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Review of PhD thesis submitted by Petr Blažek entitled:

Ecology of rhinanthoid Orobanchaceae Within- and between-site processes in metapopulations of selected grassland species

Overall

The thesis submitted has demonstrated an excellent knowledge and understanding of aspects influencing the abundance and persistence of ecologically important *Rhinanthus* species and *Melampyrum nemorosum* in agricultural landscapes, with a particular focus on the effects of grassland management. The main aim of the thesis was to explore factors affecting their population dynamics in natural habitats but also in restoration projects. Ultimately, the thesis aimed not only to provide recommendations for the preservation of hemiparasitic species in agricultural grassland, but also how they can be deployed to specifically target a problematic species. It is this aspect that I find most interesting.

Specific comments on individual Results Chapters

Chapter 2 - Establishment of hemiparasitic *Rhinanthus* spp. in grassland restoration: lessons learned from sowing experiments.

Overview - If *Rhinanthus* spp. are to be used in restoration projects to reduce productivity and increase the abundance of target species, then a detailed understanding of factors influencing their establishment success is required. This Chapter provides further support for previous findings by other authors; its main contribution to knowledge is the use of the Beals plant community index enabling predictions to be made about the suitability of a site to support *Rhinanthus* should the correct management actions also be applied.

Comment - The study consisted of a series of different studies looking at different aspects at a number of different sites. This made comparisons between different treatments difficult. For example, scarification which would also be expected to remove litter had no effect at one site, but the removal of litter at a different site was shown to increase establishment. Applying both treatments to both sites would have been beneficial to unravel these findings.

Chapter 3 - Victims of agricultural intensification: Mowing date affects *Rhinanthus* spp. regeneration and fruit ripening.

Overview - The removal of material from any plant species has the potential to influence seed production, which for annual species can be highly problematic. This study investigated the influence of mowing date on the potential for *Rhinanthus* spp. to re-grow following cutting, coupled with its impact on seed production in agricultural grassland.

Comment - In my view, a key finding of the study is that if *Rhinanthus* already has flowers there is no potential for it to re-grow and produce seed, suggesting that until this point cutting might be permitted. However, there might not be sufficient forage for farmers to utilize/benefit from this approach.

Chapter 4 - Response of two hemiparasitic Orobanchaceae species to mowing dates: implications for grassland conservation and restoration practice.

Overview - This chapter compliments Chapter 3 by investigating the effects of mowing date, but with a focus on two species that are perhaps more important with regards to conservation status. The aim was to determine the earliest suitable mowing date for these species enabling farmers to harvest nutritional hay without eradicating species of conservation concern. A key finding was that *Melampyrum* was more sensitive to cutting time than *Rhinanthus major.*

Comment - Recommendations provided for farmers are highly important if these species are to persist in the landscape.

Chapter 5 - Functional connectivity in *Rhinanthus minor* metapopulations: grassland management affects seed dispersal in fragmented landscapes

Overview - The importance of maintaining connectivity between individuals of the same species within the landscape is increasingly being recognised, particularly in the face of climate change and the ability of species to move to avoid unsuitable conditions. This study investigated the connectivity between meta-populations of *Rhinanthus minor* – populations believed to be connected within the landscape matrix, by exploring genetic similarities between individuals in relation to landscape permeability.

Comment - It is already known that *Rhinanthus* spp. are self-fertile and genetic transfer between individuals is minimal due to self-pollination. Detecting differences between populations might therefore be expected to be problematic even if connectivity in terms of a highly permeable landscape was present. However, the study found that the connectivity of *Rhinanthus minor* populations can be explained by landscape features and farm structure.

In this Chapter, it was not clear how populations were selected for study and what actually defined a "population". Due to a strong reliance on self-pollination, the study also assumed a very limited role of pollinators in genetic transfer, despite four of the 50 populations studied containing *Rhinanthus* hybrids (suggesting insect pollinators as vectors). Bumblebees are key pollinators of *Rhinanthus* spp. and many can disperse up to 4km.

It was never clear in the Chapter what was actually driving differences with regards to genetic transfer. It appears that only the physical movement of seed via farm machinery was considered possible, rather than plants moving along corridors little by little, or pollinators moving pollen over much longer distances. There is also evidence that *Rhinanthus* can disperse in water, which is perhaps a more important consideration for individuals in floodplain meadows.

Chapter 6 - Reversing expansion of *Calamagrostis epigejos* in a grassland biodiversity hotspot: hemiparasitic *Rhinanthus major* does better job than increased mowing intensity.

Overview - In most grassland restoration studies dominant species are present which can fundamentally affect the success of restoration efforts. Numerous approaches are used to reduce the dominance of such species, and the use of hemiparasitic plants is now common place.

In this study, the focus was on a highly productive species, *Calamagrostis epigejos*, and its control using *Rhinanthus major* in conjunction with cutting. The contribution from Petr to this paper is limited, but this is acknowledged accordingly.

Comment - The study revealed clear benefits for the use of *Rhinanthus major* in this situation, which might be applicable elsewhere. However, there is not sufficient detail in the methodology to allow the study to be repeated. For example, the cutting height was not specified and it was not clear whether the cuttings were removed as hay, collected immediately after cutting, or left *in situ*.

