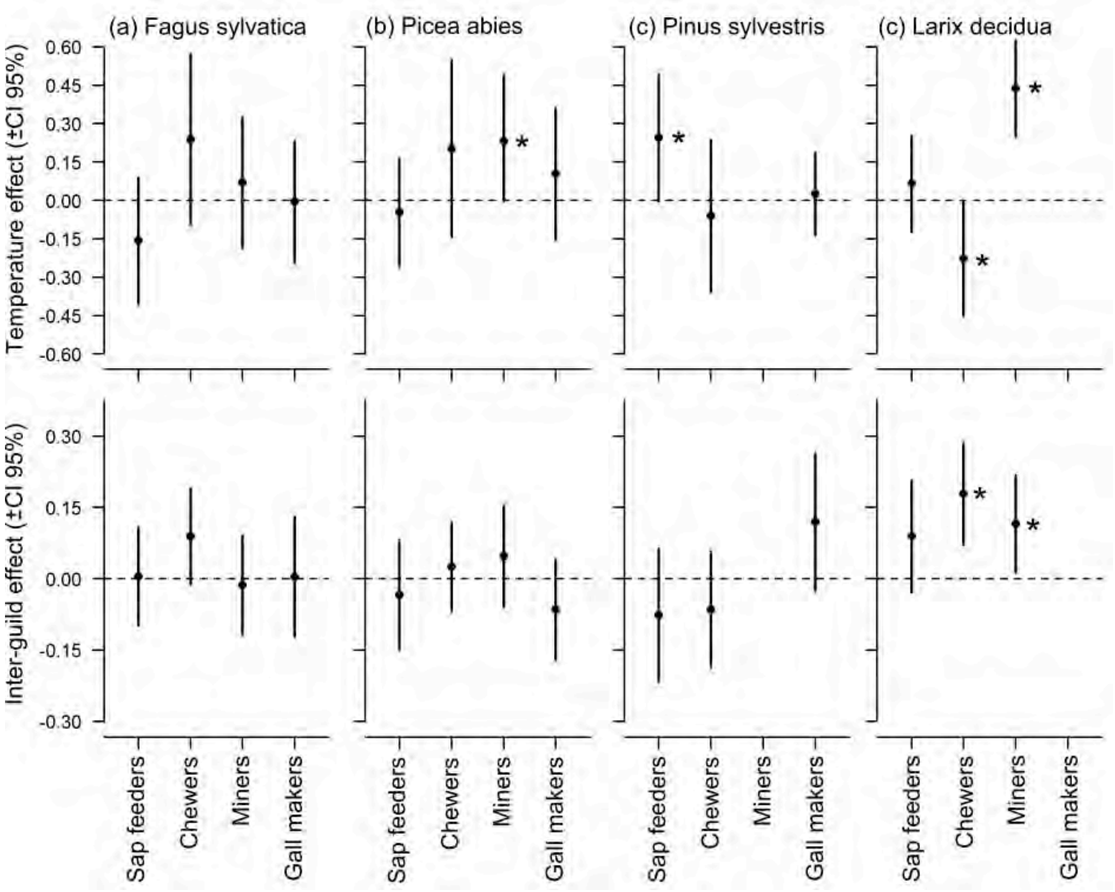


Fig. 5 Coefficient and confidence intervals (C.I. 95%) from a mixed model testing the effect of both temperature and inter-guild interaction (cumulative herbivory of all guilds but the one tested) on herbivory caused by sap feeders, chewers, miners, and gall makers. * Indicates significant effect at P<0.05.



SUPPLEMENTARY MATERIAL

Table S1 Summary table of the elevation ranges with the indication of the tree species sampled, elevation range and the number of steps. The geographical reference of the coordinates is WGS84.

Region	**Longitude**	**Latitude**	**Elevation**	**Steps**	**Leaf traits**
Fagus sylvatica					
F (Belledonne)	05°51'E	45˚06'N	1150-1952	3	No
F (Pyrenees)	00°05'W	43°05'N	131-1533	5	No
D (Schwarzwald)	07º52'E	47º55'N	580-1241	5	No
IT (Passo Pura)	12°46'E	46°24'N	768-1260	4	Yes
E (Montseny)	02°27'E	41°46'N	770-1640	4	Yes
PL (Krościenko)	20°28'E	49°25'N	500-1100	4	Yes
Picea abies					
F (Belledonne)	05°51'E	45˚06'N	1150-1950	4	Yes
D (Schwarzwald)	07º52'E	47º55'N	580-1240	5	Yes
IT (Tualis)	12˚53'E	46˚31'N	980-1700	4	Yes
IT (Pramosio)	13˚00'E	46˚34'N	930-1500	4	Yes
PL (Krościenko)	20°28'E	49°25'N	500-1100	4	Yes
SE (Dundret)	20º33'E	67°07'N	390-610	4	Yes
Pinus sylvestris					
F (Briancon)	06°26'E	44°31'N	800-1800	6	No
IT (Val Venosta)	10°47'E	46°37'N	800-1400	4	Yes
E (Barranco del Espinal)	03°27'W	37°06'N	1400-2039	5	Yes
E (Carretera)	03°27'W	37°08'N	1537-2235	4	Yes
E (Valsain)	04°01'W	40°52'N	1150-1800	5	Yes
Larix decidua					
IT (Klausberg)	11°59'E	46°59'N	1450-2070	4	Yes
CH (Goms 1)	08°17'E	46°30'N	1551-2026	4	No

CH (Goms 2)	08°21'E	46°32'N	1497-1890	4	No
CH (Engandine 3)	09°55'E	46°34'N	1751-2054	4	No
CH (Engandine 4)	09°52'E	46°30'N	1747–2054	4	No
IT (San Pietro)	12°03'E	47°01'N	1580-2070	4	Yes
F (Les Ayes)	06°39'E	44°48'N	1347-2150	7	Yes

IT: Italy, F: France, E: Spain, D: Germany, PL: Poland, CH: Switzerland, SE: Sweden

TABLE S2 List of the insect herbivores that could be identified on the trees.

Host tree species	**Insect herbivores**
Fagus sylvatica L.	**Leaf sap feeders:** *Phyllaphis fagi* Linnaeus *Typhlocyba cruenta* Herrich-Schäffer **Chewers:** Geometridae larva *Rhynchaenus fagi* Linnaeus **Gall makers:** *Hartigiola annulipes* Hartig *Mikiola fagi* Hartig **Miners:** *Lithocolletis* sp. *Rhynchaenus fagi* Linnaeus *Stigmella hemargyrella* Kollar *Stigmella tityrella* Stainton
Picea abies (L.) Karst.	**Leaf sap feeders:** *Asthenia pygmaeana* Hübner *Cinara pilicornis* Hartig *Elatobium abietinum* Walker *Physokermes* sp. *Puto antennatus* Signoret **Chewers:** *Gilpinia hercyniae* Hartig Tortricidae larva **Gall makers:** *Adelges* spp. *Sacchiphantes* spp. **Miners:** *Epinotia tedella* Clerck

Pinus sylvestris L.	**Leaf sap feeders**: *Cinara pini* Linnaeus *Eulachnus* sp. *Leucaspis loewi* Colvée *Leucaspis pini* Hartig *Leucaspis pusilla* Loew. **Chewers:** *Acantholyda sp.* *Brachonyx pineti* Paykull *Brachyderes incanus* Linnaeus *Cryptocephalus pini* Linnaeus *Thaumetopoea pityocampa* Denis & Schiffermüller **Gall makers:** *Cecidomyia pini* DeGeer *Petrova resinella* Linnaeus
Larix decidua Mill.	**Leaf sap feeders**: Aphididae **Chewers:** *Anoplonyx duplex* Serville *Anoplonyx ovatus* Zaddach *Exapate duratella* Heyden *Eupithecia lariciata* Freyer Geometridae *Pachynematus imperfectus* Zaddach *Pristiphora laricis* Hartig *Spilonota laricana* Heinemann *Zeiraphera diniana* Guenee **Miners:** *Coleophora laricella* Hübner

Chapter 3

Factors explaining the abundance of the pine processionary moth (*Thaumetopoea pityocampa* Denis & Schiffermüller) along elevational gradients in Sierra Nevada Spain

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Czwienczek E., Torres-Muros L., Marini L., Battisti A., Hódar J.A.

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I contributed to all experimental parts and to the paper writing.

Abstract

Elevational gradients can be used to explore the interaction between abiotic and biotic factors in a climate change perspective. This study compares the abundance of pine processionary moth (*Thaumetopoea pityocampa*) along two gradients in southern Spain with the patterns known in the literature. Abundance can be related to the temperature, tree quality (leaf size, leaf toughness and C/N ratio), natural enemies (egg parasitoids), and competition. Two elevational gradients of *Pinus sylvestris* in Spain (natural gradient of pine in Barranco del Espinal, 5 steps and planted gradient Carretera de la Sierra, 4 steps) were selected in 2011, because the well known presence of pine processionary moth in this field. At each step nest abundance per tree, needle toughness, needle C and N and competitors, were measured. Parasitism was also checked in Sierra Nevada in 2008 and 2009. Linear mixed models were used to test the effect of altitude and the other potential factors influencing occurence and nest abundance of pine processionary moth. The abundance of pine processionary moth nests increases with increasing altitude, which is an opposite pattern to the known trends from the literature. Possible explanatory factors were searched in stand conditions (tree density, host percentage), tree quality (tree height, needle toughness, C/N ratio, needle length), egg parasitoids, and possible competitors (leaf sap feeders and chewers). Winter temperature did not explain moth abundance, which was in turn explained by tree height, leaf C/N, but mainly by a higher realized fecundity at high elevation, likely linked to better growth conditions, and by higher egg parasitism at lower elevation.

