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# Ecology of rhinanthoid Orobanchaceae

## Within- and between-site processes in metapopulations of selected grassland species

Ph.D. Thesis

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## Annotation

The thesis is focused on the ecology of grassland hemiparasitic Orobanchaceae species, with the focus on *Rhinanthus* spp., which are vulnerable to modern landscape changes. Various aspects of their population ecology were explored to provide comprehensive information about their ecological requirements. In addition, the parasitic effect of *Rh. major* on *Calamagrostis epigejos* was explored.

## Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

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## Cover photo

*Rhinanthus major* and *Melampyrum nemorosum* in Čertoryje National Nature Reserve. Petr Blažek, 2 June 2013.

# List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

Mudrák O., Mládek J., **Blažek P.**, Lepš J., Doležal J., Nekvapilová E. & Těšitel J. (2014) Establishment of hemiparasitic *Rhinanthus* spp. in grassland restoration: lessons learned from sowing experiments. Applied Vegetation Science 17: 274–287 (IF = 2.4).

*The article is composed of seven experiments performed by the authors. PB fully designed, performed and analysed the first two experiments with the supervision of JL. PB also wrote the corresponding parts of manuscript and participated significantly on the formatting and language improvement of the whole text.*

**Blažek P.** & Lepš. J. (2015) Victims of agricultural intensification: mowing date affects *Rhinanthus* spp. regeneration and fruit ripening. Agriculture, Ecosystems and Environment 211: 10–16 (IF = 3.4).

*PB performed all steps of the work himself with the supervision of JL.*

**Blažek P.**, Lepš. J., Fajmon K. & Těšitel J. (2016) Response of two hemiparasitic *Orobanchaceae* species to mowing dates: implications for grassland conservation and restoration practice. Plant Ecology and Evolution 149: 31–38 (IF = 1.0; citations = 0).

*The experiment was designed mainly by JT and PB, and plant phenology was recorded and site management was organized by KF. PB was the leading author in all the other steps with supervision of JL and JT.*

Těšitel J., Mládek J., Fajmon K., **Blažek P.** & Mudrák O. (2018) Reversing expansion of *Calamagrostis epigejos* in a grassland biodiversity hotspot: hemiparasitic *Rhinanthus major* does better job than increased mowing intensity. Applied Vegetation Science 21: 104–112 (IF = 2.5; citations = 0).

*PB carried out the mapping survey of C. epigejos in the study area incl. drawing the map and writing corresponding parts of the manuscript. PB also participated in designing the data analysis.*

**Blažek P.**, Lepš J., Hořčicová Š., Pavlů L., Kook E., Wesselingh R. & Těšitel J. (manuscript) Functional connectivity in *Rhinanthus minor* metapopulations: grassland management affects seed dispersal in fragmented landscapes.

*PB performed all steps of the work himself with supervision of JT and JL. ŠH provided training and supervision in laboratory. LP, EK, and RW provided local support and local data in the study regions.*

# Contents

# Chapter 1

# General introduction

# Scope of the thesis

The rhinanthoid clade in the Orobanchaceae family forms a unique functional group in European flora, which is famous for root hemiparasitism. However, many of the grassland species from the target group have disappeared from most of their former sites, so the central question of this thesis is “why they have disappeared”. We performed several experiments to explore various aspects of the non-parasitic behaviour to better understand the critical points in their life cycle, mainly the seedling establishment (Chapter 2) and response to management (Chapters 3 and 4). To complete the picture of metapopulation functioning, a large-scale observational study was performed to evaluate the connectivity of populations in the landscape (Chapter 5).

In addition to the metapopulation ecology, current research focuses on the possible application of the parasitic behaviour in nature conservation for suppressing non-target species or community biomass in general. Although not the main aim of this thesis, a case study reflecting this subject is reported in Chapter 6.

Rhinanthoid Orobanchaceae include 11 genera and dozens of species (Těšitel et al. 2010b), but since it would not be possible to answer all questions for all species, we focused on our target species, which include *Rhinanthus* spp., predominantly *Rh. minor* and *Rh. major* (syn. *Rh. angustifolius*), which are the most common, and once included the rare *Rh. alectorolophus* and *Melampyrum nemorosum*.

# Life history

## Distribution changes

The target species used to be a common part of temperate grassland communities in Europe and well known weeds (Carruthers 1903; Matthies 1995a; Magda et al. 2004; Novák 2004). In recent decades, their distribution has become scattered since the species has disappeared completely from sites where fodder production is the main aim of landowners, in addition to disappearing from abandoned sites. Populations are surviving mostly in protected areas or other places with non-intensive management (road verges, mountains). Comprehensive data about the changes in the distribution of the grassland rhinanthoid Orobanchaceae species are missing, but the trend is clear and several species have appeared on the red lists of several European countries or regions. This thesis includes *Rh. alectorolophus* and *M. nemorosum* var. *praecox*, which are vulnerable and critically endangered in Czechia, respectively (Grulich 2012) and *Rh. minor,* near threatened in South Bohemia (Czechia; Lepší et al. 2013). Most other central European countries also contain some of the target species in their red lists (Zimmermann et al. 1989; Ludwig & Schnittler 1996; Garve 2004; Colling 2005; van Landuyt et al. 2006; Saintenoy-Simon et al. 2006; Király 2007; Tartes 2008; Bornand et al. 2016). Contrary to Europe, *Rh. minor* is now spreading in North America (van Hulst et al. 1987; Smith & Cox 2014).

The target species possess a combination of life-history traits (annual cycle, lack of long distance dispersal, lack of persistent seed bank, parasitism) that are quite exceptional for grassland herbs (van Hulst et al. 1987), which make them very sensitive to changes in environmental conditions and vulnerable to local extinctions (Matthies et al. 2004; Westbury 2004; Bekker & Kwak 2005; Bullock & Pywell 2005). *Rh. minor* was therefore suggested as an indicator species of habitat quality (JNCC 2004; Lindborg et al. 2005). It can be inferred from the distribution pattern that modern agricultural changes might negatively affect the species performance. In the following sections, I evaluate the sensitivity of individual life-cycle stages to various aspects of grassland management.

## Plant survival and fecundity

The target species are annuals (Těšitel et al. 2010b), but grassland communities are usually dominated by highly competitive perennials, making them generally unfavourable to annuals (Strykstra et al. 2002). As a result, special strategies must be employed for annuals to persist in this highly competitive environment.

In order to establish after germination, seedlings must get energy support from large resource-rich seeds (Irving & Cameron 2009). At this stage, the seedlings are susceptible to drought (Westbury 2004; Ameloot et al. 2006) and their growth can be limited by a thick litter layer (Svensson & Carlsson 2005). Management abandonment is associated with accumulation of litter and prevents seedling establishment. Even in managed plots, autumn management activities, especially grazing or soil-surface scarification, are recommended to create openings in litter layer to support seedling establishment (Smith et al. 1996; Leimu 2010; Hellström et al. 2011). On the contrary, litter is known to prevent soil desiccation in dry periods, so a moderate litter layer may be beneficial to reduce seedling mortality in a dry spring (Loydi et al. 2018).

Parasitic behaviour (described below) enables these species to overcome the underground competition by using the established extensive root system of perennial hosts (van Hulst et al. 1987; Strykstra et al. 2002; Westbury 2004), as well as the aboveground competition by acquiring even some organic carbon from the hosts (Těšitel et al. 2010a, 2011). However, this advantage is lost when soil resources are not limiting and the plants can get outcompeted (Matthies 1995b). As a result of agricultural intensification (namely fertilization and introduction of highly productive species), the intensity of competition for light in grassland communities has increased in the past century (Hopkins et al. 1999; Sklenička 2002; Zechmeister et al. 2003; Hodgson et al. 2005; Isselstein et al. 2005), causing lower survival of *Rhinanthus* spp. (van Hulst et al. 1987; Davies et al. 1997; Smith et al. 2000; Ameloot et al. 2006; Mudrák & Lepš 2010; Hejcman et al. 2011). Suitable management intensity and timing is important for *Melampyrum* spp. as well (Lehtilä & Syrjänen 1995; Ramula 2008; Leimu 2010).

To harvest the higher yield, mowing frequency has increased and the timing of the first cut has shifted towards spring. The new mowing date may interfere with the flowering period of the target species, reducing seed production (Smith et al. 2000; Leimu 2010). There are two basic strategies to cope with mowing: either the individuals must produce some seeds before mowing or they must resprout and flower after mowing. The response is quite complex since there is a large variation in phenology and regeneration ability between species, populations and individuals. A seasonal polymorphism was described within each of the target species, comprising a gradient from vernal to autumnal ecotypes. The names refer to a difference of several weeks in the onset of flowering but there is also a morphological difference affecting regeneration. The vernal (or aestival) types set seed before mowing and have a few long internodes below the inflorescence and few or no branches underlying poor regeneration ability. On the contrary, the autumnal types start flowering after the first mowing and have higher numbers of short internodes below the inflorescence with abundant branches determining much better regeneration ability (ter Borg 1972; Zopfi 1993a, 1993b; Štech 2000; Westbury 2004;

Jonstrup et al. 2016). In addition to the differences between ecotypes, a considerable plasticity can be observed within populations (Wesselingh 2016). A hypothesis was proposed that the ecotypes evolved as an adaptation to different management types, but the relationship is complex and not satisfactorily resolved (ter Borg 1972; Zopfi 1993b). Since the phenology varies between species, ecotypes and years, the suitable mowing date must be estimated individually (Svensson & Carlsson 2005). It is similar to the regionally historical practice of considering the meadows to be ripe for harvest when the "yellow rattle" (common name for *Rhinanthus* spp.) started to rattle (i.e. to have ripe fruits; Ivaşcu et al. 2016).

Early mowing is not only part of an intensive agricultural management, but it can be also part of conservation management. More frequent mowing (resulting in an earlier first cut) is used as a treatment in restoration projects to reduce the amount of residual nutrients, the spread of weeds and the abundance of dominant grasses (Blakesley & Buckley 2016). However, the target hemiparasites are also used as a treatment in these projects to lower the biomass of dominant grasses (see below). Proper timing and height of mowing must be carefully set to not eradicate the parasite (Blakesley & Buckley 2016).

Diversification of mowing dates within a site is applied in some protected areas as a conservation measure for protecting insects (Čížek et al. 2012) and is generally supported for plants as well (Köhler et al. 2005; Humbert et al. 2012). This can, however, include early cuts in some plots and since the target species are sensitive to the mowing date, their response needs to be carefully monitored and the position of the early mown plots adjusted to ensure their persistence.

In many nature conservation cases, the management of the sites with target species is essential for their survival. However, a publication comprehensively describing the complex management requirements is missing. The knowledge about the individual aspects of species life history and dependence of individual life stages on the management described in this chapter is often based on general assumptions not sufficiently supported by data. We, therefore, tried to identify the key limiting factors and the response of the target species to management and to land use changes.

## Seed dormancy and dispersal

To compensate for detrimental consequences of possible failure of seed production, annuals are usually able to disperse massively in space (seed dispersal) or time (seed dormancy). In case of population extinction, it can be restored by seed rain from other sites or by seeds stocked as soil seed bank in the same site. Surprisingly, neither of these adaptations is well developed in our target species (Poschlod et al. 1996; Thompson et al. 1997; ter Borg 1985; van Hulst et al. 1987; Kiviniemi & Eriksson 1999; Coulson et al. 2001; Bullock et al. 2003), probably as a cost of trade-off between these dispersal traits, which save a population in a state of collapse, and regeneration traits, which prevent a collapse from happening. The latter traits are more useful in a stable and competitive environment like permanent grassland (Kelly 1989), but they are not strong enough in the target species to withstand the conditions in intensive or abandoned grassland. The dispersal traits cannot compensate regular reproductive failure, but theycan still strongly affect metapopulation dynamics and need to be further explored.

The seed bank of the target species is described either as transient (seeds germinate in the following spring at the latest) or short-term persistent (seeds remain germinable for up to four years; ter Borg 1985; Kelly 1989; Pons 1991; Thompson et al. 1997). Massive one-year persistent seed banks have been observed occasionally in *Rhinanthus* spp. with a high number of seedlings exceeding the seed production from previous year (Kelly 1989; Blažek 2011; Hejcman M. pers. comm.; Chapter 2: Ex-arable land sowing experiment 1). It was hypothesised that prolonged dormancy can be induced by environmental factors, e.g. dry spring (Kelly 1989) or short winter stratification (ter Borg 2005). The seeds require cold stratification in temperatures close to 0°C for 2 – 3 months (Gibson & Watkinson 1991; Westbury 2004). It was shown experimentally in the laboratory that when the required span of cold temperature was shortened, the seeds did not germinate immediately, but after the next full period of cold (ter Borg 2005). Since the phenomenon of the sporadic large seed banks remains unclear, we tried to explore the effect of winter stratification in a field experiment.

As a consequence of the reduction in area of suitable grasslands in the past century, the distances between remaining ones have increased (Sklenička 2002;

Kiviniemi 2008). This complex process consisting of habitat loss and the breaking apart of habitat is described as habitat fragmentation (Fahrig 2003). In highly fragmented landscapes, long-distance dispersal becomes a key process affecting the dynamics of the whole metapopulation (Poschlod et al. 1996; Matthies et al. 2004). Despite the importance of this issue, plant dispersal within metapopulation is scarcely studied (in contrast to the spread of invasive species to new areas) due to the practical difficulties in tracking propagules. The seeds which reach long distances are very important, but also too scarce to be detected and reliably quantified (Poschlod et al. 1996). Tracking of dispersal vectors instead of individual seeds was used in some studies, but long-distance dispersal events are often caused by unusual behaviour of the standard vector or by nonstandard vectors (Nathan et al. 2008), so the quantification of the dispersal events remains unreliable. Genetic methods were proposed to overcome the practical limitations of long distance seed tracking providing a cumulative measure of past dispersal events (Cain et al. 2000; Holderegger & Wagner 2008; Kool et al. 2013). Even though this approach is powerful, the connection to the landscape structure and dispersal processes remains weak (but see Aavik et al. 2014 and DiLeo et al. 2017).

