Evaluation of the PhD thesis "Ecological constraints limiting the root-sprouting ability in wetland plant species" by Monika Sosnová.

The thesis addresses two main problems, 1) the pattern of pant clonality among different wetland communities experiencing contrasting environmental stress (submergence, salinity) and disturbances, and 2) life history and functional responses of a short-lived (annual/biannual), root sprouting wetland herb, Rorippa palustris, to disturbance and stress (submergence).

The approach to the first problem is to analyse available detailed documentation of wetland plant communities in the Netherlands in relation to information on clonal growth organs and other clonal plant traits stored in the clonal plant database CLO-PLA3 to answer novel questions on distribution of clonal growth organs among plants in different wetland communities. Predictions of clonal growth trait syndromes related to different clonal growth organs, based on the conceptual model of by Grace (1983) were tested. The results are reported in two papers (chapters II and III). The main findings are:

- 74% of wetland plant species are clonal, the majority being rhizomatous (51%), while in open water habitats (stressed by submergence) specialized clonal growth (budding, production of turions and fragments) is more common and in disturbed habitats (salt mashes) clonal growth by root derived organs (root-splitters, root-sprouters) and non-clonal plants predominated.
- With the exception of highly disturbed communities (salt-marsh), root-sprouting is underrepresented in wetland communities compared to terrestrial communities.
- Predicted clonal plant traits are not supported by the analysis, indicating that a variety of traits other than those liked to clonality differentiate plant strategies in most wetland habitats.
- Wetland habitats characterised by strong stress factors (freshwater pools and riverbeds) showed, however, distinct spectrum of clonal traits (high offspring production, good dispersability, fast-splitting clones, monocyclic shoots).

The approach to the second problem is experimental. The results are presented in three papers (chapters IV, V and VI), each reporting results from an experiment addressing specific question regarding life history and functional responses of Rorippa palustris to disturbance (injury) and/ or stress (flooding), a species common in natural or man-made moist-wet habitat experiencing periods of flooding and disturbance. The main findings are that

- *Rorippa palustris is able to tolerate sever injury by adjusting its life history. The outcome depends on the timing of germination and injury where the amount of stored carbon in roots plays a central role.*
- *R. palustris also tolerates submergence stress (7 days) while the combination of submergence and injury is detrimental (low survival)*

irrespective of the origin of the experimental populations. It is concluded that this may explain why root-sprouting plants are underrepresented in wetland communities compared to terrestrial habitats.

The thesis thus adds significantly to our knowledge of both problems addressed. The individual studies are carefully carried out and well designed and the thesis is of high quality. Three papers have been published in relatively specialised international peer-reviewed journals. I can recommend a defence of the thesis for a PhD degree.

Comments, questions and discussion topics

My main concern is that however interesting the two problems addressed by the thesis are, they do not tie very well together, i.e. they remain as two rather separate parts. It is indicated in the introduction (chapter I) as well as by the general structure of the thesis that the Rorippa studies were set out to follow up on one of the findings from the studies of clonality in wetlands, i.e. to find out what may constrain root-sprouting in wetland plants to cause the underrepresentation of root-sprouters in wetlands as demonstrated in the first part. Therefore I wonder:

- How common is Rorippa palustris in the different plant communities of wetlands in the Netherlands under scrutiny in chapters II and II?
- Why would Rorippa palustris be a good candidate to address the problem?
- Do you find that experimental studies of a single root-sprouting species already adapted to wet conditions is the best approach to provide an answer to why root-sprouters are underrepresented in wetland communities? Can you think of any better approaches as for example comparative studies of related root-sprouting species adapted to contrasting habitats? Such comparative study might also increase our understanding why is Rorippa palustris is so successful in wet / waterlogged habitats compared to most other root-sprouters in spite of all the assumed costs linked with root-sprouting in waterlogged situations.

Furthermore, the wetland clonality study showed that root sprouting was most commonly found in species of salt marsh communities (high disturbance), a habitat where Rorippa palustris is apparently not found.

- How do the root-sprouting species of salt marshes compare to Rorippa in terms of life history? Would you expect them to respond similarly to disturbance (injury) and stress (submergence)?

As discussed in Chapter III the reason why the clonal traits could not be predicted by a given community type based on Grace' conceptual model, other traits than those under scrutiny may be of overriding importance in theses wetland communities.

- If you were to identify general plant strategies for the different wetland

communities, which other traits would you take into account in the analysis?