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11) Chapter 5 - Given the high dispersal potential of many bumblebee species that visit *Rhinanthus* flowers, explain why you believe insect pollinators are not important for pollen transfer between population of *Rhinanthus*.

12) Chapter 6 - The sowing rate used was lower in years two and three due to a reduction in seed production from the original population of *Rhinanthus.* Do you think seed harvesting affected the original population? What are your recommendations for seed harvesting for future restoration projects?

13) Chapter 7 - It is stated "...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." What do you mean by "persistence" and was this actually investigated? Is there a desired density of plants in a population to enable persistence?

14) Chapter 7 - The focus of the thesis has been on grassland management and sward composition with regards to influences on the abundance and performance of hemiparasites. Can you discuss other factors that might strongly effect the persistence of large-seeded species in grassland e.g. voles eating the seed.

I am happy to recommend the thesis for Defence

14.9.18

Dr Duncan B. Westbury
Principal Lecturer in Ecology & Environmental Management
School of Science and Environment
University of Worcester (UK)

Date: 3rd August, 2018

University of Worcester

Questions to be addressed at the Defence

1) Chapter 2 - It is already known that once dormancy is broken, seed will not germinate until temperatures are conducive to growth. What do you mean by "explore the induction of a persistent seed bank"? What was the rationale for exploring sowing date and how were these dates determined?

2) Chapter 2 - Were herbivores, seed predators etc. excluded? If not, what other factors might be attributed to establishment (germination) success? How have you defined "establishment" - survival to flowering?

3) Chapter 2 - It is recommended that November should be the latest sowing date for *Rhinanthus*, but this is not supported with the data obtained; a December sowing date might also be appropriate. A simple analysis of the number of days below 4°C since sowing would have provided a greater level of detail to help explain findings. Do you still recommend a November sowing date as the cut off point?

4) Chapter 2 - At one site productivity was already very low, but *Rhinanthus* failed to establish. This aspect was not included in the discussion. Can *Rhinanthus* survive in very unproductive situations? Joshi *et al.* (2000) found R. alecto. could establish in 152 gm^{-2}, but could this be dependent on the composition of the grassland? i.e. could the presence of legumes help its establishment?

5) Chapter 2 - Litter removal helped establishment success, did it also reduce herbivory? Not discussed or mentioned. What are your thoughts on this aspect?

6) Chapter 2 - It is stated that the Beals index is a good predictor of long-term *Rhinanthus* establishment success in sowing experiments. However, *Rhinanthus* presence is expected to change floristic composition with time. As the index is a snap shot in time of meadow composition, can the index be used to account for community change enabling you to predict long-term success?

7) Chapter 3 - Focus of this chapter has been solely on the issues of cutting on *Rhinanthus* and recommendations have been based on the preservation of *Rhinanthus* spp. rather than considering implications for other species. What plant species are likely to be impacted by your recommendation of a late-June mowing?

8) Chapter 3 - Do you think sward composition has the potential to influence the potential for *Rhinanthus* to regenerate following cutting? For example, if there are lots of legumes in the sward, could these provide more nutrition to support re-growth. What about the influence of soil fertility? Are individuals more likely to be excluded after mowing on more productive swards?

9) Chapter 4 - In Chapter 3 a late-June cut was suggested but in this chapter it is state that mowing should only take place after the beginning of July. Why don't the cutting dates coincide with those recommended in the previous chapter? For this study why were the following dates selected: 7 and 18 June and 5 July? What is this based on?

10) Chapter 5 - It is stated that in Estonia, natural grasslands are mainly coastal pastures and these act as barriers because the movement of machinery is negligible. Doesn't this mean that your modelling approach is not wholly correct because you are not actually focusing on habitat suitability for *Rhinanthus* with regards to a certain vegetation type? Instead, it appears you are only interested in how land is being managed and whether there is an opportunity for seed to be transported unintentionally by machinery.

Petr Blažek – Ecology of rhinanthoid Orobanchaceae. Within and between-site processes in metapopulations of selected grassland species

PhD Thesis – Review

This thesis consists of six chapters. The first chapter is a short overview of life history and species traits mostly of the genus *Rhinanthus*. Next five chapters are published papers (4), or papers prepared for publication (1). The manuscript has in total 184 pages, which consider the broad approach of the selected topic. In general, the thesis is an essential contribution to ecology, survival and protection of *Rhinanthus* species in Central Europe. In one case it also considers another hemiparasitic species (*Melampyrum nemorosum*). As core papers, we can consider two of them – Chapter 2 (Establishment of hemiparasitic *Rhinanthus* species in grassland restorations:...) and Chapter 5 (Functional connectivity in *Rhinanthus minor* metapopulations:...).