The seeds of the target species are large (*M. nemorosum* ~ 8 mg, *Rhinanthus* spp. ~ 2 mg; Šerá & Šerý 2004) which makes their unaided dispersal extremely inefficient and they fall just below the source plant. *Melampyrum* spp. are adapted to myrmecochory (elaiosome) and *Rhinanthus* spp. to anemochory (membranous wing) to enhance dispersal, but the dispersal distance is not exceeding a few meters by these means (Coulson et al. 2001, Bullock et al. 2003, Heinken 2004). In addition, seeds are projected by mowing machinery during both the cutting and turning of hay (Bullock et al. 2003; Svensson & Carlsson 2005). Methodologically, the spread of seeds within a site was followed by counting seeds in regularly arranged ground traps (Bullock et al. 2003) or mapping the established seedlings (Heinken 2004) around an artificially established clump of source plants at an unoccupied site, which enabled a fine quantification of dispersal kernels within a several-metre radius. Bullock et al. (2008) used the fitted dispersal kernels to model the spread of *Rh. minor* population, but they admit that the application of their model is limited by the field scale.

Other dispersal means are expected to work at the landscape scale and they were studied exclusively by following the dispersal agent. Endozoochory was suggested for *Melampyrum* spp., but no clear result was achieved (Chlumský et al. 2013). The flat shape of *Rhinanthus* spp. seeds could enable epizoochory or hemerochory, when the seeds can stick to the fur of wild or farm animals or parts of machinery. The efficiency of epizoochory was experimentally tested for a grassland community with *Rh. minor*. Its dispersal parameters ranked about the 25% quantile of recorded species, and a few long-distance dispersal events were recorded (heifer: 180 m, Kiviniemi & Eriksson 1999; fox dummy: 500 m, Hovstad et al. 2009). Dispersal of seeds between sites stuck to a tractor mower was recorded for *Rh. major* (Strykstra et al. 1997). Sullivan (2018) studied genetic connectivity of *Rh. minor* populations demonstrating isolation by distance only, without a connection to landscape structure.

The recent research demonstrates the possible means of dispersal, but it does not enable the quantification of the long-distance dispersal events. We applied the genetic approach on *Rh. minor* as a model species to study the effective population connectivity in the landscape. Microsatellite markers for *Rhinanthus* spp. have been developed recently, and they have been suggested for studying genetic structure of its populations (Ducarme et al. 2008; Houston & Wolff 2009). The recent dispersal can be inferred from genetic structure of a metapopulation. Considering the means of *Rhinanthus* dispersal stated above, we expect that the isolation can be related to spatial distance as well as to other factors, such as land use (grassland with varying management intensity, other agricultural or urban land) or farm structure (more frequent dispersal by machinery within a farm property).

Genetic methods must be used with care in dispersal models, since the genetic differences are affected not only by seed dispersal, but also pollen dispersal (Cain et al. 2000) and various evolutionary processes. Our study was performed in rather small and homogenous areas where the evolution of distinct types cannot be assumed, but we need to take into account the hybridization of *Rh. minor* and *Rh. major* when they co-occur (Kwak 1980; Ducarme & Wesselingh 2005; Natalis & Wesselingh 2012). Since it may increase genetic variation with patterns not related to the studied processes, individuals of both species must be sampled to identify and exclude hybrids. On the contrary, the effect of pollen dispersal cannot be eliminated from the data and can be only discussed. However, this effect is expected to play a minor role because of the high autogamy of target species (Kwak 1980; Hargreaves et al. 2015) and the short dispersal distance of pollinators (Widén & Widén 1990).

# Parasitism

The target hemiparasites attach their roots to the roots of other plants to gain their resources, but at the same time their shoots are photosynthetic. They attack the host plants in the juvenile stage (van Hulst et al. 1987; Watling & Press 2001; Westbury 2004) through an organ called the haustorium, which mediates xylem – xylem connection (Irving & Cameron 2009). The xylem sap contains mainly water and mineral nutrients but also a certain amount of organic carbon (Irving & Cameron 2009). It was shown that over 75 % of the parasites' water (Jiang et al. 2003), over 85 % of mineral nutrients (Jiang et al. 2010), and a large portion of the parasites' organic carbon (5 – 90 %; Těšitel et al. 2010a, 2011) originates from its' host.

A certain degree of host specificity can be observed in the target hemiparasites, but this is based on host resistance, not selection by the parasite. The parasites try to attack any roots, but the hosts differ in their quality with some species completely resistant (Gibson & Watkinson 1991; Seel et al. 1993; Westbury 2004; Cameron et al. 2006; Cameron & Seel 2007), making it difficult to reliably determine the host species. Generally, grasses and legumes are better hosts, but various other plants can be parasitized as well (Gibson & Watkinson 1989, 1991; Matthies 1996; Joshi et al. 2000; Schädler et al. 2005; Svensson & Carlsson 2005; Cameron et al. 2006).

The exploitation of host resources results in the reduction of their biomass, but this loss is not compensated by the parasite's biomass. Depending on parasite's abundance, it can decrease the community biomass by up to 50 % (Matthies 1995b, 1998; Seel & Press 1996; Davies et al. 1997; Joshi et al. 2000; Keith et al. 2004; Pywell et al. 2004; Cameron et al. 2008; Mudrák & Lepš 2010; Loydi et al. 2018). For this reason, the target species were considered weeds in the past, causing serious losses in fodder produce. However, as the species disappeared from productive grasslands, opinions on their ecology changed. Parasitic suppression of hosts, which are often dominant species in grasslands, decreases the competitive pressure in the community and is supposed to promote establishment and survival of other species. The capability of the target species to affect community composition is often used in restoration projects to increase biodiversity in species poor sites (Blakesley & Buckley 2016). The vast majority of studies reported a decrease in biomass, especially of the grass and legume component, but the increase in biodiversity was sporadic (Gibson & Watkinson 1991, 1992; Davies et al. 1997; Pywell et al. 2004, 2007; Bullock & Pywell 2005; Cameron et al. 2005; Westbury et al. 2006; Westbury & Dunnet 2007, 2008; Mudrák & Lepš 2010; Hellström et al. 2011; Wagner et al. 2011; Heer et al. 2018). There is also a risk of a resistant subordinate species to prevail after a parasite's introduction, e.g. *Leucanthemum vulgare* or *Plantago lanceolata*, hindering the required increase of diversity (Westbury & Dunnett 2007; Mudrák & Lepš 2010).

One of the novel targets in restoration experiments with hemiparasites is the suppression of *Calamagrostis epigejos*. This grass species, with vigorous growth and spread, can dominate in abandoned areas, forest clearings, grassland after cessation of management and even in grassland with continuous low-intensity management in protected areas (Bravencová 2003; Klimeš et al. 2013). The combination of highly competitive above-ground biomass and the slow-decomposing litter hinders establishment and survival of co-occurring species (Somodi et al. 2008; Mudrák et al. 2013). Due to the conservative nutrient strategy with large storage of nitrogen compounds in roots (Kavanová & Gloser 2005; Gloser et al. 2007), it recovers fast after biomass removal by mowing. Standard management for controlling the cover of this grass is regular mowing twice a year, which prevents *C. epigejos* domination, but its decline is slow (Házi et al. 2011; Rebele 2014; Těšitel et al. 2017). Mowing once a year is not controlling *C. epigejos* performance sufficiently. More frequent mowing could increase the *C. epigejos* decline by depleting nutrient pools from the soil and the roots and rhizomes of *C. epigejos*, but it is more laborious and may be harmful to species of conservation interest. The use of *Rhinanthus* spp. should decrease host above-ground biomass and deplete nutrients from *C. epigejos* below-ground storage and therefore accelerate *C. epigejos* decline with low mowing frequency. First experiments (Těšitel et al. 2017) showed that the right choice of *Rhinanthus* species (not the small *Rh. minor*, which is used in British restoration studies) in combination with mowing and litter removal can really suppress even such a vigorous host. A set of about 20 experimental sites was established throughout Czechia and Slovakia, mostly in 2014, aiming to find suitable conditions for applying the *Rhinanthus* treatment. A case study with the first results of this project (established in 2013 already with the same design) is included in this thesis.

# Aims of the thesis

The central aim of the thesis is to explore the factors affecting (meta)population dynamics of *Rhinanthus* spp. and *Melampyrum nemorosum* in respect to their persistence in natural habitats as well as in restoration projects. In particular, we aimed to explore the induction of persistent seed bank (Chapter 2), environmental factors determining establishment of seedlings and persistent populations (Chapter 2) and the effect of management on population re-establishment (Chapters 3 and 4). In addition to population persistence within sites, we aimed to explore the long-distance seed dispersal in landscape (Chapter 5).

The aim of the final case study was to test whether the introduction of *Rh. major* in combination with increased mowing intensity can reverse the expansion of *Calamagrostis epigejos* in species-rich semi-natural grassland (Chapter 6).

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# Chapter 2

## Establishment of hemiparasitic *Rhinanthus* spp. in grassland restoration: lessons learned from sowing experiments.

Mudrák et al. 2014. Applied Vegetation Science 17: 274–287

# Establishment of hemiparasitic *Rhinanthus* spp. in grassland restoration: lessons learned from sowing experiments

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## Abstract

**Questions:** What are the factors limiting the establishment of hemiparasitic *Rhinanthus* spp., ecosystem engineers promoting diversity and stability of communities, in restoration of species-rich meadows? How can these constraints be overcome?

**Location:** Czech Republic, Central Europe.

**Methods:** Sowing of *Rhinanthus* seeds in grasslands of different history, biotic and abiotic properties accompanied by a variety of management practices. Followed by consequent monitoring of establishment and population dynamics and synthesis of relevant literature resources.

**Results:** Appropriate timing of seed sowing is needed for successful *Rhinanthus* introduction. *Rhinanthus* seeds must be sown at latest in November to break their dormancy (valid for Central European populations; might be different e.g. in oceanic regions). *Rhinanthus* is able to establish a viable population mainly in low- to moderately-productive grasslands with biomass production below 500 $g \cdot DW \cdot m^{-2}$. Its establishment is difficult in grasslands of higher productivity due to the effect of competition for light on the seedlings. *Rhinanthus* requires grasslands with appropriate management including mowing and removal of litter, if it accumulates. Otherwise, the litter layer strongly suppresses seedling establishment. Litter removal can allow establishment even in some productive communities such as stands of *Calamagrostis epigejos*. However, early mowing (i.e. mowing conducted before ripening of seeds) can lead to a strong decline or even local eradication of the hemiparasitic annuals. Soil conditions on the site of seed origin and the target site can play an important role, as indicated by failures of establishment of *Rhinanthus* originating from slightly acidic soils on sites with soil pH values around 8. Suitability of a given site for *Rhinanthus* introduction can be estimated on the basis of its species composition using the Beals index or less formal expert knowledge of local floristic associations of *Rhinanthus* spp. In the case of suitable conditions, sowing density of 200–500 seeds·m$^{-2}$ should be sufficient for *Rhinanthus* establishment.

**Conclusion:** Mowing or grazing, litter removal, proper timing of sowing, and use of the seeds from local seed sources should considerably increase probability of *Rhinanthus* successful introduction. However, stochastic events like adverse weather conditions or damage through herbivory make the prediction of introduction success difficult.

## Keywords

Ecological restoration, Ecosystem engineer, Grassland management, Hemiparasite, Seedling establishment, Species-rich meadows.

## Abbreviation

DW: dry weight

## Nomenclature

Kubát et al. (2002)

# Chapter 3

# Victims of agricultural intensification: Mowing date affects *Rhinanthus* spp. regeneration and fruit ripening.

Blažek & Lepš (2015). Agriculture, Ecosystems and Environment 211: 10–16.

# Victims of agricultural intensification: Mowing date affects *Rhinanthus* spp. regeneration and fruit ripening

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## Abstract

The recent population declines of annual hemiparasitic *Rhinanthus* species may be a result of changes in mowing dates associated with the intensification of grassland management, but the causal mechanisms are not well understood. We aimed to determine the dynamics of *Rhinanthus* regeneration after cutting and of fruit ripening under silage or hay making. Mowing was simulated on several dates from mid-May to mid-July in populations of a vernal ecotype of *Rhinanthus minor* and an aestival ecotype of *Rh. alectorolophus*. Survival and regeneration of clipped plants, as well as fruit ripening were monitored in the experiments. We showed that *Rhinanthus* species were capable of resprouting, albeit with high mortality, but only in early spring before the lower leaves were shed. The time of fruit ripening differed among phenological types by over a month and a considerable number of fruits ripened during hay making. If meadows are mown in the period when plants are not able to regenerate and not enough fruits have ripened, *Rhinanthus* populations could undergo a massive decline. Postponing the first cut until fruits start to ripen is necessary for the protection of these species. Current Czech agri-environmental measures (AEMs) subsidize postponing the first cut only in protected areas, which limits the distribution of *Rhinanthus*. Moreover, the earliest date for a postponed cut is mid-July, which is too late considering forage quality. We suggest implementing a late-June mowing, which would diversify the mosaic of various mowing dates within protected areas, and could be widely acceptable for farmers in nonprotected landscapes.

## Keywords

Yellow rattle, Hemiparasite, Agri-environmental schemes, Agricultural intensification, Delayed mowing.

## Abbreviation

AEMs: agri-environmental measures.

