Review of Ing. David Šebela Ph.D. Thesis

Assoc. Prof. Adam H. Sparks

September 16, 2016

Dear Prof. Petra Korcová, Head of Committee for PhD Studies in Biophysics,

Thank you for the invitation to review the PhD thesis submitted by Ing. David Šebela. I herewith submit my report to you and provide my comments and assessment with my final evaluation statement and suggested questions for the candidate.

1 Summary of thesis and its contributions

In this thesis Ing. David Šebela aims to develop a method and technological platform for non-invasive estimation of phenolic compounds in plant leaves and berries, specifically working with grapevine (*Vitis vinifera* L. subsp. *vinifera*) and rice (*Oryza sativa* L), two globally important agricultural crops with long histories of cultivation.

The thesis contains an in-depth literature review in the Theoretical Background covering plant phenolic compounds, their functions and where they occur within the plant; methods for detection of phenolic compounds; photosynthesis and related processes and pigments.

The section on estimating phenolic compounds is much more sparse than the section on the phenolic compounds. I would like to have seen more regarding the methodologies for measuring these compounds than the two to three pages only that were presented here. Since the dissertation focuses on the use of what is essentially remote sensing, some more literature should be reviewed in this area. While the work is novel, the use of remote sensing in agriculture and plant sciences is not.

The Materials and Methods section is complete describing the plant material, the optical measurements made and instruments used.

The Results are broken into several separate manuscripts that either have been peer reviewed and published or are prepared for review.

Following I present my assessments of several different criteria for this thesis. Other notes and hand-written comments may be found in the scanned PDF copy also provided for Šebela to reference when revising the thesis.

2 Assessments

2.1 Of main claims, contributions and originality of work

The thesis introduces new methods of estimating phenolic compounds in leaves and berries non-invasivly. These sorts of techniques are based on well understood processes widely used in remote sensing already. However, the application of these techniques appears to be new to this study and as such it means that this is an original work that does contribute to the fowarding of science.

2.2 Of technical work, experimental design and interpretation of results

2.2.1 Technical issues

At several points throughout the thesis, the pathogen, *Plasmopara viticola* is referred to as a fungus. This is wrong. *P. viticola* is an oomycete, a completely different kingdom, Chromista, than Fungi. This needs to be corrected throughout the thesis.

The fungus *Botrytis cinerea* is referred to in Section 3.2.2 on Page 73. The One Fungus=One Name convention would stipulate that this organism instead be referred to as *Botryotinia fuckeliana*.

The term "localized cell death" is used, this should perhaps be referred to using the more common "programmed cell death" terminology.

Section 3.2.2, Page 75 refers to a "wild range of excitation light", this perhaps is a spelling error or typo? If not, it should be clarified as to what it means.

In Section 3.1.1 Page 311, it is unclear why the reflectance spectra measurement stops at 800nm but the indices measured include the 850nm wavelength. This should be clarified in some fashion how the measurements were made using this instrument.

2.2.2 Interpretation of results

Section 3.2.1 Page 63 asserts that Šebela *et al.* 2014, proved the role of phenolic compounds in the proective role against grape vine downy mildew. I cannot find this assertion to be true in the cited paper. The idea is presented there, however, no experiment was conducted to show that these phenolic compounds did indeed protect the plant. Only detection of said compounds was achieved.

The results appear to be properly interpreted. However, there is little information on what any apparent weaknesses of this new approach might be. It would be good to include thorough, integrated overview in the conclusion of what the strengths and weaknesses of using non-invasive tools are.

The Conclusion seems week and lacking a strong statement. It closes with a statement about the health benefits of phenolics, presumably in the human diet. Little is said about the other applications that benefit producers. This seems incongruent with the papers, which seem to focus on disease detection and detection of ripeness of the fruiting bodies or the effects of temperature on

rice, which benefits breeders. I would like to see statements about what the next steps necessary to carry this forward is and who will benefit from it?

2.3 Of presentation of thesis, grammar and spelling

2.3.1 Grammar and spelling

With full understanding that English is not the native tongue of Ing. Šebela, I recommend that further attention to grammar and spelling must be given this thesis. A thorough review should be undertaken by a native English speaker to check for these types of issues, as they are predominant throughout the work.

For instance, on Page 35, "prescribed" should be "described" and "filed" should rather be "field". In the conclusion the words "even" and "though" are written as "eventhough". Many others are exhibited throughout the thesis, many I have underlined or made note of, but not all.

2.3.2 Other presentation issues

Section 3.1.2, Page 53, contains an error in the latitude and longitude coordinates given. A "V" is given in place of an "E".

Section 3.1.2, Page 54, Figure 1 contains the exact same sub-figure as previously published in Section 3.1.1, "Towards optical detection of *Plasmopara viticola* infection in the field" found on page 312. The Cab.Sauvignon portion of the figure is the same in both chapters, one of which is published, but no citation is given. This should either be cited or a unique image should be used for the figure in Section 3.1.2.

A minor note, the latitude and longitude coordinates are given in different formats depending on the chapter, perhaps due to different journal requirements?

3 Recomendation

If the comments I have raised here are addressed appropriately, I believe that the thesis submitted by David Šebela can be accepted and a doctoral degree can be awarded to him upon successfully defending.

