Report

Recommendation
Pass.

GENERAL
Petra Svetlikova has prepared a thesis examining the impact of a range of abiotic factors on the ecophysiology of root hemiparasites from the Orobanchaceae.

Novelty
The thesis provides a number of novel findings that are of significance for our understanding of physiology in this unusual group of plants. It explores the function of hydathode trichomes and their impact on the carbon balance of *Rhinanthus alectorolophus*. This is the first demonstration of the relationship between guttation from these trichomes and respiration rates in a hemiparasite.
A further advance is the demonstration of the utility of using 15N13C urea for tracing N and C movement from host plants to a hemiparasite. This technique is shown to be both simple and effective for tracing N movement.
A third contribution to our understanding is the impact of photosynthetic induction on C balance in an understorey hemiparasite, *Melampyrum pratense*. Petra has demonstrated that, particularly in summer when canopies are closed, photosynthetic induction can limit C uptake. This could result in plants having a negative C balance if they didn't also have access to heterotrophic C from hosts.
Finally, the thesis explores the water relations of *Rhinanthus alectorolophus* when grown on wheat, under a range of water conditions. Surprisingly, there has been very little research on the water relations of root hemiparasites, and this work begins to fill that gap.

Depth
The thesis explores a number of related topics in a single family of root hemiparasites, the Orobanche. However, the depth of the questions explored is certainly of a level expected for a PhD thesis.

Appropriate methods and analysis
All methods used were appropriate for the experiments and questions that were explored.

Synthesis
INTRODUCTION

- Focuses largely on Orobanche
- In the introduction you conclude by suggesting further studies of to better understand their responses to salinity and water and nutrient avaiolaiblity. Can you expand on why you think this is important. For example, what would you expect to find if comparing a 'water wasting' hemiparasite with a 'water conserving' hemiparasite?
- Are they water wasting or nutrient hungry?
- You also suggest that it would be valuable to investigate interacting effects of multiple factors. How would this help us to understand the nexus between nutrient uptake and water flow through hemiparasites?

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QUESTIONS

Ch 1
Exploration of function and cost/benefits of water movement through hydathode trichomes for maintenance of water potential gradient between host and parasite.

P34 What benefit do hydathode trichomes confer to Rhinanthoid hemiparasites? Maintenance of water potential gradient at night when VPD is likely to be low, and thus E will also be low.
Is this also a function in non-parasitic plants?

Why are they more active in juvenile plants? What happens to the hydathode trichomes in adult plants?

Respiration rates were similar in juveniles and adults. How does this relate to the activity of hydathode trichomes?

What is the likely impact on whole plant performance?
Why don't all Rhinanthoid hemiparasites use this mechanism for maintaining the water potential gradient at night?

Ch 2
New Method for tracing N/C movement between host & parasite, using 15N13C Urea. Brushing host leaves and measuring transfer to parasite.

Methods
- PFD in growth chamber was 400-500 umol m-2s-1. Could this have been a bit low? Might affect foliar uptake of urea?

Results
- Fig 2. Shows changes over time in 15N content of unlabelled parts of host (i.e. movement from labelled leaves to unlabelled parts), and also in the parasite.
- Given how easy this seems, did you consider running it again to achieve greater resolution of uptake dynamics (i.e. sampling over a shorter time 1-7 d)?
- Movement of 15N into host roots. Did you consider including uninfected wheat in the experiment?
- Field experiment? Was there any reason why you didn't test this in the field after running the growth chamber expirement?
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Did you run the reverse experiment? i.e. brushing the hemiparasite to see if any label was transferred to the host?

Ch 3

C balance of *Melampyrum pratense* in a forest understorey.

- Fluctuating, or unpredictable light, rather than unstable
- Induction response and PFD response curves. Used these to model C uptake.
- Spring/summer comparison. Open/closed canopy
- Photosynthesis is initially co-limited by induction state of Rubisco and stomatal conductance.