# Introduction

The agricultural amelioration of grasslands (i.e. fertilizer application, more frequent mowing, silage making etc.) increases the yield and quality of fodder (Zechmeister et al. 2003), but its detrimental effects on biodiversity are numerously documented (Zechmeister et al. 2003; Kleijn et al. 2009; Čížek et al. 2012). At the same time, as the demand for grassland fodder decreases (Hodgson et al. 2005), some grasslands are threatened by management abandonment (Isselstein et al. 2005; Poptcheva et al. 2009). A system of subsidies was established first to allow farmers to keep farming. Additionally, agri-environmental measures (AEMs) were introduced to motivate farmers to give up intensive farming practices by compensating for the loss in profitability. These AEMs could be a strong tool for nature conservation, but they have not been as effective in the protection of biodiversity as they could be (Coulson et al. 2001; Zechmeister et al. 2003; Hodgson et al. 2005). If AEMs are to effectively protect biodiversity and promote the survival of endangered species, they must be based on a more intimate knowledge of the needs of these species, based on real field data.

*Rhinanthus* species (Orobanchaceae) are annual hemiparasitic herbs growing in various grasslands in Europe, Asia and North America (Skála & Štech 2000; Těšitel et al. 2010). While these species can be harmful weeds in grasslands and corn fields (Carruthers 1899, 1903; Bastin 1915; Rabotnov 1956; Mizianty 1975), with *Rh. minor* even invading North America (van Hulst et al. 1987; Smith & Cox 2014), they are nowadays declining throughout Europe (Linusson et al. 1998; Westbury 2004; Ameloot 2007). The sites where they still grow are usually in areas without intensive management due to unsuitable environmental conditions or nature protection limits. They are missing in productive grasslands (ter Borg 1972; Westbury 2004), as well as in abandoned grasslands (ter Borg 1972; Lindborg et al. 2005; Ameloot et al. 2006). This distribution suggests that *Rhinanthus* species are harmed by changes in grassland management, but the reasons remain unclear.

The response of *Rhinanthus* to increased grassland productivity is rather complex due to its hemiparasitic life strategy. The plants are outcompeted when biomass is too high, but utilizing host resources, they can tolerate an increase in biomass up to about 500 $g \cdot m^{-2}$ (van Hulst et al. 1987; Hwangbo & Seel 2002; Westbury et al. 2006; Hejcman et al. 2011) and even largely reduce host biomass (Ameloot et al. 2005; Westbury & Dunnett 2007; Mudrák & Lepš 2010).

The response of *Rhinanthus* to increased mowing frequency is less understood. If mowing starts to interfere with *Rhinanthus* flowering, which can happen when the first cut is shifted to an earlier date, *Rhinanthus* seed production could be decreased. As an annual with poor dispersal (Bullock et al. 2003) and an only shortterm persistent seed bank (ter Borg 1985; van Hulst et al. 1987; Mudrák et al. 2014), *Rhinanthus* is expected to respond rapidly to a decrease in seed production (Smith et al. 2000; Westbury 2004; Bullock & Pywell 2005; Bullock et al. 2008). The impact of seed loss caused by early mowing on *Rhinanthus* population persistence has been documented (Smith et al. 2000; Magda et al. 2004), but neither of these studies described the seasonal dynamics in detail. Seed loss can be amplified by making silage instead of hay, which prevents some fruits from ripening after the cut (Smith et al. 1996; Svensson & Carlsson 2005), but the effect of this difference on seed production has not been quantified.

If *Rhinanthus* fails to produce seeds due to early mowing, it must be able to resprout and flower once more to keep its population stable. Despite being annuals without storage organs, *Rhinanthus* species have a limited regeneration capability (ter Borg 1972; Huhta et al. 2000). However, no study has investigated how the combination of both regeneration and the timing of fruit ripening could affect the persistence of *Rhinanthus* populations.

In this study we simulated mowing of meadows with *Rhinanthus* in order to ascertain the response of *Rhinanthus* to various mowing dates. We tried to answer two principal questions:(1) What is the latest mowing date for *Rhinanthus* to still be capable of regeneration, and which factors affect the regeneration rate? (2) What is the earliest mowing date for *Rhinanthus* to still produce ripe fruits, and is there any difference between hay and silage making?

# Materials and methods

## Study species and study sites

*Rhinanthus* species are known for seasonal polymorphism. A number of forms varying in plant architecture and phenology can be found within each species, ranging from small unbranched types which flower from mid-May (vernal ecotypes), through intermediate types (aestival ecotypes), to big branched types which flower from July (autumnal ecotypes; terminology according to Zopfi,1993, 2011; ter Borg 1972, 1985; Skála & Štech 2000; Westbury 2004). We deliberately put higher importance on the ecotypes than on the actual species identity. To underlie this variability, we have chosen two natural populations whose onset of fruit ripening is shifted by over a month.

The first experimental population represents the vernal ecotype of *Rhinanthus minor* L. characterized by very small and early flowering individuals (Table 1). This species is still relatively common in low-productivity meadows and pastures, and it also inhabits disturbed places such as road and path verges (Skála & Štech 2000; Westbury 2004). The experimental site was situated in a meadow near Hejdlov (Blanský les protected landscape area, South Bohemia, Czech Republic, 48°52′3.8″N, 14°14′46.4″E) at an altitude of 740 m a.s.l. on a south-east facing slope of 13°, in a low productive species rich mesic meadow (Arrhenatherion alliance).

The other population is the aestival ecotype of *Rhinanthus alectorolophus* (Scop.) Pollich, representing big branched, and phenologically intermediate forms (Table 1). This species was once thought of as a corn-field weed (Skála & Štech 2000) but is now considered vulnerable according to the Czech Red List (Grulich 2012) and is also declining in Germany (Zopfi 2011). This is one of the tallest *Rhinanthus* species, which can grow also in slightly more productive meadows, fallow land, or road verges (Skála & Štech 2000). The experimental site was situated in a meadow near Hrabětice (Jizerské hory protected landscape area, north-east Bohemia, Czech Republic, 50°46′48.3″N, 15°11′26.2″E) at an altitude of 770 m a.s.l. on a west facing slope of 6°, in a medium-productive species-rich mesic meadow (Polygono–Trisetion alliance).

Table 1: An overview of the growth habit and phenology of the studied *Rhinanthus* types. See methods for details about data collection. Data from the first clipping date in the regeneration experiment (for plant height) and the first clipping date in the fruit ripening experiment (for flowers per plant) were excluded, because the parameters did not reach final values. Note that the number of seeds per fruits refers only to seeds that fell out of fruits during haymaking simulation.

| | *Rh. minor* | *Rh. alectorolophus* |
|---|---|---|
| Ecotype | Vernal | Late aestival |
| Onset of flowering | 20 May | 20 June |
| First fruits open | 5 June | 15 July |
| Branches | 0 | 2 – 10 |
| Internodes | 5 – 8 short | 6 – 10 long |
| Plant height [cm] ± s.d. (n) | 16.3 ± 4.6 (120) | 25.7 ± 5.9 (60) |
| Flowers per plant ± s.d. (n) | 2.63 ± 1.64 (120) | 27.5 ± 22.9 (60) |
| Seeds per fruit ± s.d. (n) | 8.68 ± 2.80 (79) | 3.17 ± 1.54 (30) |

## Regeneration

The first experiment tested the response of *Rhinanthus* individuals to being clipped at various dates and heights. The experiment for *Rh. minor* was carried out in 2011. We marked out 6 points in a rectangular grid of 2 × 3 points in a place with visually homogeneous distribution of *Rh. minor*. The distance of neighbouring points was 2.5 m. We simulated mowing on 3 dates from mid to the end of May (14, 22, 28 May) around two randomly selected points (out of the six) on each of the three dates. The area around each point was split into three sectors with different clipping heights (3, 6 and 9 cm) assigned randomly to the sectors. In each sector, we labelled 10 *Rh. minor* plants, nearest to the point, with small plastic tags. In total, we labelled 180 plants: 3 dates × 2 replications (points) × 3 heights (sectors) × 10 plants. We clipped each plant to a given height and recorded its original height, the number of leaves remaining after clipping, and its phenological stage (no flower buds present, only flower buds present, flowers or fruits also present). There were no lateral shoots on the plants, only the buds. We clipped also the surrounding vegetation at the same height to the distance of about 15 cm further from the point than the furthest labelled plant occurred. Three weeks after clipping, we checked each labelled plant and counted the lateral shoots shorter and longer than 1 cm separately. At the end of June (at one date for all clipping dates), we checked the labelled plants once more and again counted the lateral shoots and flowers or flower buds on each shoot.

Data collection for the experiment with *Rh. alectorolophus* was carried out in 2013 using an analogous methodology adjusted for the different phenology of this species. We marked out 4 points and simulated mowing in two dates (18 May, 8 June) around two randomly selected points (out of the four) on each of the two dates resulting in 120 plants clipped in total.

### Fruit ripening

The second experiment focussed on the dynamics of fruit ripening in response to making hay or silage. Data collection took place in the same sites and in the same years as the above described experiment. For *Rh. minor*, in a place with visually homogeneous distribution of its individuals, we marked out 9 points in a rectangular grid of 3 × 3 points. The distance of neighbouring points was 2.5 m. We simulated mowing in 3 dates from the end of May to mid-June (28 May, 5, 13 June 2011) around 3 randomly chosen points in each date. We clipped 20 *Rh. minor* plants, nearest to the point on the given date (3 replications (points) × 3 dates × 20 plants = 180 plants in total). On each plant, we assigned each fruit to a ripeness category (Appendix 1), and put the plant into a labelled paper bag. Then we simulated hay making by drying the bags in about 3 layers at room temperature. One week after clipping, we assigned each fruit to a ripeness category again and counted the seeds that had fallen out spontaneously of the ripened fruits.

Data collection for the experiment with *Rh. alectorolophus* followed an analogous methodology adjusted for the different phenology of this species. We marked out 6 points and collected plants on 2 dates (26 June, 14 July 2013) around 3 randomly chosen points in each date, resulting in 120 plants in total. The site was mown just after 15 July.

### Data analysis

As the vast majority (88%) of *Rh. minor* shoots that were shorter than 1 cm 3 weeks after clipping died or did not overgrow the 1 cm limit, they were considered unviable and omitted from further analyses. A multiple logistic regression (i.e. a generalized linear model with binomial distribution of response variable) was used to test the relationship between the percentage of regenerated plants in the sector and the clipping date and height. We also tested the correlations between the plant characteristics at the time of clipping (i.e. plant height, number of leaves remaining after clipping, phenological stage –

the three stages transformed to numerical values 1, 2 and 3) with treatments, and intercorrelations between each pair of these characteristics. Eventually, simple logistic regressions were used to test how regeneration of individual plants depends on their characteristics, and the threshold values of these characteristics were defined for potentially successful regeneration. We considered individual plants to be independent observations in all analyses where plant characteristics were involved. As *Rh. alectorolophus* did not regenerate at all, we could only calculate the correlations of plant characteristics and treatments for this species.

Summary statistics were computed for the number of flowers per plant and seeds per fruit. The dynamics in fruit ripening were simply plotted together with the overall results of the regeneration experiment. Since the number of fruits does not change linearly with clipping date, the differences among individual date were demonstrated using analysis of variance followed by the Tukey HSD test. To evaluate which fruits were able to ripen during haymaking, a transitional matrix between fruit ripeness categories before and after haymaking was created.

# Results

## Regeneration

In total, 11% of *Rh. minor* plants regenerated (i.e. produced at least one shoot longer than 1 cm three weeks after clipping). Regeneration success increased with clipping height and decreased with clipping date (Table 2, Fig. 1). The highest regeneration (up to 80%) was found in mid-May in the highest clipping height. On the contrary, no plants regenerated at all when clipped to 3 cm. Regeneration dropped below a level which would be sufficient for keeping a population stable (i.e. over 22%; Appendix 2) around mid-May (Fig. 2).

The plant characteristics were correlated with treatments. Plant height and phenological stage increased and the number of remaining leaves decreased in time (plant height: $r = 0.51$, $p < 0.001$; phenological stage: $r = 0.50$, $p < 0.001$; remaining leaves: $r = -0.49$, $p < 0.001$; Table 3, Appendix 3). Only the number of remaining leaves increased with clipping height ($r = 0.51$, $p < 0.001$; Table 3). These characteristics were also correlated with each other (plant height vs. phenology: $r = 0.73$, $p < 0.001$; plant height vs. remaining leaves: $r = -0.47$, $p < 0.001$; remaining leaves vs. phenology: $r = -0.41$, $p < 0.001$).

Logistic regressions demonstrated that regeneration of individual *Rh. minor* plants significantly decreased with plant height ($\chi^2_1 = 9.6$, p = 0.002), and phenological stage ($\chi^2_1 = 8.8$, p = 0.003), and increased with the number of remaining leaves ($\chi^2_1 = 64.9$, $p < 0.001$). At the time of clipping, all plants that later regenerated were shorter than 20 cm, a minimum of 4 leaves remained and no flowers were present but only 39% of plants that fulfilled all of these criteria regenerated.

There were 7% *Rh. alectorolophus* plants (all 18 May, 9 cm) that fulfilled the criteria for potentially successful regeneration of *Rh. minor*, but none of them eventually regenerated. See Appendix 3 for correlations of plant characteristics and treatments.