Introduction

Climatic gradients are often used to measure the response of herbivorous insects to abiotic and biotic variations, although a number of issues have been raised on their general validity. Elevational gradients are generally considered more suitable than latitudinal gradients to measure the response at population level, as the number of confounding factors is lower (Hodkinson 2005). Insects respond to the gradient directly and through their host plants. Direct elevational effects on insect can appear through responses to decreasing temperature and oxygen availability, or increasing radiation. Elevational effects on insect host plant is related to the growth and morphology of the trees, phenology and timing, nutrient composition and availability, secondary compounds and defensive chemicals, plant age and stress, and seed size and/or production (Hodkinson 2005).

Although the general pattern of herbivory respond to increasing elevation with a decrease more or less pronounced (Garibaldi *et al.* 2011, chapter 2 of this thesis), the response of individual species may vary dramatically. For example, *Lymantria monacha* (Lepidoptera) on *Picea abies* decreases with increasing elevation (Cescatti & Battisti 1992) as well as *Cydia strobilella* (Lepidoptera) on *Picea abies* (Wermelinger *et al.* 1995), while an opposite trend was observed for *Neodiprion sertifer* (Hymenoptera) on *Pinus sylvestris* (Niemelä *et al.* 1987).

An important factor influencing insects along the gradient is the mortality by natural enemies. In general natural enemies are more active at lower elevation (M. Kenis personal communication), as shown by *Rhizophagus grandis* preying on *Dendroctonus micans* in Massif Central of France (Gilbert & Grégoire 2003). The lack of response to elevation can be also explained by natural enemies, as in the case of *Elatobium abietinum* in Straw *et al.* (2009) studies, where the highest abundance of this insects was observed at mid altitude because natural enemies (invertebrates) were the most abundant in the lowest elevation.

The pine processionary moth *Thaumetopoea pityocampa* (Denis & Schiffermüller) (Lepidoptera: Notodontidae) is an important pest of *Pinus* and *Cedrus* in the Mediterranean and in southern Europe (Devkota & Schmidt 1990; Roques *et al.* 2002). *T. pityocampa* is univoltine and its phenology largely depends on climatic conditions (Démolin 1969a). Among all abiotic factors, temperature seems to be the most important one in regulating the insect performance, and it is associated with shifting the range and outbreak development (Hódar & Zamora 2004; Battisti *et al.* 2006; Hoch *et al.* 2009). It is also known that other abiotic factors such as precipitation and radiation may play a role in population regulation (Pimentel *et al.* 2011). The pine processionary moth is also controlled by a number of natural enemies, among which the egg parasitoids *Baryscapus servadeii* Domenichini (Hymenoptera: Eulophidae) and *Oencyrtus pityocampae* Mercet (Hymenoptera: Encyrtidae) are well known to cause an important mortality (Masutti & Battisti 1990).

The aims of this study are to explore how elevation affects the population density of the pine processionary moth in a geographic area situated at the southern edge of the range of *Pinus sylvestris* in southern Spain, where the moth is becoming an important limiting factor for the survival of this forest ecosystem (Hódar *et al.* 2003). By the sampling along two gradients in the mountains of Sierra Nevada, one with native trees and the other planted, the study aims to recognise the most important abiotic and biotic factors explaining insect population abundance.

Material and methods

Study sites description

The study was carried out along two elevational gradients in Sierra Nevada in southern Spain (Barranco del Espinal and Carretera de la Sierra) (Table 1). *Pinus sylvestris* is native in Barranco del Espinal (subspecies *nevadensis*) while in Carretera de la Sierra is planted (probably subspecies *iberica*, from Central Spain, Robledo-Arnuncio *et al.* 2009). The elevational steps were set based on the local occurrence of pine trees and by keeping a minimum difference of 150 m between them. It resulted in a total of 5 steps at Barranco and 4 steps at Carretera de la Sierra. At both gradients, the trees were scattered within various types of herbaceous, shrub, and forest vegetation, resulting from historical disturbance associated with grazing and fire. This area is known for historical occurrence of the insect and for repeated outbreaks in the last decades (three population peaks since 1997) (Hódar *et al.* 2002, 2003, 2012, and unpublished observations). An historical series of temperature data was obtained from the weather station at the Jardin Botánico de La Cortijuela (monthly mean temperature 1990 – 2010) 1650 m, situated close to Barranco del Espinal. In addition, the temperature of the winter 2010 – 2011 was measured with HOBO TidbiT® UTBI – 001 temperature data loggers at three steps close to the Barranco del Espinal gradient (La Cortijuela 1650 m, Matas Verdes 1850 m, and Cerro del Mirador 2039 m). Three data loggers per step were placed on branches of adult Scots pines at 2 – 2.5 m in height. Temperature was recorded every 30 min, allowing an accurate estimate of maximum, minimum and mean temperatures per level, and thus a good estimate of variations in winter temperature along the gradient.

Nest census

The pine processionary moth builds conspicuous silk tents in winter, used by the larvae for protection and for thermoregulation (Fitzgerald & Blas 2003; Battisti *et al.* 2006), that are generally called nests. As nests are easily detectable because of the conspicuousness and the white colour of the silk, they are used for assessing population density in monitoring programmes (Jactel *et al.* 2006). Nest census was carried out at both gradients in June 2011 by inspecting the canopy from all sides of the tree. Although larvae had already left the nests at the time of the sampling, they were still detectable. At each step, 10 mature trees were randomly chosen and the number of nests on each tree was assessed. In addition, circumference and height of these focal trees were taken, as well as the number of trees in a radius of 15 m for each tree species.

Plant nutritional quality

The plant nutritional quality was assessed by measuring leaf size, leaf toughness, and C/N content of mature needles. One sample of needles was collected from several branches of each tree that were reachable from the ground with a 5 m long pruner. Needle cohorts were mixed and stored in a portable refrigerator. In the laboratory, needle toughness was measured by determining the force needed to penetrate the needles using a Dial Tension Gauge (Mitutoyo 0.05N – 1N) and measurement was given in Newton. The length of each pine needle was first measured and then it was fixed between two metal plates, and the probe (Bohemia insect pin, diameter 0.55 mm) of the penetrometer was inserted through the central part of the upper, rounded surface. Three needles of each tree were measured, and three readings were taken per needle, avoiding the distal parts.

Other needles of the same sample were used to measure the concentration of carbon and of nitrogen using Vario Macro Elementar Analyzer. Twenty needles were first dried in the oven at 60˚C for 24 hours, then grinded in a pot with liquid nitrogen. Fifty mg of the powder was poured into tin capsules with addition of 50 mg of tungsten trioxide (WO_3) and analysed.

Census of competitors and natural enemies

Herbivore density was estimated by checking two randomly selected branches per tree. For every branch the assessment was done by visual estimation of the percentage of damaged leaf area separately for chewers and sap feeders. Chewers were mainly represented by beetles (*Cryptocephalus pini*, *Brachyderes incanus*) and their activity was detected based on scars and feeding marks. Sap feeders were dominated by the leaf scales *Leucaspis* spp. and resulted in needle discoloration. Other guilds of herbivores such as gall makers and wood feeders were observed but their abundance was negligible.

The activity of natural enemies was restricted to egg parasitoids because of their relevance in population regulation and because of an easy access to field material (Zovi *et al.* 2008). It was not possible, however, to track back the egg batches from which the colonies used for the nest density were generated, because at the time of the sampling they were partly damaged or missing. At this purpose, a large data set on egg parasitism was available from a different study (Torres-Muros 2010, and unpublished results). Samples came from three altitudinal gradients in Sierra Nevada and the nearest Sierra de Baza, consisting of 200 egg batches in 2008 and 228 in 2009, collected along seven elevational steps (from 400 to 2100 m) from a total of five pine species, extending each one over 2 steps. Egg number and parasitism were estimated by removing the scales covering the eggs and counting the number of hatching holes and the number of parasitoid emergence holes, according to standard methods (Zovi *et al.* 2008).

Data analysis

To test the factors affecting egg parasitism along the elevation gradients, we run a general linear mixed model with egg parasitism as dependent variable and realised fecundity (number of eggs per batch) and elevation as explanatory variables. Sampling year (2008 and 2009) was considered as random factor.

All of the mixed models were estimated using the lme() function in the nlme package for version 2.13.0 (R Development Core Team 2011). The fit of each model in the set was then evaluated using second-order Akaike's information criterion (AICc). The best fit is indicated by the lowest AICc (AICcMIN). In a set of n models each model it can be ranked using its difference in AICc score with the best-fitting model (ΔAICci = AICci–AICcMIN). A model in a set can be considered plausible if its ΔAICc is below 2. We also computed the model weight (wi) as the weight of evidence in favour of each model being the best within the set. The weights wi represent the relative likelihood of a model. For each model, we first calculated its likelihood as exp(-0.5*ΔAICci). The weight wi for a model is its likelihood divided by the sum of the likelihoods across all models. To evaluate the relative importance of each predictor, we then summed the wi values across all of the models in the set (∑wi) in which each predictor occurred (Burnham and Anderson 2002). The multimodel inference analyses were performed using the 'MuMIn' package (Barton 2010) implemented in R version 2.13.0 (R Development Core Team 2011).

Results

The temperature during the cold period was associated with a colony survival probability around 40% for both the historical period and the experimental year. There were no major differences in temperature and expected survival along the steps of the gradients during the winter 2010-2011 (Table 2).

Nest density, however, varied strongly along the steps at both gradients (Figure 1), reaching the highest value at medium-upper elevation at both gradients. Model selection uncertainty was large with 12 plausible models (ΔAICc<2). Nest abundance resulted to be positively affected by C/N ratio and by elevation, and negatively by tree height and needle toughness. These variables were those with the highest sum of model weights. Other factors affecting positively the nest abundance were, in decreasing order of importance, chewers damage, needle length, sap feeders damage, and stand density (Table 3).

The realised fecundity increased significantly with elevation while egg parasitism decreased (Figure 2).

