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Vliv opylovačů a herbivorů na individuální přežívání a populační dynamiku druhu *Scorzonera hispanica*

Effect of plant-animal interactions on individual performance and population dynamics of *Scorzonera hispanica*

Disertační práce
Ph.D. Thesis

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 31. 5 .2016

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I thank to all my symbionts, especially the mutualists, who helped me so lot!
I thank also all those who are not offended by term seed, even though it is actually the achene with pappus.
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ABSTRACT

The population dynamics of plants with regard to plant-animal interactions is a remarkably complex topic. To look into how individual life stages are influenced in different directions by various animals is beyond the scope of a single paper. For each of the studies described below, I and my co-authors attempted to collect data that would cover as much of the plant life cycle as possible, focusing on interactions between plants and different animals during the flowering period and their consequences for the overall dynamics of the species *Scorzonera hispanica* at the local and landscape scale. Putting all the studies together allowed me to gain a better picture of the network of relationships between plant properties, animal activity and their effects on overall plant performance.

In **Chapter 1**, we focus on factors influencing germination, early growth and survival. The results show better performance in seedlings from larger seeds and from larger populations. Seed weight affected the germination rate, seedling growth within the first two months and seedling survival. Mother plant traits did not affect any of the variables studied, even though the performance of seedlings from individual mother plants did differ. Because the seed mass was the most important factor affecting germination and seedling growth, in further studies we focused our attention on factors that may affect it.

In **Chapter 2**, we discuss the results of a field experiment aimed at finding out the preferences of pollinators. Based on a comparison with open-pollinated and supplementally pollinated plants, we examined whether the plants were pollen-limited. We did not record any significant effects of pollinators on the reproduction of *S. hispanica.* Seed number, seed mass and ratio of developed seeds were connected neither with the floral visitation rate nor with supplemental pollination. The number of seeds depended solely on plant height, which was further connected with microsite characteristics, suggesting that the plants studied were likely resource-limited. Even if pollen limitation did occur, none of the flowering traits we measured correlated with the rate of pollinator visitation.

In **Chapter 3**, we report how by monitoring a population in short intervals we ascertained the preferences herbivores and the effect of herbivory on plant reproduction. We found a significant influence of herbivory on seed production whereas neither flowering in the next season nor survival of flowering plants was affected by the rate of herbivory. Flowering in the following season correlated only with plant height, which was further connected with microsite characteristics, which suggests a tendency towards resource limitation. Herbivores preferred plants with greater numbers of initial flower buds.

In **Chapter 4**, we present a dynamic, spatially explicit model to predict the prospects of the species at the landscape scale under various levels of herbivory and random population destruction. The results show that the landscape-level population dynamics under the present rate of herbivory are approximately in equilibrium allowing fluctuations of the rate of herbivory on the order of per cent. The extent of herbivory plays a large role in landscape-level population dynamics, especially when combined with disturbance events. The results of our simulation also revealed a higher survival probability of large populations than that of small ones.

Although we revealed some aspects of plant animal interactions driving population dynamics of the species, there is still room for further research into little-known processes such pollinator behaviour at the landscape scale or a direct influence of plant-animal interactions on germination and seedling performance regardless of seed mass.

INTRODUCTION

No plant is an island, entire of it selfe. The destiny of each plant is tightly linked with its entire environment. To understand why a plant grows the way it does, it is necessary to delve into the complex web of relationships with the entire environment from the moment the seed fell on to the surface of the soil, or better, from the moment it has matured on the mother plant. Why did that very egg get fertilized and give rise to a viable seed. What allowed it to develop and mature? What hardships did it have to endure? How was this very plant able to flower and produce seeds under pressure from competitors, predators, parasites and even mutualists? This brings us back to the germinating seed, the plant life cycle and interactions between plants and other organisms. We must take a step back to view the plant as a component of a population, in which all individuals fight their own little battles.

The following text does not aspire to cover the entire breadth of the topics of the plant life cycle and plant-animal interactions. As I am looking at them through the prism of my model system, I will cover some of their aspects in more depth than others that are not as important for the model system. The main topic of this thesis are plant-animal interactions, but it will take me quite a while to get to it, as I have to start from a general perspective and, more importantly, from the beginning – the seed.

The seed and its properties

The seed is where everything starts. Different plants have different strategies for survival and dispersal. These strategies come as a response to selection pressures and lead to the least possible loss to the seed set and during consequent seedling germination. In polycarpic plants, it is also necessary to consider the trade-off between current and future reproductive output (Obeso 2002).

Seeds can differ in numerous aspects such as dispersal traits, persistence or the one that is the easiest to measure – seed mass. Seed mass, for example, is a result of numerous oppositely directed forces. Probably the most basic principle is the trade-off between a small number of large offspring with a high probability of successful establishment and a large production of small seeds, of which each has a low probability of establishing (Smith & Fretwell 1974). This trade-off has been observed both within and across species (Geritz 1995, Rees & Westoby 1997, Geritz et al. 1999, Uriarte & Reeve 2003). The whole topic of seed mass nevertheless cannot be reduced to this principle (Moles & Westoby 2006).

Seed mass is also associated with dispersal and persistence, as lighter seeds enhance wind dispersal (Burrows 1975). As regards the persistence of seeds in the soil, it is unclear whether greater seed mass in this case constitutes more of an advantage (Moles & Westoby 2006) or disadvantage (Thompson et al. 1993, Bekker et al. 1998, Peco et al. 2003), or whether there is no connection (Moles et al. 2000). Probably the most fundamental, and generally acknowledged, phenomenon related to seed mass is the positive relationship between seed mass and the rate of germination, which applies both between (Moles & Westoby 2006) and within species (Counts & Lee 1991, Castro 1999, Khera et al. 2004, Lehtila & Ehrlen 2005, Van Molken et al. 2005, Benard & Toft 2007). All of these correlations have been reasonably well studied and allow us to approximately guess what will happen to the seed. Seed mass is therefore one of the key variables used when studying how various factors affect plants. But there are surely other drivers of seed mass variability that deserve to be looked into.

Seed mass varies at many hierarchical levels. On the coarsest scale, studies deal with variability among populations. Variability in seed weight among populations may be a result of differences in habitat conditions such as habitat productivity, population size or isolation of populations (Heschel & Paige 1995, Ouborg & Vantreuren 1995, Münzbergová & Plačková 2010).

At the within-population level, differences in seed weight may be caused by differences between individual mother plants (Ouborg & Vantreuren 1995, Weiner et al. 1997, Hereford & Moriuchi 2005). This variation is often related to the traits of the mother plant (e.g. depending on maternal plant biomass in Sletvold 2002).

Seed weight can vary within a single plant over the course of the season (Cavers & Steel 1984, Wolfe 1992) as well as over the life of the plant in polycarpic perennials (Herrera 1991a). It also depends on the position of the seed on the plant (Herrera 1991b, Castro 1999, Ehlers 1999). Within the subfamily Cichorioideae of the family Asteraceae, heterocarpy is a known phenomenon (Imbert et al. 1996, Gibson & Tomlinson 2002, De Clavijo 2005, Van Molken et al. 2005, Cheptou & Avendano 2006, Brandel 2007, Torices & Mendez 2010).

Seed mass variability, of course, also responds to the current situation the plant is in. Experimental studies have revealed a positive connection between seed mass and the resource or water status of the maternal plant (Gianoli 2002, Drenovsky & Richards 2005, Valencia-Diaz & Montana 2005, Breen & Richards 2008). The question remains whether seed mass and other properties of seeds have any influence on plant-animal interactions. So please bear with me, I have yet to develop my story.

Germination

Seedling establishment, its rate and timing determine the entire remaining life of the plant. More rapid germination can bring a competitive advantage (Castro 2006, De Luis et al. 2008) (Castro 2006, De Luis 2008), as is also true in the case of larger leaf area (Wulff 1986b, Ortmans et al. 2016), and seedling weight (Benard & Toft 2007) can have an impact on later life stages, affecting overall fitness (Wulff 1986a, De Luis et al. 2008, Mercer et al. 2011, Cogoni et al. 2013). This effect on the life cycle is more pronounced in perennials than in annuals (Verdu & Traveset 2005). It is these beneficial traits that tend to be positively correlated with seed mass (Wulff 1986a, Benard & Toft 2007, Ortmans et al. 2016). And this is the second good reason – besides the connection with higher germination rates – why to pay attention to seed mass.

Sometimes, however, the competitive advantages outlined above are not related to seed size (Castro 1999) or affect only the short germination stage without a clear connection with the future growth of the plant (El-Keblawy & Lovett-Doust 1998, Meyer & Carlson 2001). So what are the other factors influencing early growth of a plant and its performance in adulthood?

Further important drivers of seedling emergence and further growth are traits inherited from the mother. These can be determined genetically (Lacey 1996, Weiner et al. 1997) or by other, possibly epigenetic, maternal effects related to conditions of the mother plant’s microhabitat (Ouborg & Vantreuren 1995, Lacey 1996, Galloway 2001, Hereford & Moriuchi 2005, Latzel et al. 2009, Cendan et al. 2013) or to what happened to it during its life – most probably some misfortune, such as getting eaten (El-Keblawy & Lovett-Doust 1998, Steets & Ashman 2010).

As in the case of seed mass, germination rates and seedling performance differ depending on the population. Seedling performance is often related to genetic diversity and inbreeding depression (Lamont et al. 1993, Heschel & Paige 1995, Fischer et al. 2000, Münzbergová & Plačková 2010). This is often connected with population size (Heschel & Paige 1995, Ouborg & Vantreuren 1995, Fischer et al. 2000), albeit not necessarily (Münzbergová & Plačková 2010). Interactions with animals, which for now I am mentioning only cautiously, may also play a role – in the case of *S. hispanica* possibly pollen limitation due to a lack of pollinators – which affect seedling traits (Agren 1996, Pflugshaupt et al. 2002, Cheptou & Avendano 2006). Sometimes it is related to local adaptations, due to which offspring grow better at the maternal locality than elsewhere (Hereford & Moriuchi 2005). Plant germination and growth is affected not only by properties of the seed, the maternal plant and the locality, but also by plant-animal interactions during the life of the mother plant.

There is, of course, one additional factor that significantly affects seedling establishment – where the seed lands. The environment sometimes affects seed germination more than seed quality (Breen & Richards 2008); sometimes it determines germination to a lesser degree, but it still affects survival (Cogoni et al. 2013). At the same time, even the place where seeds germinate can influence plant-animal interactions between the herb and its pollinator (Benard & Toft 2008, Cogoni et al. 2013). And so, what was once influenced by various factors continues to affect further interactions. This, however, takes us to the next chapter in the life of the plant.

The plant life cycle and demography: a sceptical interlude

Although in the present theses I am trying to pretend that nothing happens between seedling germination and plant maturity, the opposite is true. In fact, processes taking place in individual life-cycle stages are, of course, interconnected. So, for example, plant flowering traits can be a result of selection pressures in some previous life stage (e.g. flowering phenology reviewed in Ehrlén 2015).

During ontogenesis, physiological tolerance to herbivory changes as does resource allocation to different plant organs (Boege & Marquis 2005). From the demographic point of view, however, what also changes is the susceptibility to external stress from the standpoint of the effect on the population growth rate. Altered survival during the inconspicuous stages of vegetative growth can thus have a much larger effect on the whole life cycle of a perennial plant than reduced seed production (Silvertown et al. 1993). The study of the effects of only one parameter (transition) can therefore lead to erroneous conclusions, especially when it comes to how important it is for the plant life cycle (Ehrlén 2002).

By studying the whole life cycle using transition matrix models, it is possible to trace the importance and consequences of interactions for the whole life cycle (for the first studies, see Doak 1992, Louda & Potvin 1995, for a review, see Ehrlén 2015). For example, pollen limitation might at first glance decrease plant fitness because increased pollen transport should lead to higher seed production. In fact, increased pollen transport can decrease future growth and flowering probability (Ehrlén & Eriksson 1995) or decrease seed quality (Ashman et al. 2004), which from the point of view of population growth rates can outweigh the benefits of increased seed production. The balance of these costs and benefits is not only difficult to study, but it can also fluctuate between years, depending on the current environmental context, e.g. pollen availability and recruitment conditions (Horvitz et al. 2010).

For the reasons outlined above, it is ideal to use long-term demographic data. Unfortunately, I myself was unable to collect such data, but long-term experience with the model species allowed me to neglect the effects of certain life-stages. Of course, they might still play a role, but more data would be necessary to examine it closely.

The adult plant and its flowering traits

Our seedlings are slowly reaching the life stage of adult vegetative plants. Our model system does not consider this stage interesting with regard to interactions, as we did not observe any plant-animal interactions outside anthesis, so let us skip the vegetative life-stage altogether. Let us look closer at the flowering and fruit-bearing period, the most conspicuous of the plant life cycle.

Each plant has grown in its microsite and bears certain flowering traits. Some of them are heritable, as some studies have shown, for example, in the cases of flowering phenology (Widén 1991, Mitchell & Shaw 1993, Hauser & Weidema 2000, Geber & Griffen 2003) or flower number and corolla size (reviewed in Ashman & Majetic 2006). Plant traits also reflect the properties of the microhabitat; higher resource levels, for example, correlate positively with plant height and earlier flowering (Ollerton & Lack 1998, Ehrlén & Münzbergová 2009). At the same time, the (micro)habitat itself influences the frequency of plant-animal interactions, both on the landscape (e.g. Oostermeijer & van Swaay 1998, Hirsch et al. 2003 for pollinators and Welch et al. 1990, Coulon et al. 2008 for herbivores) and locality level (e.g. Torang et al. 2006 for pollinators, Lin & Galloway 2009 for ungulate herbivores). The resulting set of flowering and micro-site traits is closely connected and mutually interacts with the activity of animals, and all of this affects plant performance. So which flowering traits am I interested in, and what bearing do they have on plant-animal interactions?

Flowering phenology – Many studies describe antagonistic pressures on flowering time that are simultaneously exerted by pollinators and seed predators (Elzinga et al. 2007, Kolb et al. 2007, Lay et al. 2011). Flowering phenology is nevertheless connected with the entire life cycle and is also influenced by selection pressures exerted outside the flowering period (reviewed in Ehrlén 2015). It can also be affected by resource availability, as earlier-flowering plants are often larger (Ollerton & Lack 1998, Ehrlén & Münzbergová 2009).

Plant height is usually mentioned as a criterion for selection by ungulate herbivores (Freeman et al. 2003, Koh et al. 2010, Fujita & Koda 2015, Prendeville et al. 2015) and, less frequently, by pollinators (Gomez et al. 2009). It also strongly correlates with available resources (Garnier et al. 2007). It is also influenced outside the flowering period, as, for example, a taller stature can also be an advantage during seed dispersal (Tremlova & Munzbergova 2007, Monty et al. 2008)**.**

Size and number of flowers (Floral display) – a trait most often mentioned in connection with attractiveness to pollinators (Abraham 2005, Lay et al. 2011, Weber & Kolb 2013) or florivores (Oguro & Sakai 2015). Only rarely is it associated with attractiveness to ungulate herbivores (Ehrlén 1997, Gomez et al. 2009), although it tends to be strongly negatively affected by their activity (Gomez 2005, Lay et al. 2011).

Floral traits often vary even on a single plant; phenologically younger flowers, for example, tend to be smaller and give rise to fewer seeds (reviewed in Diggle 1997). This effect is sometimes suppressed by herbivores if bitten off flowers are compensated for (Pilson & Decker 2002, West 2012, Aikens & Roach 2015).

Fragrance allows to precisely time advertisement to pollinators and fluctuates depending on their abundance (Theis et al. 2007). It can also attract florivores (Theis & Adler 2012). It is a unique mechanism by which herbivory can increase attractiveness to pollinators. Leaf herbivory can sometimes cause increased emissions of fragrant compounds (Effmert et al. 2008, Theis et al. 2009, Cozzolino et al. 2015). Fragrance, however, is beyond the scope of our study.

Interactions

Plant-animal interactions are an integral part of the life of each plant as well as an extensively studied phenomenon. To gain insight into their effect on the plant life cycle, it is necessary to capture and examine as many interactions as possible. Complex studies are still relatively rare, not to mention those also dealing with other biotic interactions such as with pathogens (Ehrlén et al. 2016).

Interactions between organisms are either mutualistic (i.a. involving pollinators or dispersers) or antagonistic (e.g. involving herbivores in the wide sense). These two kinds of interactions exert conflicting selective pressures on plant traits (Brody 1997, Herrera et al. 2002).

Pollinators facilitate pollen transport and thereby mating between outbreeding plants. For self-incompatible species, they are indispensable, while self-compatible species do not depend on them. Between these extremes is a whole spectrum of how important pollinators are to plants. One of the most easily observable benefits of cross-pollination is a larger seed set and greater seed mass (Lloyd 1992, Ashman et al. 2004 for a possible negative correlation between the number of seeds and seed mass in supplementally pollinated plants) or the survival of offspring (Colling et al. 2004b). Lack of pollinators and pollen transport causes pollen limitation in plants (Ashman et al. 2004, Knight et al. 2005). The causes of this imbalance between the potential of plants to produce seeds and limited pollen transport caused by a lack of pollinators can differ. Current changes in the landscape cause fragmenting and declines of plant populations and lower abundance of pollinators (Agren 1996, Milberg & Bertilsson 1997, Cheptou & Avendano 2006). The activity and abundance of herbivores in the present landscape is increasing (Meriggi et al. 2008), which decreases the abundance of flowerheads in populations, making them less attractive to pollinators (Knight et al. 2005, Lay et al. 2011). Alternatively, climate change-induced phenological shifts may occur in plant or pollinator life cycles (Fabina et al. 2009), causing the peak of flowering to not coincide with the peak of pollinator abundance. Another possible reason is the general loss of pollinators caused, among other things, by habitat loss and fragmentation, agrochemicals, pathogens and alien species (Potts et al. 2010). This is a rather long list of good reasons to expect pollen limitation in our model system.

Pollen limitation can lead to selection for floral traits (Ashman et al. 2004) because it gives an advantage to plants bearing traits that enhance pollinator attraction (Haig & Westoby 1988). To detect such selection pressures, it is first necessary to find out whether populations are pollen-limited. This is usually achieved by comparing open-pollinated and supplement-pollinated plants (García & Ehrlén 2002, Pflugshaupt et al. 2002, Andrieu et al. 2007, Ehrlén 2015). It is also necessary to look for connections between pollinator abundance and plant flowering traits such as flower display, plant height or flowering phenology. However, conditions of the microhabitat that are not directly related to the plant may also work as selection criteria for pollinators, for example, the surrounding vegetation (Ghazoul 2006, Torang et al. 2006) and the number of co-flowering individuals in the vicinity (Caruso 2002). Because pollinators play an irreplaceable role in our model system, it is necessary to ask what roles all these aspects play in plant reproduction.

Herbivory usually adversely affects plant performance (Belsky 1986, Hawkes & Sullivan 2001, Russell et al. 2001, Maron et al. 2010). In anthesis, it has the strongest direct effect on fitness via the seed set (Augustine & Frelich 1998, Maron & Crone 2006, Lin & Galloway 2009), but it also affects the survival of adult plants (Brys et al. 2011), flowering probability (Ehrlén & Van Groenendael 2001, Knight 2003, Ehrlen & Munzbergova 2009, Brys et al. 2011) and seed production in the following season (Puentes & Agren 2012). We assume that they can also affect seed properties such as mean seed mass (Meyer 2000, Pilson & Decker 2002) or germinability (Aikens & Roach 2015).

There are several types of herbivory. Insect herbivores often limit their activity to a limited time period that is strongly tied to plant phenology (Elzinga et al. 2007, Münzbergová et al. 2015). From the perspective of the relationship with the architecture of plant flowering traits, ungulate herbivory is especially interesting. Browsers preferentially feed on flowering plants (Augustine & Frelich 1998, Boege & Marquis 2005, Lin & Galloway 2009, Davalos et al. 2014). They can choose depending on plant traits such as plant height (Freeman et al. 2003, Koh et al. 2010, Fujita & Koda 2015, Prendeville et al. 2015) or number of flowers (Ehrlén 1997, Gomez et al. 2009).

Although herbivory is one of the most studied drivers of the plant life cycle (Ehrlén et al. 2016), there are still notable knowledge gaps in the study of its effects. One of them concerns the detailed aspects of herbivory such as the timing or the rate of damage and the effect on plant performance. Few studies have also attempted to look at herbivore preferences, the extent of herbivory and its effects on plant performance together. Yet this very connection is crucial for better understanding the role of plant flowering traits and plant-animal interactions, which mutually influence each other.

Flowering traits are related to reproduction success both directly and indirectly (Obeso 2002) as a proxy of resource limitation on the one hand and via animal-plant interactions on the other. As said above, plant-animal interactions can also affect seed properties and plant reproductive traits for years to come. This brings us back to the relationship between the maternal plant, seed traits and seedling performance.

Populations and their properties at the landscape scale

The network of relationships, causes and effects within a locality, even over a short time frame, is exceedingly complex. Not even a locality is an island, but part of a metapopulation (Freckleton & Watkinson 2002). It is therefore advisable to view consequences of interactions also on a broader scale. Not only because populations can influence each other through the transport of pollen or propagules, but also because the extent of plant-animal interactions often varies depending on the population (Brody 1997) and because their influence is connected with various characteristics of populations and localities. As in the previous chapters, such mutual interactions take place via different pathways and at several levels.

Just like individual plants, populations have their own characteristics, too.

Population size is probably the easiest property to measure. Yet it has many connotations. It is often related to genetic diversity: Small populations can suffer from consequences of drift load and inbreeding depression (Fischer et al. 2000), which is manifested in decreased seed production (Lamont et al. 1993, Oostermeijer et al. 1998, Luijten et al. 2000, Paschke et al. 2002), seed mass (Heschel & Paige 1995) or seedling size, the number of flowering stems and flowerheads, adult survival and total relative fitness (Luijten et al. 2000). Some studies, however, did not prove a connection between heterozygosity and population size (Ehlers 1999, Münzbergová & Plačková 2010).

Population size plays a role also in plant-animal interactions. In smaller populations, studies found both lower abundance of pollinators (Cheptou & Avendano 2006, Mayer et al. 2012) as well as pollen limitation (Agren 1996, Andersson et al. 2016). Small populations are also more prone to extinction due to stochasticity (Dornier & Cheptou 2012), which can be aggravated by disturbances or herbivore activity. However, small population size can sometimes release plants from parasites and pathogens (Colling & Matthies 2004a).

From the point of view of interacting organisms, a good measure of population size is **density of flowering plants**. Floral density positively correlates with the abundance of pollinators (Ghazoul 2006, Cheptou & Avendano 2006). Decreasing density of flowering plants due to herbivory can thus have an amplified negative effect on population fitness by decreasing the attractiveness of the population to pollinators (Vazquez & Simberloff 2004, Lay et al. 2011).

Population demography: Individual populations can differ in their dynamics, proportions of individual life stages and thus also in their susceptibility to external stress, for example, herbivory. Population dynamics can be disrupted by one-off events. This alters the importance of individual transitions in the population life cycle, and the effects of the disturbance of and plant-animal interactions can thereby amplify or cancel out each other.

Habitat properties affect both population structure (plant density, aboveground biomass or proportion of flowering individuals) and the likelihood of the population being affected by plant-animal interactions. Pollinators respond to abiotic conditions such as moisture, acidity and nutrients (Oostermeijer et al. 1998). When deciding where to graze, ungulate herbivores may also consider factors on a higher landscape scale such as the vicinity of buildings, roads, valley bottoms, woodlands (Coulon et al. 2008) or the density of habitat edges (Tufto et al. 1996, Said et al. 2005, Miyashita et al. 2008) or patch size (Welch et al. 1990). The environment also directly influences the ability of plants to compensate for consequences of herbivory. Many studies found considerable differences in the responses of plants to herbivory under high versus low resource levels (Hawkes & Sullivan 2001, Wise & Abrahamson 2007).

This is why it is better not to base a study merely on knowledge of one population. The number of populations and the length of the study period are additional shortcomings of studies of drivers of plant demography (Ehrlén et al. 2016). The structure of the landscape has been changing in recent years, as has the abundance of herbivores. However, for species that respond slowly to landscape change, changes in landscape structure may not be the most important factor influencing species dynamics. They can, however, also play a significant role when combined with factors that affect local populations in the short term. The landscape perspective of interactions can thus put findings obtained by studying populations in detail into a wider context and help better grasp their importance for the dynamics of the whole species.

Model system

As the model species for our study, we selected the polycarpic perennial grassland herb *Scorzonera hispanica* L. (Asteraceae). Each individual has a single rosette and one flowering stalk with one to seven yellow flowerheads. Although many species of the Asteraceae are autogamous or even apomictic, previous experiments done on the study species indicate that no developed seeds arise without pollen transfer. The species can thus be classified as self-compatible but not capable of spontaneous selfing, so a pollinator is needed in all cases (Banga 1961, Münzbergová et al. 2010).

Flowering stalks of *S. hispanica* are often browsed by ungulates (Hemrová et al. 2012). No other type of herbivory has been observed; however, the rate of ungulate herbivory in all populations is quite high. We have also observed the destruction of habitats of *S. hispanica* within the study region, primarily due to ploughing, the construction of solar power stations and rooting by wild boars.

Previous studies yielded certain demographic data about populations of *S. hispanica*, including eight transition matrices containing three size classes (seedling, large vegetative and flowering individuals) compiled for three different populations. Using these matrices, it was possible to roughly evaluate the importance of life-cycle transitions for the rate of population growth. In the case of our populations, the most important in terms of elasticity is survival of vegetative plants, transitions to flowering plants and seedling survival. For detailed information on the populations under study, see Table 1 in Münzbergová (2006). *S. hispanica* does not form a permanent seed bank (Münzbergová 2004).