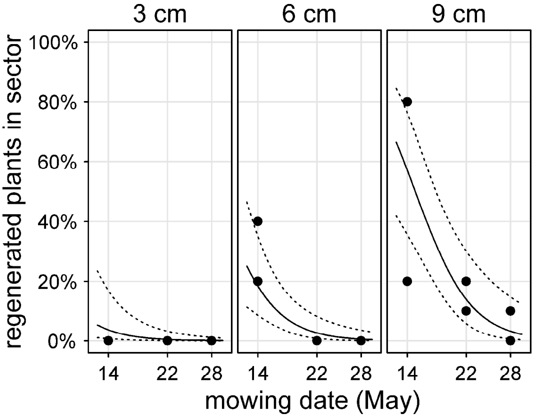

Figure 1: Dependence of percentage of regenerated *Rhinanthus minor* plants on mowing date, separate for each clipping height. Each point represents 10 plants in a sector. The result of multiple logistic regression with 95% confidence band is shown.

Table 2: Analysis of variance table of the generalized linear model with binomial distribution of response variable. Response variable: percentage of regenerated plants in the sector. Date and Height refer to clipping date and clipping height (both expressed as quantitative variables). | separates the tested effect (left) from the covariates (right), i.e. the variability explained by the first variable was tested when controlled for the second one.

| | Deviance ($\chi^2$) | Df | P |
|---|---|---|---|
| Total | 59.08 | 17 | |
| Date | 21.95 | 1 | < 0.001 |
| Height | 18.53 | 1 | < 0.001 |
| Date \| Height | 24.84 | 1 | < 0.001 |
| Height \| Date | 21.42 | 1 | < 0.001 |
| Date + Height | 43.37 | 2 | < 0.001 |
| Date * Height \| Date + Height | 1.86 | 1 | n.s. |

Table 3: Plant characteristics at individual dates: plant height, percentage of plants in individual flowering stages, and mean number of remaining leaves in each clipping height. See Appendix 3 for the regression results.

| Species (year) | | *Rh. minor* (2011) | | | *Rh. alectorolophus* (2013) | |
|---|---|---|---|---|---|---|
| Clipping date | | 14 May | 22 May | 28 May | 18 May | 8 June |
| Plant height ± s.d. [cm] | | 11.5 ± 2.9 | 15.2 ± 4.5 | 17.3 ± 4.5 | 10.9 ± 2.7 | 25.7 ± 5.9 |
| Percentage of plants with [%] | no flower buds | 58 | 33 | 18 | 100 | 100 |
| | flower buds only | 42 | 33 | 22 | 0 | 0 |
| | flowers or fruits | 0 | 33 | 60 | 0 | 0 |
| Number of remaining leaves ± s.d. | clipped in 3 cm | 1.1 ± 1.2 | 0.1 ± 0.3 | 0.1 ± 0.4 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | clipped in 6 cm | 4.2 ± 1.9 | 1.8 ± 1.9 | 0.1 ± 0.4 | 0.7 ± 1.0 | 0.0 ± 0.0 |
| | clipped in 9 cm | 4.7 ± 2.4 | 3.8 ± 1.7 | 1.4 ± 1.7 | 2.4 ± 1.7 | 0.0 ± 0.0 |

## Fruit ripening

*Rh. alectorolophus* produced about ten times more flowers per plant than *Rh. minor* and less than half the number of seeds per fruit (but not all seeds fell out of some capsules in both species), and flowering started about one month later (Table 1). A sufficient percentage of ripe fruits for keeping the population stable (i.e. over 22% and 6% for *Rh. minor* and *Rh. alectorolophus*, respectively; Appendix 2) was reached before mid-June in *Rh. minor*, and this percentage was able to ripen during haymaking around the end of May. No fruits of *Rh. alectorolophus* were ripe by mid-July, but some ripened during drying, roughly in amount needed for keeping the population stable (Table 4, Fig. 2).

The majority of large green fruits but only a small percentage of medium fruits ripened in *Rh. minor*. Ripening was lower in *Rh. alectorolophus*, but large green fruits were able to ripen at a high rate, whereas medium fruits ripened at a low rate (Table 5).

Table 4: Flower and fruit counts per plant (±s.d.) and ripe fruit percentage of total flower count. Values for each point were calculated first and these were averaged afterwards. Not all flower buds were detectable on the plant apices on the first date for both species, so that the non-zero percentage of ripe fruits could be slightly overestimated on these dates. Groups indicated with different letters differ significantly (analysis of variance, $p < 0.05$).

| Species | *Rh. minor* (2011) | | | *Rh. alectorolophus* (2013) | |
|---|---|---|---|---|---|
| Clipping date | 28 May | 5 June | 13 June | 26 June | 14 July |
| Total flowers and fruits | 2.13 ± 0.84 | 2.58 ± 0.23 | 2.68 ± 0.83 | 7.47 ± 2.69$^a$ | 27.47 ± 6.86$^b$ |
| Fruits ripe at clipping | 0.00 ± 0.00$^a$ | 0.12 ± 0.20$^a$ | 0.72 ± 0.23$^b$ | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | ( 0.0 %) | ( 4.2 %) | (26.9 %) | (0.0 %) | (0.0 %) |
| Fruits ripe after drying | 0.12 ± 0.20$^a$ | 1.62 ± 0.33$^b$ | 2.00 ± 0.60$^b$ | 0.00 ± 0.00$^a$ | 1.70 ± 0.69$^b$ |
| | ( 3.8 %) | (62.3 %) | (75.1 %) | (0.0 %) | (6.0 %) |

Table 5: The course of fruit ripening. Left – sum of fruits in the ripeness categories. Middle – a matrix summarising the transition of fruits between ripeness categories during haymaking simulation. Some categories are merged. Right – percentage of fruits in the ripeness categories within a date. See Appendix 1 for description of the ripeness categories.

*Rh. minor*

| Starting ripeness category | Count | Final ripeness category [%] | | | Clipping date [%] | | |
|---|---|---|---|---|---|---|---|
| | | Unopened | Opened unspilled | Spilled | 28 May | 5 June | 13 June |
| Flower buds, flowers | 87 | | | | 53 | 6.5 | 5.6 |
| Small fruit | 49 | 100 | 0 | 0 | 25 | 6.5 | 4.3 |
| Medium fruit | 49 | 53 | 43 | 4.1 | 13 | 15 | 5.6 |
| Large green fruit | 112 | 4.5 | 24 | 71 | 9.4 | 45 | 19 |
| Yellow unopened fruit | 70 | 0 | 5.7 | 94 | 0 | 20 | 24 |
| Opened unspilled fruit | 27 | | 3.7 | 96 | 0 | 1.9 | 15 |
| Opened spilled fruit | 50 | | | 100 | 0 | 4.5 | 27 |

*Rh. alectorolophus*

| Starting ripeness category | Count | Final ripeness category [%] | | | Clipping date [%] | |
|---|---|---|---|---|---|---|
| | | Unopened | Opened unspilled | Spilled | 26 June | 14 July |
| Flower buds, flowers | 1323 | | | | 94 | 54 |
| Small fruit | 204 | 100 | 0 | 0 | 3.8 | 11 |
| Medium fruit | 345 | 91 | 7.2 | 1.7 | 1.8 | 20 |
| Large green fruit | 226 | 30 | 27 | 42 | 0 | 14 |
| Other | 0 | | | | 0 | 0 |

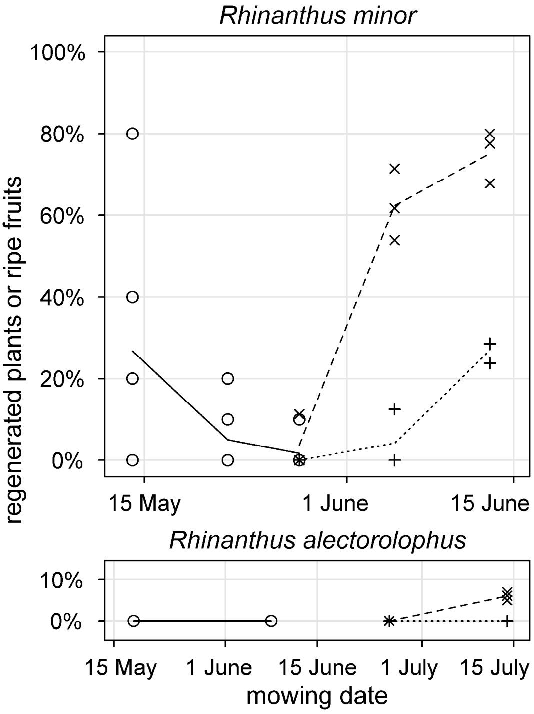

Figure 2: Dynamics in percentage of regenerated plants in each section and percentage of ripe fruits around each point. ○, solid line: percentage of regenerated plants; +, dotted line: percentage of fruits ripe at the time of clipping; ×, dashed line: percentage of fruits ripe after drying.

# Discussion

We found that there is a period in which *Rhinanthus* plants neither produce enough ripe fruits, nor resprout sufficiently. Mowing within this critical period could have a detrimental effect on the persistence of *Rhinanthus* populations. The length of this gap varies largely among species and ecotypes. It was about one month long in our early flowering *Rh. minor* population, and haymaking shortened the gap by about one or two weeks. In contrast, this gap in our late flowering *Rh. alectorolophus* population was longer than the duration of our experiment. We found no regeneration even in the first experimental date and some fruits could ripen only in hay at the end of the experiment (Fig. 2). In both sites, the usual mowing date would interfere with the critical period, but as mowing is postponed by agrienviromnental measures (AEMs) to after mid-July, both populations are persisting. Whereas the timing is very tight for *Rh. alectorolophus*, the site with *Rh. minor* could be mown one month earlier without a negative impact on its population.

## Regeneration

Despite being annuals without storage organs, regeneration of *Rhinanthus* plants after mowing was observed in this study, as well as in other studies (ter Borg 1972; Huhta et al. 2000). *Rh. minor* was able to resprout and even individuals from a population of normally unbranched plants could produce lateral shoots. Regeneration decreased with clipping date and increased with clipping height. While regeneration could be sufficient to keep the population stable (Appendix 2) until mid-May in *Rh. minor*, *Rh. alectorolophus* did not regenerate at all (Fig. 2). We cannot rule out that the *Rh. alectorolophus* would regenerate after earlier mowing, but this date would be too early for farmers. We expect that in the case of real mowing, individual plants will differ in the height where they are cut (e.g. due to soil surface relief) and this will also increase the variability in the number of remaining leaves within a single clipping date.

The effects of clipping date and height are directly interpretable in terms of agricultural practices, but the effects of these treatments are probably mediated by plant characteristics at the time of clipping. The characteristics we measured (i.e. plant height, number of leaves remaining after clipping, phenological stage) were to a large extent determined by the treatments, but they were also highly correlated with each other, so it is difficult to determine statistically which ones directly affected survival. Physiological considerations suggest that the number of remaining leaves probably plays a key role. Leaves can act as a source of energy, thereby compensating for the absence of storage organs. Huhta et al. (2000) has shown that plants that regenerated had at least one or two nodes with leaves. Our data similarly demonstrate that plants with less than four remaining leaves (i.e. 2 nodes) never regenerated. No plants with flowers at the time of clipping regenerated in our experiment (nevertheless, there were only few that had both flowers and at least four leaves remaining after clipping). The plants that have already invested in flower production have very likely no energy to invest into regeneration. The effect of plant height is probably indirect, mediated by the number of remaining leaves, because lower leaves are shed as the plants grow. ter Borg (1972) explained the differences in regeneration success by growth habit, with the focus on the length of lower internodes and branching. She found, for instance, a population of autumnal *Rh. major* (=*Rh. angustifolius* or *Rh. serotinus*) whose lower internodes were short and branched when young, making a rosette-like habit. Large parts of these plants remained after mowing and the capability of resprouting was very high. We have also found similar populations in Czechia, which were branched in lower nodes and regenerate with flowers after mowing (around Horní Planá in South Bohemia, late June 2014). We attribute the failure of our *Rh. alectorolophus* to regenerate to its long basal internodes and hence lack of remaining leaves after clipping even in the earliest date. As plant characteristics (Table 3) are more relevant for determining of the date, before which *Rhinanthus* plants resprout sufficiently, we suggest estimating whether there is still a sufficient percentage of plants (Appendix 2) which have at least four leaves remaining below the height of cutting.

### Fruit ripening

In our fruit ripening experiment, *Rhinanthus* seed production was decreased by cutting on all dates (as compared to full production without cutting), but this decrease can be to some extent compensated for by lower density-dependent mortality and higher fecundity of the next generation who will be free from intraspecific competition (Westbury & Dunnett 2007; Mudrák & Lepš 2010). The estimation of minimum seed production which will not lead to a population decline based on one-year data is provided in Appendix 2. A sufficient percentage of ripe fruits in *Rh. minor* population was attained in about mid-June if silage was made, but already by early June if hay was made. While we consider mid-June mowing to be already safe for our *Rh. minor* population, in agreement with Bullock et al. (2003), Smith et al. (2000) found it detrimental for their *Rh. minor* ecotype. *Rh. alectorolophus* had no ripe fruits even in mid-July (the cutting date postponed for the sake of subsidies), but as some fruits were already able to ripen during hay drying (about the minimum sufficient percentage), we expect fast onset of fruit production after this date.

The dynamics of fruit ripening vary not only among species and ecotypes, but also among years and regions with different climates, so we propose using plant characteristics instead of calendar dates to define cutting dates (Table 5). The date before which a population would undergo a decline must be determined individually by simply estimating if plants have already produced enough ripe fruits (Appendix 2). Where hay is made on the meadow, it is possible to count also fruits that are not yet ripe (are unopened and green), but are full size.