Discussion

In the Sierra Nevada mountains the higher density of pine processionary moth has been observed close to the upper limit of the range, where conditions are generally considered harsh. However, abiotic factors, and in particular the minimum temperature during the winter, do not seem to affect the performance of the pine processionary moth in the Sierra Nevada mountains as much as in the north, where they are the major driving factor of population establishment at the upper limit of the range (Battisti *et al.* 2005). This can be partly explained by the nature of the mountains in south – eastern Spain, where high peaks are intermixed with large valleys and basins, and phenomena of thermal inversions during winter are common. Furthermore, topography can alter the patterns of sun irradiance and temperature, thus producing severe changes in climatic conditions even in short distances (Tovar *et al.* 1995, Batlles *et al.* 2008). This may give as a result lower temperatures in lower elevations, leading to a reverse effect on performance. The phenomenon has been partly observed during the winter 2010 – 2011 between the two higher steps of the Barranco gradient, although its magnitude does not seem to be so important for the performance. As climatic inversion is also related to a change in insolation, with generally higher radiation level at higher elevation, colonies at the upper steps could also benefit of that. Unfortunately we were unable to measure solar radiation along the gradient, and this should be kept into account in future work, as it seems positively related to insect performance in both *T. pityocampa* (Robinet *et al.* 2007; Netherer & Schopf 2010) and in the closely related species *T. pinivora* (unpublished results).

Biotic factors, such as tree quality and natural enemies, have been often used to explain the performance of *T. pityocampa* in the Mediterranean mountains. Tree quality traits such as leaf toughness affects negatively larval development, especially in young instars (Zovi *et al.* 2008), while leaf N content is positively related to performance (Hódar *et al.* 2002). These studies, however, compare different pine species and are related to specific stages of development, or to short laboratory experiments. When the overall performance is measured at the end of the development, by taking for example pupal weight, or better realised fecundity, the differences tend to become small and generally do not give large support to the hypothesis of a performance driven by the tree quality (Battisti *et al.* 2006; Stastny *et al.* 2006). This can be perhaps explained by the exceptionally long developmental time of this moth, allowing compensation mechanisms to take place, reverting initially unfavourable conditions. This is probably what happened also in the gradients of Sierra Nevada, where the increase of leaf toughness with elevation, as a response to keep needle efficient during the cold winter, was actually negatively related to the number of nests but did not explain the higher abundance at high elevation. The same happened for C/N ratio, decreasing as predicted with high elevation (Reich *et al.* 1996), but having an opposite effect on nest abundance.

The idiosyncratic response of the moth to tree quality traits can also be explained by the nature of the forest stand, as Sierra Nevada is the very southern edge of the range of *P. sylvestris* in Europe. For these extreme conditions, trees grow at low density and even in plantations (gradient of Carretera de la Sierra) the distribution is quite irregular, with patches of trees intermixed with open areas with high grazing pressure. The high phenotypic diversity of individual trees under such conditions may overwhelm the attempt to find general pattern explaining insect performance with tree quality. In addition, the capacity of the insect to cope with poor tree quality, documented by the survival on needles harder and with lower N content than those of Scots pine, as for instance maritime pine (*P. pinaster*) (Hódar *et al.* 2002), can depend on thermal environment. This suggests that, along the two gradients analysed, plant quality is generally adequate and does not represent a problem for the moth. In line with his statement, egg batches are bigger at higher elevation, suggesting that larvae perform in general better when feeding on pines at high elevation. A higher number of eggs per batch is a good start to survive in cold areas, since more larvae building the nest involves bigger nest and better thermoregulation (Breuer *et al.* 1989; Fitzgerald & Blas 2003; Pimentel *et al.* 2010). The realised fecundity can be considered an adaptive trait possibly associated with the length of the developmental time, as colonies living under harsh conditions take more time to develop (Battisti *et al.* 2006) and may thus compensate with overall higher assimilation. It is interesting to observe that an increasing pattern or realised fecundity has been described as latitude increases within the whole range, by assembling data from various host plants and forest stands (Pimentel *et al.* 2010).

Natural enemies, represented here by egg parasitoids which are important players in the population dynamics of the moth (Zovi *et al.* 2008), significantly decreased their activity as elevation increased. Because of this, the colonies at low elevation suffered proportionally more the activity of egg parasitoids as they have a lower number of eggs, leading to smaller nests that are more exposed to both abiotic extremes and generalist predators such as insectivorous birds (Barbaro & Battisti 2011). This can be an important factor in the decline of the moth abundance at lower elevation, although other natural enemies not considered in this study may respond in a different way. It is, however, largely accepted that natural enemies of the insects, and especially the parasitoids, tend to have a higher reaction norm to temperature than their hosts (Berggren *et al.* 2009) and have generally higher mortality rates at lower elevation (M. Kenis personal communication).

Overall, in the studied gradients the role of natural enemies appears to explain more than plant quality the pattern of abundance, while temperature may become limiting only at the extreme steps. At low elevation temperature is adequate for moth development, but also for the development of the enemies, while competitors do not seem to play an important role. As elevation increases, the pressure exerted by natural enemies decreases, plant quality seems to be adequate anyway, and temperature is becoming more limiting. However, the moth is well adapted to low temperature, and in Sierra Nevada lethal or sublethal temperatures are rarely reached even at 2000 m. Furthermore, sun radiation is in general higher during winter, thus allowing the warming of the winter nest and an efficient processing of the food ingested during the night (Battisti *et al.* 2005). Thus, with biotic factors decreasing their negative impact and temperature increasing it with elevation, there is a point at which the combination is optimal for moth survival. Above this threshold, situated around 2000 m, conditions become very harsh and colonies usually die during winter. This is in agreement with the results found by Hódar *et al.* (2012): severe outbreaks are more frequent at high elevation in relationship with climatic factors (e.g. NAO), while lowland stands show more stable populations.

The question is how this status quo will be altered by climate change. In a first approach, increasing temperatures will favour the moth since it is clearly limited by low temperatures at high elevation, in Sierra Nevada as well as in other parts of the range. If mild winters are more and more frequent, this would allow a better development at elevations in which now the moth is limited by temperature. However, this prediction is misleading for Mediterranean mountains, since it is not clear to what extent the combination of warmer and drier winters, which is expected in the near future (López – Moreno *et al.* 2011), could affect their biota. Furthermore, in the same way than the moth is benefited, the same is true for the rest of associated arthropods, which also could migrate in elevation and limit the host development as they do now at lower elevation. According to expectations, several studies have reported that global warming is driving species ranges polewards and upwards (Parmesan & Yohe 2003, Lenoir *et al.* 2008). However, most studies have also found species range shifts opposite to theoretical predictions (Lenoir *et al.* 2010). This is because species responses to climate change are more complex than those simply dependent on temperature and precipitation. In this sense, the effect on a particular species of all the other interacting species remains largely unknown. The way in which all these interacting organisms will find a new equilibrium will depend on the individual responses to changes to temperature and precipitation, but also to food quality and interactions with predators and parasitoids.

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References

Barbaro L. & Battisti A. (2011). Birds as predators of the pine processionary moth (Lepidoptera: Notodontidae). *Biological Control*, 56, 107-114.

Battisti A., Stastny M., Buffo E. & Larsson S. (2006). A rapid altitudinal range expansion in the pine processionary moth produced by the 2003 climatic anomaly. *Global Change Biology*, 12, 662-671.

Battisti A., Stastny M., Netherer S., Robinet C., Schopf A., Rocques A. & Larsson S. (2005). Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. *Ecological Applications* 15, 2084-2096.

Berggren A., Bjorkman C., Bylund E., Ayres M.A. (2009). The distribution and abundance of animal populations in a climate of uncertainty. *Oikos*, 118, 1121-1126.

Breuer M., Devkota B., Doumapetridou E., Koutsaftikis A. & Schmidt G.H. (1989). Studies on the exposition and temperature of nests of Thaumetopoea pityocampa (Den and Schiff.) (Lep, Thaumetopoidae) in Greece. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie*, 107, 370-375.

Buffo E., Battisti A., Stastny M. & Larsson S. (2007). Temperature as a predictor of survival of the pine processionary moth in the Italian Alps. *Agricultural and Forest Entomology*, 9, 65-72.

Burnham, K. P.; Anderson, D. R. (2002), *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach* (2nd ed.), Springer-Verlag,

Cescatti A. & Battisti A. (1992). Distribution and ecology of Lymantria monacha L. and Cephalcia spp. in non-outbreak areas of Trentina (N-Italy). *Anzeiger für Schädlingskunde Pflanzenschutz Umweltschutz* 65, 92-99.

Démolin G. (1969a). Bioecologia de la processionaria del pino Thaumetopoea pityocampa Schiff. Incidencia de los factores climaticos. *Boletin del Servicio de Plagas Forestales* 12, 9 - 24.

Devkota B. & Schmidt G.H. (1990). Larval development of Thaumetopoea pityocampa (Den. And Schiff) (Lepidoptera:Thaumetopoeidae) from Greece as influenced by different host plant under labolatory conditions. *Journal of Applied Entomology*, 109, 321 – 330.

Fitzgerald T.D. & Blas X.P.I. (2003). Mid-winter foraging of colonies of the pine processionary caterpillar Thaumetopoea pityocampa Schiff. (Thaumetopoeidae). *Journal of the Lepidopterists' Society*, 57, 161-167.

Garibaldi L., Kitzberger T. & Chaneton E. (2011). Environmental and genetic control of insect abundance and herbivory along a forest elevational gradient. *Oecologia*, 167, 117 - 129.

Gilbert M. & Grégoire J.-C. (2003). Site condition and predation influence a bark beetle's success: a spatially realistic approach. *Agricultural and Forest Entomology*, 5, 87-96.

Hoch G., Petrucco - Toffolo E., Netherer S., Battisti A. & Schopf A. (2009). Survival at low temperature of larvae of the pine processionary moth Thaumetopoea pityocampa from an area of range expansion. *Agricultural and Forest Entomology*, 11, 313-320.

Hodkinson I.D. (2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews*, 80, 489-513.

Hódar J. & Zamora R. (2004). Herbivory and climatic warming: a Mediterranean outbreaking caterpillar attacks a relict, boreal pine species. *Biodiversity and Conservation*, 13, 493-500.

Hódar J.A., Zamora R. & Castro J. (2002). Host utilisation by moth and larval survival of pine processionary caterpillar Thaumetopoea pityocampa in relation to food quality in three Pinus species. *Ecological Entomology*, 27, 292-301.