One of the predictions was that persistence and carbon storage would be selected in conditions of reduced light availability, i.e. shading in deep water (submergence) and by large neighbours. There is evidence, although not based on as thorough evaluation as presented here for the wetlands, that carbon storage and persistence is common in shaded conditions of terrestrial environments like forest floors.

- Do you have any idea what may be the reason for such discrepancy?

All the Rorippa studies were done under controlled experimental conditions. Therefore I ask:

- Are any field studies available evaluating how important the ability to regenerate by root sprouting is for the maintenance of natural or semi-natural populations of Rorippa palustris? How important is root-sprouting in relation to regeneration from seed after disturbance?

In Chapter IV the effect of timing of germination and injury on life-history characteristics of Rorippa palustris is tested. Only one injury treatment was applied, i.e. all aboveground biomass was removed to make sure that regeneration was only possible by adventitious buds on the root. Such injury must be regarded as very sever disturbance.

- How common is such sever disturbance? If it is more common with less sever disturbance it might be informative to experimentally compare life history responses to different degrees of disturbance. If you agree, would you expect very different responses to less sever injuries?

In Chapter VI the effect of combined injury combined with submergence are tested.

- How common is the detrimental situation of sever injury and prolonged (7 days) total submergence?

In Chapter VII you conclude that carbon economy plays an important role in the regenerative ability of plants and because root sprouting is energetically costly it is inefficient in waterlogged situations. I am a bit confused by how that conclusion was reached based on the Rorippa experiments because they demonstrated, such great ability to regenerate from root-sprouting after injury under waterlogged (but not total submergence) situations.

- Is it perhaps the case that under natural/semi-natural situations Rorippa does not at all depend on root sprouting for population maintenance?
- If that were the case, what would be the adaptive significance of this trait?

Specific comments

In Chapters II and III the structure of the vegetation data it is not made

very clear. More details would be needed for those not with an easy access of the original data source. For example, in Chapter III it is not clear to me the difference in how the presence/absence data and the species frequency data (quantitative?) used in the two approaches were obtained.

In the experiments presented in Chapters IV, V and VI you use different sizes of pots and you apply different levels of nutrients and waterlogged situations (different water levels) in each experiment without any explanations.

- What is the reason for the different designs?
- Do you think these differences affected the outcome of the experiments?

Chapter III:

Table 3. –I presume that the values in the table Chi-square values?

Fig. 1. Table 4 is cited in the figure legend – should be Table 3?

In Chapter VI the injury and flooding treatments were applied on 9th June.

- Which developmental stage were the plants in by that time?

In the same chapter more details are needed for description of how you measured the different growth variables.

26. August, 2010

INGIBJÖRG S. JÓNSDÓTTIR

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Oponentský posudek doktorské disertační práce Moniky Sosnové

Ecological constraints limiting the root-sprouting ability in wetland plant species

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Předložená doktorská disertace je zaměřena na vybrané aspekty vegetativního rozmnožování mokřadních bylin. Zvláštní pozornost je věnována rozmnožování z pupenů na kořenech. Možná ekologická omezení tohoto způsobu rozmnožování byla studována v nádobových pokusech na modelovém druhu *Rorippa palustris*.

Práce je formálně uspořádána jako série pěti vědeckých článků (kapitoly 2 – 6), uvedená stručným, ale výstižným přehledem problematiky vegetativního rozmnožování mokřadních rostlin (kapitola 1) a uzavřená souhrnem hlavních získaných výsledků (kapitola 7). Z pěti zařazených článků tři již byly publikovány ve vědeckých časopisech (kap. 2, 4, 5) a jeden je odeslán do redakce (kapitola 3). Poslední článek (kapitola 6) je předložen v podobě rukopisu připraveného k odeslání do redakce. Práce včetně úvodní a závěrečné kapitoly je sepsána anglicky.

Vlastní experimentální práce M. Sosnové sestává ze tří kultivačních pokusů provedených na rukvi bahenní, v nichž byly postupně testovány hypotézy o vlivu různých ekologických omezení na charakteristiky životního cyklu rostliny. Všechny tři experimenty mají precizní design a elegantní statistické vyhodnocení. O kvalitě získaných výsledků svědčí publikace dvou dříve provedených pokusů v impaktových časopisech (kapitola 4, 5). Úroveň rukopisu s výsledky třetího, nejkomplikovanějšího a nejdéle trvajícího pokusu (kapitola 6) opravňuje k domněnce, že i tento článek má značnou šanci na přijetí ve vhodně zvoleném vědeckém časopise.