## Practical applications

*Rhinanthus* species have long been recognised as weeds that can reduce grassland productivity and forage quality (Carruthers 1899; Bastin 1915; Rabotnov 1959; Mizianty 1975) or even destroy cereal crops (Carruthers 1903; Mizianty 1975). It was recommended to pull out individual plants by hand or to cut them off before seeding, but not too early because of the risk of resprouting (Bastin 1915; Rabotnov 1959). The approach to grasslands has changed over the century (Isselstein et al. 2005), but the biological processes remain the same. Our results imply that intensive farming methods caused *Rhinanthus* to disappear from productive grasslands, which are mown before *Rhinanthus* fruiting. The uniform early mowing, usually done in late May or at the beginning of June, had a negative impact also on other plants flowering at the same time (Kirkham & Tallowin 1995; Zechmeister et al. 2003; Humbert et al. 2012), as well as birds during nesting (Perkins et al. 2013) and arthropods, who are dependent on various specific plant resources in all of their lifecycle stages (Konvička et al. 2008; Čížek et al. 2012; Buri et al. 2013).

Postponing the first cut until *Rhinanthus* fruits start to ripen is therefore necessary for its protection. The most important tool for regulating grassland management in Europe is agri-environmental measures (AEMs). The design of Czech AEMs for mesic meadow management, the most common habitat of *Rhinanthus* species, depends on the conservational status of a site (Ministry of Agriculture of the Czech Republic 2013). Outside protected areas, only the amount of fertilizers is regulated effectively, only indirectly affecting mowing frequency. Inside protected areas, the nature protection administration has assigned postponed mowing to individual meadows, and it is subsidized after 15 July or after 15 August.

These dates for the postponed cut are suitable for both *Rhinanthus* types in this study, which should produce enough ripe fruits by that time, but they are rather problematic for farmers. The forage quality decreases throughout season, and it is already quite low in mid-July (Kirkham & Tallowin 1995; Isselstein et al. 2005), making this measure barely acceptable at the cost of rather high financial compensation (Zechmeister et al. 2003). *Rhinanthus* suppresses nutritionally valuable grasses, and, from *Rhinanthus* plants, only the lignified leafless shoots remain in late-harvest hay, considerably decreasing its quality (Mizianty 1975; Ameloot et al. 2005). We thus suggest introducing a late-June mowing, which is a month earlier than the current date for a postponed cut, but still a month later than the usual mowing date in productive sites. This would allow for occasional control of *Rhinanthus* in case it gets overpopulated (Bullock & Pywell 2005) and it would also increase the fodder quality, so lower financial compensation should suffice. The suggested late-June cut would also be an interesting alternative for nature conservation and it could supplement current dates. The aim of nature conservation is not only to postpone, but also to differentiate the first mowing in a landscape mosaic, because there is no universal mowing date that would suit all organisms (Čížek et al. 2012; Humbert et al. 2012).

It is clear from our results that some *Rhinanthus* types would not survive in late-June mown plots, but many of them would. A thriving *Rh. minor* population in a scout campground near Frantoly, South Bohemia (pers. obs.), nicely illustrates that this date can be really favourable. The site is mown shortly before the Czech school holidays, which start at the beginning of July. Our experimental plot in Benešov (reported in Mudrák et al. 2014) is also usually mown before the end of June, and the population of *Rh. minor*, spreading vigorously throughout the locality after artificial introduction, survives more than a decade on. Not only seed production, but also seed dispersal was shown to be very limiting for *Rhinanthus* survival in the landscape (Bullock et al. 2008). Its main vector of seed transport between sites is mowing machinery, but it works only when there are still some seeds in the capsules, so the efficiency decreases over time (Smith et al. 1996; Strykstra et al. 1996, 1997; Coulson et al. 2001; Bullock et al. 2003). For instance, Coulson et al. (2001) consider mid-July to be ideal for *Rhinanthus* seed dispersal, but Bullock et al. (2003) found mid- June to be even better. Not all fruits may be ripe at the time of mowing, but a reasonable seed loss is an acceptable price for better dispersal, which is a vital process in the life of an annual (Bullock et al. 2008).

Whereas mowing can be postponed in protected areas (though the current options are not ideal), it is not regulated at all outside protected areas, which is detrimental for the whole *Rhinanthus* metapopulation. Postponing the first cut by at least a couple of weeks is essential for protection of *Rhinanthus* and so AEMs support of late-June mowing should be introduced also outside protected areas. This date should be widely acceptable for farmers, even with lower financial support. Making hay instead of silage would even increase the positive effect (Smith et al. 1996; Svensson & Carlsson 2005). We have shown that it would not suit all *Rhinanthus* types, but at least its common vernal types would be supported by mowing on this compromise date on a large scale.

Except for natural sites, *Rhinanthus* also occurs in places where species rich grasslands are being restored on formerly intensive grasslands or arable fields. *Rhinanthus* is included in sown seed mixtures as one of the desired indigenous species (Smith et al. 2000), or even as a treatment that should help with lowering biomass thanks to its parasitism (Bullock & Pywell 2005; Westbury et al. 2006; Westbury & Dunnett 2007; Mudrák et al. 2014). To keep permanent *Rhinanthus* populations in such sites, the proper timing of mowing must be applied and our results can be used as a guideline.

## Conclusions

This study not only showed that *Rhinanthus* species are seriously harmed by early mowing, but also provided details about the dynamics in crucial processes in their life cycle, highlighting the differences between phenological types. Based on this information, we conclude that the absence of *Rhinanthus* species in intensively managed grasslands and their general decline is tightly connected to changes in mowing dates and to more common making of silage. A postponed cut, which should promote plant diversity in general, is beneficial for *Rhinanthus* survival, and the grasslands where it is applied are one of its most common habitats nowadays. However, current Czech AEMs, which limit support for a postponed cut to only protected areas and support only late mowing there, can never combine both conservational effectiveness and agricultural acceptability. We suggest a compromise mowing date which should still support *Rhinanthus* survival and, at the same time, should be widely acceptable even outside protected areas, where no regulation is applied. In our opinion, AEMs are a method of cooperation with farmers, and so they should accommodate their needs in cases where it is not to the detriment of nature conservation. Making AEMs more flexible is important for diversifying the landscape mosaic and protecting plant and animal diversity.

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# Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2015.04.022.

Appendix 1 – Classification of flowers and fruits into ripeness categories.

Appendix 2 – Estimation of the amount of ripe fruits sufficient for keeping a population stable.

Appendix 3 – Relationships of plant characteristics and treatments.

# Appendix 1

Table A1.1: Classification of flowers and fruits into ripeness categories. Lower and upper boundaries are described. Only the fruits in the last category were considered ripe.

| Ripeness category | Category boundaries | |
|---|---|---|
| | Lower boundary | Upper boundary |
| Flower bud | | corolla is shorter than calyx |
| Flower | corolla is longer than calyx | corolla remains on the plant even when gently shaken |
| Small fruit | corolla falls off | capsule is oval and does not reach ripe size |
| Medium fruit | capsule is round and reaches ripe size | capsule is too flat |
| Large green fruit | capsule is as thick as in ripe fruit | capsule is green |
| Yellow unopened fruit | capsule is yellow | capsule is unopened |
| Opened unspilled fruit | capsule is opened | all seeds remain in capsule even when gently shaken |
| Open spilled fruit | at least some seeds spill out of the capsule when gently shaken | |

## Appendix 2

Reduced seed production can be compensated to some extent by performance of plants in next generation. We attempted to estimate which seed production is sufficient for keeping a population stable.

At high population densities, intraspecific competition for light and hosts causes significant reduction of plant survival, biomass, and seed production (van Hulst et al. 1987; Matthies 2003; Barham 2010; Mudrák & Lepš 2010). Lowering population density to some extent (e.g. by reduced seed production) results in lower seedling mortality, and in higher biomass and seed production per plant, which can largely balance the initial loss (Matthies 2003; Westbury & Dunnett 2007; Mudrák & Lepš 2010). However, intraspecific competition is negligible at low densities, so further lowering of *Rhinanthus* density has no positive effect on individual plant performance, which can no longer compensate for the initial decrease of density.

To keep the population size stable, the plant must produce at least as many seeds as is the reciprocal of probability that a seed develops into a mature fertile individual, also called conversion rate (Westbury et al. 2006). The relationship between a relative seed production (percentage of estimated full seed production per plant) and conversion rate when there is no change in population size can be described by the following equation:

$SP_{rel} = 1 / (CR \times SP_{tot})$

where $SP_{rel}$ is the relative seed production (~ percentage of ripe fruits ~ percentage of regenerated plants), CR is the conversion rate from seed to adult plant, and $SP_{tot}$ is potential total seed production per plant (i.e. all fruits and flowers per plant × seeds per fruit). If the seed production drops so low that the required corresponding conversion rate is higher than usual, it is not likely to be reached and the population will very probably undergo a population decline.

The usual conversion rate is about 5 – 25 % in seed-sowing experiments with low seed density ($< 500\ m^{-2}$) in suitable natural sites (Mudrák et al. 2014) and even higher in artificial sites (Westbury et al. 2006), in contrast to 1 – 10 % in stable dense populations ($> 1500$ seeds $m^{-2}$, ter Borg 1985, Kelly 1989), indicating substantial density-dependent mortality. Based on these values, we conservatively suggest that about 20 % is the highest conversion rate which is likely to be reached.

In the case of our estimated full seed production, our *Rh. minor* and *Rh. alectorolophus* populations would need the conversion rate 4.4 % and 1.1 %, respectively. A seed production corresponding to the limiting 20 % conversion rate is 22 % and 6 % of the estimated full seed production for *Rh. minor* and *Rh. alectorolophus* respectively (based on the values in Table 1). Higher seed production should still keep population stable, while lower seed production would probably lead to a population decline.

The percentage of regenerated plants can be used in a similar way to the percentage of ripe fruits, but as the damaged plants were reported to produce less fruits compared to undamaged ones (ter Borg 1972; Huhta et al. 2000), the values could be underestimated.

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## Appendix 3

Table A3.1: Results of linear regressions of plant characteristics on treatments, characterized by slope of the relationship, correlation of the response and predictor, and F-value for the analysis of variance of the regression model.

| | *Rh. minor* | | *Rh. alectorolophus* | |
|---|---|---|---|---|
| | Clipping date [day] | Clipping height [cm] | Clipping date [day] | Clipping height [cm] |
| Plant height [cm] | slope = 0.420<br>r = 0.51<br>$F_{1,178} = 63.9$<br>$p < 0.001$ | n.s. | slope = 0.707<br>r = 0.85<br>$F_{1,118} = 310.5$<br>$p < 0.001$ | n.s. |
| Phenology | slope = 0.072<br>r = 0.50<br>$F_{1,178} = 59.1$<br>$p < 0.001$ | n.s. | n.a. | n.a. |
| Remaining leaves | slope = -0.197<br>r = -0.49<br>$F_{1,178} = 57.1$<br>$p < 0.001$ | slope = 0.478<br>r = 0.51<br>$F_{1,178} = 63.2$<br>$p < 0.001$ | slope = -0.048<br>r = -0.43<br>$F_{1,118} = 26.7$<br>$p < 0.001$ | slope = 0.200<br>r = 0.41<br>$F_{1,118} = 24.4$<br>$p < 0.001$ |

## Chapter 4

# Response of two hemiparasitic Orobanchaceae species to mowing dates: implications for grassland conservation and restoration practice.

Blažek et al. (2016) Plant Ecology and Evolution 149: 31–38.

# Response of two hemiparasitic Orobanchaceae species to mowing dates: implications for grassland conservation and restoration practice

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## Abstract

**Background and aims:** *Rhinanthus* major (= *R. angustifolius*) and *Melampyrum nemorosum* are very sensitive to mowing date. As they are annuals without a long-term persistent seed bank and with a poor long-distance dispersal ability, seed loss caused by an unsuitable mowing date could lead to rapid population decline. Since their populations have disappeared from productive grasslands, they have become a focus of conservational management. *Rhinanthus* is also used in restoration projects as a treatment for reducing biomass, where its permanent populations are desired. We aimed to determine the earliest suitable mowing date for these species in White Carpathians Protected Landscape Area to support its administration to plan the management.

**Methods:** We conducted a mowing experiment with plots mown on 7 and 18 June and 5 July 2012. The number of parasites was counted in central plots before mowing and in the following growing season. The phenology of hemiparasites and co-occurring species was recorded to better understand the effects of mowing date.

**Key results:** *Melampyrum* showed a significant population decrease after mowing on 7 and 18 June, while the 5 July mowing did not inflict any significant change. The effect on *Rhinanthus* was not significant, as it was probably obscured by seed dispersal from the close surroundings.

**Conclusions:** Mowing in July is suitable for both species, while June mowing leads to population declines. Mosaic mowing (which includes early mowing in some parts of a site), could therefore gradually eradicate *Melampyrum*. *Rhinanthus* metapopulation could compensate for the seed loss by seed dispersal from neighbouring parts, but careful monitoring is necessary. When using *Rhinanthus* in restoration experiments, postponed mowing is essential to keep its population permanent. Our conclusions are widely applicable, but the particular mowing date must be determined separately for each region, species and ecotype.

## Key words

Agri-environmental schemes, Yellow rattle, Wood cow-wheat, hemiparasite, differential mowing, delayed mowing, grassland restoration, White Carpathians, conservation, endangered species.

# Introduction

Hemiparasitic plants of the family Orobanchaceae are represented in temperate European grasslands mainly by the genera *Rhinanthus*, *Melampyrum*, *Odontites*, *Pedicularis* and *Euphrasia* (Těšitel et al. 2010). They are considered ecosystem engineers because of their ability to modify competitive relations and mineral nutrient cycling in ecosystems (ter Borg 1985; Matthies 1996; Ameloot et al. 2005; Press & Phoenix 2005; Bardgett et al. 2006; Mudrák & Lepš 2010; Demey et al. 2013, 2014). Despite being very common in the past, they persisted mainly in non-intensive grasslands after the intensification of agricultural practices (ter Borg 1972, 1985; Linusson et al. 1998; Petrů & Lepš 2000; Westbury 2004; Ameloot 2007). As a result, there is a rising concern about conservation of these species (Matthies et al. 2004; Bekker & Kwak 2005; Grulich 2012) and their ecological requirements should be taken into account in conservation management planning.