In the area, the species occurs on clearly delimited patches. Some of these patches are suitable yet unoccupied, as identified by means of a sowing experiment indicating that *S. hispanica* is dispersal-limited (Münzbergová 2004). Chýlová & Münzbergová (2008) demonstrated that this species prevails in grasslands existing for at least 60 years, indicating that the dynamics of the species are quite slow.

Although *S. hispanica* is considered endangered in the Czech Republic, it is still common in the study area of dry grasslands in northern Bohemia. However, the character of the study area has rapidly changed in the last decades. In the past, there was a fine-scale mosaic of pastures and fields. At present, large areas of arable fields surround the remaining grasslands. Most of the localities are now abandoned, covered with a mosaic of grasslands and expanding shrubs and trees (Chýlová & Münzbergová 2008).

Studies in Chapters 2 a 3 were undertakenat the locality Holý vrch, which is a moderate, south-facing slope with a mosaic of open grasslands and shrubs, representing one of the largest populations in the area with ca 1,600 flowering individuals.

Aims

The aim of this thesis was to explore the network of animal-plant interactions both at the local and landscape scale, and to define its effects on plant performance from the seed to reproduction. My main questions were: (1) What is the role of source population and mother plants and seed traits in the germination and growth of offspring? (2) Which plant-animal interactions are relevant and how do they affect present and future reproduction of *S. hispanica*? (3) How is the choice of interacting animals related to individual plant traits? (4) What are the future prospects of *S. hispanica* in the current landscape, and how are they likely to be affected by herbivory?

Unfortunately, not even the answers to these questions can elucidate the whole breadth of the topic of the plant life cycle and its interactions. There are too many relationships for one doctoral thesis to grasp.

So what have we found?

In **Chapter 1** we attempted to find out how properties of the seed, the mother plant and the mother population affect germination and subsequent plant performance. By comparing seeds of different weights from different mother plants growing in several populations within the study area, we aimed to observe these three influences separately.

The most important factor affecting seedling performance was seed weight. Heavier seeds had a higher germination rate, seedling survival within six months, and the seedlings that emerged from these seeds were larger. This influence was observable for two months.

We also observed a higher germination rate and seedling survival in seeds from larger populations. Neither this nor our previous study, however, confirmed a connection between population size and genetic diversity or any other biotic or abiotic factor such as habitat suitability or plant-animal interactions.

Even though seedling performance differed between individual mother plants, this effect could not be explained by mother plant traits (height and flower number). Other characteristics of the mother plant, such as its genotype or the environment in which it grew likely play a role in determining seedling performance.

Seed weight was not provably influenced by any of the population or mother plant traits under study, and none of the factors we studied had any significant effect on the time to germination.

In **Chapter 2** we examined whether *S. hispanica* suffers from pollen limitation and which flowering traits and environmental variables pollinators base their choice on. We assessed pollen limitation by comparing the seed set of supplementally pollinated plants with that of open-pollinated ones in two consecutive experimental runs. At the same time, we identified all floral visitors at the model locality. Using path analyses, we attempted to unravel the interconnections between properties of plants, the microhabitat and pollinator visitation rates.

Pollinator choice was governed more by the micro-sites of plants than their flowering traits. Pollinators preferred solitary plants (with a smaller number of co-flowering neighbours) in the vicinity of trees. During the first experimental run, we observed two-fold greater visitation rates. Despite the large number of undeveloped seeds, we were unable to prove pollen limitation by manual supplemental pollination. Instead, the seed set and weight of seeds were correlated with plant size traits (height and flowerhead number), as larger plants produced more seeds with a higher viable/aborted seed ratio. These traits were instead correlated with micro-site characteristics such as the cover of woody species in the vegetation. This suggests that the plants under study were likely resource-limited. Mean seed mass per plant was not related to any of the variables studied.

In **Chapter 3**, we monitored the effects and preferences of ungulate herbivores. By taking regular measurements during weekly censuses, we monitored the exact timing and extent of herbivore damage. Using path analyses, we then assessed the effects of plant flowering traits, herbivore choice and intensity of herbivory on various aspects of plant reproduction.

Herbivores consistently preferred plants growing in denser vegetation and with a greater number of flower buds. Plant height was not a significant factor. The impact of herbivory on seed production was quite high; browsed plants produced several-fold fewer seeds than untouched plants. Flowering in the following season was not affected by herbivory as a factor (which is in line with the results of comparing browsed and caged plants in Chapter 4), but instead with the timing and extent of browsing. Plants that were browsed earlier and more severely had a higher probability of flowering in the following season. Losing a still developing flowering stalk probably afflicted a lower loss of invested energy than losing a proportionally small, albeit all the more valuable, organ – the flowerhead. The effect of timing and the proportion of browsing on the number of seeds was the opposite. Plants browsed at an early stage of the growth of their flowering stalk often also lost all their adventitious buds and thus also any means of compensation. Those that lost a primary or secondary flowerhead were sometimes at least partly able to compensate for these losses by opening adventitious flowerheads.

However, the most important factor affecting the probability of flowering in the following season was not herbivory, but initial stalk height, which was correlated with site characteristics – the vegetation cover. The connection between initial stalk height and seed number was never significant, in contrast to the results presented in Chapter 2, where we, however, analysed only untouched plants. The influence of herbivory therefore obviously cancels this relationship, even though plant height did not demonstrably affect herbivore choice.

Experiments described in **Chapters 1** and **2** were interesting in that they dealt only with unbrowsed plants whereas herbivory affected around 60 % of flowering individuals across all populations (**Chapter 4**). Most plants that fall victim to herbivores therefore seem to play according to different rules. While in the intact set of plants from **Chapter 2**, the number of developed seeds significantly correlated with plant height, under natural herbivore pressure, this connection disappeared. Initial plant height at the beginning of the season affected neither the number of seeds nor seed mass, and not even herbivore selection. When I compared the relationship between plant height measured after anthesis and seed number, there was an apparent difference between the treatments. While in the cases of untouched plants from **Chapter 3** and control (caged) plants from **Chapter 4**, there was a significant correlation plant height and seed number, in browsed plants from **Chapter 3**, there was no such correlation (Fig. 1). Analogously, I also compared mean seed mass, but this comparison did not reveal any demonstrable differences between the treatments.

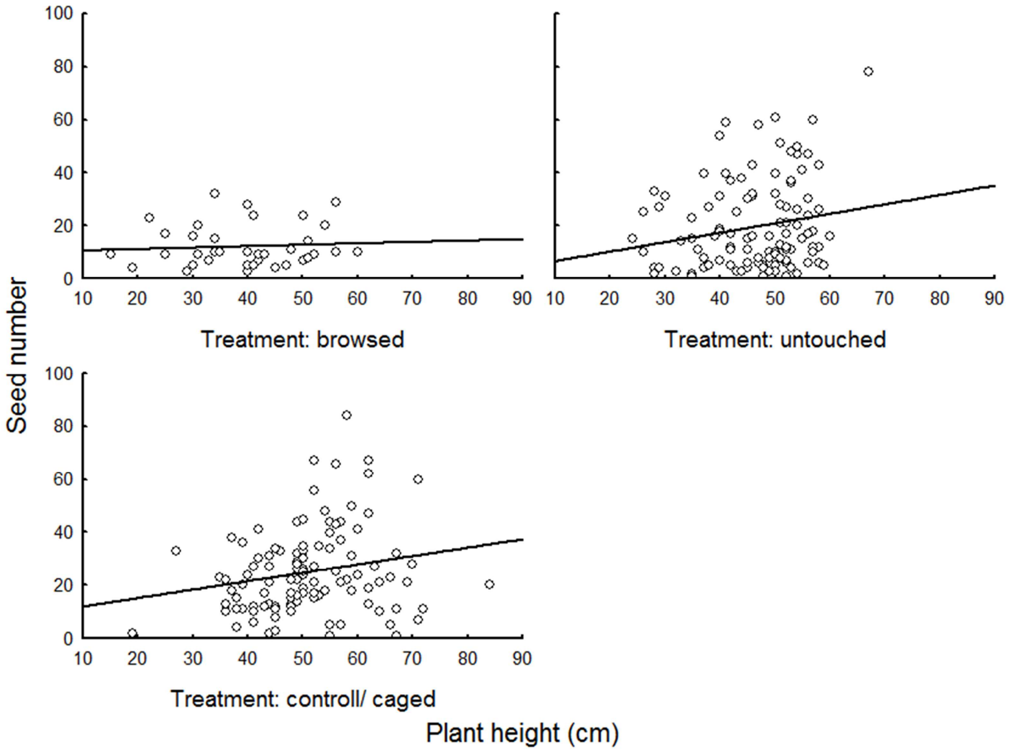

Fig. 1. Comparison of the effect of plant height on seed number between different plant treatments at the locality Holý vrch in the years 2009 and 2010.

In **Chapter 4** we modelled the effects of herbivory and occasional destruction of species' populations at the landscape-level using a dynamic, spatially explicit model. The model was based on information about the locations of patches suitable for *S. hispanica* within the study area, initial population sizes, the dispersal rate of the species and local population dynamics. We modelled local population dynamics using transition matrices and manipulated transitions, including the impact of herbivores. We manipulated these transitions based on a comparison of the reproduction of browsed and control (caged) flowering plants. These differences resided in decreased seed production and decreased production of clonal vegetative ramets in browsed individuals. Simulations were performed pertaining to the prospects of *S. hispanica* over the next 30 years under different rates of herbivory (browsing intensity) and varying frequencies of population destruction (e.g. by human activity).

Although a high rate of herbivory was detected in most populations of *S. hispanica*, the landscape-level dynamics of *S. hispanica* were approximately in equilibrium. Any decline or increase of over 20% in the rate of herbivory caused *S. hispanica* to rapidly expand or decline, respectively. This effect was much stronger in the presence of population destruction. The probable reason is that herbivory only marginally affects the survival of vegetative plants and seedlings – important transitions in the plant life cycle under stable conditions. Disturbances, however, can radically alter these contributions to changes in total fitness. Decreased seed production due to herbivory can thus complicate the regeneration of a population after its destruction.

Therefore, from the perspective of the species' dynamics in the landscape, browsing by ungulate herbivores can have a dramatic effect on its future prospects that would be impossible to predict by studying local dynamics in one or a few populations.

Conclusions

In the studies outlined above, I attempted to gain insights into the mechanisms by which plant-animal interactions affect various aspects of plant population dynamics. There were some strong connections, but others are still elusive.

In **Chapter 1**, we present evidence that the crucial factor affecting germination and seedling performance was seed mass whereas properties of the mother plant – plant height and flowerhead number – did not play any role. Therefore, in further studies, we focused our attention on factors that may affect the seed mass. In further studies, however, we did not find any connection between seed mass and plant-animal interactions.

The results presented in **Chapter 2** suggest that seed production is affected by resource limitation rather than by plant-pollinator interactions. Considering the recent changes of the landscape, climate and species composition, we expected to find pollen limitation caused by a pollinator deficit, but we were unable to confirm this in the model species.

During the study for **Chapter 3**, we found a significant influence of herbivory on seed production whereas neither flowering in the next season nor survival of flowering plants was affected by the rate of herbivory. Herbivores preferred plants with greater numbers of initial flower buds whereas plant height did not affect their choice.

In **Chapter 4** we deal with our finding that landscape-level population dynamics under the present rate of herbivory are approximately in equilibrium. The extent of herbivory plays a large role in landscape-level population dynamics, and its increase by more than 20% could lead to the species' extinction within the study area, especially if combined with disturbance events. The results of our simulation also revealed a higher survival probability of large populations than that of small ones. This effect can additionally be amplified by the observed worse performance of seedling from seeds produced in smaller populations.

Putting together the individual studies allowed me to get a basic idea about the effects of plant-animal interactions on the population dynamics of the model species. For more accurate conclusions regarding the future of the species and the factors affecting it, it would make sense to focus also on certain problems not covered by this thesis. One example is the distribution of pollinators at the landscape scale and associated phenomena that potentially threaten small populations. For deeper insights into the entire life cycle would entail the study of the direct effect of plant-animal interactions on germinantion and seedling performance regardless of seed mass.

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CHAPTER 1

Seed mass and population size affect germination and seedling performance in *Scorzonera hispanica*

Zita Červenková and Zuzana Münzbergová

ABSTRACT

Seed weight is one of the most important factors determining the performance of offspring and this effect is often combined with the effect of the mother plant. While both the effect of seed weight and the effect of mother plants on plant performance were previously studied, the effects of one of the factors is usually confounded by the effects of the other factor. We thus still do not know the extent of the effect of seed weight on seedling performance when properties of mother plants are taken into account. To explore this, we studied the effects of seed weight, mother plant and site on seedling performance in the perennial herb *Scorzonera hispanica* (Asteraceae).

In our dataset, the wide ranging seed weight could not be explained by population size even the mother plant traits. Seed weight positively affected germination probability seedling growth and seedling survival. The seeds from larger populations had higher germination rate and seedling survival. The mother plant traits did not affected any studied variable, despite the performance of seedlings from individual mother plants was different.

Key words: germination, population size, seed mass, mother plant

INTRODUCTION

Seed production and seedling recruitment are among the key periods in the plant life cycle (Harper 1977). Seed germination and the further life of seedlings is influenced not only by properties of the seeds, such as their weight and morphology (Counts & Lee 1991, Van Molken *et al.* 2005), but may be also affected by the identity of the mother plant or by population characteristics (Kirkpatrick & Lande 1989). To understand the factors driving seedling recruitment, we need to estimate the importance of different properties of seeds, mother plants and sites for seedling performance and separate their effects.

Variation in seed weight has been studied in many species and at many hierarchical levels. On the coarsest scale, many studies deal with variability among populations. Variability in seed weight among populations may be a result of differences in habitat conditions such as habitat productivity, population size or isolation of populations (Valencia-Diaz & Montana 2005, Münzbergová & Plačková 2010). The effects of population size and isolation might be a consequence of lower genetic diversity and inbreeding in small populations (Heschel & Paige 1995, Oostermeijer *et al.* 1998) or possibly a result of the Allee effect (Lamont *et al.* 1993, Pflugshaupt *et al.* 2002).

At the within-population level, differences in seed weight may be caused by differences among single mother plants (Ouborg & Vantreuren 1995, Weiner *et al.* 1997, Hereford & Moriuchi 2005). This variation can often be related to mother plant traits (e.g. depending on maternal plant biomass in Sletvold 2002). Comparisons of seed weight among single plants may be complicated by within-plant variability. Seed weight within a single plant can vary during the season (Cavers & Steel 1984, Wolfe 1992, Buide 2004) as well as over the life of the plant in polycarpic perennials (Herrera 1991a). Seed weights also depend on the position of the seed on the plant (reviewed in Herrera 1991b, Diggle 1997, 2003). Several studies found this sort of variability at the level of inflorescences or at the level of flowers within inflorescences (Buide 2004, Zeng *et al.* 2009). Within the subfamily Cichorioideae of the family Asteraceae, heterocarpy or seed weight variability within the inflorescence is a known phenomenon (Imbert *et al.* 1996, Van Molken *et al.* 2005, Brandel 2007, Torices & Mendez 2010).

Numerous studies have shown that seed weight is positively correlated with the probability of germination (Counts & Lee 1991, Lehtila & Ehrlen 2005), time prior to germination and seedling growth (Meyer & Carlson 2001, Khera *et al.* 2004). Nevertheless, seed weight is not the sole predictor of seedling development. Many other factors are assumed to affect this initial phase of plant life; the identity of the mother plant, for example. The effect of mother plant identity on seedling development can be attributed to genetic effects (Lacey 1996, Weiner *et al.* 1997) or other, possibly epigenetic, maternal effects related to microhabitat conditions in which the mother plant was growing (Ouborg & Vantreuren 1995, Lacey 1996, Galloway 2001, Hereford & Moriuchi 2005). Seedling development was also shown to depend on seed shape and position of the seed within the flowerhead (Brandel 2007), but see Van Molken et al (2005), who found no such effect. It is also known that germination can differ between populations (Counts & Lee 1991).

Despite the high number of studies on the relationships between plant traits, habitat characteristics, seed weight and seedling performance, we still lack studies that would explicitly separate the effects of seed weight from other effects such as the effect of the mother plant or of the environment of their origin. This is because seed weight is largely affected by properties of both mother plants and populations (sites), which make it difficult to separate the effect of seed weight from all other possible determinants of seed performance. To achieve this, we need to study the performance of seeds of different weights originating from the same mother plants and compare the effect of seed weight among different mother plants coming from different populations (sites). When performing such a comparison, it is not possible to separate the effect of maternal environment and effects of specific genotype of the mother plant.

The aim of our study is to describe the factors affecting seed weight and subsequent growth of seedlings in the model plant species *Scorzonera hispanica*, Cichorioideae, Asteraceae. Specifically, we aim to separate the effect of seed weight from the effect of mother plant traits and the effect of habitat conditions on seedling performance. We asked the following questions: (i) What is the effect of site conditions and mother plant traits on seed weight in natural populations of *S. hispanica?* (ii) What is the effect of seeds size, mother plant and site characteristics on seed germination and subsequent growth of seedlings?

In contrast to some other Asteraceae species the outer and inner seeds within a flowerhead in *S. hispanica* are not clearly morphologically differentiated. There is only a short gradient from straight seeds (in the middle) to slightly curved seeds (at the periphery) but there can be a large variation in seed weight within a flowerhead and between flowerheads within the same plant. The seeds are not dormant and the species does not form persistent seed bank (Münzbergová 2004).

In a previous study, Münzbergová & Plačková (2010) studied the effect of seed weight, population characteristics and their interactions on the performance of *S. hispanica* by studying seeds of the same range of weights sampled from 20 different sites. In this study, we expanded this approach by comparing the performance of seeds of the same range of weights coming from individual mother plants from different populations. We are thus able to separate the effect of seed weight, the effect of the mother plant and the effect of the site.

MATERIALS AND METHODS

Study system

Scorzonera hispanica L. (in English known as black salsify or Spanish salsify**,** Asteraceae) is an allogamous perennial herb growing rarely in dry grasslands of central and southern Europe being common in the Iberian Peninsula. In the Czech Republic, it occurs in central and northern Bohemia and southern Moravia. It has a single rosette and a single flowering stalk with one to seven flowerheads. It is occasionally cultivated for its edible rootstock and is naturalized locally (Chater 1976). In the study area, it is, however, considered native.

The populations included in this study are dispersed in an area of dry grasslands in northern Bohemia, Czech Republic, covering about 150 sq. km. In the past, the area was covered with a fine-scale mosaic of pastures and fields that were largely interconnected by grazing cattle (Chýlová & Münzbergová 2008). At present, large areas of arable fields surround the remaining grasslands. All the study sites are abandoned at the present time and comprise mosaic of homogeneous grasslands and expanding shrubs. The plants are grazed by ungulates (European Roe deer, *Capreolus capreolus* L.) at natural sites; no other type of herbivory was observed (Hemrová *et al.* 2012). For this study, we used 7 of the 21 populations that were found in a field survey of the landscape. The selected populations range from 48 to 2500 flowering individuals (Table 1). Genetic variability in the field is rather high; Nei's genetic diversity of the populations ranges from 0.04 to 0.32

indicating that all the populations are genetically variable (Münzbergová & Plačková 2010).

Tab. 1: Geographic positions and population sizes (estimated as number of flowering individuals in 2009) of sites included in the study.

	Longitude	Latitude	Population size
1	13° 58' 39.6"	50° 29' 45.9"	48
2	14° 14' 6.7"	50° 32' 2.9"	296
3	14° 5' 21.9"	50° 32' 58.2"	600
4	14° 13' 59.3"	50° 32' 11.6"	740
5	14° 14' 13.9"	50° 31' 46.6"	1600
6	14° 5' 16.4"	50° 32' 25.0"	1920
7	14°15' 12.6"	50° 31' 44.6"	2500

Seed sampling

The main goal of the study was to estimate the effect of mother plant and site on seed weight and the effect of mother plant, site and seed weight on germination and subsequent seedling growth. To study the relationship between sites, mother plant traits, seed weight and

subsequent plant growth, we sampled 10 flowerheads, one per plant, in each of seven sites at the time of fruiting in July 2007. We selected the seven sites with the aim to cover the range of population sizes across all populations and to make this range as wide as possible. We preferentially collected non-grazed plants because in grazed plants it is difficult or impossible to measure mother plant traits and collect developed seeds for further experiments. Flowerheads within plants were selected randomly. We noted the height of each plant and its number of flowerheads.

Mean seed weight per flowerhead was calculated as the total weight of all developed (visually full) seeds in the flowerhead divided by the number of developed seeds. When measuring seed weight, we used whole achenes, *i.e.*, fruits including pappus, in all analyses. The weighting was performed using analytical balance with precision 0.01 mg. In the whole text we use the term “seed” for the whole achene.

We selected 5 seeds from each of the 10 flowerheads from each site. The seeds were selected with the aim to evenly cover the whole range of seed weight in each flowerhead. All seeds in the inflorescence were weighed, and five seeds corresponding in weight to the median, upper and lower quartiles and both extremes were chosen from each plant. The minimum seed weight across all plants and sites in the data set was 2.57 mg, and the maximum was 22.06 mg (SE = 0.18 mg). The mean range between the lightest and the heaviest seed within one mother plant across all the plants was 6.80 mg (SE = 0.31 mg). This sampling strategy was used to facilitate separation of the effects of site, mother plant and seed weight on seed germination and seedling performance.

Common garden experiment

For each of the selected seeds, we recorded its weight and planted it individually in a pot into soil collected at one locality within the study area in a greenhouse at the beginning of March 2008. The seeds were watered regularly, and germination was recorded in 3-day intervals over the period of seed germination (until the 26th day, 8 measurements in total). The first seed germinated nine days after planting (this day is referred to as ’start of germination' in the subsequent text), and we used time since this start of germination as a measure of the time to germination of each seed.

The seedlings were later measured at 1-week intervals until the 55th day and then they were transplanted to single pots in the common garden and measured four times until the 173rd day at the end of August. In the first periods, we measured length of cotyledons until they dried (22 days since start of germination). Afterwards we measured the length of the longest leaf. A plant was counted as germinated when its cotyledons reached at least 1 mm in length. Seeds that did not germinate within two months were considered non-viable. Subsequent monitoring of the germination pots confirmed that these seeds did not germinate even later. The plants were further monitored during the next field season to estimate the effect of the mother plant, site and seed weight on subsequent growth. Most of the plants did not flower in the following season, so only vegetative parameters were analysed (number of leaves, and length and width of the longest leaf). In all the subsequent analyses we compare size of plants measures at the same occasion, i.e. at the same day, independent when the given seed really germinated. Due to this, the information about time to germination for each seed is partly correlated with the information on plant size (plants that germinated later and younger and thus smaller). We use this measure of plant size, rather than a measure comparing plant size of plants of the same age as it is more relevant to competition in natural conditions.

Statistical analyses

We used the data to explore the effect of the mother plant traits (height and flowerhead number) and population size on seed weight and of seed weight, mother plant traits and population size on seed germination and seedling growth. The relationships among mother plant traits and population size the seed weight, and the germination and growth of the plants were analysed using mixed effects models in lme4 package (Bates *et al.* 2012) in R (R Core Team, 2012).

To study the factors affecting seed weight, we entered mother plant height and flower number and (log)population size as fixed effects and population and mother plant as random effects into the model. P-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question.

To study the factors affecting germination, survival and offspring growth, we used seed weight, mother plant height and flower number and (log)population size as fixed effects and population and mother plant as random effects. The dependent variables were germination of seeds (germinated or not), germination time (expressed as the number of days since start of germination), seedling survival (survived until the 173rd day or not) and seedling size (cotyledon and later leaf length) in all the above mentioned time periods. The effect of all the mentioned independent variables on the germination and survival was tested using generalized mixed-effect model with binomial distribution of the dependent variable and the normal distribution of the dependent variable in germination time and seedling size, respectively. The residuals of cotyledons and leaf length were normally distributed, so no transformation was necessary. Time to germination was log transformed before analyses.

Because the plants were measured repeatedly over time (15 measurements of seedling size in total), we first used the whole data set and tested the effect of time and seed weight, mother plant height and flower number and (log)population size in interaction with time in a single test. Population, mother plant and seed were used as random effects in these tests. In case of any significant interaction between the plant or population characteristics and time, we repeated the tests separately for each time period. This allowed us to see the persistence of the effects over time.

RESULTS

In the plants under study, the mother plant traits (plant height and flowerhead number) had no effect on mean seed weight (p = 0.938, Df error = 6, Dev = 0.1 for plant height and p = 0.54, Df error = 6, Dev = 0.4 for flowerhead number, respectively). Seed weight was also independent of population size (p = 0.16, Df error = 6, Dev = 2.0).

In the common garden experiment, seed weight had a significant effect on germination rate of the seeds and seedling survival up to 6 months, but not on time to germination (Table 2). Specifically, heavier seeds germinated better and seedlings from those seeds also had higher survival rate. Also population size affected the germination rate and seedling survival up to 6 months, but did not have any effect on time to germination (Table 2). Specifically, seeds from larger populations germinated more often (Fig. 1) and more of them survived. The mother plant height and flowerhead number did not have any significant effect on seed germination, time to germination and seedling survival (Table 2).

Seedling growth over time was significantly affected by seed weight and time, but not by mother plant height, flower head number and population size. The seedlings from heavier seeds were larger and they became even larger over time. Also there was a significant interaction between time and seed weight and time and population size (Table 3). No other interactions were significant (Table 3).

When tested separately for each time period, seed weight positively affected the seedling growth between 9th to 66th day (Table 4) and the significant impact of population size occurred only once in the 6th day since germination (p = 0.041, Dev = 4.18, Df error = 6, Dev = 4.2). The seedlings coming from heavier seeds were larger (Fig. 2 and 3).