Ve srovnání s výsledky experimentů má z hlediska přínosu pro širší vědecký obor možná ještě větší význam studie, kterou autorka provedla při své stáži v Holandsku v rámci programu Erasmus. Studie řeší otázku zastoupení různých forem klonálního šíření rostlin v různých typech mokřadních společenstev Holandska. Je založena na vyhodnocení dat o způsobech vegetativního rozmnožování klonálních rostlin v databázi CLO-PLA 3 a publikovaných údajích o vegetaci různých typů mokřadních biotopů Holandska. Výsledky studie jsou obsahem kapitol 2 a 3, z nichž kapitola 2 již byla publikována.

Práce zdařile rozvíjí odborné téma klonálního šíření rostlin, jímž se školitelka J. Klimešová dlouhodobě zabývá. Autorka také vhodně využila odborný potenciál pedagogů a kolegů Přírodovědecké fakulty JU, zejména při volbě metod statistického zpracování. Podobně při své zahraniční stáži vytěžila maximum z možnosti kontaktu se zkušenými zahraničními kolegy jak po odborné, tak i jazykové stránce. Množství provedené práce a také podíl již publikovaných výsledků je umožněn mimo jiné i tím, že autorka se ekologií rukve bahenní (s hlavním zaměřením na vegetativní rozmnožování z pupenů na kořenech) zabývá již od své bakalářské práce. Výsledkem je disertace mezinárodní úrovně, která autorce slouží ke cti a jistě těší i vedoucí práce.

K práci mám několik drobných dotazů a připomínek, které jsou míněny spíše jako příspěvek do diskuse než jako kritika.

1. V seznamu literatury v kapitole 1 chybí citace práce van Groenendala et al. 1996 – výjimka potvrzující pravidlo o mimořádné pečlivosti provedení celé práce včetně formální stránky!

2. Proč byla při statistickém hodnocení výsledků v kapitole 6 (str. 97) zvolena metoda GLM s binomiální distribucí? Vzhledem k tomu, že jde o pokročilou statistickou metodu, s níž nemusí být mnozí čtenáři obeznámeni, stálo by zato věnovat více prostoru popisu metodiky (např: jak byly formulovány statistické pracovní hypotézy?) a také podrobněji popsat výsledky získané touto metodou (našla jsem k ní pouze jednu větu na straně 99).
3. V disertaci se několikrát odkazuje na větší energetickou náročnost tvorby pupenů na kořenech ve srovnání s růstem již založených pupenů na oddencích, což může znevýhodňovat způsob rozmnožování pupeny na kořenech u mokřadních rostlin díky deficitu kyslíku v zaplaveném substrátu. Tuto úvahu je možno doplnit ještě o význam vnitřního provětrávání, jehož kapacita je v oddencích zpravidla větší než v kořenech (byť aerenchymatických). Vzhledem k tomu, že dělení buněk nemůže probíhat bez kyslíku, nemohly by v anaerobním prostředí růst ani nové oddenky, byť z pupenů již dříve vytvořených, pokud by neměly zajištěn stálý a dostatečný přísun kyslíku aerenchymem.
4. V práci mi chybí souhrnná diskuse, která v podobně strukturovaných disertacích bývá zařazena jako závěrečná kapitola. Souhrnná diskuse dává autorovi možnost zdůraznit souvislosti mezi jednotlivými dílčími aspekty vlastní práce, oddělenými do samostatných kapitol, a svou práci jako celek pak začlenit do kontextu mezinárodního výzkumu diskusí relevantních prací jiných autorů. Umožňuje tak překlenout riziko tematické roztříštěnosti, kterému jsou práce koncipované jako soubor článků vystaveny. Toto riziko bývá patrné i u obhajoby, pokud ji autor pojme jako lineární výčet informací o provedených dílčích studiích. Předložená práce bezesporu obsahuje dostatek materiálu pro syntézu (byť rozčleněnou na experimentální studie Rorippy na straně jedné a spíše teoretickou studii výskytu různých forem klonálního šíření na holandských mokřadních biotopech na straně druhé). Bude přínosné, pokud ji autorka zařadí do obhajoby své práce.

Závěrem lze konstatovat, že předložená disertace v plné míře splňuje podmínky na takovou práci kladené, a s potěšením ji doporučuji k obhajobě.

V Českých Budějovicích 10.8.2010

Doc. RNDr. Hana Čížková, CSc.