The decline in the distribution of the hemiparasitic species in grasslands has been driven by intensive agricultural practices, mainly by fertiliser application and an increase of mowing frequency. Fertilizer application increases biomass production and is known to increase competition for light (Hautier et al. 2009). This decreases the establishment of hemiparasite seedlings and also the densities of their populations (van Hulst et al. 1987; Karlsson 1984; Matthies 1995; Fibich et al. 2010; Hejcman et al. 2011; Těšitel et al. 2013). By contrast, the effect of mowing regime on hemiparasitic species has received much less attention. Populations of hemiparasites were shown to be seriously harmed if mowing is applied during flowering (Smith et al. 2000; Blahník 2013; Blažek & Lepš 2015), but the problem is more complex. There is a variety of morphological types within each species differing in the onset of flowering (vernal, aestival, autumnal types), the length of basal internodes and branching frequency, affecting regeneration potential (ter Borg 1972, 1985; Zopfi 1993, 1998; Štech 2000; Westbury 2004; Blažek & Lepš 2015). These ecotypes react differently to the same mowing dates, so the most suitable mowing date must be determined separately for each species and its ecotypes.

Hemiparasites are mostly found at sites where mowing is applied in summer as a conservation management measure (Isselstein et al. 2005; Humbert et al. 2012). In recent years, a mosaic mowing regime has received substantial popularity in nature conservation. Under this regime, various mowing dates are applied to different plots within the same site, and the assignment of a plot to individual mowing dates is changed each year. This is especially important for arthropods, which require constant availability of plant resources (Konvička et al. 2008; Čížek et al. 2012; Buri et al. 2013), and also for those plant species for which the single uniform mowing date is considered not suitable (Humbert et al. 2012; Valkó et al. 2012). However, some plots under the mosaic mowing regime are inevitably mown early. This might be an issue since some plant species may react negatively to early mowing (Humbert et al. 2012). This applies in particular to those species with a short life span, without a persistent seed bank or without an efficient dispersal mechanism, which would help the metapopulation to compensate for an occasional decrease in some plots. Unfortunately, the hemiparasitic Orobanchaceae display a combination of all these traits (to an extent depending on the species) making them especially sensitive to early mowing (Westbury 2004; Bekker & Kwak 2005; Bullock & Pywell 2005; Kleyer et al. 2008; Těšitel et al. 2010).

The mowing regime is not only a concern at sites where a current population of hemiparasites exists. Maintaining metapopulation dynamics requires the occurrence of suitable unoccupied sites where plants can establish (Hanski 1998, 1999). Therefore, the mowing regime allowing existence of populations of hemiparasites should be applied also to sites where their populations are currently absent, but their occurrence would be plausible or desirable from a conservation perspective. This, however, raises the question how to choose a suitable mowing regime at such sites where the phenology of hemiparasitic species cannot be taken as a guideline and individual sites within a region can notably differ in climatic conditions resulting in shifts in plant phenology (Blažek & Lepš 2015). We suggest instead to use the phenology of cooccuring species as a useful indicator for suitable mowing dates.

*Rhinanthus* species are also used in projects where species- rich grasslands are being restored on formerly ameliorated grasslands or arable fields to help with lowering the community biomass (Bullock & Pywell 2005; Westbury et al. 2006; Pywell et al. 2007; Westbury & Dunnett 2007). To keep permanent populations in such sites, a suitable mowing date should be used.

Here, we aim to determine a suitable mowing date for two annual hemiparasitic species in the Orobanchaceae. Our study is based on an experimental application of mowing on different dates, and on monitoring the phenology of both target species and co-occurring perennial species.

# Materials and methods

## Study species and study site

Annual hemiparasites *Rhinanthus major* L. (referred to in some ecological papers using the synonyms *R. angustifolius* C.C.Gmel. and *R. serotinus* (Schönh.) Oborny, hereafter referred to as *Rhinanthus*) and *Melampyrum nemorosum* L. (hereafter referred to as *Melampyrum*) were used for this study. Both species have rather scattered distributions in central Europe. Although they may be still quite common in some regions, they both have undergone a substantial decline. Moreover, in case of *Melampyrum*, our study deals with its variety *M. nemorosum* var. *praecox* Štech, which is included in the Red List of vascular plants of the Czech Republic and considered critically endangered (Štech 2000; Grulich 2012). The studied populations of both species belong to the respective vernal ecotypes with peak flowering in the first half of June (Table 1).

The study was conducted in the Čertoryje National Nature Reserve, White Carpathians (Bílé Karpaty) Protected Landscape Area (hereafter PLA), Czech Republic. The reserve is mainly formed by regularly mown dry to mesic meadows. Grasslands form a mosaic with single or grouped trees or small forests (Jongepierová 2008). It is one of the most valuable grassland reserves in the Czech Republic, which is famous also for several world records in vascular plant species richness in plots sized between 16 and 49 m$^2$ (Merunková et al. 2012; Michalcová et al. 2014; Chytrý et al. 2015). Due to the high species richness and occurrence of many rare and protected species, the PLA administration tries to apply the best management considering also the requirements of arthropods (Čížek et al. 2012). The mosaic mowing might however be in conflict with the requirements of the hemiparasitic species growing on multiple sites in the reserve. In addition, extensive grassland restoration projects are conducted in the Čertoryje surroundings using mainly regional seed mixtures (Prach et al. 2015) and *Rhinanthus* population in the Čertoryje reserve can be used as a local seed source for facilitating grassland restoration using this hemiparasite.

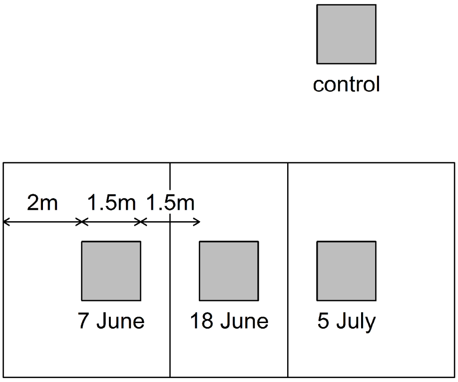

Figure 1: Arrangement of the experimental plots. Mowing was done in the whole plot (white) on the specified dates, hemiparasites were counted only in the central plots (grey). There were two such blocks for *Melampyrum*, two for *Rhinanthus* and three for both species combined. The position of the control plot varied.

## Experimental design and data analysis

We selected plots with visually even distribution of individuals of one or both study species for our study in summer 2012. We established two blocks with *Rhinanthus*, two blocks with *Melampyrum* and three blocks containing both species (i.e. five blocks per species; 48°51′22″–48°51′47″N, 17°24′48″–17°25′11″E). Each block consisted of four permanent plots 1.5 m × 1.5 m, where parasite individuals were counted on 1 to 3 June. Three of the plots, together with the buffer zone (Fig. 1), were mown on 7 and 18 June and 5 July, 33 respectively, and hay was dried on site. The control plot was not mown on any of these days and it was located further away, so it was not influenced by the experimental mowing. The plots were mown once more in late July, when the whole area was mown by tractor-mounted machinery. The parasite plants were counted again between 31 May and 3 June 2013.

The phenology of the hemiparasites together with co-occurring species was recorded to allow for a generalization of the mowing-date recommendations between years and sites within the region. Since there is no single dominant species, we monitored fifty subdominant species. Only species which were found on most dates and showed a reasonable trend are presented. Some of these are also used in local restoration projects as sown or target species (Jongepierová et al. 2007; Prach et al. 2015).

Population change between years (i.e. count in 2013 / count in 2012) was used as the response. It was log-transformed before computations, back-transformed values are presented in figures. The effect of treatment on species response was tested for each species separately using an analysis of variance

(ANOVA) with mowing date and block identity as the main effects. When the effect of mowing date was significant, the Tukey test was performed to determine significantly different pairs of dates. As the population size of most hemiparasites is prone to large inter-annual fluctuations (de Hullu et al. 1985; Ameloot et al. 2006), the comparison of the population change in treated plots with the change in control plots is of the main interest, not the absolute change.

## Results

The number of *Melampyrum* individuals mostly decreased between years in control plots, while there was no change on average in the *Rhinanthus* population (Table 2, Fig. 2). The response of *Melampyrum* differed significantly among treatments ($F_{3,12} = 12.1$, $p = 0.001$; Fig. 2). Whereas the early mowing dates (7 and 18 June) resulted in a significant decrease in population size by 90.3% and 80.5%, respectively, when compared to the control plots, the population change in the plots mown on 5 July was not different from the control (pairwise comparisons using Tukey test: 7 June vs. control: $p = 0.003$, 18 June vs. control: $p = 0.032$, 5 July vs. control: n.s.). The effect of mowing date on the *Rhinanthus* population was not significant ($F_{3,12} = 0.22$, n.s.; Fig. 2).

Both *Rhinanthus* and *Melampyrum* were in a flowering stage on the June mowing dates, possibly with small unripe fruits. Most individuals already finished flowering in July, with almost ripe fruits able to ripen during drying of the hay, or even sporadically with some ripe fruits (Table 1). In unoccupied sites, plants that finished flowering at the same time can be used as good indicators of suitable mowing dates, such as *Cirsium pannonicum*, *Dianthus carthusianorum*, *Digitalis grandiflora*, *Geranium sanguineum*, *Knautia kitaibelii*, *Thesium linophyllon*, as well as other plants that develop their flowers or fruits during this time period (Table 1).

Table 1: Phenology of hemiparasites and of co-occuring species on the mowing dates. Species showing no or weak trend were omitted. If two values are shown, it is a difference between NE and SW slopes. Target species of restoration projects are classified to sown, spontaneously established and other target species (Jongepierová et al. 2007; Prach et al. 2015). Red List classification is also indicated (CR = critically threatened taxa, EN = endangered taxa, VU = vulnerable taxa, NT = lower risk – near threatened; Grulich 2012). Nomenclature: Danihelka et al. (2012).

| Code | Description |
|---|---|
| 0 | sterile plants without visible flower buds |
| 1a | flower buds start to appear |
| 1b | clearly visible but small flower buds |
| 1c | flower buds just before flowering, some individuals could start flowering |
| 2a | most individuals started flowering |
| 2b | peak of flowering |
| 2c | end of flowering |
| 3a | most plants just finished flowering (some plants or parts of inflorescence can still have some flowers) |
| 3b | plants after flowering with almost ripe fruits |
| 3c | plants with fruits, seeds fall out |

| Species | Red List | Target | 7 June | 18 June | 5 July |
|---|---|---|---|---|---|
| *Agrostis capillaris* | | | NA | 2a | 3b |
| *Agrostis vinealis* | | other | NA | 2b | 3b |
| *Allium carinatum* | VU | other | NA | NA | 1c |
| *Anthericum ramosum* | NT | spont. | NA | NA | 2a |
| *Arrhenatherum elatius* | | sown | 2c | 3b – 3c | 3c |
| *Asperula tinctoria* | | | 2b | 2b | 3a |
| *Astrantia major* | | other | 1c | 2a | 2b |
| *Avenula pubescens* | | spont. | 3b | 3c | 3c |
| *Betonica officinalis* | | sown | 1b | 1c | 2b |
| *Briza media* | | sown | 2b | 3b | 3c |
| *Bromus erectus* | | sown | 3b | 3c | 3c |
| *Calamagrostis arundinacea* | | | 1b | 2b | 3c |
| *Centaurea jacea* | | sown | 1a | 1b | 2a |
| *Centaurea scabiosa* | | sown | 1a | 1c | 2a |
| *Centaurea stenolepis* | | | 0 | 0 | 1c |
| *Cirsium pannonicum* | VU | sown | 2a | 2a | 3a |
| *Dianthus carthusianorum* | | sown | 2a | 2b | 3a |
| *Digitalis grandiflora* | | | 1c | 2b | 3a |
| *Elymus hispidus* | | | NA | 1c | 2b – 2c |
| *Galium verum* | | sown | 1a | 1b | 2b |
| *Geranium sanguineum* | NT | other | 2b | 2b | 3a |
| *Inula salicina* | | spont. | 0 | 1b | 2b |

| Species | Red List | Target | 7 June | 18 June | 5 July |
|---|---|---|---|---|---|
| *Iris variegata* | EN | | 2b | 3a | 3b |
| *Knautia kitaibelii* | NT | sown | 2a | 2b | 3a |
| *Lathyrus niger* | | other | 2b | 3a | 3b |
| ***Melampyrum nemorosum* var. *praecox*** | **CR** | **other** | **2b** | **2b – 2c** | **3a** |
| *Molinia arundinacea* | | other | NA | NA | 1c |
| *Orobanche alba* | VU | | NA | 2a | 2c |
| *Peucedanum cervaria* | NT | spont. | NA | 1a | 2a |
| *Phleum phleoides* | | other | 1c | 2b | 3b |
| *Prunella grandiflora* | VU | other | NA | NA | 2a |
| ***Rhinanthus major*** | | | **2b** | **2c** | **3a** |
| *Scorzonera hispanica* | VU | other | 2a | 3b | NA |
| *Serratula tinctoria* | | spont. | 1a | 1b | 1c |
| *Stachys recta* | | other. | 2b | 2b | 2c |
| *Tanacetum corymbosum* | | sown | 1b | 2b | 2c |
| *Thalictrum simplex* subsp. *galioides* | CR | | 0 | 1c – 1a | 2b |
| *Thesium linophyllon* | VU | spont. | 2b – 2c | 2c – 3a | 3a |
| *Trifolium alpestre* | | spont. | 2b – 2a | 2b – 2c | 3b |
| *Trifolium montanum* | | sown | 2a | 2b | 2c |
| *Trisetum flavescens* | | sown | 2b | 3b – 3c | 3c |
| *Valeriana stolonifera* subsp. *angustifolia* | NT | spont. | 2c – 2b | 3b | 3c |
| *Vicia tenuifolia* | | | 2b | 3a | 3b |