Jactel H., Menassieu P., Vétillard F., Barthélémy B., Piou D., Frérot B., Rousselet J., Goussard F., Branco M. & Battisti A. (2006). Population monitoring of the pine processionary moth (Lepidoptera: Thaumetopoeidae) with pheromone-baited traps. *Forest Ecology and Management*, 235, 96-106.

Lenoir J., Gégout J.C., Marquet P.A., de Ruffray P., Brisse H. (2008) A significant upward shift in plant species optimum elevation during the 20th century. *Science* 320, 1768

Lenoir J, Gégout J.C., Guisan A., Vittoz P., Wohlgemuth T., Zimmermann N.E., Dullinger S., Paulis H.,Willner W., Grytnes J.A., Virtanen R., Svenning J.C. (2010) Cross-Scale

Lenoir J, Gégout J.C., Guisan A., Vittoz P., Wohlgemuth T., Zimmermann N.E., Dullinger S., Paulis H.,Willner W., Grytnes J.A., Virtanen R., Svenning J.C. (2010) Cross-Scale Analysis of the Region Effect on Vascular Plant Species Diversity in Southern and Northern European Mountain Ranges. *PLoS ONE* 5(12): e15734.

Lopez - Moreno J.I., Vincente - Serrano S.M., Moran - Tejeda E., Zabalza J., Lorenzo - Lacruz and Garcia - Ruiz J.M.(2011) Impact of climate evolution and land use changes on water yield in the ebro basin. Hydrol. Earth Syst. Sci., 15, 311 - 322

Masutti L. & Battisti A. (1990). *Thaumetopoea pityocampa* (Den. & Schiff.) in Italy. Bionomics and perspectives of integrated control. *Journal of Applied Entomology*, 110, 229-234.

Netherer S. & Schopf A. (2010). Potential effects of climate change on insect herbivores in European forests-General aspects and the pine processionary moth as specific example. *Forest Ecology and Management*, 259, 831-838.

Niemelä P., Rousi M. & Saarenmaa H. (1987). Topographical delimitation of Neodiprion sertifer (Hym., Diprionidae) outbreaks on Scots pine in relation to needle quality. *Journal of Applied Entomology*, 103, 84-91.

Parmesan C. & Yohe G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421, 37 - 42.

Pimentel C., Calvão T. & Ayres M.P. (2011). Impact of climatic variation on populations of pine processionary moth Thaumetopoea pityocampa in a core area of its distribution. *Agricultural and Forest Entomology*, 13, 273-281.

Pimentel C., Ferreira C. & Nilsson J. (2010). Latitudinal gradients and the shaping of life-history traits in a gregarious caterpillar. *Biological Journal of the Linnean Society*, 100, 224-236.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria ISBN 3-900051-07-0, URL http://wwwR-projectorg

.

Reich P.B., Oleksyn J. & Tjoelker M.G. (1996). Needle respiration and nitrogen concentration in Scots pine populations from a broad latitudinal range: a common garden test with field – grown trees. *Funct Ecol* 10, 768 – 776.

Robinet C., Baier P., Pennerstorfer J., Schopf A. & Roques A. (2007). Modelling the effects of climate change on the potential feeding activity of Thaumetopoea pityocampa (Den. & Schiff.) (Lep., Notodontidae) in France. *Global Ecology and Biogeography*, 16, 460-471.

Roques A., Boivin S. & Auger – Rozenberg M.A. (2002). Douglas – fir vs. Lodgepole pine in Europe: colonization of exotic conifer species by native phytophagous insects depends on taxonomic isolation. Pacific Temperate Conifers International Symposium. *IUFRO Working Parties S2.02.05 ans S2.01.06 Orléans, France, 27 – 30 May 2002*.

Stastny M., Battisti A., Petrucco-Toffolo E., Schlyter F. & Larsson S. (2006). Host-plant use in the range expansion of the pine processionary moth, Thaumetopoea pityocampa. *Ecological Entomology*, 31, 481-490.

Straw N.A., Timms J.E.L. & Leather S.R. (2009). Variation in the abundance of invertebrate predators of the green spruce aphid Elatobium abietinum (Walker) (Homoptera: Aphididae) along an altitudinal transect. *Forest Ecology and Management*, 258, 1-10.

Wermelinger B., Hirschheydt J.v. & Fecker B. (1995). Abundance and emergence of spruce cone insects in different parts of Switzerland. *Journal of Applied Entomology*, 119, 9-15.

Zovi D., Stastny M., Battisti A. & Larsson S. (2008). Ecological costs on local adaptation of an insect herbivore imposed by host plants and enemies. *Ecology*, 89, 1388-1398.

TABLES AND FIGURES

Table 1. Description of the elevational gradients used in the study.

Gradient	Step elevation (m)	Longitude	Latitude	Tree density (n / ha)	Pinus sylvestris %
Barranco del Espinal	1400	3°27'30.35"W	37°6'13.15"N	296.3	81.3
	1560	3°27'26.16"W	37°6'3.72"N	188.7	24.1
	1750	3°27'21.68"W	37°5'43.35"N	196.8	92.4
	1895	3°27'17.7"W	37°5'28.08"N	94.0	92.5
	2039	3°27'1.14"W	37°5'15.36"N	68.4	100
Carretera de la Sierra	1537	3°27'26.82"W	37°8'20.58"N	105.4	77
	1825	3°25'43.92"W	37°7'51.72"N	111.1	97.5
	2050	3°26'13.68"W	37°7'10.56"N	52.7	100
	2235	3°24'59.04"W	37°6'39.72"N	109.7	77

Table 2. Mean of the minimum temperature during the cold period (1 December – 28 February) from historical data (1990 - 2010) and from data loggers in 2010 - 2011, with the indication of the expected colony survival based on the model of Buffo et al. (2007)

Site	Elevation (m)	Period	T min cold period	Expected survival (%)
Cortijuela	1650	1990-2010	-1.03	38.7
Cortijuela	1650	2010-2011	-0.80	40.3
Matas Verdes	1850	2010-2011	-1.33	36.5
Cerro del Mirador	2040	2010-2011	-0.89	39.6

Table 3. Likely models (ΔAICc<2) explaining pine processionary moth (*Thaumetopoea pityocampa*) nest distribution on pines along the Sierra Nevada elevational gradients. Models are in the columns and they are ranked from the left to the right site according to their ΔAICc value. Variables in the rows are ranked regarding to their Σw_i. Parameter estimate, model weights (w_i), degrees of freedom (df) are reported.

		Mod1	Mod2	Mod3	Mod4	Mod5	Mod6	Mod7	Mod8	Mod9	Mod10	Mod11	Mod12
	ΔAICc	0	0.17	0.19	0.81	0.9	0.99	1.16	1.61	1.62	1.66	1.72	1.93
	Df	9	9	8	8	7	7	9	7	6	10	10	8
Σw_i	Model w_i	0.078	0.072	0.071	0.052	0.05	0.048	0.044	0.035	0.035	0.034	0.033	0.03
-	Intercept												
1	Tree height	-0.2556	-0.2688	-0.2237	-0.2979	-0.303	-0.2486	-0.2673	-0.2281	-0.2495	-0.286	-0.2759	-0.2788
1	C/N	0.1085	0.1107	0.1108	0.1035	0.102	0.09906	0.1123	0.1067	0.09682	0.1097	0.1089	0.1088
0.812	Toughness	-3.6490	-3.537	-3.488	-3.748	-3.662	-3.579	-3.61	-3.364	-3.427	-3.717	-3.63	-3.549
0.714	Elevation	0.003833	0.003619	0.003324	0.0027	-	0.002977	0.003095	-	-	0.003604	0.003867	-
0.598	Chewers damage	0.5248	0.515	0.4185	-	-	-	0.352	0.3545	-	0.4648	0.5581	0.2807
0.401	Needle length	-	-	-	0.01694	0.01834	-	0.0134	-	-	0.01063	-	0.01564