Report on the PhD. thesis of Monika Sosnová

The thesis is a set of five papers, two of which deal with the analysis of clonal organs of wetland species in the Dutch flora, and three with life history of a short-lived annual/perennial plant with root resprouting. Although links between the two parts are scarce (more below), I must say I like the thesis and appreciate the work that has been done. The research reported here is based on good and fresh ideas, has been well performed (as far as I was able to judge), data are well analyzed and summarized into papers.

Strong points of the thesis

The analysis (in the first) paper of the relationship between CGO types and clonal growth traits. These two aspects have often been confused in the literature. This thesis represents one of the first analyses that links the morphologically/developmentally defined CGO types with their possible ecological functions.

Systematic analysis of the life history of a root-sprouting plant, *Rorippa palustris*. The three papers on *Rorippa* address the key features of its life history and the role of injury by disturbance/flooding. It proceeds in a systematic fashion from testing ontogeny/injury timing interaction, through analysis of carbon storage to long-term fitness effects of the injury. I like the approach that made possible the separation of the ontogenetic stage, season and injury (in particular, discussion of the paper 3 is very good).

The last paper collected really valuable data on the ultimate fitness effects of root sprouting under normal conditions and under injury. This required a rather long-term experiment. Such approach is really laudable as most similarly conceived ecological studies use only indirect fitness measures (such as size) in order to get results during a short-term experiment.

Weak points of the thesis

Description of methods/data analysis in most papers. At many places it is difficult to learn what was done, and whether statistical tests used are appropriate or not. Presentation of the statistical results is also rather heterodox. In some cases, meaning of statistical tests are not clear (e.g. in Tab. 2, p 64), number of degrees of freedom is often not given (let alone the denominator d.f. which is indicative of the ANOVA design/correctness). Sometimes it is unclear whether "ANOVA" refers to general linear model or to the function `anova` in R/Splus (Tab. 2, p 64, Tab. 3, p. 66). In most cases, I give the author benefit of the doubt, but much clearer description must be required for further publications.

Almost completely missing discussion of the evolutionary implications of the experimental findings. The author(s) carefully discuss(es) ecological processes, but almost invariably avoids mentioning the bearing that these ecological processes have on selective forces that act on the plants.

Splitting the analysis of the Dutch wetland flora into two papers. I (hope I) understand the different messages of both papers, but I am convinced that putting all the analyses together would result in a much stronger paper.

The introduction is too general and does not do a good job in providing the specific contexts and motivations for the study. I would like to hear more general considerations on root sprouting, and more general motivation for the whole study, and links between its individual parts. On the other hand, there is too much stress on anoxia in the introduction. I understand that anoxia is dealt with (indirectly) in the last paper, but still the stress on it here is undue.

Specific questions

Chapters 2 and 3 (papers 1 and 2)

The author says that hypogeogenous rhizomes might be more suitable in permeable waterlogged soil of wetlands (p. 30). What about hypoxia, which may act in opposite direction and prevent rhizomes to stay too long in the anoxic conditions?

If I understand it properly, alliances (sets of ecologically similar releves) were units in the multivariate analysis. As the author herself admits, number of releves in individual alliances differed. Did the analysis take this into account (e.g. by differential weighting). If not, why? If yes, why?

Phylogenetic correction is mentioned in the first paper, but not in the second paper. Could you please comment why it was done only for the part of the research that fell into the first paper?

The analysis shows that some traits are favoured in some habitat types, but their prevalence is not absolute, and in almost all cases plants bearing less-favoured traits are also present. This indicates that while environment selects to some degree, there are other factors that contribute to trait richness of communities. Could you please comment on which factors may contribute to maintaining the trait richness?

In the analysis of the whole flora as to species traits, there are many species that possess clonal growth organ of more than one type. This poses difficulties for the analysis, which the author handled by dealing with one CGO type for each species only. While the author says that she used the one with most cells filled, it is unclear whether it refers to the amount of information available on the particular CGO, or to the parameters of the clonal growth type. I would also appreciate more information on the potential error introduced by deliberate exclusion of some clonal growth organs. Could you comment on this?

The two parts of the thesis (analysis of clonal traits in the Dutch flora and population biology of root-sprouting short-lived perennials) are only weakly connected to each other. The focus of the second part of the thesis requires asking what specific traits are associated with root sprouting. The author says that root sprouting is restricted in wetlands relative to other habitat types, but then she chooses a wetland species as an example of root sprouting. This seems odd. Is root sprouting specific in terms of the traits associated with it (compared with other types of clonal growth organs) or not? How does *Rorippa* exemplify these traits? Could you be more specific on the motivations/advantages of this particular choice?