Methods & Results

- PFD response curves. Starting at high PFD could result in downregulation of photosynthesis, and thus underestimation of QY. This is important for the modelling of C balance in understorey light conditions. Ask Petra whether she considered this?
- How did you determine Amax and QY? QY should have units (e.g. umol CO^2 umol photons^{-1}).
- N=5 for PFD response curves
- Explain how dark respiration measurements were made? What is meant by 'ten zero PFD values were logged at the end of measurements'. Dark respiration measurements made after PFD response curves? How long were plants left in darkness before dark resp was recorded?
- Induction curves. Did you also record stomatal conductance? Water loss due to guttation might interfere with gs calculations. Did you confirm the presence of hydathode trichomes? Estimations of water loss from hydathode trichomes?
- Induction curves. N=5 and 7 in Spring and Summer, respectively. Was this enough?
- Greater variability in PFD and induction curves in Spring than in Summer. Can you offer a reason for this?
- Model parameters used during the period 7 May-22 July. Explain how you decided whether to use the spring or summer parameters. i.e. when did Spring become Summer?

Discussion

- What do you mean by 'efficient photosynthesis'?
- How confident are you that transpiration rates are a good proxy for stomatal conductance? Especially given the possibility of guttation interfering with the estimation, and that you didn't control temperature or RH?
- Would you expect induction to acclimate to the closure of the canopy during summer? i.e. shouldn't plants have faster induction responses in summer than in spring?
- Given that hydathode trichomes are mostly active at night, how much would you expect them to contribute to transpiration during the day?
- How soon after germination does M. pratense form haustorial connections with host plants?
- Have you also considered the impact of photoinhibition on C uptake, especially following highlight sunflecks?
- Given that most parasitic plants have high stomatal conductances, would you expect this to be different for *M. pratense*? Especially in summer when it appears to become more dependent on host derived C?

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Ch 4

Water stress in *Rhinanthus alectorolophus*.

Introduction

- Cell wall elasticity also enables plants to maintain turgor as water content declines. Remember that Ψp is also a component of Ψ.
- Would help to explain why you measured stable C and O isotopes and what you expected to find.

Methods

- Can you provide an explanation of $\Psi_{\pi \text{ gas exchange}}$?
- P-V curves. How is the 'squeeze' method different from standard approaches to obtaining P-V curves?
- More details of how Hofler diagrams were obtained would be helpful. A reference would suffice.
- What was the O isotope composition of the water used to irrigate plants?

Discussion

- No difference in water relations parameters between low and high water treatments. Lack of osmotic adjustment may suggest that transpiration rate is the main mechanism for maintaining water potential gradient?
- Explanation in discussion is that osmotic potential at full turgor is already lower than most other plants.
- Higher rates of photosynthesis in W- parasites relative to W+ at same osmotic potential. Could this be related to increased stomatal conductance? Difficult to ascertain from the transpiration values. But treatment had no significant effect on E.
- Suggestion that host may have allocated more resources to roots in W- treatment. Was there any evidence to support this?
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I would like to have seen a more comprehensive conclusion drawing all the findings together and commenting on how they have contributed to our understanding of hemiparasite ecophysiology, and providing suggestions for areas of further study.

General comments

Terminology needs tightening up in a few places.

- PPFD should replace intensity. Intensity is the rate of emission from a source, whereas PPFD is the photon flux density per unit area per unit time.
- Translocation occurs in the phloem, transpiration in the xylem
- Induction state, not inductive state.

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ÚSTAV EXPERIMENTÁLNÍ BIOLOGIE

DOCTORAL THESIS REVIEWERS´ REPORT

Doctoral Candidate: **Petra Světlíková**

Doctoral Thesis: **Effect of abiotic fators on hemiparasitic plants**

Supervisor: doc. RNDr. Jakub Těšitel, Ph.D.

Reviewer: doc. RNDr. Vít Gloser, Ph.D., Masaryk University, Brno

Petra Světlíková devoted her Ph.D. work to ecophysiology of hemiparasitic plants. This area represents a very interesting research space with multidisciplinary outreach to ecology or agriculture. So the subject of the thesis is topical and also relevant for longer term research activity. Her thesis is presented as a set of four scientific manuscripts accompanied with a general introduction (review) focused on several aspects of ecophysiology of hemiparasitic plants.