The first impression from the thesis (which is completed from high-quality papers) is a bit chaotic. Most of the thesis is focused mainly on the genus *Rhinanthus*, but not all species of this genus are included. All studies work with one or two species, and it is not so easy to recognize, how general the results are. Authors within their papers sometimes did not offer the level of generality of their results to the reader explicitly. There is an evident lack of broader general introduction and overview of the genus and species growing in Europe, their taxonomical and ecological differences, phylogenetical history, genetic variability, phytosociology and phenological plasticity, which could be done in the first introductory chapter of the thesis. It would be much easier to understand next results presented in papers. Such a chapter would be a big advantage in cases when the topic is not so consistent as here. Especially the **Chapter 2** (Petr Blažek is not the first author of this study) generalises results in Discussion frequently on the whole *Rhinanthus* genus. However, obtained results needn't fit all *Rhinanthus* species perfectly. E.g. the time span of seed stratification for the dormancy interruption may be different for *R. minor* (shorter period, higher winter temperatures), which is widely distributed across the oceanic part of Europe, than for some other species. Species also may differ in their sensitivity to pH and productivity. There is also some level of intraspecific biological and environmental variability, which was not mentioned and compared. The text must be studied by the reader very carefully concerning species, which were selected for experiments. From the paragraph about the productivity (pg. 40, bottom lines) we can conclude from indirect information mentioned in the text that *R. minor* grows in more productive vegetation than *R. alectorolophus*. Is it the truth?

Chapter 3 – Absolute dates of phenological stages (flowering, ripening) used in this paper are misleading. Both compared localities are in similar altitude but different regions. Also, as we could see this year, the spring was phenologically much faster and earlier than before. The phenological difference was 2-3 weeks from average phenological observations. Therefore, more general results published in the paper should be, e.g. in cumulative sums of average daily temperature over 10°C.

Rhinanthus species are dietary food for grazers. Is the effect of accidental grazing of the population similar to cutting, or is it more destructive?

In the end, the authors propose postponing of grassland mowing of nature protected meadows 'after 15th July or after 15th August'. Where (in which conditions), for which *Rhinanthus* species and in which vegetation? Sometimes it need not be in agreement to the management organised due to other species (e.g. mowing in May due to the elimination of *Arrhenatherum* expansion). How to solve these conflicts?

Chapter 4 has found no response of *Rhinanthus minor* to the mowing date expressed by the relative change in population density. It is in contrast to the Chapter 3, where *R. minor* has shown a clear dependence of regeneration on the mowing date. How do you explain these two contrasting results? Was the number of replications sufficient (5 blocks) in respect to high differences between individual counts recorded at each plot?

Chapter 5 – You have found hybrids of two *Rhinanthus* species with intermediate genetic characteristics. Were they fertile? Is there a possibility of their additional hybridisation? How do you explain their distinct pattern in ordination diagrams (Appendix 1)? Text at page 114 says that you have found 20 *R. minor x R. major* hybrids. They are divided in the tab. 1 to 18 for *R. minor* and 2 for *R. major*. Why did you split hybrids according to one of the parent species?

As for the Mantel test used for isolation by distance, please pay attention to the paper Legendre et al. 2015. Should the Mantel test be used in the spatial analysis? *Methods in Ecology and Evolution* 6: 1239-1247.

The conclusion "...dispersal effect of pollinators (mostly bumble bees) was much lower than the lack of machinery movement in those sites." seems too simplified. Bumblebee species differ in their foraging range from the nest. Some species collect nectar and pollen to the distance of 100 to 300 m while others may be efficient to the distance of almost 2 km. Therefore, it could also depend on the diversity, and population structure of pollinators and the obtained result need not be too general.

Appendix 1, Fig. 1 and 3 – is there PCA (Principal Component Analysis), or PCoA (Principal Coordinate Analysis)? Is D_{ps} genetic distance or dissimilarity? Would it be possible to project Emin, Cmin and Bmin (Emaj, Cmaj and Bmaj) in one ordination diagram? It would be interesting to see genetic variability within the species over three distinct areas. Why there is no ordination diagram for Belgium localities. As I understand, the ordination diagram from Estonia identified 4 hybrid individuals. However, the table 1 indicates that 17 hybrids were found from Estonian data. Where is the rest within the diagram?

Chapter 6 – This chapter could be more focused on changes in the whole vegetation structure. The chapter Discussion does not discuss almost any other structural changes than those related to *Rhinanthus* and *Calamagrostis*.

One type of experiment could appear there additionally – we can see results of summer, summer+autumn mowing and there is a lack of information about the only autumn mowing combined with the sowing of the *Rhinanthus*. The effect of late autumn mowing may be different from others, because it removes mainly litter and green part of grasses, while summer mowing affects both grasses and dicots. We started to apply "winter mowing" as an alternative to winter grazing in fringe vegetation with no change of vegetation structure in diagnostic species.

Pg. 159, Fig. 3B – What does the Proportion of *Brachypodio-Molinietum* mean? Is it the proportion of the absolute total cover? The chart shows the increasing target vegetation. Was it also in absolute numbers or *Calamagrostis* reduced cover more intensively than other species?

Despite my comments and questions, I would consider this PhD thesis as a scientific material of high quality. Generally, I can conclude that the whole manuscript can be accepted as a PhD thesis of the School of Doctoral Studies in Biological Sciences at the University of South Bohemia.

Brno, 7th September 2018

Doc. Mgr. Lubomír Tichý, Ph. D.