Chapters 4 to 6 (papers 3 to 5)

In the last paper, I miss further analysis of cumulative fitness of plant individuals. I assume that regenerating plants (since they regenerate from root buds) may form several "ramets", each of which may bear seeds. Cumulative fitness should take into account this, but it should also account for the fact that timing (and hence fitness contribution) of seed production of injured plants is changed. (Fitness is affected not only by the number of offspring, but also by generation time which is longer in injured plants.) This would also call for a deeper "economic" analysis of tradeoffs between generative and vegetative reproduction.

In the fourth paper (carbon storage), control plants were analyzed as to the starch content throughout their development, while injured plants were analyzed only at the end of the experiment. Why? Could you be more specific on the information that you could gain if injured plants were also sampled longitudinally?

I would be curious to know how the starch levels found in *Rorippa* compare with levels in truly perennial plants and in plants that are strict annuals.

Do you have an idea how *Rorippa* behaves when winters are milder (and therefore less starch resource is required for survival)?

Assessing the role of individual traits in plants is typically hindered by the fact that traits are difficult to manipulate. This can be circumvented by a comparative approach used in the thesis, e.g. by comparison of different life-stages/phenologies, and of plants from different habitats, but this is either confounded with other effects (life stages) or having very little effect (habitats). Have you even consider true manipulation of the root sprouting capacity by e.g. hormonal means?

Minor comments

Chapters 2 and 3 (papers 1 and 2)

The first two papers revolve around the conceptual model of clonality in wetland plants of James Grace. It is really odd that the first paper, although mentioning the model, is very unspecific about which hypotheses to examine or test.

p. 23: the description of species selection is unclear and mixes species selection used in Schaminee et al. and the present paper.

Table 2 (p. 27). the P-levels are not independent of each other.

p. 43: how good was the coverage of the Dutch wetland flora by CLO-PLA3? Was the coverage similar over all vegetation units? (Systematic bias in coverage might have affected the result.)

p. 44, data analysis. I am not fully satisfied with the analysis by contingency tables and CCA. For the contingency tables, I would first expect an overall tests (as the individual tests are interdependent). This test might be a CAA-like test, but not the current CCA, as this is based on different null hypothesis than the contingency tables (it takes into account also species frequencies).

Description to the phylogenetic correction is very incomplete. How was it done? What source data were used?

Chapters 4 to 6 (papers 3 to 5)

p. 64, table 2. I do not understand what is tested here.

p. 79: Fixed effect (treatment) is nested within a random factor (container)?

p. 78: "For implementation of injury, the appearance of the plant rather than age was important.": I do not understand. I assume that injury was applied to plants independently of their size, based only on the design of the experiment.

The paper heavily uses concept of "Total starch", but the Methods do not say anything about how it was calculated.

In the third paper, the key results should be tests of the interactions between treatment/injury and cohort/ontogeny. Possibly I am a bad reader, but I was not able to find it in the results.

Fig. 2, p. 81: It is doubtful to plot transformed values of a variable - the reader is interested in seeing the relationship between untransformed values. (Transformations should be reserved for analysis and testing.) More details on the polynomial regression used are also needed.

Table 4, p. 84: residual d.f. are missing. This is especially important in hierarchical ANOVA reported in the table. (This is a common omission in the thesis.)

p. 86, the para "The storage pattern…": This is going too far. Root sprouting is not the only process that can turn an annual plant to a short-lived perennial; therefore one cannot ascribe the survival pattern found in this species to root sprouting.

p. 87: the author notes here that biomass and starch allocation to roots do not match well, but does not develop the idea further. Did biomass allocation and starch accumulation take place at the same time, or is the biomass allocation just a carryover from the growth phase that preceded starch allocation? When would biomass allocation to roots late in the season be ecologically meaningful?

Paper five: was submergence for 7 days sufficient to induce anoxia in the roots?

p. 98: I do not understand why the factorial experiment was not analyzed in a factorial fashion (i.e. anoxia and injury as separate orthogonal factors).

Conclusion

In summary, the thesis demonstrates the ability of the candidate to perform research in plant ecology. It is based on a solid amount of laboratory and analytical work, it shows her understanding of the subject and her ability to present her results in the form of scientific papers. The number of questions I am asking (and I am looking forward to pose further questions not listed here during the defence) is largely due to the thought-provoking nature of

the subject, and does not imply bad quality of the work. Some results presented would call for further analysis/interpretation and I hope the author will have the chance to conclude her research by doing it.

I recommend the thesis for defence.

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