0.353	Sap feeders damage	-	0.1059	-	-	-	-	-	-	-	-	0.07078	-
0.271	Density	0.002394	-	-	-	-	-	-	-	-	0.00214	0.001663	-

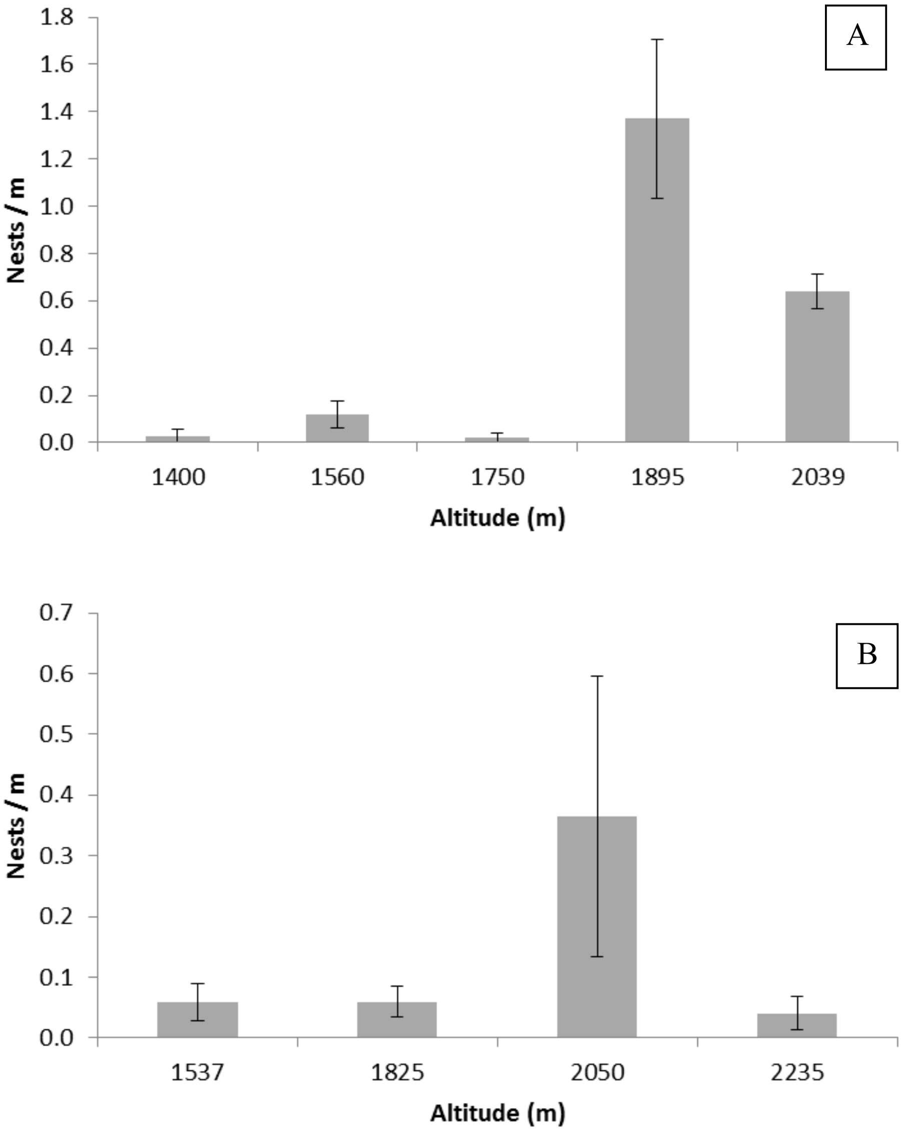

Figure 1. Mean number of nests, standarsided by the height of the tree, along elevational gradients in Sierra Nevada. A) Barranco del Espinal, B) Carretera de la Sierra. Bars indicate SE.

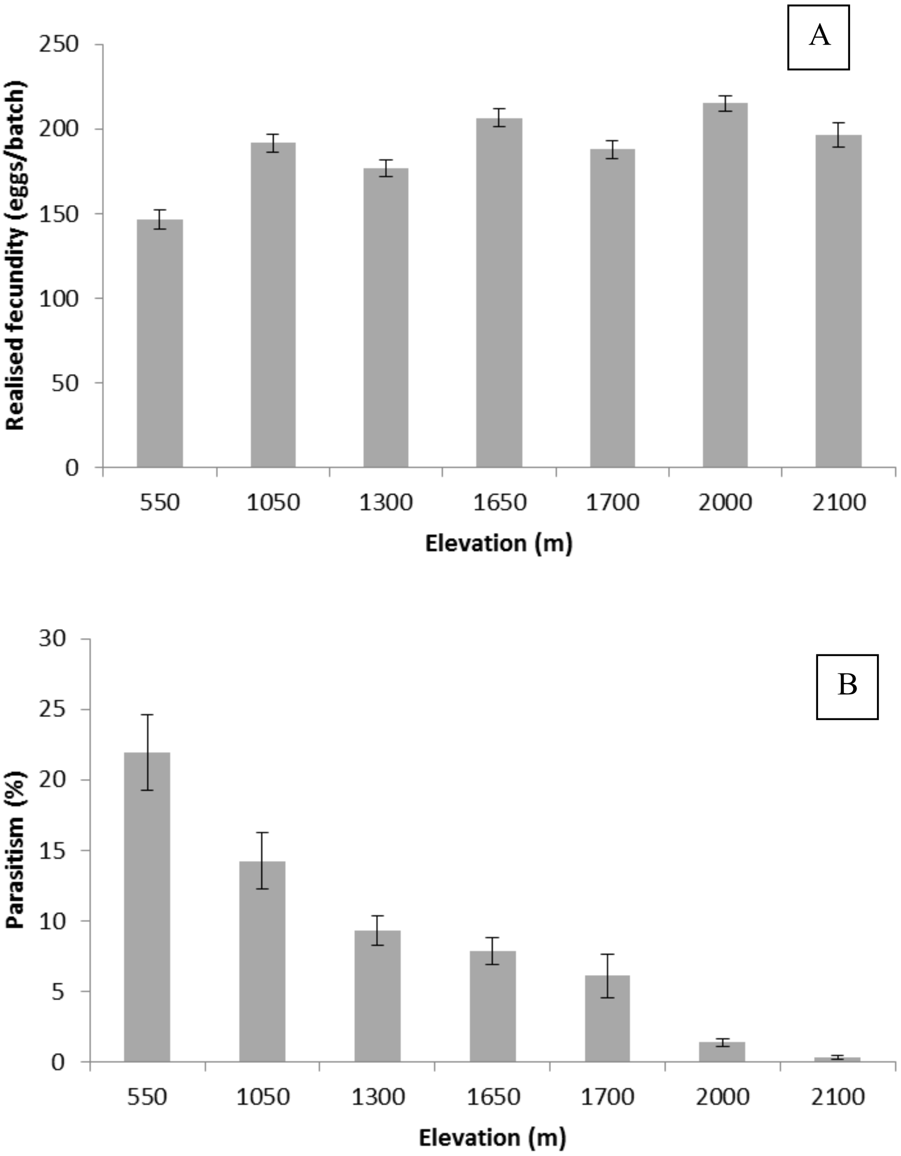

Figure 2. Realised fecundity measured as the mean number of eggs per egg batch (A) and egg parasitism measured as the percentage of eggs killed by egg parasitoids (B) along an elevational gradient in Sierra Nevada. Egg batches were collected from five pine species in 2008 and 2009 (sample size 40, 59, 102, 59, 58, 66, 48, respectively). Bars indicate SE.

Chapter 4

Abundance of Norway spruce cone insects along alpine elevational gradients

spruce cones in Belledonne (France)

Manuscript in preparation as:

Czwienczek E., Battisti A. Abundance of Norway spruce cone insects along alpine elevational gradients

I contributed to all experimental parts and to the paper writing.

Abstract

Aims: To verify if the abundance of cone and seed insects associated with Norway spruce (*Picea abies*) respond to elevation. The lapse rate of temperature related to elevation shift can be used as a proxy for climate change.

Methods: Four steep elevational gradients were chosen in the Southern Alps, at the southern edge of Norway spruce range. At each gradient, mature cones were sampled from the ground at four elevation steps, over a range of about 600 m.

Results: Four species of cone insects were detected (*Cydia strobilella*, *Dioryctria abietella*, *Ernobius abietis*, *Strobilomyia anthracina*). Cone tissue feeders (*D. abietella* and *E. abietis*) did respond negatively to increased elevation while specialized seed feeders (*C. strobilella* and *S. anthracina*) did not. Interaction analysis showed that the specialist *C. strobilella* is positively associated with generalist species.

Conclusions: Generalist species may take benefit from favorable temperature conditions at low elevation and from a larger amount of resource available, while specialized seed feeders are more dependent on the availability of the resource, irrespective of temperature and quantity.

Introduction

Coniferous female reproductive structures (cones) provide an important base of food for insects, mammals, and birds in the forest environment. As insects may reduce the number of viable seeds by 60 to 80% (Roques *et al.* 1983), they may limit the reproductive potential of the forest. As insects respond positively to temperature, their activity could be increased by global warming, becoming even more limiting to tree regeneration. The studies with use of elevational gradients, as a proxy for changing temperature conditions, have been lately explored all around the world to study different organisms including also the insects. (Erelli *et al.* 1998; Hodkinson 2005; Mühlenberg & Stadler 2005; Lara *et al.* 2006; Körner 2007; Garibaldi *et al.* 2011; Haynes *et al.* 2012). There are also a few studies regarding cone insects in the mountains (Skrzypczyńska *et al.* 1994; Wermelinger *et al.* 1995; Kozioł 1998) which can be useful for climatic studies.

Seed cone production and also abundance vary a lot in space and time (Brockerhoff *et al.* 1999, Turgeon *et al.* 1994). The insects depend on the source of the food – that means that when there is more food (cones) – there should be more insects feeding on them (density dependence). Kozioł (2007) observed that the population dynamics of cone insects is largely dependent on the availability of the cone through time. He marked also that for the population dynamics of cone insects, the parasitoids do not play a major role, which is instead linked to the availability of the food (that mean cones and seeds) and endogenous parameters like fertility of females and diapause. It is also possible that there are other factors influencing the patterns of insects, like temperature: indirectly (while the cones are still on the tree) and directly by temperature.

In the case of Norway spruce (*Picea abies*), elevation had a significant effect on the number of insect species (Kozioł 2007a). Based on a 3 grade scale to estimate cone production in 42 stands of spruce, insect abundance was compared between years of high and low cone crop. In the years of high fertility there was higher abundance and diversity of spruce cone insects than in the years of low cone density. In the high mountains cones appeared every year on the same trees, that means a very good food base for the insects, while at lower elevations the cone crop was very variable through time, leading to pronounced decline of insects abundance (Kozioł 2007). Kozioł (1998) also showed that the cone midge *Kaltenbachiola strobi* in Tatra Mountains was more abundant in spruce stands of the upper mountain (1300 – 1500 m) forest zone than in the lower mountain (1000 – 1200 m). Kozioł (1998) compared his results with those from literature: the same pattern was described by Cankov (1974) in Bulgaria, while an opposite trend was found by Eide (1927) in Norway. Kozioł (2000) showed also that mean length of cones and mean numbers of seeds in cones are significantly decreasing with increasing elevation.

Wermelinger *et al.* (1995) studied 29 Swiss locations for spruce cone insects, with the aim to investigate species spectrum, abundance, distribution, and also emergence dynamics of cone insects. They found that abundance of *K. strobi* and its parasitoid *Torymus* sp. are positively correlated with elevation. Wermelinger *et al.* (1995) noticed also that *Cydia strobilella* has a slight negative dependence on altitude. There was no negative correlations (competition) between species abundance (Wermelinger *et al.* 1995). Kišija (2010) investigated in Norway abundance of cone insects during a period of 39 years (data of X rays photos from the Norwegian Seed Authority) also in relation to factors influencing them, without clear results about elevational and latitudinal effect.