The time to germination was not affected by any of measured variables (see Table 2).

Tab. 2. Effects of seed weight and mother plant and population characteristics on plant performance. Proportion of variability in germination rate and survival (Df error = 6) and time to germination (Df error = 7) explained by seed weight, mother plant traits and population size. Proportion of variability in seed weight (Df error = 6) was explained by mother plant traits and population size. Dev. indicates deviance explained by the model.

	Seed weight		Germination		Survival		(Log)time to germ	
	p	Dev	p	Dev	p	Dev	p	Dev
Seed weight			**<0.001**	129.39	**<0.001**	82.98	0.796	0.18
Mother plant height	0.938	0.1	0.660	0.19	0.756	0.10	0.674	0.06
Mother plant flower no	0.54	0.4	0.678	0.17	0.417	0.66	0.803	0.02
(Log) population size	0.16	2.0	**0.015**	5.87	**0.036**	4.41	0.380	0

Tab. 3. Impact of time, seedling weight, mother plant traits (plant height and flowerhead number), population size and their interactions on seedling growth over the time. Dev. indicates deviance explained by the model.

	Seedling size		
	p	Dev	Df error
Time	**<0.001**	2685.8	9
Seed weight	**<0.001**	14.9	24
Mother plant height	0.7617	0.09	24
Mother plant flower no	0.5526	0.23	24
(Log) population size	0.1775	1.82	24
Seed weight*time	**<0.001**	68.11	73
Mother plant height*time	0.996	4.01	73
Mother plant flower no.*time	0.774	11.02	73
(Log) population size*time	**<0.001**	44.54	73

Tab. 4. Proportion of variability (Df error = 7) in seedling size explained by seed weight in different periods (no. of days since germination). For days 0-22, the dependent variable is the length of cotyledons. Further on, it is the length of the leaves. The effects of mother plant traits (plant height and number of flowerheads) were not significant in any case and are thus not shown. The 6th day since germination also occurred the significant effect of population size ($p = 0.041$, $\chi 2(1Df) = 4.18$, Df error = 6, Dev = 4.2).

Days since germ.	p	Dev
3	0.340	0.91
6	0.137	2.20
9	0.007	7.33
12	< 0.001	16.42
15	< 0.001	19.83
19	< 0.001	19.71
22	< 0.001	18.52
26	< 0.001	31.09
32	< 0.001	31.61
41	< 0.001	14.65
55	< 0.001	11.64
66	0.017	5.71
79	0.179	1.82
101	0.382	0.80
173	0.682	0.21

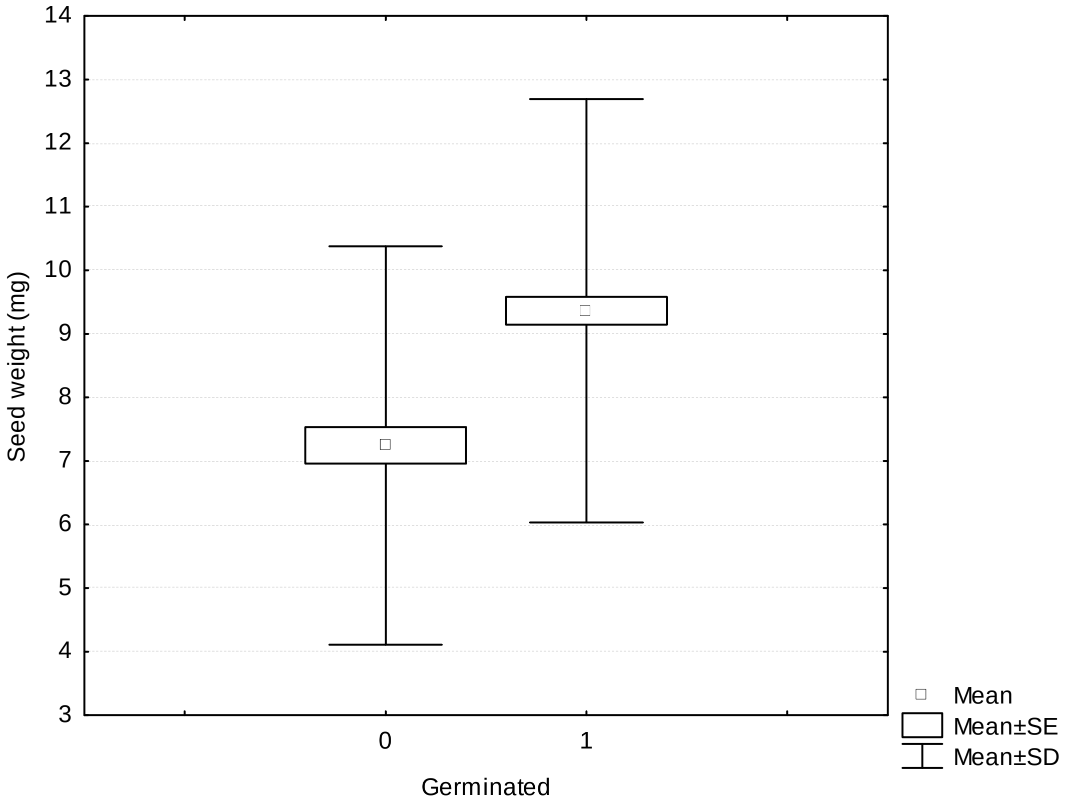

Fig. 1. Weights of germinated and not germinated seeds. Heavier seeds germinated significantly better. N = 350 (232 germinated and 118 not germinated)

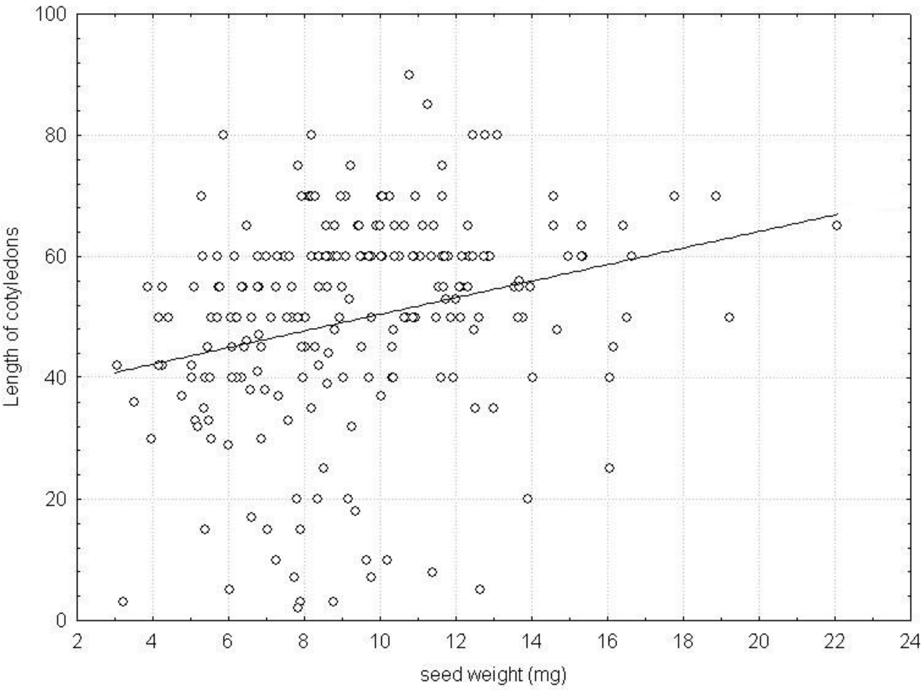

Fig. 2. Relationship between length of cotyledons and seed weight. Cotyledons 20 days after start of germination. N = 217

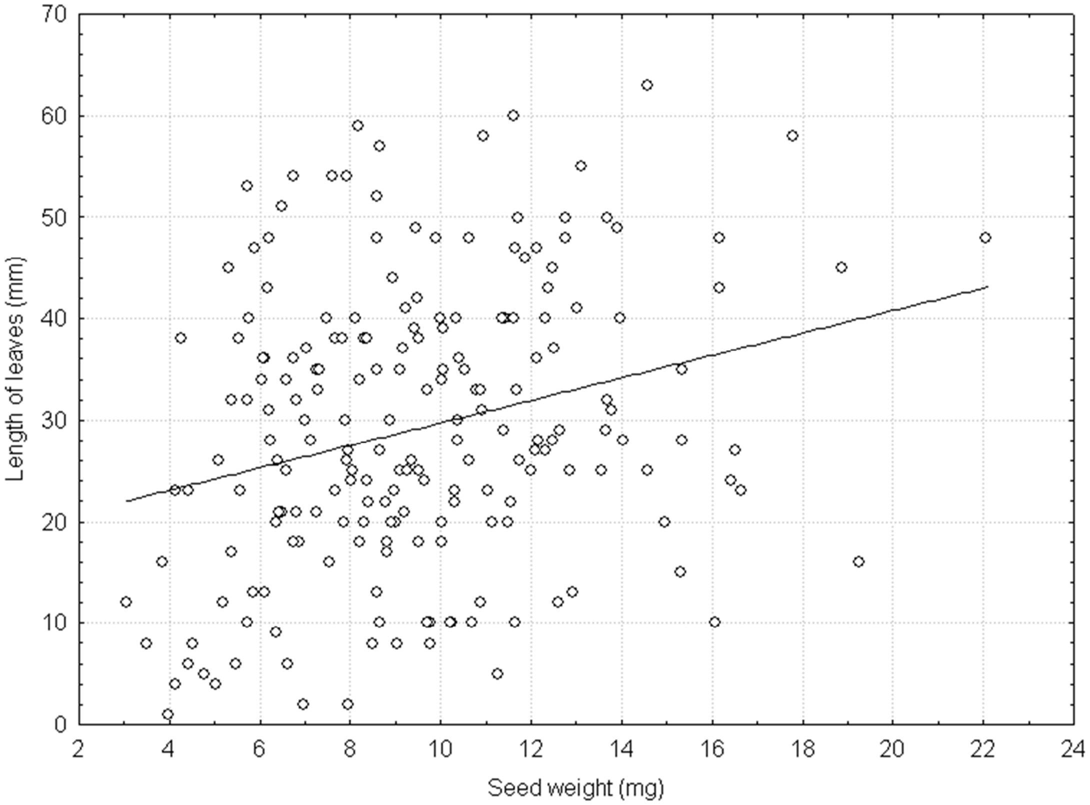

Fig. 3. Relationship between length of seedling leaves and seed weight. Seedlings 27 days after start of germination N = 209.

DISCUSSION

The results of this study demonstrate that seed weight is an important predictor of plant performance in early stages of plant development, but the effect disappears over time. Also the effects of population size are significant only in the early stages of plant development. In contrast, mother plant height and number of flower heads did not have any significant effect on plant performance throughout the whole study.

The main aim of the study was to test the effect of seed weight, mother plant and population characteristics on seedling performance in the exposed plants. The sampling design allowed us to separate the effects of seed weight from the effects of site and mother plant and observe their strength in different phases of plant growth. The strong influence of the seed weight on germination was corresponding to, *e.g.*, Counts and Lee (1991), Lehtila and Ehrlen (2005) and also to previous germination experiments on *S. hispanica* (Münzbergová 2006). In whole process we found no effect of plant height and flowerhead number on plant performance, despite there were differences in offspring performance among single mother plants. The differences in seed and seedling performance among mother plants were often observed (Weiner *et al.* 1997, Castro 1999, Ortmans *et al.* 2016), but rarely connected to mother plant size (Sletvold 2002).

The effect of population size emerged in germination and survival, while the effect of seed weight was evident for several weeks, starting six days after germination. The higher germination rate and survival in the seeds from larger populations is in accordance with some previous studies (Heschel & Paige 1995, Luijten *et al.* 2000, Vergeer *et al.* 2003, Ortmans *et al.* 2016). The population size tends to be connected with lot of factors; e.g. the genetic diversity (Ouborg & Vantreuren 1995, Oostermeijer *et al.* 1998, Hensen *et* *al.* 2005, Leimu *et al.* 2006), habitat suitability (Oostermeijer *et al.* 1998, Hegland *et al.* 2001, Busch & Reisch 2016) or plant-animal interactions, both mutualistic and antagonistic (Cheptou & Avendano 2006, Mayer *et al.* 2012, Andersson *et al.* 2016). In our dataset, the population size did not significantly correlate with no of such available data. However, it could be because of the low number of localities.

None of the measured factors affected the time to germination (see Castro 1999, Ortmans *et al.* 2016 for similar results). This contrasts to previous study on germination of seeds of the same species under comparable conditions in the greenhouse with the same frequency of measurements indicating that heavier seeds germinate faster (Münzbergová & Plačková 2010). The time period between the first and the last germination was about 20 days, but very few seeds germinated after 10th day. In contrast, the seeds in the study of Munzbergová and Plackova (2010) germinated over a period of 30 days and the study was thus more likely to detect differences between seeds of different size. This difference can be probably attributed to the climatic conditions, as in both cases the germination took place in greenhouse with limited temperature regulation and under natural sunlight.

Even a 10 day difference in time to germination can have, however, significant effect on seedling growth (reviewed in Verdu & Traveset 2005, Castro 2006) up to plant fecundity several years later (De Luis *et al.* 2008, Mercer *et al.* 2011). Despite we found no significant driver of this process.

As the seedlings grew, the differences in size appeared in 6th day after germination, when the effects of seed weight and population size on plant size were detected (Table 3). The higher germination rate in heavier seeds had been found in *S. hispanica* in the previous studies (Münzbergová 2006, Münzbergová & Plačková 2010). The positive effect of seed weight on performance of plants in early stages of their development is in agreement with many studies on various species (e.g. Ouborg & Vantreuren 1995, El-Keblawy & Lovett-Doust 1998, Castro 1999; but see also Zimmerman & Weis 1983, Wulff 1986, Latzel *et al.* 2009). In previous studies, the effects of seed weight disappeared within a few weeks (Meyer & Carlson 2001), whereas in our study the effects on seedling growth ended more than two months later and influenced even the seedling survival.

The lack of significant effect of the mother plant traits (height and number of flowerheads) on anything might be also biased because of grazing. At natural localities the taller plants with more flowerheads are more likely to be grazed by roe deer (pers. obs.). In the experiment, we preferably chose the non-grazed plants, because the seed set might be affected or lost due to herbivory in the grazed plants. We thus might not include the whole spectra of mother plants traits and therefore, the effect of the plant height and flowerhead number could not be detected.

Growing plants were further monitored during the following field season, but no effects of seed weight, mother plant and site were observed. This may be due to the fact that the plants were grown under favourable common garden conditions, so the initial differences that would have had strong consequences under natural conditions did not matter, as all the plants had enough resources and did not have to struggle for survival. We attempted to reduce this bias by growing the plants in soil from natural stands, but biotic factors such as competition among plants were not simulated. Alternatively, the absence of mother plant and seed weight effects in latter stages of plant development can be explained by the fact that more advanced stages of plant development tend to vary less among various treatments, as shown, *e.g.*, by Aarssen and Burton (1990) and Han and Lincoln (1997).

CONCLUSIONS

The present study shows that seed weight varies at multiple hierarchical levels ranging from the within-flowerhead to inter-population differences. Despite this high variation, it was possible to select seeds of a similar range of sizes from multiple mother plants from multiple sites and thus separate the effect of seed size from other possible determinants of seed germination and seedling performance.

The results showed better performance in seedlings from heavier seeds and from larger populations. The seed weight affected germination rate, seedling growth within 2 months and seedling survival. The seeds from larger populations had higher germination rate and seedling survival. The mother plant traits did not affect any studied variable, despite the performance of seedlings from individual mother plants was different. There are probably other unmeasured mother plant properties, such as genotype or individual history, including habitat conditions from which the plant originated and plant-herbivore interactions, which plays role in seedling recruitment of *S. hispanica*.

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CHAPTER 2

Pollen limitation and pollinator preferences in *Scorzonera hispanica*

Zita Červenková & Zuzana Münzbergová

ABSTRACT

The plant life cycle is often affected by animal–plant interactions. In insect-pollinated plants, interaction with pollinators is very important. When pollen transfer due to a lower abundance of pollinators limits seed production, selection pressures on plant traits related to plant attraction to pollinators might occur, *e.g*. on flowering phenology, height or number of flowerheads. Landscape changes (*e.g*. habitat fragmentation or changed habitat conditions) may cause plant-pollinator systems to lose balance and consequently affect population dynamics of many plant species. We studied the relationship between measured plant traits, environmental variables and pollinator preferences in *Scorzonera hispanica (*Asteraceae), a rare perennial, allogamous herb of open grasslands. We estimated the pollen limitation by comparing seed set of supplemental-pollinated plants with that of open-pollinated ones. Pollinators selected plants based on position within the locality (isolated plants close to trees) rather than on their traits. In spite of a high proportion of undeveloped seeds on the plants, we demonstrated that they are not pollen limited. Instead, seed set and weight of seeds was correlated with plant size traits (height and flowerhead number), with larger plants producing more and larger seeds. This suggests that the studied plants are likely resource limited. Overall, the results suggest that pollinators are not a selection factor in this system, in contrast to studies on various plant species, including self-compatible species of the Asteraceae. The lack of any effect of pollinators in the system may be caused by a strong negative effect of ungulate herbivores, which could play a decisive role in functioning of the system.

Keywords: Evolution; floral display; generative reproduction; heritability; outcrossing.

INTRODUCTION

Biotic and abiotic interactions during the flowering period may influence plant flowering strategy due to selection pressures on plant reproductive traits (Elzinga *et al.* 2007; Ehrlén & Münzbergová 2009). Floral visitors, for example, exert important selection pressure on flowering traits in insect-pollinated plants (Ashman *et al.* 2004). One necessary condition for the evolution of a plant trait due to pollinators is that the trait is heritable and at the same time relevant for the plant–pollinator relationship. The occurrence of pollen limitation in a species is a second necessary condition for the existence of selection pressure (Ramsey 1995; Knight *et al.* 2005). Pollen limitation arises when seed production of the maternal plant is limited by pollen receipt (reviewed in Ashman *et al.* 2004; Knight *et al.* 2005). Pollen limitation is expected to be more common in obligate outcrossers than in self-compatible plants (Knight *et al.* 2005). Pollen limitation is also expected in species with specialised pollinators when the species occur in small populations (Agren 1996; Milberg & Bertilsson 1997; Cheptou & Avendano 2006). However, in some cases, pollen limitation may arise even in large populations, *e.g.* as a consequence of a reduction in abundance of flowerheads by herbivores and in species with generalist pollinators (Pilson 2000 in Liliaceae; Knight 2003 in Asteraceae).

Pollen receipt is not the only factor limiting seed production. Resource availability within a microsite and resource acquisition by a single plant or single inflorescence may also affect seed set (Herrera 1991; Mustajarvi *et al.* 2001; reviewed in Diggle 1997, 2003).

To understand pollinator selection pressures on plant flowering, we need to separate the effect of limited resource availability from the effect of pollen limitation and determine the role of the pollen limitation. However, studies seldom separate these factors (Agren 1996; Pflugshaupt *et al.* 2002; Sandring & Agren 2009). The methodical principle is based on comparing open-pollinated plants with those that receive supplemental hand-pollination. Plants not suffering from pollen limitation do not have extra resources available for maturation of ovules fertilised after supplemental pollen receipt, so their seed set does not differ from that of open-pollinated plants. When plants suffer from pollen limitation, supplemental-pollinated plants have a larger seed set than open-pollinated plants (reviewed in Knight *et al.* 2005).

Pollen limitation combined with pollinator preference exerts selection pressures on flowering traits. Many studies have found significant relationships between attraction of pollinators and floral display, both in field studies (Willson 1979; Ohashi & Yahara 1998) and in manipulation experiments (Andersson 1996; Abraham 2005) for a wide range of plant families (*e.g.* Asteraceae, Malvaceae). Pollinator abundance often varies over the flowering season, and several studies found pollen limitation especially in the later part of the flowering period (Ramsey 1995 in Liliaceae; Santandreu & Lloret 1999 in Ericaceae; Elzinga *et al.* 2007 in Polemoniaceae).

There is an assumption that during the evolution of flowering, plants evolved towards an optimal strategy, in which the costs of attraction balance the benefits for seed maturation. It might thus be expected that no strong selection pressures on flowering traits act at any given time. However, when the long- term balance between plants and their pollinators is disrupted, for example due to habitat fragmentation or species diversity loss, pollen limitation may occur and plant-pollinator interactions may represent strong selection pressures (Ghazoul 2005 in Asteraceae; Steffan-Dewenter & Tscharntke 1999 in Brassicaceae). The aim of this study was to identify selection on traits related to flowering in a rare perennial, allogamous plant species *Scorzonera hispanica* L. (Asteraceae). Narrow specialist plant-pollinator relationships are rare in the Asteraceae, and pollen transfer by generalist pollinators is more common (Ghazoul 2005; Ellis & Johnson 2009). Nevertheless, several studies have found pollen limitation or pollinator-mediated selection pressures in the Asteraceae, Pilson (2000) in *Helianthus annuus* and Cheptou & Avendano (2006) in *Crepis sancta*, but see the experimental studies on *Heterotheca subaxillaris* (Olsen 1997) and *Centaurea scabiosa* (Ehlers 1999).

Scorzonera hispanica in our study area of fragmented dry grasslands typically has a high proportion of aborted seeds. Previous studies in the system demonstrated that the populations are highly genetically variable and that the high proportion of aborted seeds is thus probably not a consequence of inbreeding (Münzbergová & Plačková 2010). These facts raise the question of whether undeveloped seeds within flowerheads are caused through pollen limitation or a lack of resources. Pollen limitation in the system is likely to arise due to strong turnover of land use in the area in the last century, leading to high levels of fragmentation and thus isolation of the single habitats (Chýlová & Münzbergová 2008). Many species, including *S. hispanica*, that are restricted to fragments of dry grasslands are currently rare in the landscape, with only a few populations in the region (Knappová *et al.* 2012). In addition, the selection on flower- ing traits in the system is likely affected by high and variable levels of mammalian herbivory, the abundance of which has recently increased in the area (Hemrová *et al.* 2012). Simultaneously, there is a high variability in plant traits (mainly height and number of flowerheads), which might affect attraction of plants to pollinators. To identify the potential selection pressures in this system, we asked the following questions: (i) is seed set of *S. hispanica* limited by pollen receipt or resource availability; and (ii) what are the criteria for pollinator choice and, consequently, potential selection pressures on species reproductive traits, including floral display and flowering phenology? To answer these questions, we studied the relationship between measured plant traits and pollinator preferences on tagged plants in the field. To estimate the rate of pollen limitation, we compared supplemental-pollinated plants with the open-pollinated ones.

MATERIALS AND METHODS

Study system

The study system is situated in an area of dry grasslands in northern Bohemia, Czech Republic. In the past, the area was covered with a fine-scale mosaic of pastures and fields. At present, large areas of arable fields surround the remaining grasslands. Most of the localities are now abandoned, forming a mosaic of grasslands with expanding shrubs and trees (Chýlová & Münzbergová 2008). The flower stalks of *S. hispanica* are often grazed by ungulates (Hemrová *et al.* 2012).

The study population is situated at Holy vrch, which is a mild, south-facing slope with a mosaic of open grasslands and shrubs, and represents one of the largest populations in the area with *ca.* 1600 flowering individuals. The dry grasslands can be classified as belonging to the Bromion erecti Koch 1926 community (Ellenberg 1988), and are undergoing a slow succesional process towards oak or hornbeam forest (Chýlová & Münzbergová 2008). The locality is seldom visited by people and provides enough space and plant individuals for manipulative experiments.

Scorzonera hispanica L. (Asteraceae) is a perennial herb, the centre of its distribution range being the Iberian Peninsula, with scattered occurrence in dry grasslands in Central and Southern Europe. Occasionally it is cultivated for its edible root and is locally naturalised (Chater 1976). In the study area it is, however, considered native. Rare occurrences of distinct popu- lations in private gardens are possible, but we do not have any information on this.

The plant has a single rosette and one flowering stalk with one to seven yellow flowerheads, opening successively from the uppermost to the lowest. The flowers within flowerheads open from the outer towards the centre over 3–5 days, depending on the weather (personal observation). The flowering period in the study region, northern Bohemia, Czech Republic, is from late May to the beginning of July, with a peak of flowering in June. In the study area, the most common flower visitors are beetles (*Mordellidae, Buprestidae, Dasytidae, Oedemeridae, Cerambycidae* and *Chrysomelidae*) and bees (*Apidae, Megachilidae and Halictidae*; Červenková & Münzbergová personal observations). While many species of the Asteraceae may be autogamous or even apomictic, previous experiments with the studied species indicate no developed seeds arise without pollen transfer. The plant can thus be classified as self-compatible but not capable of spontaneous selfing, so a pollinator is needed in all cases (Banga 1961; Münzbergová & Plačková 2010).

Field experiment

In the peak of the flowering period, from 10 to 18 June 2010, we chose and tagged 204 fertile plants in the same phase of opening of the upper (first opening) flowerhead. A total of 99 plants were monitored in the first run (10–13 June), and a second group of 105 plants was monitored in the second run (14– 18 June). We started the experiment on the first day of opening of the upper flowerheads. Because of the possibility of different fecundity of flowerheads within the plant (personal observation; reviewed in Herrera 1991; Diggle 1997, 2003), we only worked with the upper flowerheads (one flowerhead per plant). For each plant, we recorded height, number of flowerheads, length of the upper flowerhead (measured as length of the whole closed flowerhead including the ovary), number of open flowerheads of *S. hispanica* within 1 m, height (cm) and cover (%) of surrounding herbs within a 0.5-m radius and occurrence of trees or shrubs within a 1.0-m radius. Different radii were chosen for herbs or shrubs and trees because of their different heights and thus different assumed impacts on microclimate, plant performance and pollinator preference. The very rare co-occurrences of a shrub and a tree within the radius around a plant were recorded as ‘tree’. For the number of surrounding open flowerheads, we used the mean number for the whole 3-day period of the observation.