Table 2: Summary of plant counts per plot. Median and range is shown. There were five replicates per species and treatment.

| | *Rhinanthus* | *Melampyrum* |
|---|---|---|
| 2012: | | |
| Control | 54 (45 – 550) | 114 (42 – 350) |
| All treatments | 57 (36 – 305) | 157 (51 – 350) |
| 2013: | | |
| Control | 117 (42 – 136) | 67 (30 – 89) |
| Mown on 7 June | 68 (37 – 164) | 6 ( 2 – 11) |
| Mown on 18 June | 111 (68 – 112) | 14 ( 8 – 44) |
| Mown on 5 July | 103 (44 – 153) | 78 (56 – 168) |

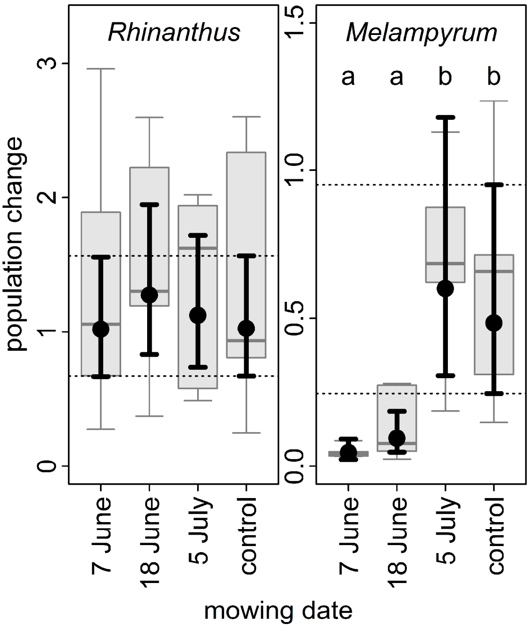

Figure 2: Response of *Rhinanthus* and *Melampyrum* to mowing date, expressed as the relative population change from the first to the second year (one means no change). Grey boxplots show median, quartiles and range of original data. Points and thick bars show means and 95% confidence intervals based on ANOVA models for log-transformed data and back-transformed for plotting. Letters indicate groups which differed significantly in Tukey tests. There were five replicates in each group.

# Discussion

We have demonstrated that the survival of *Melampyrum* growing in the Čertoryje meadows is strongly affected by mowing date. Its population size changed similarly to the control treatment after the July cut, but it was strongly reduced in the plots mown in June (Fig. 2), when the fruits were not ripe yet (Table 1). We expected similar trends for *Rhinanthus*, because it was shown in a previous study that it is harmed by early mowing (Blažek & Lepš 2015) and its phenology was very similar to *Melampyrum* (Table 1), but there was no such trend in our data (Fig. 2).

The lack of the treatment effect on *Rhinanthus* can be attributed to the "safety mechanisms" which annual plants use to compensate for occasional seed loss: seed dormancy and dispersal. The data on seed dormancy are scarce for both species, but they are considered to form only a transient seed bank (the seeds remain dormant to the first autumn or early spring) or a very scarce short-term persistent seed bank (ter Borg 1985; Pons 1991; Thompson et al. 1997). There are also sporadic observations of good *Rhinanthus* spp. population establishment with a one-year delay and it was suggested that this is caused by environmental conditions. An insufficiently long period of cold stratification or dry weather in early spring may prevent some seeds from germination, which then remain dormant (Kelly 1989; ter Borg 2005; Mudrák et. al. 2014). This could also have been the case in our experiment, as there was a dry spring in the first year (precipitation from February to April 2012 reached only 38% of the long-term mean in the region), and we observed *Rhinanthus* establishment to be postponed by one year also in a seed-sowing experiment on restored grasslands nearby.

*Melampyrum* seeds are ant-dispersed and *Rhinanthus* seeds wind-dispersed, but for both species, the natural dispersal distance is usually shorter than 1 or 2 m (Adamec 2012; Coulson et al. 2001). *Rhinanthus* dispersal can however be largely enhanced by mowing machinery within a site (Strykstra et al. 1996, 1997; Bullock et al. 2003). When the whole meadow was mown in the end of July including our plots, the heavy seeds of *Melampyrum* were not able to surpass the buffer zone, while the much lighter, winged *Rhinanthus* seeds from the surroundings might have been able to reach the central plots. Smith et al. (2000) also reported the spread of *Rhinanthus* between experimental plots. Although *Rhinanthus* was able to compensate for the local seed loss in our small-scale experiment, early mowing still presents a threat to species persistence as the species cannot rely on irregular seed dormancy, and seed dispersal is still limited to several meters within a mown area.

Until recently, postponed mowing (after 15 July) was mostly applied in Čertoryje meadows, because it is supported by agri-environmental measures (Ministry of Agriculture of the Czech Republic 2013). This was favourable for the populations of both studied species, which form stable populations in the reserve. However, the PLA administration has recently introduced a mosaic mowing scheme to preserve continual resource availability for arthropods (Konvička et al. 2008; Čížek et al. 2012). As a result, some sections of the reserve are mown early in the season and the early mowing is applied to various sections each year to maintain management heterogeneity. A possible adverse impact of this management could however be a gradual decline of hemiparasites, because of the seed loss on early-mown parts. *Rhinanthus* would be probably able to compensate for occasional seed loss and keep a persisting metapopulation, but the effect on *Melampyrum*, whose population decreased by 80–90% in the June-mown plots in our experiment, would be detrimental. We suggest, therefore, that plots with *Melampyrum*, which is more susceptible and has higher conservational priority (the vernal ecotype is considered critically endangered; Grulich 2012), should never be assigned the early cut, so they will always be mown after the beginning of July. *Rhinanthus* probably does not require special treatment, but it needs to be carefully monitored. Some of the unoccupied patches suitable for the establishment of hemiparasites should be also treated in the same way to allow for their potential spread. Proper mowing dates can be identified using the phenology of other species (Table 1) in such patches.

While the continuity of grasslands in the Čertoryje reserve was not interrupted, many semi-natural grasslands in the surroundings were destroyed by agricultural improvement or conversion to arable fields in the second half of the 20th century, and they are now being restored (Jongepierová et al. 2007; Jongepierová 2008; Prach et al. 2013, 2014, 2015). *Rhinanthus* species were shown to help in such projects, because they can lower the community biomass, mainly by suppressing grasses (Bullock & Pywell 2005; Westbury et al. 2006; Pywell et al. 2007; Westbury & Dunnett 2007). After the establishment of a sown *Rhinanthus* population (Mudrák et al. 2014), it is desirable to keep the population permanent. If seed from the studied area is used, the restored areas should be mown after the beginning of July. In addition, a finer adjustment of mowing date can be achieved on the basis of the phenology of *Rhinanthus* or the correlation with co-occuring species on the target site (Table 1) even before sowing of *Rhinanthus* in the target plots. In contrast to permanent populations, no recovery from seed bank or by natural spread from surroundings can be expected, because there are no seeds in the soil and the long-distance dispersal of *Rhinanthus* is poor (Bullock et al. 2003).

Not only are our results useful for the local nature conservation authorities, but they can also be used as a guideline case study elsewhere. The described problems and biological principles are general, only the recommended mowing date will undoubtably differ among regions with different climates and among species and their ecotypes with different phenologies (Svensson & Carlsson 2005). To compensate for this and for the variable and changing climate, which affects the phenology of hemiparasites, the phenological data on the host vegetation (Table 1) can provide a reasonable guideline for a precise setting of the mowing regime. We encourage people in charge of management planning either to at least check the hemiparasites' phenology (Svensson & Carlsson 2005; Blažek & Lepš 2015) or even to arrange a similar simple experiment as in this study to determine the earliest possible mowing date, so that a proper conservation management for hemiparasites can be applied.

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# Chapter 5

# Functional connectivity in *Rhinanthus minor* metapopulations: grassland management affects seed dispersal in fragmented landscapes

Blažek et al. (manuscript).

# Functional connectivity in *Rhinanthus minor* metapopulations: grassland management affects seed dispersal in fragmented landscapes.

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## Abstract

**Context:** Recent biodiversity decline in Europe includes several hemiparasitic Orobanchaceae species, which are adapted to traditional grassland management and have disappeared from most of their former sites due to agricultural intensification. In addition to changes in the site conditions, habitat fragmentation also contributes to this trend.

**Objectives:** We aimed to explain the connectivity within metapopulations of an annual hemiparasitic plant *Rhinanthus minor* by modelling the permeability of landscape features.

**Methods:** Genetic dissimilarities of individual populations (corresponding to their isolation) from three regions of Europe with contrasting landscape structure (Belgium, Czechia, Estonia) were used to parametrize landscape-resistance raster for computing effective spatial distances. The effect of farm identity, representing farm machinery movements, on the genetic structure was tested.

**Results:** Straight distance accounted for 2.6% of genetic variation between populations while the effective distance in best landscape models accounted for

15.6%. Corridors were formed mainly by grasslands with suitable management type in all regions, while coastal pastures in Estonia and intensive grasslands in Belgium formed barriers. Forests and urban areas formed barriers consistently in all three regions and the effect of roads varied. Interestingly, *Rh. minor* populations managed by identical farmer were also significantly more similar that those managed by different farmers in some regions.

**Conclusions:** Landscape structure has a profound impact on *Rh. minor* population connectivity. Grassland management affects not only habitat suitability but also other processes underlying the metapopulation dynamics. Our data support the hypothesis that seed dispersal is facilitated by farm-machinery movement.

## Key words

Least-cost distance, landscape resistance, effective spatial distance, plant long-distance dispersal, temperate grasslands, root hemiparasites.

*This chapter is a manuscript prepared for submission. The full version is archived by the Faculty of Science, University of South Bohemia in České Budějovice.*

## Chapter 6

# Reversing expansion of *Calamagrostis epigejos* in a grassland biodiversity hotspot: hemiparasitic *Rhinanthus major* does better job than increased mowing intensity.

Těšitel et al. (2018). Applied Vegetation Science 21: 104–112.

# Reversing expansion of *Calamagrostis epigejos* in a grassland biodiversity hotspot: Hemiparasitic *Rhinanthus* major does a better job than increased mowing intensity

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## Abstract

**Questions:** Can hemiparasitic *Rhinanthus* major originating from a local population suppress the competitive clonal grass *Calamagrostis epigejos* and reverse its expansion in species-rich semi-natural grasslands? Does sowing seeds of *R. major* facilitate restoration of target meadow vegetation? Is *R. major* more beneficial for biodiversity restoration/conservation than increased mowing intensity, a conventional measure to suppress *C. epigejos*? Location: Čertoryje National Nature Reserve, Bílé Karpaty (White Carpathians) Protected Landscape Area, Czech Republic.

**Methods:** We conducted a before-after- control- impact experiment in meadow patches heavily infested by *C. epigejos*: eight blocks, each containing four plots with four treatment combinations: (1) traditional management, i.e. mowing once in summer, (2) mowing in summer and autumn (3) mowing in summer and seed sowing of *R. major*, (4) mowing in summer and autumn and seed sowing of *R. major*. Above-ground biomass of *C. epigejos* and vegetation composition of each of the plots were monitored every year from 2013 to 2016.

To assess the effects of treatments, we analysed biomass production of *C. epigejos*, herb layer cover and vegetation composition.

**Results:** Both sowing *R. major* and an additional autumn meadow cut significantly suppressed *C. epigejos*. Their effects were additive and of comparable size. Both treatments also had significant but markedly different effects on community composition. *Rhinanthus* major facilitated directional community composition change towards the regional Brachypodio-Molinetum meadows. In contrast, increased mowing intensity significantly decreased frequency of threatened species, which however may have also been influenced by *R. major*.

**Conclusions:** Sowing of autochthonous *R. major* seeds was demonstrated as an efficient tool to suppress *C. epigejos* and facilitate community restoration. It can be combined with an additional meadow cut to further accelerate decline of the grass. The additional cut should however be used as a short-term practice (1–2 years) only to minimize potential negative effects of its long-term application on some threatened plant species. The effects of *R. major* are comparable to those of *Rhinanthus alectorolophus* reported previously. As a species occurring naturally in species-rich dry grasslands, *R. major* has a broader and longer-term application potential than *R. alectorolophus* in ecological restoration and conservation of these communities.

## Key words

*Calamagrostis epigejos*, clonality, competition, conservation management, dry grassland, ecological restoration, hemiparasite, mowing, *Rhinanthus*, White Carpathians.

## Nomenclature

Danihelka et al. (2012) for plants; Chytrý (2010) for plant communities

# Chapter 7

# General conclusions

# General conclusions

The target species (*Rhinanthus* spp. and grassland *Melampyrum* spp.) have a set of life-history characteristics which makes them simultaneously unique and vulnerable. Among these characteristics, the response to variable timing of mowing seems to be the key factor limiting population survival in otherwise suitable sites. We performed two studies describing the species performance, one with a detailed focus on individual plants' response to mowing simulation (Chapter 3), the other with a whole population response to real mowing (Chapter 4). Both studies demonstrated that mowing at the time of flowering, which often corresponds to the most common mowing date under modern grassland management, strongly reduces seed production and subsequently the population persistence. Later mowing, shortly after the seed set, is the recommended management for conservation of the target species. We also emphasised that the specific mowing date must be set individually for each site, species and ecotype because of the large phenological variation. For instance, the vernal ecotype of *Rh. minor* sets flowers at the end of May and the late aestival ecotype of *Rh. alectorolophus* at the end of June in our study, and even later flowering autumnal ecotypes are known. However, the latter are usually adapted to mowing through high regeneration ability to set seeds after mowing.