The introduction shows well organized collection of recent information about response of plant hemiparasites to abiotic environmental factors and also a short summary of some ecological facts about this plant group. It is written clearly in good English language with only rare typing errors. Over hundred and fifty cited references in this chapter also leave no room for doubts that Petra did this review thoroughly with a lot of effort. In the end of this section she also indicates several prospective directions and interesting experimental questions for future research in her area of interest. I have following (mostly conceptual) comments on this section.

The presented papers are surely nice and complementary in their topics and followed by summary of results at the end thesis. However, I miss a short introductory explanation that would integrate the ideas behind to the whole set of separate manuscripts.

I should appreciate that Petra in her work also applied some methodological approaches that are rather new in application on hemiparasitic plants (e.g. double isotopic labelling via leaf, mathematical modelling of productivity). The contribution of Ph.D. student to development and modification of methods is always very good and desirable. Hence, I am missing a short section in the first part of the thesis focused on methodology. It could have highlighted the contribution of candidate in this area and potential methodological challenges and problems that are difficult to describe elsewhere.

The scientific value of all presented experimental data is very good. All four included manuscripts represent well conducted and presented experimental work with appropriate and up-to date methodology. Three of the manuscripts were already published in well recognized international scientific journals.

In chapter one is presented a functional study of specialized hydatode trichomes on leaves that clearly confirmed an active role of these structures in regulation of water flux in *Rhinanthus* plants. It considerably contributes to knowledge of mechanisms in resource acquisition and its regulation in hemiparasitic plants.

Chapter two shows a novel method for identification (and to some extent also quantification) of nitrogen fluxes between host and hemiparasite. Easy use and rapid, reliable results both in the lab and in the field belong to the most significant contributions of presented method.

The problem of seasonal adjustment of hemiparasitic species in understorey habitats is addressed in the chapter three. Evidence for functional adjustments of leaves to variable light conditions and detailed modelling of carbon balance over the vegetation season represent the most valuable parts of this manuscript.

Finally, the chapter four addresses the problems of water relations of hemiparasitic plants more in detail. Functional analysis of gas exchange and processes affecting stomatal conductance of hemiparasite under the reduced water availability in soil suggests considerably different regulatory mechanisms when compared to the host plants. These useful results open a new view on functional combination of mechanisms involved in regulation of water use in this plant group.

I have following questions for discussion related to the content of the thesis.

Can you please explain more closely the mechanisms behind the "extracting" diluted mineral nutrients from the sap of host plant in quantities sufficient for growth of parasite? How can be appropriate balance among nutrients achieved when the composition of sap provided by host fluctuates in time? How can parasite prevent excessive accumulation of some ions?

In the chapter 4 you describe the measurement of P-V curve but the description is rather complicated also due to specific problems you had to solve. Can you please briefly describe the procedure, the problems you were facing and their solution?

I think it is a bit problematic to conclude something specific about carbon transfer between host and parasite based on the application of ^{13}C labelled urea. Did you consider use of some other compounds as a tracer for these experiments (e.g. double labelled glycine)?

In results of chapter 4 you showed surprising finding that photosynthetic rate of water stressed *Rhinanthus* plants was higher than those in well-watered treatment. Can you please present hypotheses (or at least speculations) that would explain this unexpected outcome?

In conclusion, it is impressive that in all presented manuscripts is Petra the first author who considerably contributed to both experimental work and manuscript preparation. This clearly exceeds standards of Ph.D. candidates at Czech universities and proves the potential of Petra for creative research activities.

The thesis clearly shows that the author is able to plan, conduct and evaluate experiments independently. Petra showed that she can successfully present her experimental work in manuscripts that are published in respected scientific journals which is a key prerequisite of bright career any scientific area. Therefore, it is my pleasure to recommend the thesis of Petra Světlíková to be defended.

Brno 18. 6. 2018