The aim of our study was to explore the direct effect of elevation on the abundance of spruce cone insects (*Cydia strobilella, Dioryctria abietella*, *Ernobius abietis*, *Strobilomyia antracina)* in four elevational gradients (3 in Italy and one in France) used for a more general sampling of herbivory (Marini et al., this thesis) and possible confounding effects of environment, by checking the damage on the mature cones. Mature cones dropped to the ground can be a useful tool in the case of Norway spruce, because the signs of attack by different insect species can be easily detected (Roques 1983). In addition, as they stay for several years without being decomposed, their sampling can give a good picture of the general insect abundance through time, becoming less dependent on the yearly variation of cone crop and its direct effect on the presence of the different insect species.

Materials and methods

Cone collection and damage estimation

Mature spruce seed cones were collected in 3 gradients in Northern Italy (Tualis, Pramosio and Passo Mauria (June 2011) and in one gradient in Southern France –

Belledonne (July 2011). The elevations considered were: Passo Mauria (930, 1140 m), Pramosio (930, 1200, 1300, 1500 m), Tualis (980, 1260, 1500, 1700 m), and Belledonne (1150, 1400, 1700, 1950 m). All the forests were mature and had relatively homogenous tree composition and density along the elevation gradients, and were without symptoms of disease or pest outbreak.

Ten cones per tree were collected from the ground under the canopy projection of 10 trees per elevational step – for a total of 100 cones per elevational step. Each transect had 4 elevational steps, except Passo Mauria where only 2 steps were considered. In total, around 4000 cones were collected and analyzed. The length of each cone was taken and the damage made by insect was estimated by dissection in two ways: presence/absence of feeding marks by *D. abietella, C. strobilella,* and *S. anthracina*, and proportion of area occupied by *E. abietis*. The way by which the feeding marks were attributed to the species is given below.

Cydia strobilella (L.) (Tortricidae, Lepidoptera) spruce cone moth larvae is yellowish or white with light brown head (Brauns 1976). The eggs are laid in May and June on young green cones of spruce. The larvae emerge in June and firstly feed on only seeds: eating internal part of cones and damaging scales of cones. Larvae left two round – shaped holes and internal part of seed full of excrements (Brauns 1976). After that few (around 10) larvae stay in the core of cone and feed there until the end of summer. The central part of the cone core is filled by the compacted mass of excrements (Brauns 1976). This "compacted mass" is finishing with elongated chamber which is entering to the seed scale. Only one larva is overwinter in the chamber like this, and after this chamber becomes the place where in spring the larva will pupate. The flight of adults is from April until the end of May. *C. strobilella* is univoltine, but sometimes can be bivoltine (Brauns 1976). The group of natural enemies of *Cydia* is big, with many Hymenoptera and Diptera.

Dioryctria abietella (Pyralidae, Lepidoptera), spruce cone worm, is poliphagous species appearing on spruce, fir and sometimes also pine and larch. The grown larva is 20 mm long, brownish and reddish, longitudinally striped. *D. abietella* can appear on the young trees, damaging the shoots, but it is also very important insects damaging cones. Inside the cones larvae they can drill wide tunnels fully filled and easy to recognize (conspicuous) with big brownish connected excrements (they are also visible outside) and also they do very characteristic damage marks on the base of the cone scales in the shape similar to anchor (Brauns 1976). Also the seeds are damaged externally, but the core of the cone covered by resin and excrements, is not touched by larvae (Brauns 1976). The cones with *D. abietella* can be C – bended, can fall down earlier from the tree, they can change color untimely. The eggs are laid June and July (Brauns 1976). The larvae emerged in July and they overwinter until feeding in April, and they have metamorphosis between April and May (Brauns 1976). They are exciting the cone in autumn (October) through the round-shaped hole to go to the ground for overwintering in cocoon under the litter, probably in pronymph stadium. This species can be bivoltine, because the different developmental stages can be found all year (Brauns 1976). Sometimes, where there is not enough spruce cones, the caterpillar can feed on the Adelges galls (Brauns 1976). To natural enemies of *D. abietella* belong to families Ichneumonidae, Tachinidae and Rhaphidiidae.

Ernobius abietis (Anobiidae, Coleoptera) is a small beetle (around 3.5 mm length), brownish (Kiełczewski 1967). The larvae are cream – white and they have on the head bifurcated line, which is bigger and wider in older larvae. *Ernobius* lays several dozen eggs inside the female spruce cone and after that the larvae emerged and they feed in the core of the cone and on the base of the scales of cone (Kiełczewski 1967). According to Brauns (1976) female lay eggs all the vegetation season (especially spring and autumn) to the hanging cones on the tree, that is why it is possible to see all larval stadiums simultaneously. The feeding tunnels are full of small and flat excrements which can pour outside the cone. In the spring larvae made inside the cone long spiral tunnels in the direction down – top of (from base of the cone until the top), avoiding the most tough bases of scales of cone (Brauns 1976). Still alive core of the cone can react realizing resin (Brauns 1976). In the cones attacked later (autumn, after releasing seeds), it is possible to see damaged, firstly on the bigger scales of cones in the softer parts, after that inside the core of cone (Brauns 1976). Brauns (1976) marked also that in the digestion process of *E. abietis* fungi are involved. Pupation happens usually in the fallen cones. Starting from August it is possible to see pupae inside the cones. Beetles drill the scales to go out and they leave 1 – 1.5 mm exit holes (Brauns 1976). They are univoltine but depending on the weather they can be bivoltine (Kiełczewski 1967). It is known that *E. abietis* appears usually on the cones already fallen on the ground or on the mature and dried cones, damaged on the trees by the other insects (Kiełczewski 1967), but according to other authors (Stocki *et al.* 2008) feeding larvae of *E. abietis* can damage seeds and make the cones fall dawn earlier so in this way has an economic importance. The beetles are going out through the holes (1mm diameter hole). During the sampling it is possible to see also the natural enemies of *E. abietis* like family Rhaphidiidae and some other beetles like Cleridae and Cantharidae.

Strobilomyia anthracina (Anthomyiidae, Diptera) spruce cone maggot, cause characteristic C bend of cone while is inside with flow of resin outside (Brauns 1976). The rest of damage is very similar to the one of *C. strobilella*. *Strobilomyia* spp. are "conophytes" (Turgeon *et al.* 1994) and this mean that "larvae feeds exclusively on developing seed and seed cone tissues" (Brockerhoff *et al.* 1999). Adults emerge in the spring and after lay eggs in the seed cones in the time of pollination (Brockerhoff *et al.* 1999).

Statistical analysis

We used Generalized Linear model (two types): one with adding cone length as a factor, and the second only the fixed effect of elevation on the damaged cones. Step within the region (gradient) was considered as a random factor. We checked also the direct effect of elevation on all of the variables (like damage and cone length). Interactions among species (*C. strobilella*, *D. abietella*, *E. abietis*, *S. anthracina*) were also tested.

Results

Cone length decreased significantly with increasing elevation across all gradients (Fig. 1). The abundance of larvae of *E. abietis* was significantly and negatively related to elevation but positively related to cone length, meaning that with increasing elevation the number of larvae of *E. abietis* is strongly decreasing (Fig. 2 and table 1).

C. strobilella and *S. anthracina* had neutral relationship with elevation and also with cone size (Fig. 2 and Table 1). Damage made by *D. abietella* had a strongly negative relationship with elevation but did not respond to cone size (Fig. 2 and Table 1)

The interaction analysis indicates that *C. strobilella* is positively associated with both *D. abietella* ($p<0.01$) and *E. abietis* ($p<0.05$) while other interactions were not significant.

Discussion

The responses of main cone insects of Norway spruce in alpine elevational gradients are of two types, the first being typical of the cone specialists (seed feeders) such as *C. strobilella* and *S. anthracina*, which do not respond at all to both elevation and to decrease of cone size with elevation. The second type includes generalists (cone tissue feeders) such as *D. abietella* and *E. abietis*, which responded negatively to the increase of elevation and also to reduced cone size, at least for the latter species. The difference can be associated with an opportunistic behavior of the generalist, tissue – feeding species which may take benefit from favorable temperature conditions at low elevation and from a larger amount of resource available, while seed – feeding species are more dependent on the availability of the resource, irrespective of temperature and quantity. This pattern conforms to the general rules of resource partitioning among generalist and specialist herbivores in insect ecology (Speight et al. 2008). As cones are a resource highly dependent on plant reproductive cycle, it appears that specialist cone and seed insects have evolved in order to adapt to it at best, being then less dependent on the abiotic conditions. This observation is further confirmed by the endophagous habit of cone and seed insects, being protected from temperature extremes that my hit for example folivores (Turgeon et al. 1994).

As the assessment was done on mature cones dropped to the ground, it is possible that some damage went undetected because partial decomposition of cone tissues. By sampling dropped cones, however, it was possible to observe the impact of the most important species, while a few others (Roques 1983) could not be retrieved because they leave the cone when it is still on the tree, without leaving visible signs on the cone tissues. This is the case for example of the cone midge *K. strobi*, a specialist which was previously described to respond positively to elevational gradient (Kozioł 1998, Wermelinger et al 1995). By sampling mature cones which were likely produced during several years, it was possible to balance the potential effects of a variable cone crop and insect population density.

The interaction analysis showed that the seed feeder *C. strobilella* is positively associated (facilitation) with tissue feeders *D. abietella* and *E. abietis*. This may result from a cross talk of the early coloniser *C. strobilella* and the the generalists *D. abietella* and *E. abietis*, which may be facilitated in exploiting the cone tissues. Interestingly, this does not happen with the other seed feeder *S. anthracina*, which probably makes the cone unsuitable to the generalist species because of tissue deformation and resin flow. The prediction that cone insect could respond in the similar way to the elevation/temperature gradient as general herbivorous insects (Bale *et al.* 2002), that means increasing damage with increasing temperature, can be confirmed by the data set but only for generalist cone feeders. Specialists are probably independent from temperature, as well as from other abiotic factors, likely because they are more dependent on the resource availability. One specialist, *C. strobilella*, has been described in the literature as responding slightly negatively to elevation in a Swiss gradient (Wermelinger 1995) and this aspect should be further explored perhaps extending the sampling to very extreme elevation for spruce, which was not the case in the gradients considered in this study. As cone size decreases strongly with elevation, it is possible that spruces growing at timberline may show a reduced cone crop, in addition to reduced cone size, and this could have a negative feedback also on the populations of specialist cone feeders. In a climate change perspective, it appears that the major player is the host plant and its reproductive response to an increase of temperature. Specialist cone feeders would likely track the potential change in cone crop irrespective of elevation, while an increase of damage is expected for generalist species. The observations that a generalist species such as *D. abietella* is a major pest in seed orchards, generally located at low elevation in order to exploit more favorable temperature conditions, gives strength to this hypothesis.