The vulnerability to timing of mowing is dangerous particularly in combination with other plant characteristics and environmental factors. We confirmed that *Rh. minor* does not form a persistent seed bank, which would provide a backup at occasional seed-set failure (Chapter 2). We tried to show if the seed bank can be induced by environmental factors, e.g. a short winter stratification period. Although there are sporadic observations of massive one-year seed banks, our experiment did not provide definitive proof.

The life history of annuals makes it hard for the target species to coexist with the perennial competitors. Large seeds and parasitic nutrient acquisition provides a certain degree of competitive advantage. However, under high plant litter (abandoned sites) or productivity (intensively managed sites), the advantage is not sufficient and the survival of early life-stages is poor (Chapter 2). In agricultural practice, artificially increasing site productivity is combined with frequent mowing, reducing both survival and fecundity of target species, and the short-term persistent seed bank cannot compensate for the regular failures caused by intensive management.

In addition to processes within sites, seed dispersal between sites within a metapopulation is another important factor for species persistence. Based on the spatial models, we demonstrated that suitable grassland management enhances population connectivity of *Rh. minor* (Chapter 5). This is mainly a result of direct seed dispersal by farm machinery and by creating suitable habitats for stepping-stone populations. This implies that management is not only a concern at sites where current populations of the target species exist, but also at unoccupied sites potentially suitable for the establishment of new populations. We also highlight the importance of seed dispersal at the landscape scale and the lack of knowledge about plant dispersal in general. This gap must be filled as a matter of urgency to provide reliable information for nature-conservation practitioners so that they can properly handle the connectivity of valuable habitats.

Finally, we demonstrated that *Rh. major* is able to suppress *Calamagrostis epigejos* in grassland restoration projects (Chapter 6). The effect of the parasite is comparable to the effect of additional mowing and these two treatments can be successfully combined. Moreover, the presence of parasite supported species of the target community, so its use as an additional treatment in grassland restoration is recommended. To achieve this, suitable conditions must be created for the parasite based on knowledge of its population establishment and survival (Chapters 2, 3 and 4), especially initial litter removal, establishment of regular management with suitable mowing dates and prevention of deer herbivory. Papers resulting from the same project that include more details about the use of *Rhinanthus* spp. for the suppression of *C. epigejos* are to be published soon, adding a landscape context.

The target species are representatives of a unique functional group in temperate grasslands and, due to the changes in landscape management, several of them have been classified as endangered. At the same time, the common species are used to help with grassland restoration. This thesis provides comprehensive information about their ecological needs, which can be used for effective protection of rare species and for a successful application of hemiparasitic treatment.

# Chapter 8

# Curriculum vitae

# Curriculum vitae

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|---|---|
| Date of birth: | 22 March 1987 |
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## Education

| | |
|---|---|
| 2002 – 2006: | Secondary education: Grammar school of Zikmund Winter, Rakovník. |
| Thesis: | Jilmová alej podél silnice z Rakovníka do Pavlíkova a její zdravotní stav [Elm tree line along the road from Rakovník to Pavlíkov and its health condition.] (2005) |
| 2006 – 2010: | Bachelor study programme: Biology. Faculty of Science, University of South Bohemia, České Budějovice. |
| Thesis: | Čím je omezováno rozšíření poloparazitické rostliny kokrhele menšího (*Rhinanthus minor*)? [Which factors limit the distribution of the hemiparasitic plant *Rhinanthus minor*?] (2009) |
| 2010 – 2012: | Master study programme: Botany. Faculty of Science, University of South Bohemia, České Budějovice. |
| Thesis: | Faktory ovlivňující populační dynamiku poloparazitické rostliny kokrhele menšího (*Rhinanthus minor*). [Factors affecting population dynamics of the hemiparasitic plant *Rhinanthus minor*.] (2011) |
| Since 2012: | Doctoral study programme: Botany. Faculty of Science, University of South Bohemia, České Budějovice. |

## Publications in journals with impact factor

Mudrák O., Mládek J., **Blažek P.**, Lepš J., Doležal J., Nekvapilová E. & Těšitel J. (2014) Establishment of hemiparasitic *Rhinanthus* spp. in grassland restoration: lessons learned from sowing experiments. Applied Vegetation Science 17: 274–287.

**Blažek P.** & Lepš. J. (2015) Victims of agricultural intensification: mowing date affects *Rhinanthus* spp. regeneration and fruit ripening. Agriculture, Ecosystems and Environment 211: 10–16.

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Světlíková P., **Blažek P.**, Mühlsteinová R. & Těšitel J. (2016) Tracing nitrogen flow in a root-hemiparasitic association by foliar stable-isotope labelling. Plant Ecology and Evolution 149: 39–44 (not included in this thesis).

Těšitel J., Mládek J., Fajmon K., **Blažek P.** & Mudrák O. (2018) Reversing expansion of *Calamagrostis epigejos* in a grassland biodiversity hotspot: hemiparasitic *Rhinanthus major* does better job than increased mowing intensity. Applied Vegetation Science 21: 104–112.

## Unpublished manuscript

**Blažek P.**, Lepš J., Hořčicová Š., Pavlů L., Kook E., Wesselingh R. & Těšitel J. Functional connectivity in *Rhinanthus minor* metapopulations: grassland management affects seed dispersal in fragmented landscapes.

## Other contribution

Těšitel J., Těšitelová T., **Blažek P.** & Lepš J. (2016) Parasitism and mycoheterotrophy. – www.pladias.cz.

**Blažek P.** & Lepš J. (2016) Symbiotic nitrogen fixing. – www.pladias.cz.

## Conferences and thematic meetings

| | |
|---|---|
| 9 – 11 May 2013 | PopBio2013, Tartu, Estonia. |
| Talk: | How does *Rhinanthus minor* respond to early mowing? |
| 12 – 15 September 2013 | Third symposium on the biology of non-weedy parasitic plants, Namur, Belgium. |
| Talk: | Response of grassland rhinanthoid Orobanchaceae to different mowing dates. |
| 31 January 2014 | Botanická sekce při Správě CHKO Bílé Karpaty [Botany Division of the Administration of the Protected Landscape Area White Carpathians], Veselí nad Moravou, Czechia. |
| Talk: | Reakce rhinanthoidních poloparazitů na různé termíny seče. [Response of rhinanthoid hemiparasites to different mowing dates.] |
| 29 – 30 November 2014 | Česká botanická společnost [Czech Botanical Society], Praha, Czechia. |
| Poster: | Reakce poloparazitů z čeledi Orobanchaceae na různé termíny seče. [Response of hemiparasites from the family Orobanchaceae to different mowing dates.] |
| 14 – 16 May 2015 | PopBio2015, Tübingen, Germany. |
| Poster: | Metapopulation ecology of *Rhinanthus*, reconstructing population connectivity. |
| 23 – 25 October 2015 | Česká společnost pro ekologii [Czech Society for Ecology], České Budějovice, Czechia. |
| Poster: | Metapopulation ecology of *Rhinanthus*, reconstructing population connectivity. |
| 13 – 16 December 2015 | British Ecological Society, Edinburgh, United Kingdom. |
| Poster: | Metapopulation ecology of *Rhinanthus*, reconstructing population connectivity. |
| 21 – 23 October 2016 | Seventh Meeting of PhD students in Plant Ecology and Botany, Tihany, Hungary. |
| Talk: | The interaction of hemiparasitic *Rhinanthus* spp. with their host communities. |

## Internship

University of Tartu, Department of Botany, Macroecology workgroup led by prof. Meelis Pärtel. Tartu, Estonia, 30 June 2012 – 6 February 2013.

## Teaching

Biostatistics (tutorials, CZ, 2013 – 2016)
Biostatistics (lectures and tutorials, EN, 2012, 2017 – 2018)
Field work I "Vomáčka" (excursion, CZ, 2013 – 2016)
Field work III - 2 "Mohelno" (excursion, CZ/EN, 2013, 2015, 2017)
Occasional participation in other statistical and field courses.

## Thesis supervision

Šímová Š. (2017) Porovnání různých typů obhospodařování luk v předhůří Novohradských hor. [Comparison of different types of grassland management in the foothills of the Novohradské Mountains. B.Sc. thesis, in Czech.] – p. 27, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

# Chapter 9

# Shrnutí

[Summary, in Czech]

# Shrnutí

Práce se zabývá ekologií vybraných druhů z čeledi zárazovitých (Orobanchaceae), konkrétně druhy rodu kokrhel (*Rhinanthus*) a černýš (*Melampyrum*). Tato skupina druhů je známá svým parazitickým chováním, které však není hlavním tématem této práce. Mnoho těchto druhů bylo v minulosti velmi hojných, ale z většiny svých původních stanovišť vymizely. Hlavní otázkou této práce proto je „proč vymizely“.

Problematiku přežívání populací objasňuje série experimentů zaměřených na vliv prostředí na cílové druhy v různých fázích jejich životního cyklu, především uchycování semenáčů (kapitola 2), reakce na obhospodařování stanovišť (kapitoly 3 a 4) a šíření semen v krajině (kapitola 5).

Ukázali jsme, že hlavním důvodem pro absenci zájmových druhů na jinak vhodných lokalitách je jejich reakce na různé termíny seče. Seč v době květu, která odpovídá nejběžněji používanému termínu v dnešním zemědělství, významně snižuje produkci semen a následné přežívání populací (kapitoly 3 a 4). Pro zachování populací se obecně doporučuje pozdější seč, konkrétní termín je však vzhledem ke značné mezidruhové i vnitrodruhové fenologické variabilitě nutné vždy určit individuálně.

Časná seč ohrožuje populace zájmových druhů zejména v kombinaci s dalšími vlastnostmi rostlin a prostředí. Potvrdili jsme, že kokrhel menší netvoří trvalou semennou banku, která by mohla tvořit pojistku pro občasné selhání produkce semen (kapitola 2). Do tohoto schématu však nezapadají občasná pozorování masivního výskytu semenáčků převyšujícího produkci semen v předchozím roce. Pokusili jsme se objasnit, zda může semenná banka vzniknout na základě specifických podmínek prostředí, například po příliš krátké chladové stratifikaci, avšak neúspěšně.

Luční společenstva jsou tvořena konkurenčně silnými druhy a pro zájmové druhy, které jsou jednoleté, je těžké mezi nimi přežít. K tomu jim pomáhají velká živinami bohatá semena a parazitický příjem živin, ale jen do určité míry. V prostředí s vysokým množstvím stařiny (opuštěná stanoviště) nebo vysokou produkcí nadzemní biomasy (intenzivně využívaná stanoviště) jsou podpůrné mechanizmy neúčinné a přežívání mladších životních stádií nízké (kapitola 2). V zemědělské praxi je zvýšená produktivita úzce svázána s častou (a tedy první časnou) sečí, které snižují jak přežívání rostlin, tak produkci semen, a krátkodobá semenná banka může jen stěží kompenzovat tyto pravidelné ztráty způsobené intenzivním hospodařením.

Přežívání druhů kromě procesů uvnitř stanovišť ovlivňuje také šíření semen mezi jednotlivými populacemi. Na základě prostorových modelů jsme ukázali, že vhodný způsob lučního hospodaření podporuje vyšší propojení populací kokrhele menšího (kapitola 5). To je způsobeno hlavně přímím šířením semen přichycených k zemědělským strojům, a také vytvořením vhodných biotopů pro zhuštění sítě populací. Z toho plyne, že vhodný způsob hospodaření je potřeba udržovat nejen na lokalitách stávajících populací, ale také na lokalitách vhodných pro založení nových populací. Je potřeba také zdůraznit, že šíření semen v krajině je zásadní proces pro přežívání jednotlivých populací, ale vzhledem k obtížnosti jeho studia je nedostatečně prozkoumaný. Na rozdíl od pohybu živočichů je prakticky nemožné přímo sledovat pohyb jednotlivých semen na velké vzdálenosti.

Jak už bylo zmíněno, zájmové druhy jsou nad zemí zelené a fotosyntetizují, avšak pod zemí svými kořeny napadají kořeny hostitelských rostlin a získávají tak vodu a živiny paraziticky. Fyziologie i ekologie parazitických interakcí jsou díky intenzivnímu studiu celkem detailně známé. V současnosti je snaha o využití schopnosti druhů rodu kokrhel (*Rhinanthus*) snižovat biomasu trav za účelem obnovy ochranářsky cenných společenstev. V této práci je zařazena jedna případová studie (kapitola 6) testující použití kokrhele pro potlačení expanzivní trávy třtiny křovištní (*Calamagrostis epigejos*), která působí značné problémy v praktické ochraně přírody a běžnými prostředky jí lze jen obtížně omezit. Výsledky ukazují, že při použití vhodného druhu (kokrhel větší nebo luštinec; *Rh. major*, *Rh. alectorolophus*) a zajištění vhodných podmínek pro uchycení semenáčků (vhodné kosení, odstranění a narušení opadu) jsou poloparazité velmi účinným prostředkem k rychlému omezení i tak silného druhu, jako je právě třtina křovištní. Aby bylo použití parazitů jako zásahu úspěšné, i zde je potřeba zohlednit ekologické nároky zájmových rostlin (kapitola 2).

Zájmové druhy reprezentují unikátní funkční skupinu travních ekosystémů mírného pásma. Některé druhy se kvůli změnám v hospodaření v krajině staly ohroženými a některé se využívají při ochranářské obnově luk. Tato práce shrnuje současné znalosti o jejich ekologických nárocích, které mohou být využity pro účinnou ochranu vzácných druhů i pro úspěšné použití parazitů v ekologické obnově.

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