The flowerheads opened every day from about 06:30 to 11:00 h, depending on the weather, which was sunny or partly cloudy throughout the whole experiment. During that time, each flowerhead was observed for pollinator visits every 1.5 h for a total of 30 s (three times per day per flowerhead on average). The number and species (or higher taxonomic group) of visitors were recorded. Flowerhead visitors were identified in the field or collected for later identification. The visitation rate was estimated as the number of observed visitors per 30-s period.

To estimate the impact of pollen limitation, half of the tagged plants were randomly selected for supplemental hand- pollination. The plants received hand-pollination every day of the observation in the period in relation to the highest length and wetness of the stigma, which indicates its receptivity. The pollen was transferred (after the 30 s observation period) to the stigmas using a paintbrush, from at least three pollen donor plants randomly chosen among plants 2–10 m from the focal plant.

The pollinator monitoring and supplemental hand-pollination was repeated every day until all flowers in the flowerheads had withered (3–4 days). At the end of the flowering, the seeds were left to mature and collected 3 weeks later. Thereafter, we recorded the number of developed (visually full) and aborted seeds and the weight of developed seeds per flowerhead. Mean seed weight was estimated on the basis of the number and weight of developed seeds within each flowerhead. When measuring seed weight, we used whole achenes, *i.e.* seeds including carpels (pappus). We were unable to analyse 32 out of the 204 studied plants because there were browsed by ungulate herbivores.

Statistical analyses

The role of single variables in the study system was tested with path analyses (structural equation modelling) using the AMOS 5 software (Small Waters Corp., Chicago, IL, USA). We designed three models for the number of developed seeds, proportion of developed seeds and mean seed weight as dependent variables. The causal relationship among plant height, number of flowerheads, length of upper flowerhead, number of open flowerheads of *S. hispanica* within 1.0 m, height and cover of the surrounding vegetation within a 0.5-m radius, occurrence of trees or shrubs within a 1.0-m radius, insect visitation rate and the supplemental pollination, and plant performance (number of developed seeds, proportion of developed seeds and mean seed weight) were estimated. Simultaneously, vegetation cover, individual plant height and flowerhead number were each affected by a latent variable representing the residual variation. For the structure of models, see Fig. 1. The significance of the relationships was estimated using the generalised least squaresmethod because the residuals were over-dispersed. Length ofthe flowerhead was not significant in any model, so it wasremoved from the final models. The surrounding vegetationheight and vegetation cover were closely correlated. As vegetation cover performed better in the model, we did not incorporate vegetation height into the final models.

Because the model for the number of developed seeds andproportion of developed seeds gave very similar results, onlyresults for the number of developed seeds is shown in theresults. See the Supplementary information for other diagrams showing results for mean seed weight and proportion of developed seeds. In addition to the above-described tests, we performed several other tests. Their results, however, did not differ from the results of the above tests and are thus not presented. Specifically, we included insect visitation rate separately for different pollinator functional groups (Coleoptera + Heteroptera, Hymenoptera and Diptera) in the model. We also performed all the tests only for plants from open grasslands,excluding plants under the woody cover from the data set (64 out of 168 plants were excluded). Finally, we also performed allthe tests separately for the single observation runs and single observation days.

RESULTS

The flowerheads contained from 0 to 90% developed seeds (37 ± 2%, mean ± SE); the remaining the seeds was aborted. No seeds appeared damaged by insect seed predators. In total, 24 different insect species were observed visiting flowers of *S. hispanica* at the locality (Table S1). Because of flowerhead morphology, all the insect species were considered as potentially effective pollinators.

The number of developed seeds per flowerhead and proportion of developed seeds per flowerhead were significantly affected by plant height (Figs 1 and 2) and by the number of flowerheads. Neither the insect visitation rate nor the supplemental pollination had any significant impact on the seed set. However, both plant height and flowerhead number were affected by vegetation cover and the occurrence of trees and shrubs. This might be evidence for a limitation from resource availability rather than from pollen transfer (Fig. 1). The insect visitation rate was negatively affected by the number of sur-rounding flowerheads of *S. hispanica* (Figs 1 and 3). The visitation rate was higher in

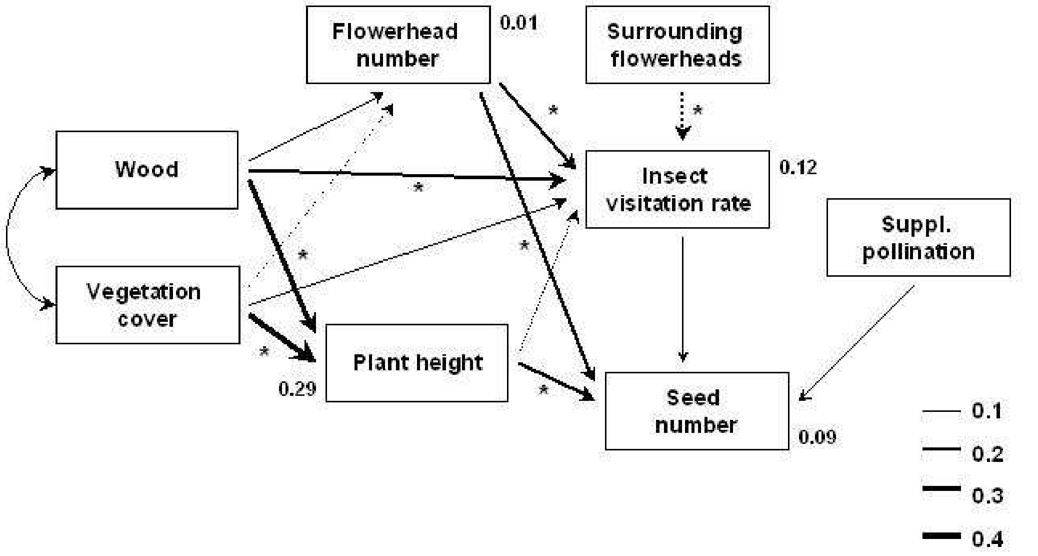

Fig. 1. Path model depicting the hypothesised causal relationships between environmental variables, plant traits, pollen transfer and resulting seednumber. Width of each arrow is proportional to the standardised path coefficients (see legend for scale), and dotted lines indicate negative paths. Asterisks indicate values significantly different from 0 ($P < 0.05$). Numbers in bold are estimates of the proportion of total variance explained by all relationships (squared multiple correlations) for each dependent (endogenous) variable. Variables and paths representing unmeasured residual variation are not included for simplicity.Themodel fit was significant ($\chi 2 = 35.5$, df = 14, P = 0.01)

flowerheads close to a tree. In theproximity of shrubs or in open vegetation, the visitation ratewas lower (Fig. 4).

Insect visitation rate was independent of plant height andslightly positively affected by flowerhead number. The overallmodel with number of developed seeds per flowerhead was significant ($\chi^2 = 35.5$, df = 14, $P = 0.01$). The model explaining theproportion of developed seeds (Figure S1) had a very similar fit ($\chi^2 = 35.6$, df = 14, $P = 0.01$), and causal relationships were thesame, with very similar standardised path coefficients, exceptfor the non-significant impact of the number of flowerheads on the proportion of developed seeds. The mean seed weight per flowerhead was not significantlyaffected by any related variable (Figure S2). As in previous models, plant height and flowerhead number were affected bythe vegetation cover and occurrence of trees and shrubs; moreover, the vegetation cover and occurrence of trees and shrubs were correlated. The causal relationships among independent variables and insect visitation rate were the same as in the previous models. The visitation rate differed between the first and second experimental run, with a 2.2-fold higher visitation rate in the first experimental run ($R^2 = 0.055$, $P < 0.001$; Figure S3).

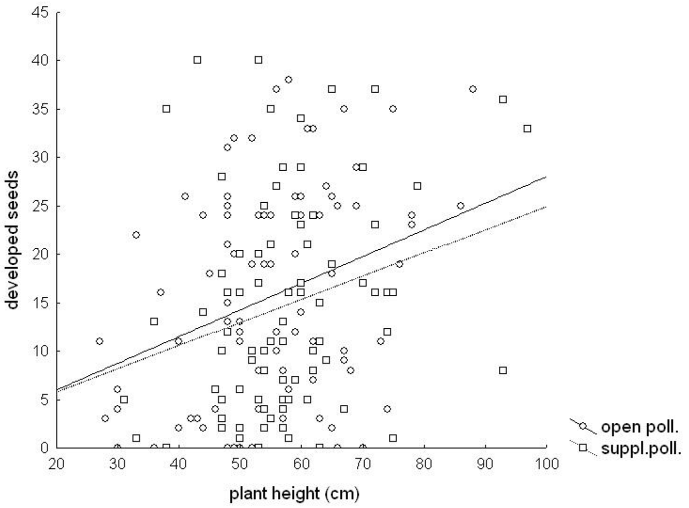

Fig. 2. Impact of plant height on the number of developed seeds per flowerhead. There is no significant difference between open-pollinated and supplementary-pollinated plants.

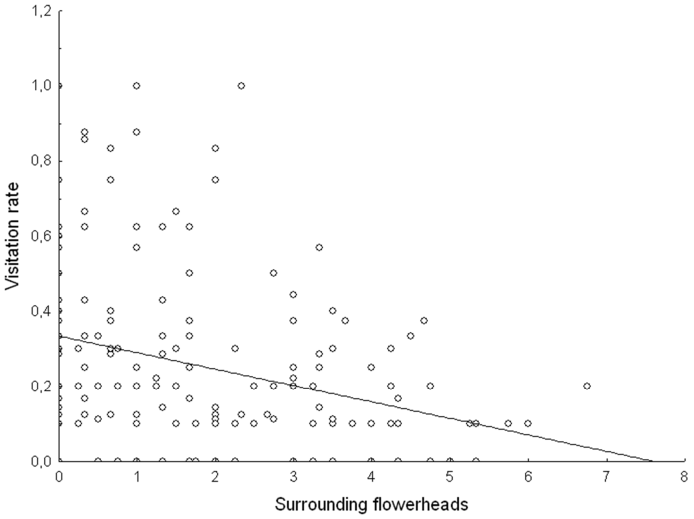

Fig. 3. Impact of surrounding flowerheads (mean number of the open flowerheads within a 1-m radius for the whole 3-day period of the observation) on pollinator visitation rate. The graph shows all the plants in the experiment, even those that were browsed later and then excluded from further analysis.

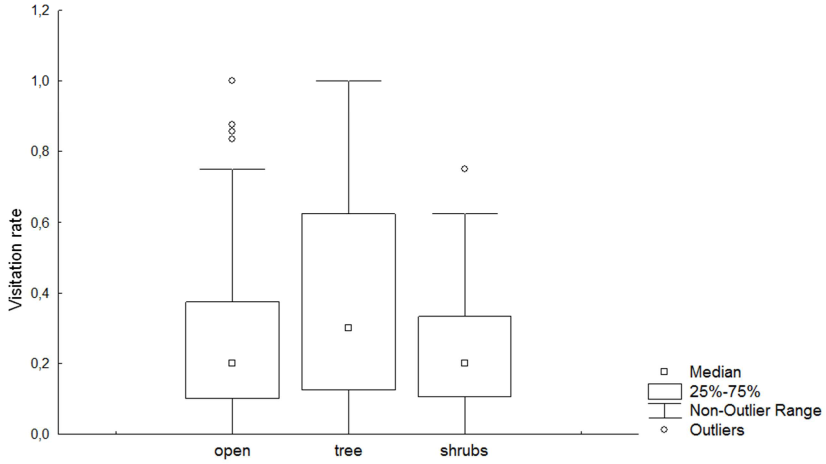

Fig. 4. Impact of the woody cover on insect visitation rate; visitation rate of the flowerheads under trees (n = 27) was higher than in flowerheads under shrubs (n = 44) or in open grassland (n = 133). The visitation rate was estimated as the number of observed visitors per 30-s period. The graph shows all plants in the experiment, even those that were browsed later and then excluded from further analysis.

DISCUSSION

This study demonstrated several significant relationships among the environmental variables, plant traits and seed set. The plant visitation by pollinators was affected by several environmental variables, but pollinators did not have any direct impact on seed set. On the basis of the positive relationship between resource availability (evaluated as surrounding vegetation cover) and plant height and flowerhead number per plant, we suggest that seed set in the system is resource-limited rather than pollen-limited. This conclusion is also supported by the fact that hand-pollination did not increase seed set in *S. hispanica*. Pollinators thus probably do not represent an important selection agent in this system.

The results also suggest that directional selection on theflowering traits does not occur here. *S. hispanica* does not suffer from pollen limitation, and the pollinator interactions with plant traits are rather weak. These results contradict results ofstudies using similar experimental methods to detect pollenlimitation in *Lythrum salicaria* (Lythraceae), *Prunus mahaleb* (Rosaceae) and *Arabidopsis lyrata* (Brassicaceae), respectively(Agren 1996; Pflugshaupt *et al.* 2002; Sandring & Agren 2009). On the contrary, the results are in agreement with Ehlers(1999), who found no pollen limitation in *C. scabiosa* (Asteraceae), or Olsen (1997), who excluded pollen limitation in *Heteroteca subaxillaris* (Asteraceae) using the hand-pollination method. This may indicate that species from the Asteraceae are less likely to be pollen-limited than plants from other families due to their generalist flower morphology.

Pollen limitation is less common in self-compatible speciesthan in species that are self-incompatible (Mustajarvi *et al.* 2001). Milberg & Bertilsson (1997), however, confirmed pollenlimitation even in a self-compatible species. *S. hispanica* is self-compatible but it is not capable of spontaneous self-pollination, so pollinators are required for successful selfing. Pollen limitation could thus theoretically also occur in the self-compatible *S. hispanica*. Reasons for the lack of such limitationare discussed below.The diversity of pollinators in our system was quite high. Franzen & Larsson (2009) found opposite impact of different groups of pollinators on *Knautia arvensis* (Dipsacaceae); therefore we also separately tested the effect of each group of pollinators on *S. hispanica*, but no difference between pollinator groups was detected. As stated above, this may be related to the generalist flower morphology within the Asteraceae.

In spite of the failure to detect pollinator limitation in thesystem, varying abundance of flower visitors was observed during the experiment. Several studies have shown that pollenlimitation varies within a season, being highest either at the beginning (Ramsey 1995) or at the end of the flowering period (Widen 1991; O'Neil 1999; Santandreu & Lloret 1999; Elzinga*et al.* 2007; Weber & Kolb 2011). This variation is likely because of variations in pollinator abundance, abundance ofco-flowering species or abundance of antagonists (Ehrlén & Münzbergová 2009). Although the number of pollinators inour study decreased during the season, we do not considerselection for earlier flowering because pollinators do not seem to be a limiting factor in the system.In our system, pollinators responded to the surrounding environment (*i.e.* the surrounding flowerheads or surrounding vegetation cover) rather than to the traits of the plants. The pollinator preference of more isolated plants with less sur-rounding flowerheads contrasted with similar studies thatfound an opposite pattern (Caruso 2002; Torang *et al.* 2006). However, our conclusion concerning the importance of sur- rounding vegetation for the pollinator visitation rate is congru- ent with conclusions in the review of Ghazoul (2005): that in areas with a lower density of flowerheads, pollinators spend more time on a single plant, and therefore the probability of recording the pollinator on a flowerhead is higher. In addition, the longer time spent on an individual flowerhead may translate into the possibility of increased pollen removal (Harder 1990; Neff & Simpson 1990), more pollen deposition on stigmas and/or more florets per flowerhead being successfully pollinated (Neff & Simpson 1990).

The absence of pollen limitation and pollinator-mediated selection pressure could indicate a balance in the plant–pollinator system at the locality studied. This explanation is tenable because the locality is still rather undisturbed, large and species-rich. However, the changes in the surrounding landscape are striking: an expanding urbanised area, development of photovoltaic plants, changes in the amount of intensively Pollination in *S. hispanica* managed fields and succession in abandoned places (see also Chýlová & Münzbergová 2008; Knappová *et al.* 2012). These processes drive changes in the ambient environment and population dynamics at many localities of *S. hispanica* and also present a serious also for the system at the locality. On the other hand, the absence of pollen limitation and pollinator-mediated selection could also be caused by a coincidence of mutualistic and antagonistic animal–plant interactions in the system, as the plants are heavily grazed by ungulates (Hemrová *et al.* 2012).

Opposing selection from mutualists and antagonists has been found, by Ehrlén *et al.* (2012). According to their study, some plant traits, including inflorescence height, can influence the total seed production both positively and negatively via pollina- tor and seed predator preferences. In another study, on *Erysimum mediohispanicum* (Cruciferae) grazed by ibex, a significant selection on flowering traits (e.g. flower number, plant height, petal length) was observed when the grazing ungulates were absent. When the ungulates were present, selection on floral traits completely disappeared (Gomez 2003). In our study system, the rate of ungulate herbivory (mainly by roe deer) is high. The role of pollinators could thus theoretically change between localities with different herbivore pressure. Vanhoenacker *et al.* (2013) suggest a decreasing role of selection mediated by pollinators with an increasing intensity of interaction, whereas selection mediated by antagonists increases together with the intensity of the interaction. According to this study, corroborated in the results of Hemrová *et al.* (2012), who found a rate of herbivory between 40 and 100% among localities of *S. hispanica*, we suppose that pollen limitation or pollinator-mediated pressure occurs in less browsed localities. A follow-up study at the landscape level would be needed to explore this.

The high proportion of undeveloped seeds in our study can most likely be explained by limited resource acquisition of the plant. This finding is supported by the significant relationship between the number of developed seeds and maternal plant height, and also between mean seed weight and flowerhead length in this study (Herrera 1991 for similar findings). In addition, Münzbergová & Plačková (2010) demonstrated, in the same system, that seed weight in *S. hispanica* was significantly affected by habitat conditions, while Münzbergová (2006) demonstrated that seed number increased with site productivity. This expectation is also confirmed in the significant decrease in seed number and mean seed weight per flowerhead from the uppermost to the lower flowerheads (personal observation). In general, our results suggest that *S. hispanica* does not experience any selection pressure on the part of pollinators. Resource limitation is likely stronger than pollen limitation in this system. The realized preferences can hardly cause any selection pressure because pollinator choice was not affected by plant traits, but simply by the ambient environment. This situation is not static, however; any shift in population density, the rate of herbivory or the slightest change in landscape dynamics can disrupt the present functioning of the system.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

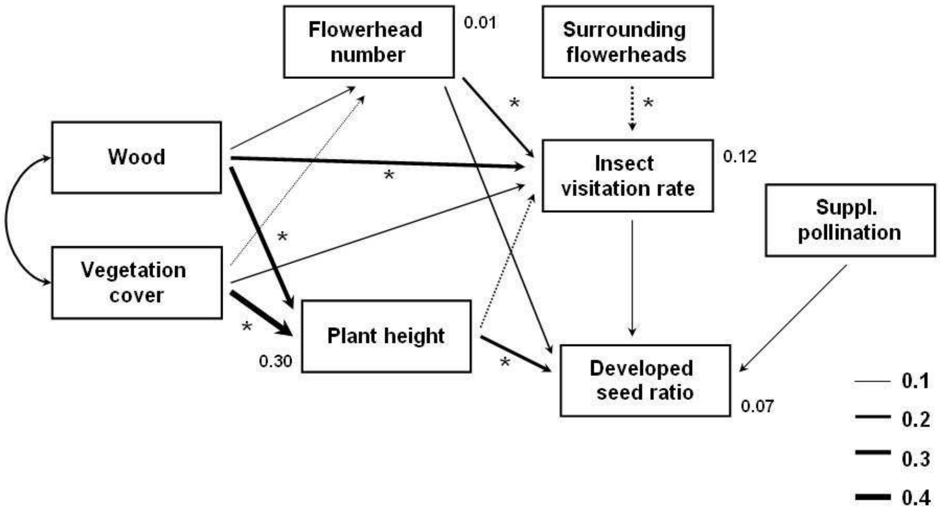

Fig. S1. Path model depicting the hypothesised causal relationships between environmental variables, plant traits, pollen transfer and resulting developed seed ratio.

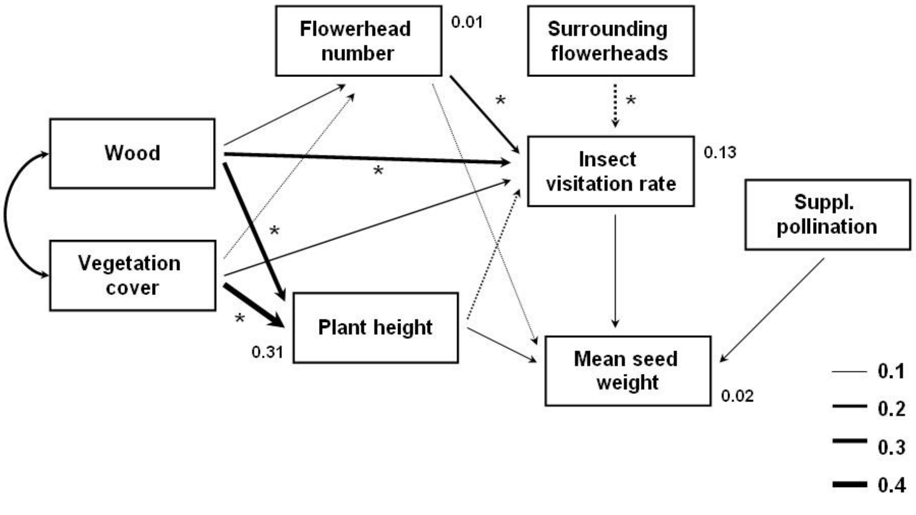

Fig. S2. Path model depicting the hypothesised causal relationships between environmental variables, plant traits, pollen transfer and resulting mean seed weight.

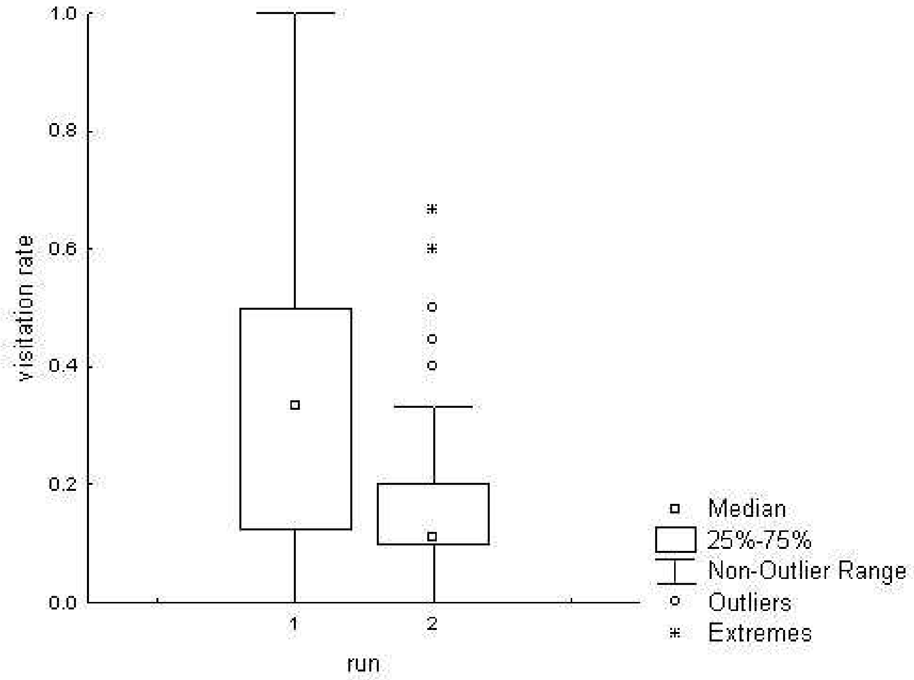

Fig. S3. Comparison of insect visitation rate between the two observation runs.

Tab. S1. List of the natural floral visitors on S. hispanica, showing the number and capture date of the determined individuals.

Family	Species	No. specimens and date captured
Heteroptera: Rhopalidae	*Stictopleurus punctatonervos* (Goeze, 1778)	1 – 10/6/2010
Coleoptera: Buprestidae	*Anthaxia* (s.str.) *nitidula* (Linnaeus, 1758)	3 – 10/6/2010
Coleoptera: Nitidulidae	*Meligethes aeneus* (Fabricius, 1775)	1 – 18/6/2010
Coleoptera: Mordellidae	*Mordellistena* (s.str.) *secreta* Horák	1 male – 10/6/2010
	Mordellistena (s.str.) *kraatzi* Emery	1 male – 15/6/2010
Coleoptera: Dasytidae	*Dasytes* sp.	1 – 15/6/2010
	Dasytes sp.	1 – 10/6/2010
Coleoptera: Oedemeridae	*Oedemera podagrariae* (Linnaeus, 1758)	1 – 10/6/2010
	Oedemera podagrariae (Linnaeus, 1758)	2 – 15/6/2010
	Oedemera podagrariae (Linnaeus, 1758)	2 – 18/6/2010
	Oedemera lurida (Marsham, 1802)	2 – 10/6/2010
Coleoptera: Cerambycidae	*Stenopterus rufus* (Linnaeus, 1767)	1 – 15/6/2010
	Stenurella melanura (Linnaeus, 1758)	1 – 15/6/2010
	Stenurella melanura (Linnaeus, 1758)	1 – 18/6/2010
	Pachytodes erraticus (Dalman,1817)	1 – 15/6/2010
Coleoptera: Chrysomelidae	*Cryptocephalus violaceus* Laicharting, 1781	2 – 15/6/2010
	Cryptocephalus sericeus (Linnaeus, 1758)	1 – 15/6/2010
	Cryptocephalus hypochaeridris (Linnaeus, 1758)	1 – 10/6/2010
Diptera: Tephritidae	*Myopites inul*	1 – 10/6/2010
		1 – 15/6/2010
Diptera: Ulidiidae	Sp.	1 – 10/6/2010
Lepidoptera: Coleophoridae	Sp.	1 – 10/6/2010
Hymenoptera: Megachilidae	*Hoplosmia spinulosa*	2 male – 10/6/2010
	Neosmia bicolor	1 – 15/6/2010
Hymenoptera: Halictidae	*Lasioglossum puncticolle*	2 – 15/6/2010

CHAPTER 3

Effect of ungulate herbivory on reproduction of *Scorzonera hispanica*

Zita Červenková & Zuzana Münzbergová

ABSTRACT

Of the great number of studies dealing with plant-herbivore interactions, only very few have collected detailed data on the timing and intensity of herbivory in connection to plant fitness. Our knowledge of the criteria for herbivore choice and the effects of herbivory of varying intensity on plant performance over time is thus still very limited. The aim of this study was to assess the relationship between plant flowering traits, herbivore choice, and the intensity and timing of herbivory and plant reproduction. Herbivores consistently preferred plants growing in denser vegetation and with a greater number of flower buds whereas plant height was not a significant factor. The effect of herbivory on seed production was quite high, whilst flowering in the following season was only weakly affected by the timing and intensity of browsing. Resource limitation was thus probably a more important factor affecting plant performance in the next season than herbivory. Still, herbivory seems to exert consistent selection pressure on plant flowering traits via seed production.