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References

Bale J.S., Masters G.J., Hodkinson I.D., Awmack C., Bezemer T.M., Brown V.K., Butterfield J., Buse A., Coulson J.C., Farrar J., Good J.E.G., Harrington R., Hartley S., Jones T.H., Lindroth R.L., Press M.C., Symrnioudis I., Watt A.D. & Whittaker J.B. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, 8, 1 - 16.

Brauns A. (1976). Taschenbuch der Waldinsekten Ökologische Freiland - Differential - diagnose Bildteil (Band 2). Stuttgart: Gustav Fischer.TX - 0020

Brockerhoff E., Turgeon J. & Brockerhoff A. (1999). Ovipositional Phenology and Behavior of the Spruce Cone Fly, Strobilomyia neanthracina (Diptera: Anthomyiidae). *Journal of Insect Behavior*, 12, 47 - 65.

Erelli M.C., Ayres M.P. & Eaton G.K. (1998). Altitudinal patterns in host suitability for forest insects. *Oecologia*, 117, 133 - 142.

Garibaldi L., Kitzberger T. & Chaneton E. (2011). Environmental and genetic control of insect abundance and herbivory along a forest elevational gradient. *Oecologia*, 167, 117 - 129.

Haynes K., Liebhold A. & Johnson D. (2012). Elevational gradient in the cyclicity of a forest-defoliating insect. *Population Ecology*, 54, 239 - 250.

Hodkinson I.D. (2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews*, 80, 489 - 513.

Kišija S. (2010). Cone and seed insects in Norway spruce [Picea abies (L) Karst.]. *Radovi Šumarskog Fakulteta Univerziteta u Sarajevu* 40, 1 - 13.

Kozioł M. (1998). Influence of altitude on adult emergence and dynamics of Kaltenbachiola strobi (Winnertz) (Diptera: Cecidomyiidae) populations and its parasites in the Tatra National Park, Poland. *Anz. Schädlingskde., Pflanzenschutz*, 71, 121 - 127.

Kozioł M. (2000). Cono - and seminiphagous insects of Norway spruce Picea abies (L.) Karst. And their parasitoids in lower and upper montane zone of the Tatra National Park in Poland. *J. Appl. Ent.* , 124, 259 - 266.

Kozioł M. (2007). Diversification of entomofauna of Norway Spruce Picea abies (L.) Karst. cones in the aspect of variable spruce stands masting in the Tatry National Park. *Forest Research Papers*, 4, 29 - 46.

Kozioł M. (2007a). Cone entomofauna of Norway spruce Picea abies 9L.) Karst. in selected stands of the Tatra National Park in Poalnd. *Electronic Journal of Polis Agricultural Universities*, 10.

Körner C. (2007). The use of 'altitude' in ecological research. *Trends Ecol Evol* 22, 569 - 574.

Lara A., Fernandes G. & Goncalves - Alvim S.J. (2006). Tests of hypotheses on patterns of gall distribution along an altitudinal gradient. *Tropical Zoology, North America*, 15.

Mühlenberg E. & Stadler B. (2005). Effects of altitude on aphid-mediated processes in the canopy of Norway spruce. *Agricultural and Forest Entomology*, 7, 133-143.

Roques A., Martinez M. & Delplanque A. (1983). Les Diptères Anthomyiidae du genre Lasiomma Stein. ravageurs des cônes et graines de Mélèze d'Europe (Larix decidua Mill.) en France. *Zeitschrift für Angewandte Entomologie*, 95, 429 - 439.

Skrzypczyńska M., Kożuch J. & Gibas S. (1994). Conophagous and seminiphagous insects of Norway spruce Picea abies (L.) Karst. in the Babia Góra National Park and its surroundings. *J. Appl. Ent. Blackwell Wissenschafts-Verlag, Berlin*, 118, 321 - 326.

Turgeon J.J., Roques A. & de Groot P. (1994). Insect fauna of coniferous seed cones: Diversity, host plant interactions and management. *Annu. Rev. Entomol.*, 39, 179 - 212.

Wermelinger B., Hirschheydt J.v. & Fecker B. (1995). Abundance and emergence of spruce cone insects in different parts of Switzerland. *Journal of Applied Entomology*, 119, 9 - 15.

TABLES AND FIGURES

Table 1 Effect of elevation on cone damage by insects and the cone length from a mixed model including elevation as fixed effect and step within gradient as random effect. For each insect species a separate model was run.

	Estimate	**SE**	**P**
(a) Ernobius abietis(n gradients: elevation=4; with cone length=3)			
Elevation	-1.256e-04	7.444e-05	0.09
Cone length	3.426e-02	1.258e-02	0.007
(b) Cydia strobilella (n gradients: elevation=4; with cone length=3)			
Elevation	-6.676e-06	2.515e-05	0.79
Cone length	-1.592e-03	3.106e-03	0.60
(c) Strobilomyia anthracina (n gradients: elevation=4; with cone length=3)			
Elevation	3.223e-06	4.793e-06	0.50
Cone length	-9.008e-04	7.579e-04	0.23
(d) Dioryctria strobilella (n gradients: elevation=4; with cone length=3)			
Elevation	-1.921e-04	6.894e-05	0.006
Cone length	5.823e-03	1.097e-02	0.59

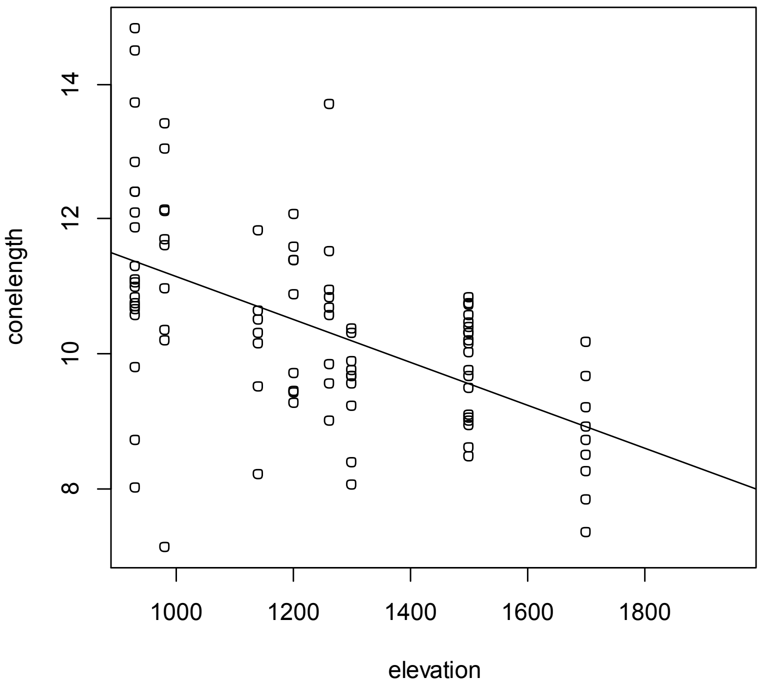

Fig. 1 Effect of elevation (m) on spruce cone length (cm) by pooling cones from all gradients.

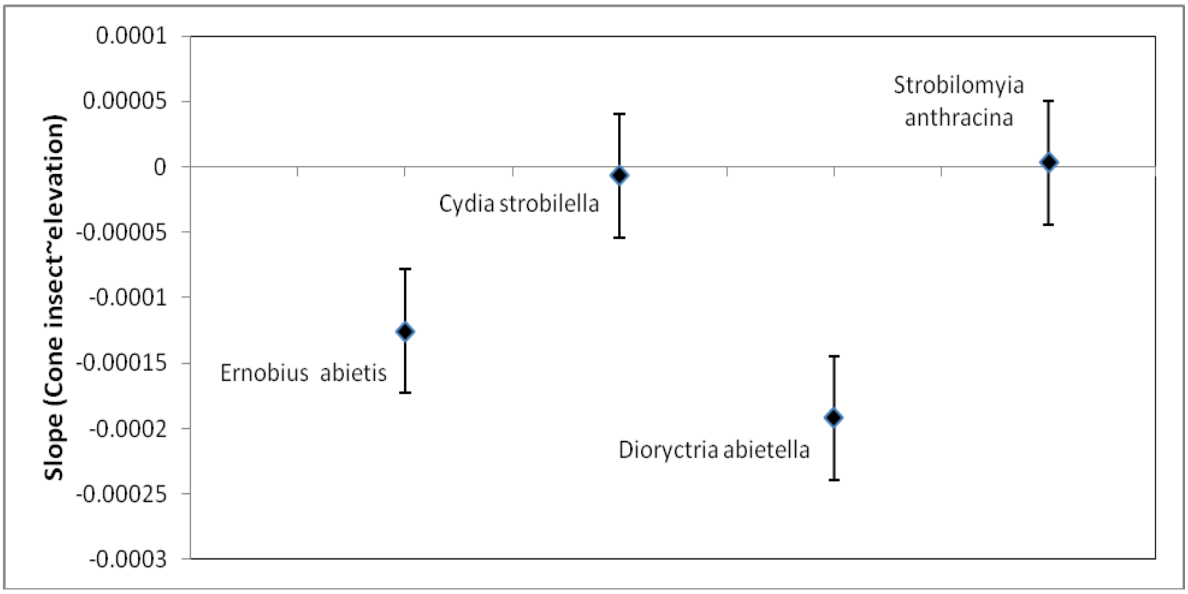

Fig. 2 Elevation effect on each spruce cone insect (*Ernobius abietis*, *Cydia strobilella*, *Dioryctria abietella*, *Strobilomyia anthracina*) with standard error from mixed model, including elevational step within gradient as random effect.