INTRODUCTION

Plant-animal interactions represent one of the key drivers of the performance of many plant species. These interactions may include interactions such as pollination, seed dispersal or herbivory. Herbivory – in contrary to other interactions – usually has negative effects on plant performance (Belsky 1986, Bergelson & Crawley 1992, Hawkes & Sullivan 2001, Russell et al. 2001, Maron & Crone 2006). The need to compensate for tissue damage and ensuing decreased fitness exerts selection pressures on the development of tolerance or avoidance strategies.

Herbivory can interfere with the life cycle through a wide range of mechanisms. A reduction of the number of seeds is usually considered having the strongest direct effect on fitness (Augustine & Frelich 1998, Maron & Crone 2006, Lin & Galloway 2009, Jacquemyn et al. 2012). Other types of herbivory can affect seed germination via seed quality (Aikens & Roach 2015) or seedling survival by damaging seeds in the seed bank (McKenna & McKenna 2006) or seedlings (Gomez 2005, Becerra & Bustamante 2008). Herbivores feeding on plant parts in perennial species may strongly affect the transition to the fertile life stage or survival to the next season by reducing the resources available to the plant (Knight 2003, Ehrlén & Münzbergová 2009) . The reduction of available resources and the ability of the plant to compensate for the tissue lost are affected by the timing (García & Ehrlén 2002, Obeso 2002, Knight 2007) as well as the intensity of the interaction (Knight 2003, Leimu & Lehtila 2006). In spite of this, most studies looking at plant-herbivore interactions census plants only once a year (Gomez et al. 2009, Koh et al. 2010, Fujita & Koda 2015) , and our knowledge of the effects of timing, intensity of herbivory and interaction of these factors is thus limited. Nevertheless, details, such as the timing or rate of herbivory within each flowering individual, can be important for characterizing the interaction or assessing its effect.

The timing and intensity of herbivory differs between vertebrate and invertebrate herbivores. In contrast to vertebrate herbivores, insect herbivores are present only for a limited part of the field season (Elzinga et al. 2007, Münzbergová et al. 2015), and their occurrence is often correlated with plant flowering phenology. Studies on vertebrate herbivory usually focus on large (ungulate) herbivores, be it browsers or grazers, and demonstrate that vertebrate herbivory is more consistent over time and space and that its impact is usually higher (Gomez et al. 2009). Several studies also demonstrate that flowering individuals are more commonly selected by vertebrate herbivores than non-flowering ones (Ehrlén 1997, Augustine & Frelich 1998, Lin & Galloway 2009, Davalos et al. 2014).

Browsers selection of flowering plants within a locality may be affected by a wide range of characteristics of the plants as well as of their surroundings. Several studies have found found that herbivores prefer earlier-flowering plants, which causes a shift in the timing of the flowering peak and may lead to pollen limitation of the plants (Widén 1991, Vazquez & Simberloff 2004, Elzinga et al. 2007, Ehrlén & Münzbergová 2009). Other studies have shown that herbivores prefer taller, more vigorous plants (Freeman et al. 2003, Koh et al. 2010, Fujita & Koda 2015, Prendeville et al. 2015). Therefore, simple comparisons of browsed and untouched plants may lead to seemingly higher fitness of plants damaged by herbivores (Freeman et al. 2003). On the other hand, in the case of overcompensation, a herbivore attack can actually enhance at least some components of fitness, as shown in several studies (Paige & Whitham 1987, Lennartsson et al. 1998, Nilsen et al. 2004, West 2012, Cozzolino et al. 2015). Moreover, more detailed studies at the population level enable us to estimate the secondary effects of herbivory, as it often interacts with other biotic or abiotic factors such as plant diseases (Ericson & Wennstrom 1997) parasites (Puustinen & Mutikainen 2001) and pollinators (Widén 1991, Vazquez & Simberloff 2004, Elzinga et al. 2007, Ehrlén & Münzbergová 2009). To assess the role of herbivory in the complex net of relationships occurring during the life of plants and to ascertain the strength and directions of potential selection pressures, we need to take into account all the possible aspects that enter every plant-herbivore interaction, such as plant traits and the timing and rate of herbivory.

In previous studies, we observed a hight rate of ungulate herbivory in most populations of our model species *Scorzonera hispanica*. Herbivory is almost exclusively restricted to flowering individuals from the emergence of the flowering stalk to the wilting of the flowerheads, and it also strongly negatively affects seed production (Hemrová et al. 2012). From the perspective of population dynamics, however, it is also important to deal with other components of fitness, such as survival and the probability of flowering in the next year. Another potential factor affecting reproduction is infestation by the smut fungus *Ustilago scorzonerae* (Ustilaginales). Plants within a population also differ in their flowering traits, which can affect fitness components both directly and indirectly as a herbivore preferences criterion.

The goal of our recent study was to identify the factors affecting herbivore choice and the impact of herbivory on plant reproduction within a population. The main questions we attempted to address were: (1) Which plant flowering traits affect herbivore choice? (2) What is the impact of herbivore attack, including its timing and strength, on the individual plant reproduction? (3) How are plant flowering traits, herbivory and incidence of smut related to the reproductive cycle?

By monitoring plant traits of single plants over three years, we identified the factors affecting the probability of herbivore attack and its impact on future prospect of individual plants as to seed production and flowering in the next season. Thanks to the frequent monitoring of plant growth, we are able to assess the effect of timing and proportion of herbivory within each plant. Such frequent censuses in connection with both criteria of herbivore choice and impact of herbivore attack on plant performance are very rarely included in population studies. By comparing the various fitness components of browsed and untouched plants, we were able to estimate the relationships between herbivory and reproductive traits and to reveal the possible selection pressures imposed by large herbivores.

MATERIALS AND METHODS

Study system

Scorzonera hispanica L. (Asteraceae) is a perennial allogamous herb with a scattered distribution in dry grasslands in Central and Southern Europe and frequently occurring in Iberian Peninsula. Occasionally it is cultivated for its edible rootstock and is locally naturalized. In the study area it is, however, considered native (Chater 1976).

The plant has a single rosette and one 20 - 80 cm tall flowering stalk with one to seven yellow flowerheads. The flowering period in the study region, northern Bohemia, Czech Republic lasts from late May to the beginning of July with a peak of flowering in June.

The study system is situated in an area of dry grasslands in northern Bohemia, Czech Republic. In the past, the area was covered with a fine scale mosaic of pastures and fields that were probably largely interconnected by grazing animals. At present, large areas of arable fields surround the remaining grasslands. Most of the localities are abandoned at present, formed by mosaic of grasslands and expanding shrubs and trees (Chýlová & Münzbergová 2008). The flowering stalks of *Scorzonera hispanica* are often browsed by ungulates (Hemrová et al. 2012). No other type of herbivory has been observed. Roe deer, mouflon and wild boars are very common in the landscape, whereas fallow deer and red deer occur only rarely (Municipality Litoměřice, Department of Environment).

The study population is situated at locality Holy vrch. The locality is a south-facing slope with mosaic of open grasslands and shrubs, and represents one of the largest populations in the area with about 1600 flowering individuals. The population suffers from the relative high herbivory rate (Hemrová et al. 2012) and some plants in the population are infected by smut fungus *Ustilago scorzonerae* (Ustilaginales). The locality is seldom visited by people and provides enough space and plant individuals for manipulative experiments. For more detailed description of the locality, see Münzbergová (2006).

Field experiment

To observe performance of individual plants in response to browsing, we established 2 transects with tagged plants at the locality in 2008. Transects were 10 and 27 meters long and each consisted of more than 200 tagged plants. We tagged all the flowering plants within 90 cm distance from the transect and monitored them for 3 ongoing years 2008 - 2010. If a new flowering plant occurred in the monitored area in the next seasons, we add the tag at the beginning of the season. The plants transitioned to vegetative stage were not monitored in a given year, as we found only negligible herbivore damage on them. The survival of all plants was closed to 100 % for all 3 years. We started the monitoring at the moment when flowering stalks were visible (about May 15th). For each plant we recorded number of leaves, stalk height, number of flower buds and height and cover of the surrounding vegetation within 0.5 m and the occurrence of trees or shrubs within 1 m radius during the first census. The plants were then monitored every 5-7 days. In each following census we recorded stalk height, number of flower buds, number of open flowerheads, and incidence of smut and herbivory. At the end of the flowering period, the seeds were left to mature and seeds from each flower head were collected in a separate paper bag. Thereafter, we recorded the number of developed (visually full) and the mean seed weight of the developed seeds per plant. The timing of herbivory was counted as a number of days from the first census to the census, when the incidence of herbivory was recognized. The proportion of browsing was estimated on the basis of difference between the stalk height before and after herbivore attack. The incidence of herbivory was factorial variable (browsed / untouched plant). The number of monitored flowering plants was 398 in 2008, 134 plants in 2009 and 200 plants in 2010.

Statistical analyses

The role of the single variables in the study system was tested by path analyses (structural equation modelling) using the AMOS 5 software package (Small Waters Corporation, Chicago, IL, USA). We designed three models for the number of the developed seeds and the flowering in the next season as the dependent variables. The causal relationship among the initial plant height, initial number of flower buds, initial number of leaves and cover and height of the surrounding vegetation within 0.5 m radius, the incidence of the smut fungus and the incidence or timing of herbivory or proportion of browsing were estimated. Simultaneously, the vegetation cover, initial plant height and the flower bud number were affected by a latent variable 'resource availability'. As the variables timing of herbivory and proportion of browsing did not perform well in the analysis, we created a combined variable “degree of herbivory”. Degree of herbivory was estimated as an interaction of centred variables timing of herbivory and the proportion of browsing. It should describe the rate of damage, which the plant suffered. In the earlier stages the flower buds are usually concentrated on the top of the flowering stalk, which is often cut by the herbivore; therefore the entire flowering possibility for appropriate season can be destroyed. On the other hand, the later herbivory is related to higher energy investment in reproductive tissues. All the three variables – the original timing of herbivory and the proportion of browsed part and their centred interaction – were incorporated in the models. In the result diagrams we show the combined variable only. To estimate the impact of the incidence of herbivory and the degree of herbivory separately, we performed 2 sets of analyses. We performed the partial analyses for each year and summary analyses combining data from all the 3 years. In results we present only four total models combining data from all the 3 years. For the results of all partial analyses for single years (standardized path coefficients, significance and explained variance), see the Supporting information. The structure of the models was the same, except the dependent variable and factor of herbivory (see Figure 1-4). In total we performed 14 models (see the tables 1 – 8 in Supporting data). The significance of the relationships was estimated using Generalized Least Squares method, since the residuals were overdispersed. Number of leaves was not significant in any of the models, so it was removed from the final models. The surrounding vegetation height and the vegetation cover were closely correlated. As the vegetation cover performed better in the models, we did not incorporate the vegetation height into the models.

Moreover, we performed the same sets of path analysis with dependent variable “mean seed mass”. As there was no significant relationship between mean seed mass and incidence or degree of herbivory, we do not present the results of this part of analysis.

RESULTS

The rate of herbivory strongly varied between years (374 grazed plants out of 398 (93 %) in 2008, 114 out of 134 (85 %) in 2009 and 103 out of 200 (53 %) in 2010). Average rate of herbivory over all 3 years was 78.2 %. All summary models showed strong positive relationship between vegetation cover and initial stalk height and no effect of presence smut on any dependent variable (Fig 1-4). Herbivory was more common in denser vegetation (Fig 5) and in plants with more flower buds (Fig. 1, 3). The seed number was always affected by herbivory – both by its presence as well as degree (Fig 1, 2). Flowering in the next season was positively affected only by the initial stalk height; no effect of herbivory was found (Fig 3, 4).

The first model explaining the number of developed seeds per plant in relation to herbivore attack showed that the browsers preferred plants with higher initial number of flower buds and plants surrounded by denser vegetation (Fig.1, Fig. 5). Initial stalk height of the plants was higher in denser vegetation. The browsed plants produced significantly lower number of developed seeds (Fig 1, Fig. 6). The number of developed seeds was also positively affected by the initial number of flower buds (Fig 1). The effect of vegetation cover on herbivore attack and the effect of herbivore attack and the initial flower bud number on seed number were consistent in its direction and significance over all three years. The overall model with number of developed seeds per plant was significant ($\chi^2 = 163.7$, df = 6, $p < 0.001$).

The second model explaining the flowering in the next season in relation to herbivore attack showed very similar relations among vegetation cover, initial stalk height and initial flower bud number and herbivory as the first model (Fig. 3). However, flowering in the next season was not significantly affected by herbivory (nor in the partial models, see the supporting information), but it was positively correlated just with the initial stalk height. Only the effect of vegetation cover on herbivore attack was significant in both years. The effect of herbivory on flowering in the next year was never significant. The overall model with flowering in the next season was significant ($\chi^2 = 218.9$, df = 11, $p < 0.001$).

In the third model the degree of herbivory was negatively affected by the initial bud number and affected the seed number negatively (Fig.2). Vegetation cover had a significant impact on all the three variables – initial flower bud number (neg.), initial stalk height and degree of herbivory (pos.). The relationships in the models differed between single years in their direction or significance. The overall model corresponds best with the model from the season 2008. The model from the third season (2010) did not show any significant relationships. The overall model with number of developed seeds per plant was significant ($\chi^2 = 219.6$, df = 6, $p < 0.001$).

The fourth model showed weak positive significant impact of the degree of herbivory on the flowering in the next season. The initial flower bud number affected the degree of herbivory negatively. The initial stalk height positively affected flowering in the next season (Fig 4). The relationships in the models differed between single years in their direction or significance. The overall model corresponds better with the model from the season 2008. The overall model with flowering in the next season was significant (χ^2 = 165.2, df = 11, $p < 0.001$).

The relationships in the models differed between years. The overall models corresponded best with the model from 2008, however the number of the significant relationships in the overall models were lower.

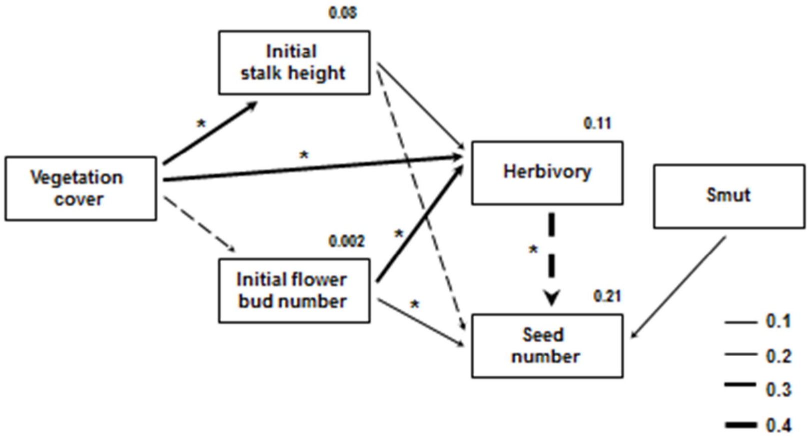

Fig. 1. Path model depicting the hypothesized causal relationships between environmental variables, plant traits, incidence of herbivory and resulting seed number. Width of each arrow is proportional to the standardized path coefficients (see legend for scale), and dashed lines indicate negative paths. Asterisks indicate values significantly different from 0 (P < 0.05). Numbers in bold are estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable. Variables and paths representing unmeasured residual variation are not shown for simplicity.

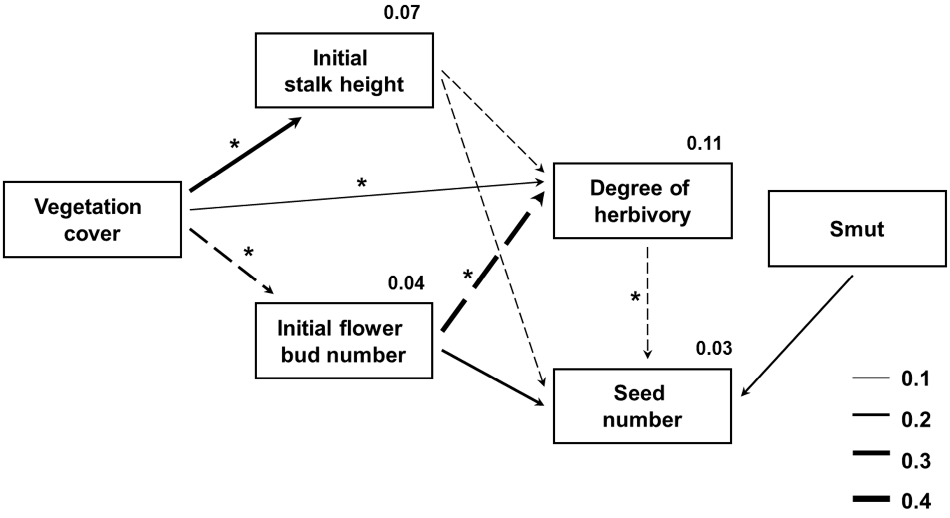

Fig. 2. Path model depicting the hypothesized causal relationships between environmental variables, plant traits, degree of herbivory and resulting seed number. Width of each arrow is proportional to the standardized path coefficients (see legend for scale), and dashed lines indicate negative paths. Asterisks indicate values significantly different from 0 (P < 0.05). Numbers in bold are estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable. Variables and paths representing unmeasured residual variation are not shown for simplicity.

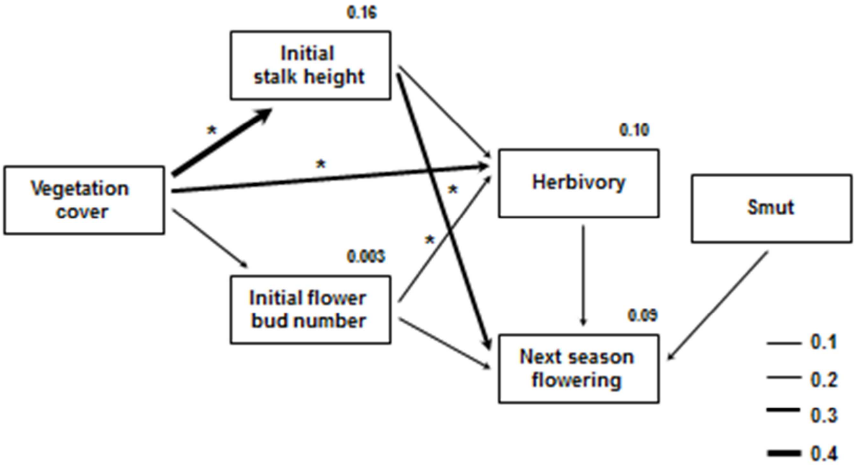

Fig. 3. Path model depicting the hypothesized causal relationships between environmental variables, plant traits, incidence of herbivory and flowering in the nest season. Width of each arrow is proportional to the standardized path coefficients (see legend for scale), and dashed lines indicate negative paths. Asterisks indicate values significantly different from 0 (P < 0.05). Numbers in bold are estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable. Variables and paths representing unmeasured residual variation are not shown for simplicity.

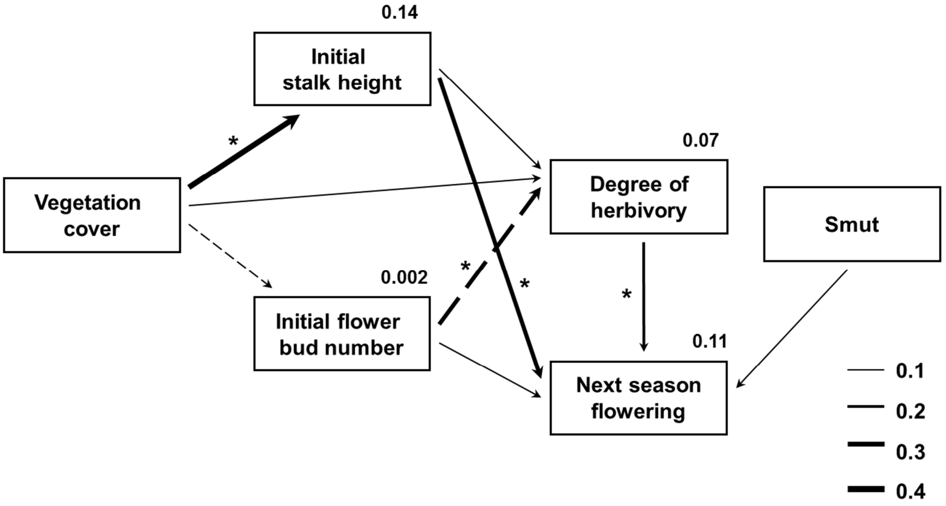

Fig. 4. Path model depicting the hypothesized causal relationships between environmental variables, plant traits, degree of herbivory and flowering in the nest season. Width of each arrow is proportional to the standardized path coefficients (see legend for scale), and dashed lines indicate negative paths. Asterisks indicate values significantly different from 0 (P < 0.05). Numbers in bold are estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable. Variables and paths representing unmeasured residual variation are not shown for simplicity.

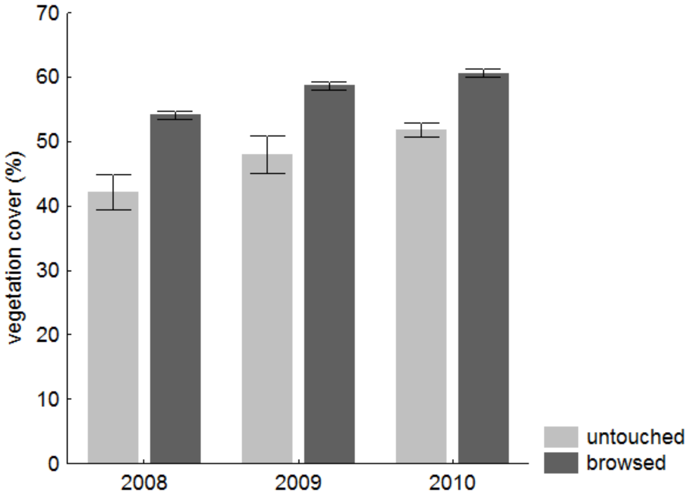

Fig. 5. The impact of surrounding vegetation cover on the rate of herbivory separately for each year. Mean + SE

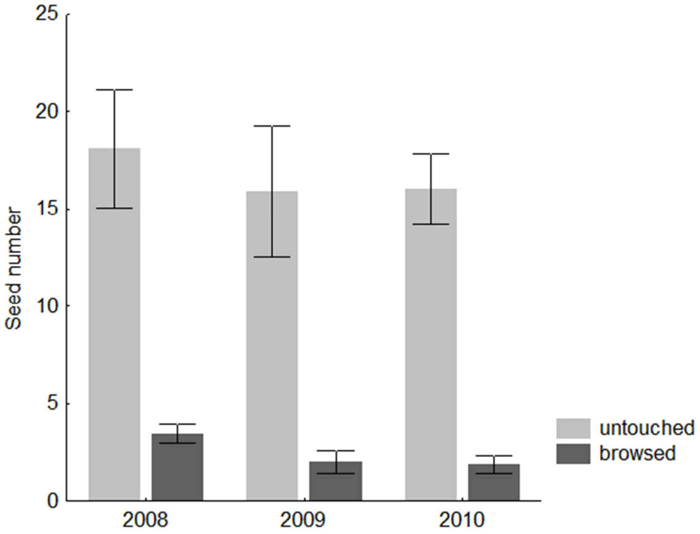

Fig. 6. Variability in number of developed seeds in untouched and browsed plants separately for each year. Mean + SE

DISCUSSION

Ungulate herbivory strongly influences the reproduction of *S. hispanica*, especially by decreasing the seed set of fertile plants. Thus, herbivores exert a selection pressure on the plant life strategy through seed production. By contrast, flowering in the next season was mainly affected by resource availability within the microsite. There was no significant relationship with the incidence of herbivory. However, in browsed plants, flowering in the following season was affected by the timing and proportion of browsing.

Several studies have found a negative effect of herbivory (presence/absence) on flowering probability in the next season (Knight 2003, Ehrlén & Münzbergová 2009, Brys et al. 2011), and some, such as Augustine and Frelich (1998) and our present study have not. The weak positive effect of the degree of herbivory in our study means plants browsed earlier during the growing season and to a higher proportion were more likely to flower in the next season. This result is the opposite of that reported, for example, by Knight (2003), who found a negative effect of early timing of herbivory on the probability of flowering in the next season. The most likely mechanism is that earlier and more seriously damaged plants can save resources to the next season better than plants which were damaged later, often with already developed flowers or almost matured seeds. Browsing later during the flowering season affects a smaller fraction of the flowering stalk but leads to higher energy loss.

The timing and proportion of browsing had the opposite effect on seed number. Plants browsed early often lost all their adventive flowerbuds and thereby all means of compensation. Those that were browsed later, and therefore lost of one or more flowerheads, were sometimes able to compensate from the remaining adventive buds.

Herbivory can be expected to significantly affect plant survival (Ehrlén 2003). The effect of herbivory on the survival of adult plants could, however, not be tested because of very low numbers of dead individuals during the study. We suppose that herbivory is not the main driver of the survival (see also Puentes & Agren 2012, Lehndal & Agren 2015) and that survival is mainly affected by disturbances such as random activity of wild boars or changes in the landscape (MacDonald & Kotanen 2010, Hemrová et al. 2012).

There was no relationship between herbivory and mean seed mass per plant, in accordance with some other studies (Meyer 2000, Pilson & Decker 2002). This result is supported by our comparison of browsed plants and control plants protected from browsing (using cages) within the same locality in the same year. There was also no significant difference in mean seed mass between the sets of plants (Cervenkova, unpublished results).

In the sense of seed number, the difference in reproductive success between browsed and untouched plants was striking. One of the factors enhancing the probability of herbivore damage was higher initial flower bud number. On the other hand, the initial flower bud number directly positively affected the number of seeds. Therefore, we suppose that herbivores preferred more fertile plants. So, this estimate of herbivore impact can be biased in this aspect, like in many natural populations (Stowe et al. 2000). However, thanks to path analyses, we were able to reveal the part of variability in seed set explained by the initial number of flower buds.

Our continuous three-year study allowed us tu identify certain consistent criteria of herbivore choice. Surprisingly, initial plant height did not have any impact on herbivore's choice, in contrast to what has been found in other species (Freeman et al. 2003, Fujita & Koda 2015, Prendeville et al. 2015). Initial plant height was strongly positively affected by the vegetation cover, pointing to a relationship with resource availability. Taller plants tended to flower more often in the following season (e.g. Ehrlén & Van Groenendael

2001, or Andrieu et al. 2007), but see Pfeifer et al. (2006) or Min (2014) for opposite results. Initial plant height could also be connected with flowering phenology, which has also been shown as a criterion for choice of plants by large herbivores (Knight et al. 2008). Flower bud number and plant height were correlated with each other in the model. We suppose that both variables reflect the condition of the plant, but in a slightly different direction and with different consequences.

Resource limitation within the micro-site is probably highly important in this species. This factor played the key role even in the previous study at the same locality, which dealt with the impact of pollinators on the life cycle. We found no pollen limitation in this species. The seed set was related only to plant height and flowerhead number (Červenková & Münzbergová 2014). Resource limitation was previously shown to interact with herbivory in other species in both directions – in the sense of weakening or strengthening the herbivore effects (Hawkes & Sullivan 2001, Fornoni 2011, Salgado-Luarte & Gianoli 2012, Davalos et al. 2014). In our study, the resource limitation – connected with overall plant condition – seems to be the main driver of flowering in the next season. We derived this fact from the strong positive relationships between “vegetation cover”, “initial stalk height” and “next season flowering” in the appropriate models.

The high importance of resource limitation in the life cycle of *S. hispanica*, together with the low tolerance to herbivory, shows low level of adaptation to ungulate herbivory. This contradicts studies on perennial (Paige & Whitham 1987) as well as in monocarpic species (Lennartsson et al. 1998), showing a high level of compensation. In the previous study, we found that the rate of herbivory strongly varied among localities (Hemrová et al. 2012) and simultaneously varied over time. This high variability of the intensity of disturbance may not allow the evolution of the adaptation and may lead to the higher phenotypic plasticity. Simultaneously, different responses to herbivory across the population have already been found in several studies (Banga 1961, Brody 1997, Prendeville et al. 2015). However, Prendeville (2015) found the local-scale factors of herbivory to be more important than the landscape-scale factors.

The incidence as well as effects of herbivory has also been shown to vary from year to year. Inter-annual variability is common in long-term studies (Doak 1992, Ehrlén 2003), leading to opposing selection on flowering traits in different years (Dominguez & Dirzo 1995). However, differences in the directions and strengths of the effects could be related to the varying proportion of flowering plants between seasons.

In our case study, herbivory varied strongly among years even though herbivore preferences were consistent. The impact of herbivory on seed production was quite high, while the overall performance in the next season was affected rather weakly. Resource limitation probably plays a more important role in affecting flowering in the following season.

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SUPPORTING INFORMATION

		2008		2009		2010		overall	
dependent variable	independent variable	Estimate	P	Estimate	P	Estimate	P	Estimate	P
bud number	veg.cover	0.065	0.392	-0.177	0.169	**0.394**	**0.005**	-0.049	0.415
init.height	veg.cover	**0.384**	***	-0.054	0.669	**0.451**	***	**0.277**	***
attacked	bud number	**0.177**	**0.021**	0.088	0.483	0.01	0.948	**0.228**	***
attacked	veg.cover	**0.197**	**0.001**	**0.51**	***	**0.453**	**0.001**	**0.243**	***
attacked	init.height	0.005	0.947	**0.312**	**0.008**	0.05	0.738	0.006	0.926
seed number	init.height	0.002	0.98	0.181	0.103	-0.042	0.617	-0.007	0.899
seed number	smut	0.074	0.153	0.076	0.429	-0.086	0.161	0.029	0.391
seed number	bud number	**0.179**	**0.007**	**-0.239**	**0.029**	**0.212**	**0.012**	**0.126**	**0.019**
seed number	attacked	**-0.341**	***	**-0.443**	***	**-0.509**	***	**-0.465**	***

Tab. S1: Standardized path coefficients and p-values for the single relationships in the partial path analyses testing the effect of herbivore attack on the seed number.

	2008	2009	2010	overall
init.height	0.148	0.003	0.203	0.077
bud number	0.004	0.031	0.155	0.002
attacked	0.076	0.333	0.232	0.106
seed number	0.13	0.245	0.279	0.208
$\chi2$	148.9	65.3	82.0	256.8

Tab. S2: Estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable and Chi-square for each partial path analysis testing the effect of herbivore attack on the seed number.

dependent variable	independent variable	2008 Estimate	2008 P	2009 Estimate	2009 P	overall Estimate	overall P
bud number	veg.cover	**0.221**	**0.003**	-0.172	0.181	0.057	0.377
init.height	veg.cover	**0.504**	***	-0.05	0.695	**0.402**	***
attacked	bud number	**0.174**	**0.027**	0.087	0.489	**0.152**	**0.02**
attacked	veg.cover	**0.205**	**0.012**	**0.508**	***	**0.239**	***
attacked	init.height	-0.003	0.977	**0.311**	**0.008**	0.049	0.501
flowering	init.height	**0.205**	***	0.233	0.06	**0.284**	***
flowering	smut	0.014	0.793	0.003	0.978	0.013	0.788
flowering	bud number	**0.288**	***	-0.046	0.706	0.016	0.777
flowering	attacked	0.033	0.565	0.059	0.584	0.052	0.309

Tab. S3: Standardized path coefficients and p-values for the single relationships the partial path analyses testing the effect of herbivore attack on the flowering in the next season

	2008	2009	overall
init.height	0.254	0.161	0.143
bud number	0.049	0.003	0.001
attacked	0.087	0.096	0.086
flowering	0.145	0.089	0.091
$\chi 2$	155.7	65.2	218.9

Tab. S4: Estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable and Chi-square for each partial path analysis testing the effect of herbivore attack on the flowering in the next season.

		2008		2009		2010		overall	
dependent variable	independent variable	Estimate	P	Estimate	P	Estimate	P	Estimate	P
init.height	veg.cover	**0.411**	***	0.075	0.604	0.141	0.431	**0.269**	***
bud number	veg.cover	0.04	0.607	-0.029	0.829	-0.566	0.652	**-0.187**	**0.003**
degree of herbi.	veg.cover	**-0.19**	**0.05**	-0.113	0.404	0.453	0.551	**0.099**	**0.023**
degree of herbi.	bud number	**0.38**	***	**0.439**	***	0.521	0.273	**-0.306**	***
degree of herbi.	init.height	**0.545**	***	0.211	0.106	0.046	0.723	-0.012	0.849
seed number	smut	0.049	0.373	0.087	0.388	-0.038	0.734	0.051	0.252
seed number	init.height	0.082	0.511	0.317	0.375	-0.188	0.345	-0.013	0.849
seed number	degree of herbi.	**-0.213**	**0.04**	0.044	0.743	0.025	0.94	**-0.109**	**0.017**
seed number	bud number	**0.232**	**0.038**	0.261	0.699	-0.058	0.782	0.109	0.148
proportion of herbi.	bud number	0.064	0.389	0.841	***	-0.216	0.235	0.271	***
proportion of herbi.	init.height	0.192	0.019	0.42	0.002	0.133	0.216	0.163	0.009
proportion of herbi.	veg.cover	-0.045	0.556	-0.143	0.31	-0.141	0.64	-0.059	0.17
timing of herbi.	bud number	0.425	0.358	-0.669	***	-1.885	0.507	0.306	***
timing of herbi.	veg.cover	-0.065	***	0.064	0.497	-1.153	0.691	-0.054	0.218
timing of herbi.	init.height	0.563	***	-0.014	0.88	0.256	0.126	0.201	0.002
seed number	proportion of herbi.	-0.041	0.556	-0.442	0.517	-0.163	0.237	-0.03	0.513
seed number	timing of herbi.	-0.053	0.603	0.122	0.4	0.17	0.749	0.014	0.773

Tab. S5: Standardized path coefficients and p-values for the single relationships in the partial path analyses testing the effect of degree of herbivory on the seed number. The relationships in the lower part of the table entered the analyses as a component of interaction of “degree of herbivory” and thus they are not demonstrated in the path diagrams.

		2008		2009		overall	
dependent variable	independent variable	Estimate	P	Estimate	P	Estimate	P
bud number	veg.cover	0.141	0.069	-0.012	0.929	-0.041	0.52
init.height	veg.cover	**0.486**	***	0.096	0.506	**0.372**	***
degree of herbi.	veg.cover	**0.45**	***	-0.133	0.326	0.075	0.155
degree of herbi.	bud number	**-0.558**	***	**0.434**	***	**-0.239**	***
degree of herbi.	init.height	**-0.515**	***	0.214	0.101	0.029	0.667
flowering	smut	0.03	0.562	-0.019	0.855	0.022	0.642
flowering	degree of herbi.	**0.222**	**0.008**	0.184	0.195	**0.177**	***
flowering	init.height	**0.231**	**0.003**	-0.148	0.702	**0.274**	***
flowering	bud number	**0.367**	***	-0.671	0.345	0.07	0.295
proportion of herbi.	bud number	0.349	***	0.842	***	0.316	***
proportion of herbi.	veg.cover	-0.246	***	-0.171	0.225	-0.099	0.058
proportion of herbi.	init.height	0.502	***	0.431	0.002	0.239	***
timing of herbi.	veg.cover	0.333	***	0.075	0.426	-0.009	0.872
timing of herbi.	init.height	0.029	0.743	-0.018	0.847	0.147	0.03
timing of herbi.	bud number	-0.08	0.304	-0.668	***	0.24	***
flowering	proportion of herbi.	-0.031	0.694	0.564	0.446	-0.04	0.443
flowering	timing of herbi.	0.103	0.13	0.074	0.618	-0.094	0.053

Tab. S6: Standardized path coefficients and p-values for the single relationships in the partial path analyses testing the effect of degree of herbivory on the flowering in the next season. The relationships in the lower part of the table entered the analyses as a component of interaction of “degree of herbivory” and thus they are not demonstrated in the path diagrams

	2008	2009	2010	overall
init.height	0.169	0.006	0.02	0.072
bud number	0.002	0.001	0.321	0.035
degree of herbi.	0.393	0.249	0.213	0.114
seed number	0.054	0.092	0.085	0.034
timing of herbi.	0.478	0.454	2.481	0.131
proportion of herbi.	0.036	0.901	0.049	0.1
$\chi 2$	225.6	60.6	68.0	219.6

Tab. S7: Estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable and Chi-square for each partial path analysis testing the effect of degree of herbivory on the seed number.

	2008	2009	overall
init.height	0.236	0.009	0.138
bud number	0.02	0	0.002
degree of herbi.	0.523	0.247	0.067
flowering	0.142	0.112	0.108
timing of herbi.	0.12	0.453	0.078
proportion of herbi.	0.314	0.912	0.149
$\chi 2$	228.9	60.7	165.2

Tab. S8: Estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable and Chi-square for each partial path analysis testing the effect of degree of herbivory on the flowering in the next season.

CHAPTER 4

The effects of large herbivores on the landscape dynamics of a perennial herb

Lucie Hemrová, Zita Červenková & Zuzana Münzbergová

ABSTRACT

Models assessing the prospects of plant species at the landscape level often focus primarily on the relationship between species dynamics and landscape structure. However, the shortterm prospects of species with slow responses to landscape changes depend on the factors affecting local population dynamics. In this study it is hypothesized that large herbivores may be a major factor affecting the short-term prospects of slow-responding species in the European landscape, because large herbivores have increased in number in this region in recent decades and can strongly influence local population dynamics. The impact of browsing by large herbivores was simulated on the landscape-level dynamics of the dry grassland perennial polycarpic herb *Scorzonera hispanica*. A dynamic, spatially explicit model was used that incorporated information on the location of patches suitable for *S. hispanica*, local population dynamics (matrices including the impact of large herbivores), initial population sizes and dispersal rate of the species. Simulations were performed relating to the prospects of *S. hispanica* over the next 30 years under different rates of herbivory (browsing intensity) and varying frequencies of population destruction (*e.g.*, by human activity). Although a high rate of herbivory was detected in most populations of S. hispanica, current landscape-level dynamics of *S. hispanica* were approximately in equilibrium. A decline or increase of over 20% in the herbivory rate promoted rapid expansion or decline of *S. hispanica*, respectively. This effect was much stronger in the presence of population destruction. Browsing by large herbivores can have a dramatic effect on the landscape dynamics of plant species. Changes in the density of large herbivores and the probability of population destruction should be incorporated into models predicting species abundance and distribution.

Keywords: Bromion erecti, grazing, landscape-level modelling, large ungulates, roe deer.

INTRODUCTION

Rapid changes in the landscape in recent years have resulted in increased levels of habitat fragmentation for many plant species. These changes have provoked discussion about the prospects of such species in the future agricultural landscape (*e.g.*, Saunders *et al* 1991; Collinge 1996; Bastin and Thomas 1999; Lindborg and Eriksson 2004). Several authors have emphasized the importance of describing species dynamics at the landscape level to estimate the future prospects of species (*e.g.*, Eriksson 1996; Husband and Barrett 1996; Bastin and Thomas 1999; Hanski 1999).

Modelling studies that simulate species dynamics at the landscape level focus primarily on the impact of changes in landscape structure (*e.g.*, With *et al* 1997; Hanski and Ovaskainen 2000; Herben *et al* 2006; Alados *et al* 2009). However, for species that respond slowly to landscape change (*e.g.*, long-lived species with limited dispersal; *e.g.*, Mildén *et al* 2006), changes in landscape structure may not be the most important factor influencing species dynamics. Instead, the factors that affect local population dynamics are probably crucial in influencing landscape-level species dynamics over the short term.

Browsing by large herbivores is one of the most important factors affecting local population dynamics of plant species (*e.g.*, Bergelson and Crawley 1992; Augustine and Frelich 1998; Russell *et al* 2001; Rooney and Waller 2003). Browsing can strongly influence local population dynamics by affecting the components of the plant life cycle, such as seedling survival (Paige and Whitham 1987; Knight *et al* 2008), plant seed production (Knight *et al* 2008; Ehrlén and Münzbergová 2009; Lin and Galloway 2009) and the probability of flowering in the next season (Knight *et al* 2008; Ehrlén and Münzbergová 2009). In addition to these negative effects, large herbivores can have positive effects on long-distance dispersal (reviewed in Nathan *et al* 2008) and thus on species colonization. Large herbivores can also positively affect plant population growth rate by enhancing seedling recruitment (reviewed by Maron and Crone 2006). The number of large herbivores, such as roe deer, has been increasing in the agricultural European landscape in recent decades (Meriggi *et al* 2008). Increased herbivory and dispersal rates due to a higher number of large herbivores can have both negative and positive effects on the prospects of plant species in the landscape.

In the present study, we estimated the prospects of a grassland polycarpic perennial herb, *Scorzonera hispanica*, at the landscape level, incorporating the effect of browsing by large herbivores on the species dynamics. Specifically, the aims of the present study were to model the landscape dynamics of *S. hispanica* in northern Bohemia (Czech Republic) and to simulate the prospects of this species in the near future. Although *S. hispanica* is considered endangered in the Czech Republic, it is common in the study area. In the area, the species occurs on clearly delimited patches. Some of these patches are suitable but unoccupied, as identified by means of a sowing experiment indicating that *S. hispanica* is dispersal-limited (Münzbergová 2004). Chýlová and Münzbergová (2008) demonstrated that this species prevails in grasslands established for at least 60 years, indicating that the dynamics of the species are quite slow. *S. hispanica* does not form a permanent seed bank (Münzbergová 2004). Recolonization is thus only possible by means of long-distance dispersal. All the above properties indicate that *S. hispanica* fulfils the criteria for possessing metapopulation dynamics (Freckleton and Watkinson 2002). Information on landscape-level dynamics of this species can thus be generalized to other species fulfilling the same criteria with slow response to landscape changes. The identified patterns could thus be generalized for many grassland and forest-understorey long-lived perennial herbs.

Browsing by large herbivores has been observed in most *S. hispanica* populations. We have also observed the destruction of habitats of *S. hispanica* within the study region, due primarily to ploughing, the construction of solar power stations or rooting by wild boars. Therefore, in the present study, we evaluated the effects of both browsing by large herbivores and population destruction on the landscape-level dynamics of *S. hispanica*, as they both may influence its future prospects.

To understand the future dynamics of *S. hispanica* in the landscape, we asked the following questions: (i) What are the future prospects of *S. hispanica* in the current landscape and under the current rate of herbivory (i.e. browsing by large herbivores)? (ii) What is the effect of herbivory on the future prospects of *S. hispanica*? (iii) What are the combined effects of herbivory and population destruction on the prospects of *S. hispanica*?

To answer these questions, we parameterized a model of landscape dynamics for S. hispanica based on available information on the distribution of suitable habitats, the local population dynamics (including the current rate of herbivory and the risk of population destruction), dispersal ability and current population sizes. We then simulated the prospects of the species after 30 years under a wide range of herbivory rates and with different levels of risk of population destruction. We assumed that the landscape would not change dramatically over such a short period and that the response to landscape changes would be slow. Under these assumptions, browsing by large herbivores is expected to be the primary factor influencing the prospects of *S. hispanica*. Model credibility was tested by performing sensitivity analyses of the model parameters.

METHODS

Study species and study area

Scorzonera hispanica L. (Asteraceae) is a rare, allogamous, polycarpic perennial herb inhabiting the dry grasslands of central and southern Europe. It has a single rosette and a single flowering stalk with one to seven yellow flowerheads. It is occasionally cultivated for its edible rootstock (Chater 1976). The fruits of *S. hispanica* are achenes with a pappus. The presence of the pappus enables dispersal by wind and exozoochory. The species does not form a persistent seed bank; the seeds, which do not germinate, decompose within 2 years (Münzbergová 2004).

In the Czech Republic, *S. hispanica* is a native species and is considered endangered. It occurs in central and northern Bohemia and in southern Moravia, occupying calcareous dry grasslands (alliance Bromion erecti of Ellenberg 1988). To model the prospects of S. hispanica under different rates of herbivory and population destruction, we focused on a typical agricultural landscape with a common occurrence of both *S. hispanica* and large herbivores. All study populations are browsed by ungulates. No other type of herbivory has been observed. Roe deer, mouflon and wild boar are very common in the landscape, whereas fallow deer and red deer occur only rarely (Municipality Litoměřice, Department of Environment). Only browsing by roe deer, common herbivores of numerous plant species in both grassland and forest-understorey (*e.g.*, Gill *et al* 1996; Jepsen and Topping 2004; Hewison *et al* 2007), has been observed in *S. hispanica* populations. However, we consider that the other large herbivores in the landscape can also occasionally browse S. hispanica.

The study area (4.39 × 4.39 km) was situated in northern Bohemia in the Czech Republic (50°33′26″N, 14°12′45″E, to 50°31′21″N, 14°17′3″E). Calcareous dry grasslands are typical of the landscape. These grasslands form distinct patches surrounded by shrubs and large agricultural fields. These formerly maintained grasslands are now unmanaged and therefore experience very slow succession of shrubs and trees. Population sizes range from three to 2500 flowering individuals. Genetic variability in the field is high; Nei's genetic diversity values range from 0.04 to 0.32, indicating that all populations are genetically variable (Münzbergová and Plačková 2010). Large herbivores favour the flowering stalks of *S. hispanica*. Our long-term field observations indicate that the flowering stalks of *S. hispanica* are browsed extensively without signs of leaf herbivory on the browsed individuals or on the surrounding vegetation (Z. Münzbergová, pers. obs.).

Field data collection

All dry grassland patches (73 in total, from 48.7 to 214 396.3 m^2) in the study area were located within the region studied by Chýlová and Münzbergová (2008). In their study, a digital map of dry grassland patches was created and the presence of *S. hispanica* and 65 other species (Supplementary Data Table S1) were recorded at each patch. We added data from 12 populations (patches) of *S. hispanica* outside the study area to the present dataset to increase sample size. All external patches were ≤ 30 km from the study area and ranged in size from 882.9 to 62 365.9 m^2. The external patches all hosted the same dry grassland vegetation (i.e. Bromion erecti, Ellenberg 1988) as the patches within the study area. At each external patch, we recorded the presence of the 65 selected species of dry grassland vegetation. We counted the number of flowering *S. hispanica* individuals at all patches. We surveyed 85 patches of dry grassland, 35 of which hosted *S. hispanica*. The external patches were used to improve the predictive power of models of patch suitability and of the herbivory rate at each patch. External patches were not used to simulate the prospects of S. hispanica in the study area.

To model the impact of large herbivores on *S. hispanica* landscape-level dynamics, we incorporated the effect of herbivory on performance of *S. hispanica* into transition matrix models of the local population dynamics of the species. We used a set of eight transition matrices containing three size classes (seedling, large vegetative and flowering individuals) to simulate local population dynamics. These eight matrices were constructed for a previous study (Münzbergová 2006) and included data collected between 2001 and 2004 in three populations over three transition intervals (population nos. 16, 18 and 20 in Table 1 in Münzbergová 2006). Population size ranged between 1632 and 2464 individuals, with at least 150 individuals marked in each population; see Münzbergová (2006) for additional details. Two populations (nos. 16 and 20) are found within the present study area; the third is nearby and occurs in the same type of habitat. This latter population is among the 12 external populations described above. We considered these populations to be representative as they contain a sufficient number of individuals for studying population dynamics and exhibit habitat conditions typical of other populations in the area. Two populations have been largely stable over the last 10 years. However, all marked plants in the third population were destroyed by wild boars during the last transition period; therefore, no transition matrix could be built from these data.

The plants used for matrix construction experienced browsing by large herbivores; however, browsing intensity was not quantified. It was thus necessary to identify those transitions within the matrices that were affected by herbivory and to replace these transitions by probabilities with a quantified rate of herbivory. Z. Červenková (unpubl. res.) found that only flowering stalks were browsed; there was very little herbivore damage to vegetative plants. Z. Červenková (unpubl. res.) also estimated the impact of large herbivores on performance of flowering *S. hispanica* in a field experiment (Supplementary Data Appendix S1). Specifically, she protected selected plants from browsing using cages and compared the performance of intact and browsed plants. She found that herbivory decreased the seed production and the production of clones by flowering plants. No other impact of herbivory was found. In addition, Münzbergová (2004) demonstrated that the recruitment and survival of seedlings and adult *S. hispanica* plants are not affected by the above-ground biomass at the localities. Seedling recruitment and survival are also unaffected by the presence of open spaces in the vegetation (Z. Münzbergová, unpubl. res.). These findings suggest that neither biomass removal nor an increase in canopy openness due to herbivory affect the reproductive success of *S. hispanica*. We therefore focused only on the impact of large herbivores on flowering individuals in the present study.

To estimate the rate of herbivory of flowering stalks, we collected data on intensity of browsing from 21 *S. hispanica* populations of varying size in 2009 and 2010. Ten populations were within the study area, and 11 were external. In all 21 populations, we recorded the total number of browsed and intact flowering plants. In populations comprising fewer than 150 flowering individuals, we recorded browsing data from all flowering plants present. In the larger populations, we collected data from approx. 150 flowering plants sampled along randomly selected transects. The study was conducted at the end of the flowering period (mid-July), when herbivory on *S. hispanica* ends but faded flowerheads are still present on the stalks (the herbivores browse flowering, not mature, flowerheads).