Chapter 5

General conclusions

The climate is changing and there are many evidences of it in the literature (Williams & Liebhold 1995; Menéndez 2001; Bale *et al.* 2002; Parmesan & Yohe 2003; Hodkinson 2005; Adger *et al.* 2007; Laštůvka 2009; Netherer & Schopf 2010; Cornelissen 2011). Current spatio – temporal environmental changes are expected to alter interactions between plants and insect herbivores in forests all around the world. Temperature is expected to be a main driver of forest damage for its direct and positive effect on herbivory. For this reason, the use of elevation gradients has been suggested to study the effect of different temperature conditions (Hodkinson *et al.* 2005; Garibaldi *et al.* 2011), although confounding factors as changes in radiation and oxygen partial pressure may partly limit this approach. In this thesis, some of the most common European tree species were selected in the experiments because of their importance in forestry, namely *Fagus sylvatica, Larix decidua, Picea abies*, and *Pinus sylvestris*.

The conclusions will be broken into 3 parts: insect herbivory along gradients in a pan – European study (Chapter 2), the pine processionary moth (*Thaumetopoea pityocampa*) study in Sierra Nevada in Spain (Chapter 3), and Norway spruce cone and seed insects along alpine altitudinal gradients (Chapter 4).

1. Elevational patterns of insect herbivory across European forests

The study of herbivory was carried out along 21 gradients in Europe. The leaf traits vary with temperature, with leaf size increasing in two host trees (*Fagus sylvatica, Picea abies*) out of three (in the case of *Pinus sylvestris* the size of the leaves decreased with temperature and for *Larix decidua* measures were not done). The toughness decreases with increasing temperature in 3 cases out of 4. Leaf toughness of *Larix decidua* remained stable along the temperature gradient. Generally evergreen conifers had tougher leaves at higher elevation – lower temperature. Also higher radiation at higher elevation may influence leaf toughness in the way that the cuticle will become thicker and leaf tougher to prevent the damage of the internal part of the tissues.

The study of C/N ratio along the temperature/elevational gradients did not show a clear pattern for the species with except of *Fagus sylvatica*, where C/N ration tends to decline with increasing temperature. C/N ratio varies in an unpredictable way with temperature for both *Picea abies* and *Pinus sylvestris*.

The problem with the confounding factors (the factors which may influence the analysis but were not taken into account because of difficulty with the sampling in the fields) may affect the C/N ratio analysis. Soil fertility, for example, was not controlled for potential confounding effect on nutrient availability.

The direct effect of increasing temperature on herbivory was positive or neutral, depending on host plant species. General herbivory of *Picea abies* and *Larix decidua* decreases with increasing temperature, while general herbivory of *Pinus sylvestris* and *Fagus sylvatica* had a neutral relationship with temperature. General herbivory on *Larix decidua* and *Picea abies* exhibited a stronger sensitivity to temperature than insect herbivory on *Fagus sylvatica* and *Pinus sylvestris*. This can be important for forest managers, who should seek for effective measures to mitigate potential climate change effects. When guilds are used instead of general herbivory to assess the relationships with temperature, it looks like that in the group of sap feeders there is a positive pattern with temperature in only one tree species (*Pinus sylvestris*). Chewers has a negative relationship with temperature in 1 case out of 4 (*Larix decidua*) and leaf miners had a positive relationship with temperature in 2 cases (*Larix decidua* and *Picea abies*) out of 3, where *Pinus sylvestris* was not included. Gall makers did not show any pattern in all the 3 host plant considered, when *Larix decidua* not included. High variability in the response to temperature of different groups was observed. Mixed responses can be derived from idiosyncratic responses of major species in each guild.

Interactions between guilds were examined too and no clear effect was found. Only two significant interactions, facilitation of miners and chewers in the case of *Larix decidua*, were observed.

The effect of increasing temperature can be associated with an increase of herbivory but this cannot be generalised and need to be tested in each species of tree, because the response of herbivore communities probably depends on the tree species. The indirect effect of tree quality on herbivory was not significant or marginally significant.

The overall message is that herbivore communities of coniferous trees seem to respond more to temperature than those of broadleaved trees. As elevational gradients are known as a good proxy of temperature increase with global change, they can be further exploited for the future study of climatic changes.

2. Factors explaining the abundance of the pine processionary moth (*Thaumetopoea pityocampa* Denis &Schiffermüller) along elevational gradients in Sierra Nevada Spain

In the Sierra Nevada mountains the higher density of pine processionary moth has been observed close to the upper limit of the range, where conditions are generally considered harsh and some factors which may cause this pattern were investigated.

Like in the case of first study, the elevational gradient was used to test the effect of temperature. Direct response of temperature as well as indirect response through the host plant quality (needle length, toughness, C/N ratio, thee height) and through the natural enemies (egg parasitism) were checked. The density of the pine forest was also taken into account. The nest abundance in the year of the survey (2011) reached the highest value in the mid– high elevation at each of the two gradients considered. Nest abundance was affected positively by C/N ratio and elevation (proxy for the temperature) and negatively by tree height and needle toughness. Other factors were positively affecting the abundance of pine processionary moth nests, mentioned in the decreasing order of importance: chewers damage, needle length, sap feeders damage and stand density.

The realized fecundity increased significantly with elevation while egg parasitism decreased.

The minimum winter temperature did not seem to affect the performance of pine processionary moth in both gradients because the lower thresholds for feeding (Battisti *et al.* 2005) are rarely met in this area. The abundance of pine processionary moth in Sierra Nevada can be explained by the nature of this mountains and thermal inversions during the winter, allowing perhaps colonies at upper elevations to benefit of stronger solar radiation. The higher realized fecundity under these conditions is also a good indicator of the higher performance. The plant quality (toughness and C/N ratio) did not explain the abundance of nests at higher elevation.

Natural enemies instead can explain more than tree quality the pattern of abundance of pine processionary moth, as much higher egg parasitism was observed at lower steps in a companion survey carried out in 2008 and 2009. Temperature could become more limiting only at extreme steps, in the timberline area.

3. Norway spruce cone insects along alpine elevational gradients

Cone insects are a very specialized group of insects and the idea was to investigate them in relation to their response to temperature, like it has been done in the Chapter 2 with leaf herbivory.

Appearance of cone insects depends highly on the host plant's reproductive cycle, being less dependent than some folivores on abiotic conditions, because of the cone isolation. The host plant can also indirectly affect cone insects by responding to the changing temperature, for example modifying the reproductive capacity.

The direct effect of temperature was checked on the detected species of cone insects (*Ernobius abietis*) or the marks left by other insects (*Cydia strobilella*, *Dioryctria abietella*, *Strobilomyia antracina*). It was possible to distinguish two different types of the responses, namely that of generalist cone tissue feeders (*Ernobius abietis* and *Dioryctria abietella*) and that of specialists seed feeders (*Cydia strobilella* and *Strobilomyia antracina*). The generalist cone feeders responded in similar way to the leaf herbivores, i.e. with increasing elevation, abundance of the insects decreased. The seed feeding specialists did not respond likely because they developed an adaptation to harsh conditions, being more restricted by the source of food. The specialists did not respond also to the reduced cone size, while *Ernobius abietis* for example responded negatively. Generalists can take the advantage of the increase of temperature while specialists seem to be more dependent on the availability of the food.

Generally, this thesis is trying to answer a few of the questions related to the impact of climate change on forest insect herbivores. If on one side it was possible to show some patterns of the responses, consisting of a slight increase of the herbivory with increasing temperature, on the other side there are several uncertainties related to responses of individual guilds of herbivores, the level of specialization of the species, and the nature of the host plant. If one considers that natural enemies are important players as well, being mostly insects and responding in the same way to temperature, it may results quite a puzzling situation. More research will be definitely needed to answer the questions left open, and to indicate the possible mitigation measures to be adopted to limit the impact of climate change on forest ecosystems.

References

Adger N., Aggarwal P., Agrawala S., Alcamo J., Allali A., Anisimov O., Arnell N., Boko M., Canziani O., Carter T., Casassa G., Confalonieri U., Cruz R.V. & e a.a. (2007). IPCC 2007 climate change 2007: impacts, adaptation and vulnerability. Working Group II Contribution to the Intergovermental Panel on Climate Change Fourth Assessment Report. Cambridge University press., P 23.

Bale J.S., Masters G.J., Hodkinson I.D., Awmack C., Bezemer T.M., Brown V.K., Butterfield J., Buse A., Coulson J.C., Farrar J., Good J.E.G., Harrington R., Hartley S., Jones T.H., Lindroth R.L., Press M.C., Symrnioudis I., Watt A.D. & Whittaker J.B. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, 8, 1 - 16.

Battisti A., Stastny M., Netherer S., Robinet C., Schopf A., Rocques A. & Larsson S. (2005). Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. *Ecological Applications* 15, 2084-2096.

Cornelissen T. (2011). Climate change and its effects on terrestrial insects and herbivory patterns. *Neotropical Entomology*, 40, 155 - 163.

Hodkinson I.D. (2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews*, 80, 489 - 513.

Laštůvka Z. (2009). Climate change and its possible influence on the occurrence and importance of insect pests. *Plant Protect. Sci.*, 45.

Menéndez R. (2001). How are insects responding to global warming? *Tijdschrift voor Entomologie*, 150, 355 - 365.

Netherer S. & Schopf A. (2010). Potential effects of climate change on insect herbivores in European forests—General aspects and the pine processionary moth as specific example. *Forest Ecology and Management*, 259, 831 - 838.

Parmesan C. & Yohe G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421, 37 - 42.

Williams D.W. & Liebhold A.M. (1995). Herbivorous insects and global change: potential changes in the spatial distribution of forest defoliator outbreaks. *Journal of Biogeography*, 22, 665 - 671.

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