Habitat characteristics

To estimate the suitability of individual grassland patches for *S. hispanica* and to identify the factors affecting the herbivory rate, we recorded data on 26 characteristics at each patch. These included both local habitat characteristics and characteristics describing landscape structure (Table 1), *e.g.*, the location of individual patches in the landscape. To obtain data on the local habitat characteristics, we first constructed digital elevation models (DEMs) with a 5-m grid size. DEMs were derived from digital contours (1:10 000, 2-m vertical distance between contours) provided by the Czech Office for Surveying, Mapping and Cadastre. DEMs were constructed for the entire study area and the 12 external patches in ArcGIS 9.2 (Environmental Systems Research Institute Inc., Redlands, CA, USA). Based on these models, we created grids of slopes and potential direct solar irradiation (PDSI) for the 21st day of the month from December to June using ArcGIS 9.2, and created grids of topographic wetness index (TWI) using SAGA GIS 2.0.4. (SAGA User Group Association, Hamburg, Germany). For each patch, we then calculated the logarithm of the total area, the mean values of slopes, PDSI (from December to June) and TWI (Table 1).

Other local habitat characteristics were calculated using the presence of the 65 selected species from our species list (Supplementary Data Table S1). First, we calculated the Beals index, which expresses the probability of a species presence at a patch using the number of joint occurrences with other species (Beals 1984; Münzbergová and Herben 2004). We used the presence of all plant species from the species list in all patches for this calculation. Second, we calculated Ellenberg indicator values of light, temperature, moisture, nutrients, soil reaction and continentality (Ellenberg 1988; Table 1) for each patch using all species recorded at the patch.

To obtain the parameters describing landscape structure around the patches, we calculated the nearest distance between each patch and shrubs, forests, roads and villages; we also recorded the amount of shrub and tree cover surrounding each patch (Table 1). We used digital maps of shrubs, forests, roads and villages for these calculations. The digital map of shrubs and forests based on NATURA 2000 mapping was provided by the

Agency for Nature Conservation and Landscape Protection of the Czech Republic. The digital maps of roads and villages were created by combining information on the latest online cadastral and orthophotomaps provided by the Czech Office for Surveying, Mapping and Cadastre and by the Czech Environmental Information Agency, respectively. Using the digital maps, we first calculated the areas of both shrubs and forests within both a 500-m and 1-km radius of each patch. We then calculated the distance between each focal patch and (1) the nearest shrub, (2) the nearest forest, (3) the nearest road and (4) the nearest village (Table 1). Distances were calculated between centre points of each patch to the boundaries of these objects using ArcGIS.

Tab. 1. – List of recorded habitat characteristics used to develop predictive models of (1) patch suitability for *S. hispanica* ($P < 0.001$, $F = 16.57$, d.f. error = 73, $R^2 = 0.75$) and (2) herbivory rates at patches ($P < 0.001$, $F = 88.61$, d.f. error = 15, $R^2 = 0.78$). +/– represents the positive/negative effect of characteristics included in the final model (significant values at $P < 0.05$ are in bold type), n indicates characteristics not included in the model and x indicates characteristics excluded from the test.

		Patch suitability R^2		Herbivory rate R^2
Local habitat characteristics				
Area		n	–	0.022
Slope		n		x
TWI	–	0.018	–	0.004
PDSI				
December		n		x
January		n		x
February	–	0.143		x
March	+	0.147		x
April	–	0.128		x
May		n		x
June	+	0.113		n
Beals index	+	0.483		x
Ellenberg indicator values				
Light	–	0.042		x
Moisture		n		x
Soil reaction	+	0.032		x
Nutrients	+	0.146		n
Temperature	+	0.048		x
Continentality	+	0.045		x
Landscape structure				
Forest 1 km		x		n
Shrub 1 km		x	–	0.359
Forest 0.5 km		x		n
Shrub 0.5 km		x		n
Nearest forest		x		n
Nearest shrub		x		n
Nearest village		x	+	0.033
Nearest road		x	–	0.025
Population size		x		n

Data analysis

To estimate patch suitability, we tested for the effects of habitat characteristics on the occurrence of *S. hispanica*. We used a generalized linear model (GLM) with a binomial distribution of the dependent variable (presence/absence of *S. hispanica*) in this test. We excluded data on landscape structure from our independent variables, as they related to patch availability not to patch suitability. We used data from all 85 patches, 35 of which hosted *S. hispanica*. To simplify the model (correlation matrix in Supplementary Data Table S2), we used step-wise both-direction regression starting with the maximal model. We identified those habitat characteristics that best explained *S. hispanica* presence using the Akaike information criterion (AIC; Crawley 2002). Based on this model, we calculated the probability of *S. hispanica* presence at each patch (Crawley 2002). These probabilities were used to identify suitable unoccupied patches for *S. hispanica* (see 'Simulation plane' below).

Our investigation of the factors determining *S. hispanica* herbivory rate involved a small number of observations (21 populations). Therefore, we primarily selected those landscape and habitat characteristics expected to influence herbivory rate (Table 1). Specifically, we used the area of forests and shrubs surrounding each patch within a radius of 0.5 and 1 km and the distances to the nearest forest, shrub, village and road as possible factors influencing the behaviour of large herbivores and the subsequent herbivory rate (*e.g.*, Welch *et al* 1990; Tufto *et al* 1996; Hewison *et al* 2001; Nilsen *et al* 2004; Coulon *et al* 2008). We also evaluated factors related to site vegetation, including *S. hispanica* population size, TWI, PDSI in June (i.e. in the growing season), the Ellenberg indicator value for nutrients and the Beals index (Table 1).

To identify the characteristics influencing herbivory rate, we used the mean rate of herbivory in 2009 and 2010 as the dependent variable (herbivory rates did not differ significantly between years, data not shown). The total numbers of browsed and intact plants over both years were used as the dependent variables with a binomial distribution in a GLM. As we had three similar measures of the impact of forest and shrubs, we made three partial tests, using: (1) the amount of cover of shrubs and forests within 1 km, (2) the amount of cover within 500 m and (3) the proximity of the nearest shrub and forest. In each test, we performed step-wise, bothdirection logistic regression (using AIC; Crawley 2002) starting with the maximal model. We then chose the best model (i.e. the model with the lowest number of independent variables and the highest explanatory power). Using the selected model, we calculated the predicted rate of herbivory (Crawley 2002) at each patch in the area. All analyses were performed in S-Plus Professional Release 2 (MathSoft, Inc., Seattle, WA, USA).

Model description and estimation of model parameters

To simulate the dynamics of *S. hispanica* in the landscape, we used a dynamic, spatially explicit landscape-level model presented in previous studies by Münzbergová *et al* (2005), Herben *et al* (2006) and Mildén *et al* (2006), following similar methods. This model does not assume equilibrium between species extinction and colonization; this assumption is important because disequilibrium species dynamics have often been observed in long-lived species following rapid changes in landscape structure (*e.g.*, Matlack 1994; Eriksson 1996; Brunet *et al* 2000; Lindborg and Eriksson 2004; Herben *et al* 2006). The model uploads (1) the information on location and size of suitable patches (habitats) for a species, (2) the initial habitat occupancy including local population sizes, (3) a set of matrices simulating local population dynamics and (4) the coefficients of dispersal curves (exponential and/or hyperbolic functions) and proportion of seeds dispersed independent of distance (for model details see Supplementary Data Appendix S2).

Simulation plane. Suitable patches were identified on the grid (5-m cell resolution) by the probabilities of *S. hispanica* presence. Patches were classified as either suitable or unsuitable by finding the lowest calculated probability in the set of patches that, in the actual study area, host *S. hispanica*. We then considered all patches of the same or higher probability to be suitable for the species assuming that *S. hispanica* occurred on suitable patches only. We also found probability thresholds using methods recommended by Liu et al. (2005). As the threshold we took either the prevalence of the model-building data or the average predicted probability of the model-building data. Compared with the original threshold, these two thresholds identically identified three more unoccupied patches as unsuitable for *S. hispanica*. The lower number of suitable unoccupied patches was then used to estimate the sensitivity of the model to landscape structure. However, the changed model provided similar results to the original and is not discussed further.

Local population dynamics, herbivory and population destruction. Suitable patches were classified according to the predicted herbivory rate into 11 categories of habitat quality, corresponding to proportions of browsing of 0–100% at 10% step intervals. We then used the eight available transition matrices of the Münzbergová (2006) study to build 88 additional matrices (11 from each matrix). Each set of eight matrices included the rate of herbivory corresponding to specific herbivory rate (ranging from 0–100 %, 10% step intervals). Specifically, in each of the eight matrices, we substituted those transitions significantly affected by browsing with the weighted mean of transition values in browsed and intact plants found by Z. Červenková (Supplementary Data Appendix S1). The set of the eight matrices with specific rate of herbivory was assigned to each patch according its predicted herbivory rate.

Ploughing, construction and rooting by wild boars occasionally occurring in the landscape can cause destruction to varying extents. Therefore, we included the probability of population destruction in the model. We had no reliable estimates of the probability and extent of population destruction. However, we assumed that some individuals could survive during the destruction. We thus used the 88 transition matrices described above and decreased all transition probabilities by 90% to obtain a set of 88 modified matrices. In this way 90% of all individuals that would have survived into the next year did not survive. Modified matrices were used at various frequencies to original ones. In all simulations, except those modelling the impact of population destruction on prospects of *S. hispanica*, we used a frequency of one disturbance matrix per 29 original matrices, i.e. one population destruction per 30 years per population. This proportion was chosen based on the observation of Münzbergová (2006) and our subsequent monitoring of the populations in the area.

Initial population size. The numbers of seedlings and vegetative individuals at each patch were calculated from the numbers of flowering individuals (counted in the field) according to the mean stable stage distribution occurring under a specific rate of herbivory. However, stable stage distributions are reached only in populations with stable local population dynamics. To estimate the sensitivity of the model to this assumption, we used half the numbers of seedlings and vegetative individuals calculated from the stable stage distribution. The results of this alternative model were, however, very similar to the original and are therefore not reported further.

Density-dependence. To simulate density-dependence, we estimated the maximum population density at any patch, based on the number of *S. hispanica* individuals at each patch and patch size. The calculated maximum was 0.97 individuals m -2 . Based on our field experience, we assumed that the maximum density of seedlings was four times higher than the maximum density of vegetative or flowering individuals. Thus, seedlings had one- quarter the competitive effect of flowering and vegetative individuals. We also performed a sensitivity analysis of this parameter, using 0.97/3 or 0.97 × 3 individuals m^{-2} during the simulations.

Dispersal. We assumed no incoming diaspores to the simulation plane, as the study area was somewhat isolated from other *S. hispanica* populations (the nearest flowering population was 4.3 km from the area border). Therefore, low numbers of incoming diaspores could be expected. Outgoing diaspores during the simulations were considered to be lost. *S. hispanica* was expected to disperse by wind and exozoochory. Wind dispersal was modelled as distance-dependent using a negative normalized exponential function (Münzbergová *et al* 2010):

$$y = \alpha * \exp(-\alpha * x),$$

where y is the proportion of seeds dispersed to distance x and α is the coefficient indicating the shape of the curve. We used the exponential model due to its simple normalization, which was necessary for calculation of the dispersal coefficient of the curve using data on wind speed and species traits. Commonly used dispersal models (including the exponential) tend to underestimated long-distance dispersal (*e.g.*, Bullock and Clarke 2000; Nathan *et al* 2002). We thus modelled *S. hispanica* landscape dynamics under several scenarios of dispersal ability (including different exponential curves). We then checked whether higher/lower dispersal ability influenced the results of the model. Specifically, dispersal coefficient a was calculated as $1/D$, where D is the mean dispersal distance of the seeds calculated from the formula (*e.g.*, Augspurger 1986; Soons and Heil 2002; Tremlová and Münzbergová 2007)

$$D = wh/t,$$

where w is wind speed (m.s^{-1}), h is the height of inflorescence (m) and t is the terminal velocity (m.s^{-1}) of the fruits. For the measure of wind speed w, we used the maximum daily mean wind speed over June and July, as detected by the Czech Hydrometeorological Institute at the nearby meteorological station in Doksany from 2005 to 2010 (9.6 m.s^{-1}). Wind speeds detected during this period ranged from 3.4 to 32.6 m.s^{-1}. Inflorescence height h was estimated from 2007 data on 70 plants in seven populations (ten from each population, range = 0.17–0.83 m, mean = 0.47 m). We used the mean terminal velocity of *S. hispanica* fruits (1.78 m.s^{-1}) estimated by Münzbergová (2004). Due to the lack of data on the range of terminal velocities, we chose a range of 1.78 m.s^{-1} ± 33%. Based on the ranges of all parameters (w, h and t), we calculated a dispersal distance range of 0.24–22.80 m and a mean dispersal distance of 2.51 m. In the simulations, we used the mean dispersal distance. Minimum and maximum dispersal distances were used to perform sensitivity analyses of the dispersal parameter.

We did not have an estimate of the proportion of seed dispersed via animal fur in the field as obtaining realistic estimates of such a value is difficult (Nathan *et al* 2008). Therefore, we assumed that only 0.1% of all seeds were dispersed by exozoochory and by rare events (see also Münzbergová *et al* 2005). As herbivores attack individuals during flowering and not when the seeds are mature, the proportion of damaged flowerheads with mature seeds is very low. Therefore, endozoochory was not considered in our simulations. Exozoochory was assumed to affect primarily the long-distance dispersal of *S. hispanica*. It was modelled as independent of distance. Although this assumption seems to be unrealistic, it was used in previous studies (*e.g.*, Münzbergová *et al* 2005; Mildén *et al* 2006). In our study, dispersal was modelled within small study area. This does not suggest that dispersal is independent of distance at any scale, but rather that the animals can easily cross the whole model landscape within a short period of time. The sensitivity analysis of distance-independent dispersal was performed using 1% and 0.01% of the dispersed seeds.

Simulations All forecasts were run for 30 steps (30 simulation years), with each forecast replicated 100 times. We ran the simulations for up to 30 years, assuming that the landscape would not change dramatically over such a period. However, running the model for 100 years provided qualitatively very similar results, with the time frame having no impact on our conclusions (data not shown).

We estimated the impacts of the range of the model parameters on the prospects of S. hispanica under different herbivory rates. First, we simulated the prospects of the species under the current rate of herbivory predicted for individual patches in the area. We then simulated a gradual (at 10% intervals) decrease (or increase) in the predicted rate of herbivory over the entire study area, until the herbivory rate (*i.e.* the number of browsed flowering individuals) decreased to 0% (or increased to 100 %) in all patches. In this way, we obtained 17 different simulations of different herbivory rates.

To assess the effect of the frequency of population destruction on the prospects of S. hispanica, we simulated different rates of population destruction under different herbivory rates. The frequencies of population destruction ranged between 0.5 and 10 disturbances per population per 30 years. We obtained 11 different simulations of the impact of disturbance regime on the prospects of *S. hispanica* under 17 different rates of herbivory.

RESULTS

Determinants of patch suitability and herbivory rate

The step-wise regression identified 11 of 17 local habitat characteristics as significant predictors of the presence of *S. hispanica* in a patch (Table 1). These include PDSI in various months, the Beals index and the Ellenberg indicator values. Based on the model, we identified 31 patches as suitable for *S. hispanica*, eight of which were unoccupied. Five of 12 landscape and local habitat characteristics were selected in the step-wise regression as significant predictors of the rate of herbivory in a patch (Table 1): patch area, TWI, shrub cover within 1 km of the patch, proximity of the nearest village and proximity of the nearest road. The predicted rate of herbivory ranged from 40 to 100% (mean 77 %, median 80 %) among single patches.

Impact of herbivores at the landscape scale

Our simulations revealed a strong effect of large herbivores on the long-term prospects of *S. hispanica*. Simulation using the predicted herbivory rate showed an equilibrium in the number of *S. hispanica* individuals in the area (mean population size after 30 years = 86 371, s.d. = 23 632; initial population size = 78 462, Fig. 1A). Under high rates of herbivory, *S. hispanica* tended to go extinct; under low rates, population size increased substantially. Similarly, herbivory rates had a negative effect on patch occupancy (Fig. 1B). Using the current rate of herbivory in our simulations, the patches hosting small populations experienced higher turnover of patch occupancy than the patches hosting large populations (Fig. 2).

Sensitivity analyses of dispersal parameters (Supplementary Data Figs S1a,b and S2a,b) revealed an effect of both wind dispersal and exozoochory on the number of occupied patches under low rates of herbivory at the end of the simulations (Supplementary Data Fig. S2a,b). Sensitivity analysis of maximum population density (Supplementary Data Figs S1c and S2c) showed a strong effect on the total number of individuals under low rates of herbivory (Supplementary Data Fig. S1c). In both cases, parameter effects disappeared under high rates of herbivory, which indicates that increased herbivory reduces the positive effects of longer dispersal rates and higher carrying capacity (Supplementary Data Figs S1 and S2). Our simulations also revealed that not only herbivory rates but also frequencies of population destruction had a strong effect on the landscape-level dynamics of *S. hispanica*. Rates of population destruction higher than those observed in the field (i.e. one per 30 years in a patch) led to a considerable decrease in the number of individuals and the number of occupied patches in the area (Fig. 3). The pattern was observed for all rates of herbivory. A rapid decline of the number of *S. hispanica* individuals was observed when high frequencies of population destruction were combined with high rates of herbivory.

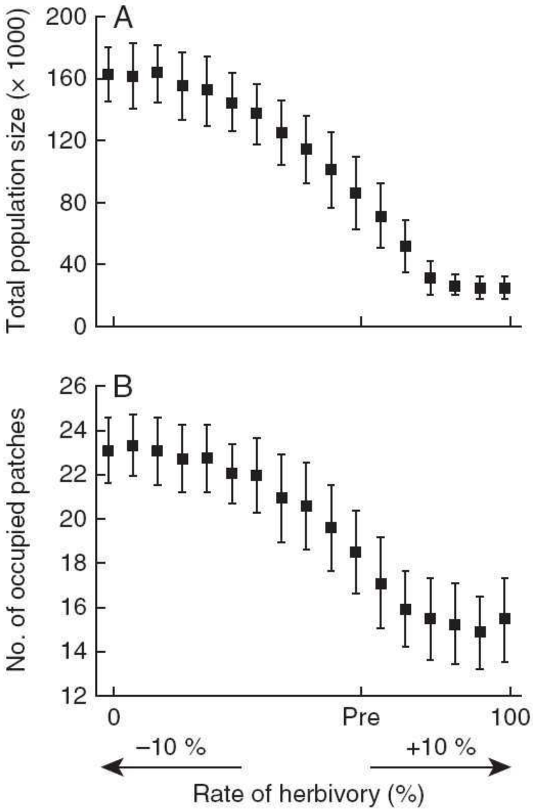

Fig. 1. – The negative effects of increasing herbivory rates on (A) the total number of *S. hispanica* individuals and (B) the number of patches occupied by *S. hispanica* at the end of simulations. The mean ± s.d. (obtained from 100 repetitions of each simulation) are shown for all simulations. 'Pre' shows the current rate of herbivory predicted in each patch in the area. The rate of herbivory was increased or decreased incrementally by 10% for all patches until it reached 100% or 0 %, respectively.

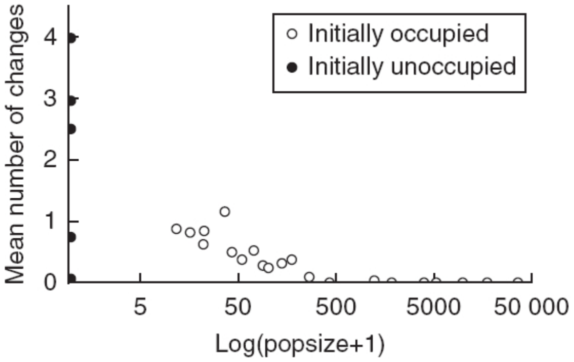

Fig. 2. – The relationship between initial population size and tuzrnover of patch occupancy during the simulation, using the predicted current rate of herbivory in the area. The mean number of changes (i.e. extinctions and colonizations) over all 100 repetitions of the simulation for each of 31 suitable patches in the area is plotted.

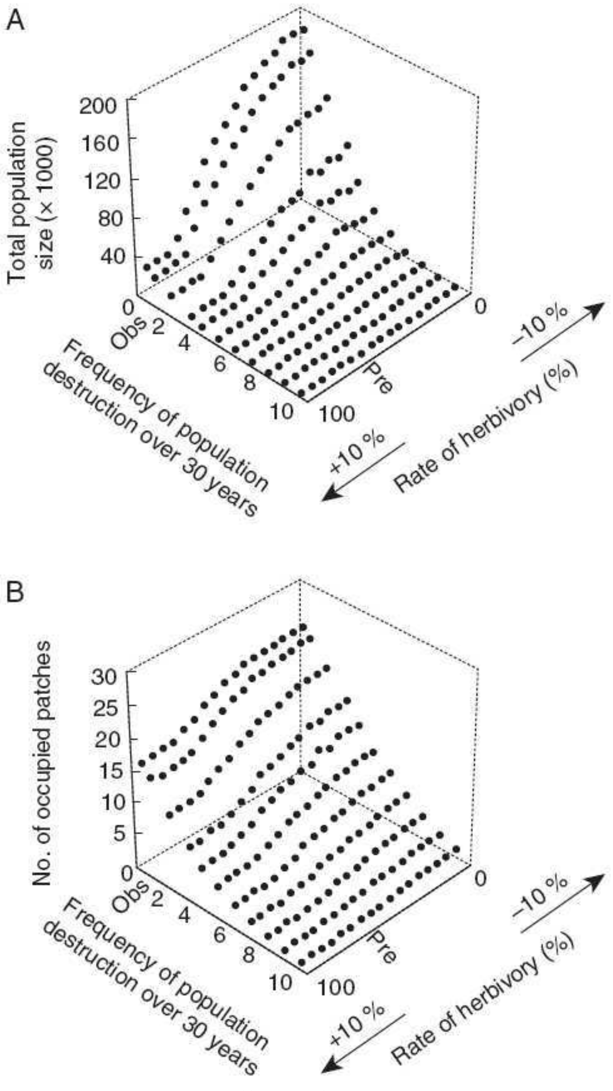

Fig. 3. – The negative effects of increasing herbivory rates and the frequency of disturbances in the area on (A) the total number of *S. hispanica* individuals and (B) the number of patches occupied by the species at the end of simulations. 'Pre' shows the current rate of herbivory predicted in each patch in the area. The rate of herbivory was increased or decreased incrementally by 10% for all patches until it reached 100% or 0 %, respectively. 'Obs' shows the observed frequency of population destruction in the area (one per population per 30 years).

DISCUSSION

In this study, we demonstrated the importance of browsing by large herbivores on the landscape-level dynamics of *S. hispanica*. Despite the negative effects of browsing on the performance of *S. hispanica*, the landscape-level dynamics of *S. hispanica* are currently at equilibrium. The future prospects of this species, however, depend on the prospects of large populations. We also found that the potential effects of large herbivores on landscape-level dynamics may be considerable. Simulated declines or increases in the rate of herbivory throughout the landscape by more than 20% often led to the rapid expansion or decline of *S. hispanica*. A similar effect of herbivory rate was observed in the sensitivity analyses of various model parameters. These findings indicate that herbivores can be among the major drivers of landscape dynamics of long-lived perennial herbs.

The results of the simulations demonstrated relatively high turnover of the local populations, especially those that are small. This indicates that despite being a long-lived perennial, *S. hispanica* exhibits features of metapopulations, as we expected. The metapopulation framework (Hanski 1999) is thus a suitable approach for modelling the dynamics of *S. hispanica* and other similar species. More specifically, the high turnover of the small populations and high survival of the large ones suggests that *S. hispanica* is a likely representative of species with mainland–island metapopulation dynamics (Harrison 1991). From a conservation point of view, survival of large populations is crucial for survival of the whole metapopulation. These large populations may, however, be threatened in the landscapes by factors such as human-induced population destruction as well as an increased rate of herbivory.

Effect of herbivores on landscape-level dynamics

We expected *S. hispanica* to decline by the end of simulations using the current rate of herbivory because of (1) the negative effects of large herbivores on performance of S. hispanica, (2) its slow dynamics and dispersal-limitation (Münzbergová 2004) and (3) the increased fragmentation of the landscape over the last 60 years (Chýlová and Münzbergová 2008). Unexpectedly, our simulations of the prospects of *S. hispanica* following 30 years of current landscape conditions (herbivory and disturbance) suggest that the total number of individuals in the study area is largely stable. We can, however, expect slight declines in future habitat occupancy. *S. hispanica* was unable to establish new large populations during the simulations due to the high risk of extinction of small populations (as demonstrated also in the field by Münzbergová 2006).

The maintenance of landscape dynamics of *S. hispanica* near equilibrium (except the extinction of small populations) can be explained by the type of its local population dynamics. Population dynamics are very stable over time (even with the current high rate of herbivory) due to the high survival probability of individuals and occasional clonal reproduction. Nevertheless, a simulated 20% decline in the current herbivory rate markedly increased the number of seeds produced, resulting in higher seed dispersal and more successful colonization. In contrast, a 20% increase in the current rate of herbivory led to a serious population decline. This decline was due primarily to the large changes occurring in the most abundant populations. Sensitivity analyses of the dispersal parameters revealed that habitat occupancy depended partly on the estimation of the dispersal parameters. However, the dispersal parameters did not influence overall decline/increase of S. hispanica under higher/lower than current rates of herbivory. In addition, changes in dispersal rates had only negligible effects on landscape-level prospects of *S. hispanica* under high rates of herbivory. This indicates that the negative effects of herbivory on performance of species, specifically on generative reproduction (*e.g.*, Knight *et al* 2008; Ehrlén and Münzbergová 2009; Lin and Galloway 2009), can be much stronger than the possible positive effects of herbivores as dispersal agents (Fischer *et al* 1996; Nathan *et al* 2008). The effect of herbivory was enhanced significantly when combined with population destruction (resulting from large disturbances). The importance of population destruction to species landscape dynamics has been previously demonstrated, for example by Münzbergová *et al* (2005). Such a clear negative effect of herbivory and population destruction on species dynamics is caused by the absence of any positive effects of these activities on plant performance. If overcompensation (Paige and Whitham 1987) or enhanced seedling recruitment (Gomez 2005) was found in the case of *S. hispanica*, the effect of herbivory rate and population destruction on its landscape-level dynamics would be much less clear.

The current equilibrium state of the *S. hispanica* metapopulation may reflect several factors. One possibility is that the expansion of *S. hispanica* has been constrained in the past (*e.g.*, by cattle grazing and mowing). This hypothesis is supported by the fact that many *S. hispanica* populations occur in former pastures (Münzbergová 2004). In such a scenario, current patch occupancy should reflect high past landscape connectivity. Alternatively, landscape connectivity may still be the same, but with an ongoing increase in the rate of herbivory and the frequency of large disturbances in the area. This would result in reduced growth rates of local populations and in higher probability of extinction of small populations.

Patterns of herbivory

First, it is important to note that the proportion of browsed individuals in a population does not necessarily relate to the frequency of visits by large herbivores, as the herbivores can readily pass over patches without browsing. We found less herbivore damage in larger patches and in patches surrounded by high shrub cover. This pattern corresponds to the results of previous studies of the habitat preferences of large herbivores. For example, roe deer prefer small patches (Aulak and Babinska-Werka 1990; Welch *et al* 1990) with rich ground vegetation (Welch *et al* 1990), and the density of deer increases with increasing habitat heterogeneity (Kie *et al* 2002) and the density of habitat edges (*e.g.*, Tufto *et al* 1996; Saïd *et al* 2005; Miyashita 2008). According to Lamberti *et al* (2006), roe deer prefer open habitats (*e.g.*, orchards and fields) to woodlands and scrublands. This latter observation suggests that the presence of shrubs or forest may decrease the attractiveness of patches to herbivores.

Another important factor affecting behaviour of large herbivores is human activity in the landscape, especially developed areas and roads. Several studies have found that these factors negatively impact deer density (*e.g.*, Hewison *et al* 2001; Coulon *et al* 2008). In our study, populations near villages were browsed less heavily whereas the proximity of roads had the opposite effect. The increased rate of herbivory near roads may result from the use of roads as corridors by large herbivores. The affinity of large herbivores to individual patches may have been also affected by landscape topography and surface (Coulon *et al* 2008). We found a higher rate of herbivory in the drier patches above valleys than in the wetter patches closer to valley bottoms.

Estimated parameters and model credibility

Several things should be kept in mind in interpreting our simulation results. The sensitivity analyses indicated that dispersal ability and carrying capacity had an effect on the total number of *S. hispanica* individuals under low, but not high, rates of herbivory. Similarly, these parameters had a greater effect on habitat occupancy under low than under high herbivory rates (Supplementary Data Figs S1 and S2). Similar results were found when using smaller initial population sizes and running the simulations for 100 years (data not shown). These results indicate that our conclusions regarding the effects of large herbivores on the species landscape dynamics are independent of the parameter estimates.

To simulate the prospects of *S. hispanica* in the landscape, we set the initial patch occupancy at 74%, corresponding to the observed occupancy of this species in the study area. This occupancy level is significantly higher than the 32% found by Münzbergová (2004), who used sowing experiments to identify patches suitable for, but unoccupied by, *S. hispanica*. However, in the study by Münzbergová (2004) patches were distributed over a larger area (approx. 400 km 2) than in our study (approx. 20 km 2), but within the same landscape. In addition, in the study of Münzbergová (2004), the predictions of patch suitability were based on seedling establishment, which does not necessarily reflect patch suitability for adult plants (Ehrlén *et al* 2006). In our study, suitable patches were identified using a combination of abiotic conditions and species composition. These types of factors have been shown previously to explain species distribution (*e.g.*, Dupré and Ehrlén 2002; Münzbergová 2004; Ehrlén *et al* 2006; Chýlová and Münzbergová 2008). As suggested, for example, by Tájek *et al* (2011) and observed in the present study, the combination of these two types of factors provides the best predictions of habitat suitability for a species. Specifically, we identified drier, shaded, basic, nutrient-rich patches as more suitable for *S. hispanica* in dry grasslands. Patch preference may reflect both specific abiotic conditions and past land use (particularly as pastures). Nevertheless, the sensitivity analyses revealed that a reduction in the number of suitable patches had little effect on the model results.

In our simulations, local population dynamics was assumed to be the same among patches (except for the impact of herbivory) and largely stable over time. We simulated demographic stochasticity representing random changes in local population dynamics over time. There was no indication for a gradual change in environmental conditions. In our simulations we thus assumed that among-year variation in the local population dynamics was caused only by environmental stochasticity. Environmental stochasticity was simulated by drawing a random transition matrix (for each population, in each step) from a set of matrices. The matrices were very similar and thus their random sample had little effect on the local population dynamics. Although differences between patches and changes in local population dynamics over time could occur, we argue that these factors are unlikely to strongly affect our conclusions. First, all our populations occur within a small area under very similar habitat conditions, minimizing potential differences among populations. Second, our simulations extended only 30 years into the future, making it unlikely that habitat conditions will change dramatically. Third, it is likely that the responses of *S. hispanica* to any landscape changes would be slow, and thus minimal over this period. Therefore, browsing by large herbivores is probably the primary factor influencing the prospects of *S. hispanica*.

Finally, we assumed a stable rate of herbivory at each patch during the individual simulations. However, the incidence of browsing by large herbivores fluctuates between years due to changes in their abundance. For example, 22% more roe deer were recorded in the landscape in 2008 than in 2007 (Municipality Litoměřice, Department of Environment). However, we suggest that fluctuations in herbivory rate are unlikely to alter the main conclusions of the model.

CONCLUSIONS

Our field observations indicate that over 60% of flowering *S. hispanica* individuals are damaged by large herbivores in most populations each year. Our simulations, however, suggest that current dynamics of *S. hispanica* are approximately in equilibrium under the current rate of herbivory and frequency of large disturbances (one per 30 years per population). The simulation results also revealed a higher survival probability of large populations than that of small ones. Therefore, under current landscape conditions, the prospect of *S. hispanica* in the landscape depends heavily on the prospects of large populations.

Simulations of the effect of herbivory rate on the dynamics of *S. hispanica* indicated that a decline or increase in the herbivory rate of more than 20% over the entire landscape could lead to a rapid expansion or decline of the species. This effect is predicted to be much stronger under the additional occurrence of disturbance. These results confirm our hypothesis that browsing by large herbivores can have dramatic effects on the landscape dynamics of species if important components of the life cycle are strongly affected by these herbivores.

Finally, as concluded in other studies, our study suggests that the probability of population destruction should be incorporated into models predicting changes in species distributions. Incorporating the effect of large herbivores and population destruction into models of species landscape dynamics should be a major endeavour of future metapopulation studies.

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SUPPORTING INFORMATION

Appendix S1. – Collecting and testing the data on the impact of large herbivores on performance of S. hispanica.

In 2009 and 2010, Zita Červenková conducted a field demographic study of one large *S. hispanica* population (no. 16 in Table 1 in Münzbergová 2006). Two sets of tagged flowering plants were compared. The first set was exposed to browsing by large herbivores (140 individuals), whereas the second set was caged and thereby fully protected from browsing (70 individuals). In both seasons, Z. Červenková recorded plant height and flowerhead number; in the plants exposed to browsing, she also recorded the presence of herbivory (1/0). Mature seeds of all plants were collected and counted. For the exposed plants, Z. Červenková collected performance data only from those plants that experienced herbivory (119 individuals). Z. Červenková then compared the performance of browsed and intact individuals. Specifically, she tested for differences in seed production, the production of flowering or vegetative clones, transitions from flowering to vegetative stages and the stasis of flowering individuals.

Z. Červenková identified two transitions that were significantly affected by browsing. First, clonal reproduction of flowering stalks was absent among browsed plants and occurred in 10% of intact plants (df error = 185; F = 14.64; *P* = 0.001). Second, she observed significant differences between browsed and intact plants in seed production. Intact plants produced an average of 92.0 ± 6.2 SE seeds, while plants damaged by large herbivores produced only 4.3 ± 1.2 SE seeds per plant (df error = 185; F = 314.39; *P* < 0.001). There was no significant difference between browsed and intact plants in the transition stasis of flowering plants (df error = 185; $\chi^2 = 214.70$; *P* = 0.93), in the transition from flowering to large vegetative plants (df error = 185; $\chi^2 = 211.50$; *P* = 0.90) nor in the production of vegetative clones (df error = 185; F = 0.65; *P* = 0.42).

Appendix S2. – Detailed description of the landscape-level model.

Simulations are performed on a grid representing a study area, with suitable patches explicitly located and surrounded by unsuitable areas. Multiple cells can be coded as part of the same patch. To simulate the population dynamics of a species, a population vector containing the number of individuals in each size class is assigned to each patch. Population growth at the k-th patch is simulated using transition matrices as

$$x'_k(t+1) = A_{Q(k)} \times x_k(t),$$

where x_k is a population vector on patch k at time t, and A_Q is a transition matrix of quality Q (e.g., corresponding to the rate of herbivory at patch k). Density dependence within patches is modelled using a logistic growth equation; the difference between total (N) and maximum (K) population size at each patch is used as the logistic term. The population vector $x'_k(t+1)$ resulting from the matrix multiplication is then replaced by the following term:

$$x''_k(t+1) = x'_k(t+1)\frac{1}{\lambda}\left[1+(\lambda-1)\left(1-\frac{N_k(t)}{K_k}\right)\right],$$

where λ is the dominant Eigenvalue of the matrix A_Q, $N(t)$ is the total population size (sum of all stages) at patch k at time t (before the matrix multiplication) and K is the capacity of patch k (*i.e.* the maximum number of individuals at the patch of the given size). This form of density-dependence affects all transitions equally. Demographic stochasticity is simulated by replacing each vector element by a Poisson-distributed deviate with a mean of x''_{ik}. A new population vector $x_k(t+1)$ is created by combining these numbers.

Environmental stochasticity is simulated using several matrices for each habitat quality. At each step, one matrix from a set of matrices assigned to the given patch is randomly chosen with a probability assigned to the matrix. Environmental stochasticity is assumed to be uncorrelated over space and time; the random matrix is therefore chosen independently for each patch. Such an assumption could be used in case of S. hispanica, because there was very small among-year and among-population variation in the transition matrices. The choice of specific matrix has little effect on the population dynamics at the patch.

The model simulates distance-dependent and distance-independent dispersal. For distance-dependent dispersal, seeds produced at each patch are divided equally among the cells that comprise that patch and dispersed from these cells according to either a negative exponential function, or a negative hyperbolical function, or their summation. Distance-independent dispersal is simulated by removing a fraction of seeds at each step from each patch, then randomly redistributing them over the whole simulation plane. The seeds arriving at all cells within a patch are summed to yield the seed input at that patch. Seeds that fall into the space between patches are considered lost. Model setting enables either absorbing or periodic boundaries of the simulation plane, such that diaspores passing over the boundary are either lost or appear across the plane, respectively. At each step, transitions within patches are simulated to yield a seed crop before performing any dispersal.

Tab. S1. – List of selected dry grassland species.

Agrimonia eupatoria
Anemone sylvestris
Anthericum ramosum
Anthyllis vulneraria
Asperula cynanchica
Asperula tinctoria
Asteramellus
Aster linosyris
Astragalus cicer
Astragalus glycyphyllos
Brachypodium pinnatum
Bromus erectus
Bupleurum falcatum
Campanula glomerata
Campanula rotundifolia
Carex flacca
Carex humilis
Carex tomentosa
Carlina vulgaris
Centaurea jacea
Centaurea rhenana
Centaurea scabiosa
Cirsium acaule
Cirsium eriophorum
Cirsium pannonicum
Coronilla vaginalis
Coronilla varia
Eryngium campestre
Festuca rupicola
Fragaria viridis
Gentiana cruciata
Geranium sanguineum
Gymnadenia conopsea
Helianthemum nummularium subsp.g
Hieracium pilosella
Inula salicina
Laserpitium latifolium
Leontodon hispidus
Linum flavum
Linum tenuifolium
Listera ovata
Lotus corniculatus
Medicago falcata
Melampyrum arvense
Melampyrum cristatum
Melampyrum nemorosum
Ononis spinosa
Peucedanum cervaria
Plantago media
Potentilla arenaria
Potentilla heptaphylla
Prunella grandiflora
Salvia pratensis
Salvia verticillata
Sanguisorba minor
Seseli hippomarathrum
Sesleria albicans
Stachys recta
Tanacetum corymbosum
Thesium linophyllon
Thymus praecox
Trifolium medium
Trifolium montanum
Veronica austriaca subsp.teucrium

Note: Nomenclature follows Tutin et al. (1964-1983).

Tab. S2. – Correlation matrix of habitat characteristics recorded at dry grassland patches. Significant correlations (P < 0.05) are in **bold**.

		Log (area)	Slope	TWI	PDSI							Beals index	Ellenberg indicator values						Fo 1 km	Sh 1 km	Fo 0.5 km	Sh 0.5 km	Near fo	Near sh	Near vill
					De	Ja	Fe	Ma	Ap	Ma	Ju		Light	Moist	Soil re	Nutr	Temp	Cont							
	Slope	-0.02																							
	TWI	0.09	**-0.65**																						
PDSI	De	**0.23**	-0.18	0.15																					
	Ja	**0.24**	-0.21	0.17	**1.00**																				
	Fe	**0.23**	**-0.25**	0.21	**0.99**	**1.00**																			
	Ma	**0.23**	**-0.30**	**0.24**	**0.99**	**0.99**	**1.00**																		
	Ap	**0.22**	**-0.38**	**0.28**	**0.97**	**0.98**	**0.99**	**1.00**																	
	Ma	**0.22**	**-0.48**	**0.34**	**0.94**	**0.95**	**0.97**	**0.98**	**0.99**																
	Ju	0.21	**-0.54**	**0.38**	**0.91**	**0.93**	**0.95**	**0.96**	**0.98**	**1.00**															
	Beals index	**0.25**	**0.43**	**-0.32**	-0.20	-0.21	**-0.23**	**-0.25**	**-0.28**	**-0.32**	**-0.34**														
Ellenberg indicator values	Light	**0.48**	-0.02	-0.08	**0.25**	**0.24**	**0.24**	**0.24**	**0.24**	**0.23**	**0.22**	-0.03													
	Moist	**-0.28**	-0.10	**0.23**	-0.20	-0.20	-0.20	-0.19	-0.18	-0.15	-0.14	**-0.47**	**-0.30**												
	Soil re	**0.26**	**0.31**	-0.18	-0.05	-0.05	-0.07	-0.08	-0.10	-0.13	-0.15	0.21	**0.35**	-0.03											
	Nutr	-0.10	**-0.28**	0.19	0.16	0.16	0.18	0.19	0.21	**0.24**	**0.25**	**-0.60**	0.13	**0.56**	**-0.24**										
	Temp	**0.25**	**-0.38**	0.20	**0.52**	**0.52**	**0.54**	**0.55**	**0.57**	**0.58**	**0.58**	-0.09	0.16	-0.19	**-0.27**	0.10									
	Cont	-0.02	**-0.24**	-0.03	0.17	0.17	0.18	0.19	0.21	**0.22**	**0.23**	**-0.28**	0.12	**-0.28**	-0.07	-0.13	**0.37**								
	Fo 1 km	-0.15	**-0.35**	**0.36**	**0.25**	**0.25**	**0.26**	**0.27**	**0.30**	**0.33**	**0.35**	-0.09	**-0.31**	**0.23**	**-0.27**	0.19	0.20	-0.12							
	Sh 1 km	0.16	0.11	-0.09	-0.16	-0.16	-0.16	-0.16	-0.16	-0.17	-0.17	**0.22**	-0.21	0.07	-0.21	-0.16	**0.24**	-0.09	0.05						
	Fo 0.5 km	**-0.25**	**-0.38**	**0.39**	0.01	0.03	0.04	0.06	0.09	0.14	0.17	-0.09	**-0.41**	0.18	**-0.26**	0.08	0.05	-0.07	**0.83**	-0.08					
	Sh 0.5 km	**0.30**	0.16	-0.08	-0.11	-0.12	-0.12	-0.13	-0.14	-0.15	-0.16	0.10	0.02	0.08	0.06	-0.07	0.10	-0.14	-0.11	**0.70**	**-0.24**				
	Near fo	0.21	0.20	-0.09	0.00	0.01	-0.01	-0.02	-0.04	-0.06	-0.08	-0.07	**0.26**	-0.01	**0.31**	-0.07	-0.18	0.06	**-0.55**	-0.20	**-0.59**	-0.06			
	Near sh	0.01	-0.21	**0.29**	0.19	0.19	0.19	0.20	0.21	**0.23**	**0.25**	0.01	-0.02	-0.08	-0.16	-0.02	0.06	-0.03	**0.45**	**-0.40**	**0.56**	**-0.38**	**-0.22**		
	Near vill	-0.13	-0.20	**0.22**	**-0.23**	**-0.23**	**-0.23**	**-0.21**	-0.19	-0.16	-0.13	-0.03	**-0.32**	0.13	**-0.27**	-0.04	-0.04	-0.10	**0.37**	**0.26**	**0.43**	**0.34**	**-0.27**	0.19	
	Near ro	0.07	-0.10	0.18	-0.11	-0.11	-0.10	-0.09	-0.08	-0.06	-0.04	-0.02	**-0.21**	0.08	**-0.22**	-0.11	0.07	-0.15	0.19	**0.40**	**0.26**	**0.50**	-0.14	0.17	**0.70**

Abbreviations: Log = logarithm; TWI = topographic wetness index; PDSI = potential direct solar irradiation; De-Ju = December-June; Moistr = moisture; Soil re = soil reaction; Nutr = nutrients; Temp = temperature; Cont = continentality; Fo = forest; Sh = shrub; Near = nearest; vill = village; ro = road.

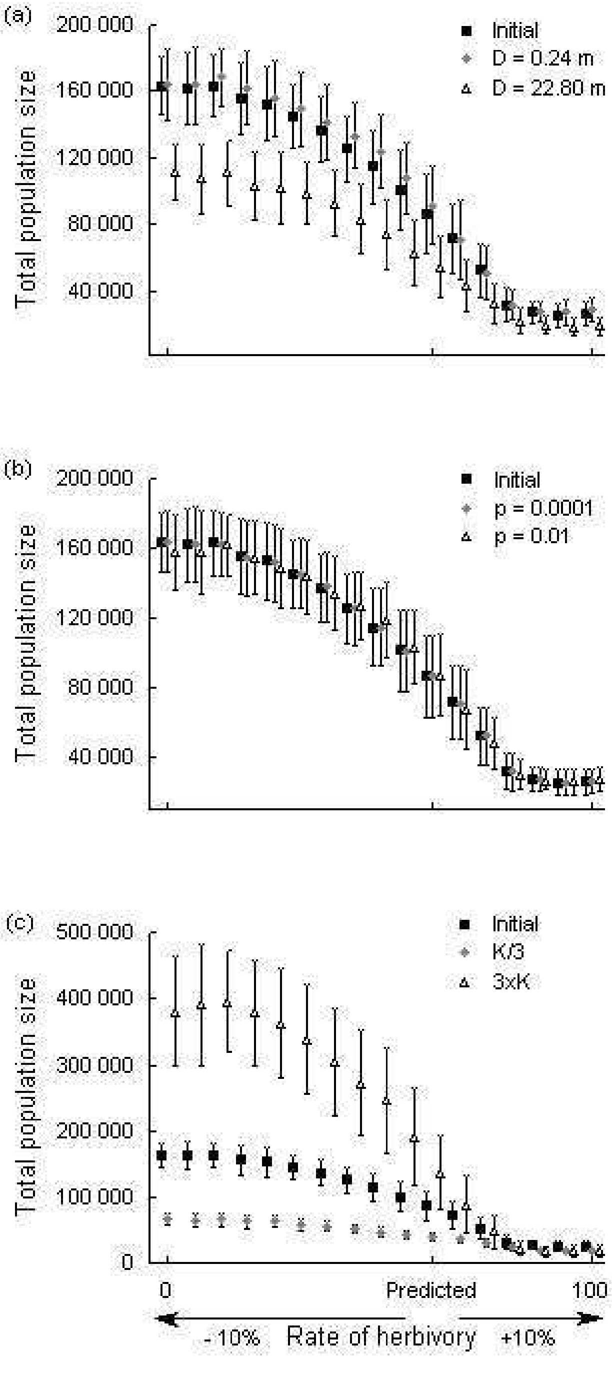

Fig. S1. – Sensitivity analysis of the model: the negative effect of increasing herbivory rates on the total number of *S. hispanica* individuals in the simulation area at the end of simulations, using different values of the model parameters: a) distance-dependent dispersal *(D)*, b) distance-independent dispersal *(p)*, and c) maximum population density *(K)*. Mean ± SD are shown for all simulations. Initial model parameters were set at D = 2.51 m, p = 0.001, K = 0.97.m $^{-2}$. "Predicted" shows the current rate of herbivory predicted in each patch in the area. The rate of herbivory was increased or decreased incrementally by 10% for all patches until it reached 100% or 0%, respectively.

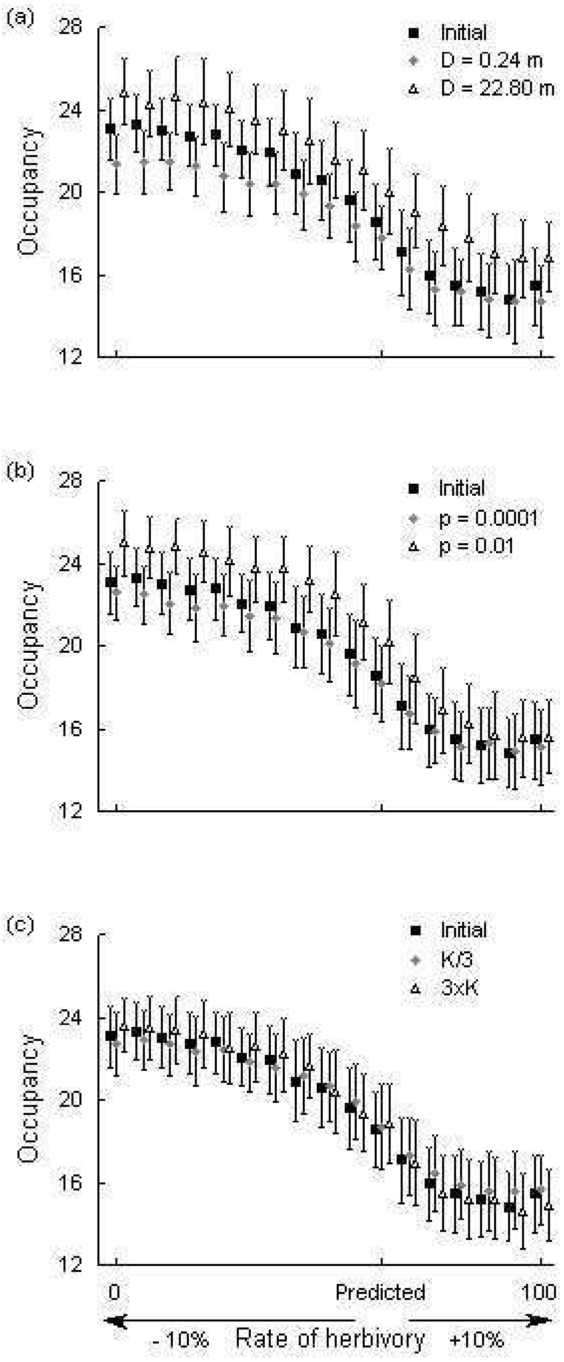

Fig. S2. – Sensitivity analysis of the model: the negative effect of increasing herbivory rates on patch occupancy by *S. hispanica* in the simulation area at the end of simulations, using different values of the model parameters: a) distance-dependent dispersal *(D*), b) distance- independent dispersal *(p)*, and c) maximum population density *(K)*. Mean ± SD are shown for all simulations. Initial model parameters were set at *D* = 2.51 m, *p* = 0.001, *K* = 0.97.m $^{-2}$. "Predicted" shows the current rate of herbivory predicted in each patch in the area. The rate of herbivory was increased or decreased incrementally by 10% for all patches until it reached 100% or 0%, respectively.

CONTRIBUTIONS

Contribution of Zita Červenková to the papers with co-author included in the thesis

Červenková Z & Münzbergová Z. *manuscript*. Seed mass and population size affect germination and seedling performance in *Scorzonera hispanica.*

idea: **ZČ**, ZM
data collection: **ZČ**
data analyses: **ZČ** with the help of ZM
writing: **ZČ** with the help of ZM

Červenková Z & Münzbergová Z. 2014. Pollen limitation and pollinator preferences in *Scorzonera hispanica*. *Plant biology* 16(5): 967–972

idea: **ZČ**, ZM
data collection: **ZČ**
data analyses: **ZČ** with the help of ZM
writing: **ZČ** with the help of ZM

Červenková Z & Münzbergová Z. *manuscript*. Effect of ungulate herbivory on reproduction of *Scorzonera hispanica*

idea: **ZČ**, ZM
data collection: **ZČ**
data analyses: **ZČ** with the help of ZM
writing: **ZČ** with the help of ZM

Hemrová L, **Červenková Z**, Münzbergová Z. 2012. The effects of large herbivores on the landscape dynamics of a perennial herb. *Annals of Botany* 110:1411–1421

idea: LH, **ZČ**, ZM
data collection: **ZČ** (data on herbivores impact on local dynamics and proportion of attacked flowering individuals in populations), LH (list of species on external patches, data on habitat characteristics of all patches)
data analysis: **ZČ** (determinants of herbivory rate in landscape), LH (habitat suitability for species),
simulations of species prospect: LH
writing: LH, **ZČ** (all parts concerning herbivores’ behavior and herbivores’ effect on local species dynamics in Introduction, Materials and methods, Results and Discussion)