4 Suggested questions

1. In Šebela *et al.* 2014, the author suggests that the methodology developed and tested offers faster implementation of effecient strategies for vineyard management. How much sooner can management decisions be made using these techniques? What advantage does this offer?

2. If disease is detected earlier than traditional methods by using these methods, what other conditions should be accounted for before deciding to recommend a treatment?

3. How many plants/vines need to be measured for an accurate assessment to be made before deciding to apply a treatment?

4. What level of infection of grapevine powdery mildew would warrant treatement?

5. How would the results be different for a necrotrophic pathogen than a biotrophic pathogen like *P. viticola*? How would the methodology change?

6. Since wheat has recently been shown to exhibit a grain-specific C4 photosynthesis pathway, is it possible that rice also could harbour this pathway or is it still necessary to try to develop it in rice as the C4 Rice Project is attempting to do?

Thank you for the opportunity to act as an external reviewer for this thesis. I feel that it is advancement to the field of science and an important piece of work. Please feel free to contact me for any further questions or concerns regarding my report.

With warmest regards,

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Opponent´s review of Ph.D. Thesis
Candidate: Ing. David Šebela
Title: The development of methodological and technological platform for noninvasive estimation of phenolic compounds in leaves and berries

Topicality of thesis

Development of non-destructive methods for evalution of important physiological characteristics of plants belongs to one of topical research areas in plant science (physiology, ecophysiology). Presented thesis is focused mainly on the application of fluorescence techniques (both Chl-a fluorescence and UV-induced fluorescence of plant phenolics) and reflectance spectra for the analysis of „physiological status" and contents of phenolic substances in grapevine leaves and berries. The presented results contributed: 1, to methodologies of the application of spectral optical properties of leaves and berries for the purposes mentioned above; 2, to our knowledge of factors that influence ripening of grape berries and development of rice panicles.

Theoretical background

This section (31 pages) deals mainly with plant phenolics (their classification, biosynthetic pathways, variability of roles of phenolic compounds in plants, their presence in different plant organs with emphasis on phenolics content in grapes. Further methods for direct (biochemical) and non-direct (optical) estimations of contents of individual phenolics are briefly mentioned. This section includes also brief description of photosynthetic apparatus and reactions followed by basic characterization of native fluorophores in plants and interpretation of both UV excited blue-green fluorescence and red chlorophyll a fluorescence.

This part is generally well written, contains valuable information related to the topic of disertation thesis. English is mostly correct, both stylistically and grammatically. The amount of not-precise statements or jerky passages is not high (yet there are some). I do not understand why almost the entire paragraph on the first half of page 26 is written in *italics.*

Comments and questions to this part of thesis:

Page 26, row 24: You state: „This transmembrane multi-protein structure (ATP synthase) utilizes protons coming from OEC." ***OEC is the only source of protons contributing to formation of ΔpH acros thylakoid membrane?***

Page 26, rows 28-29: You write: „To simplify, during first `light` phase of the photosynthesis, the water is broken in the chlorophyll molecule into H+ and OH- ions in the presence of light." ***I do not understand this simplification. Can you make it more realistic?***

Page 30, rows 19-23: You postulate: „Since in the photosynthetic apparatus, both Chl-b and carotenoids have a role of accessory pigments transferring their energy to Chl-a, also UV radiation can be used to induce Chl-F. To complement the formulation of Chl-F, it is not emitted only from LHC of PSII."

a, Can you explain the first sentence? Does it mean that excitation of Chl-F in UV region is (mainly) due to UV-absorption by Chl-b and carotenoids?

b, May be „formulation" is not the best word to express meaning of the second sentence. Does the PS II fluorescence in vivo (intact leaves) at room temperature really origin from the LHC II?

Materials and methods, Results and Conclusion

In section Material and methods the brief recapitulation of plants, experimental design and techniques used was given (3 pages).

The section Results (59 pages) contains 5 manuscripts, two of them already published in journals with IF (in both of them candidate was the first author) and three declared as prepared manuscripts (in two of them candidate is the first author).

A brief survey of main achievements (particularly regarding developed methodological approaches) is presented in section Conclusion (2 pages). However, I am missing more general discussion of the main results together with formulation of possible open hypotheses for the next research. I think that doubling the abstracts (once before the paper and/or manuscript and once within the paper) has no meaning.

As all the three „prepared manuscripts" are dedicated to PREMIVM project (that was closed already more than 3 years ago), it should be explained why these manuscripts were not submitted to review earlier.

Comments and questions to this part of thesis (only those concerning the non-published manuscripts):

Chapter 3.1.2

I find the results presented in this manuscript very interesting.

It has been confirmed that infection of grapevine leaves by *Plasmopora viticola* is connected with accumulation of phytoalexins (trans-resveratrol, pterostilbene and recently identified 2,4,6-trihydroxyphenanthrene-2-O-glucosides – the highest concentrations of the latest compound were found in infected leaves) in both resistant and susceptible vine cultivars. Whereas there was a considerable difference in spreading of the infection between the resistant and susceptible varieties (determined by F_V/F_M imaging) no straightforward difference in profile of induced phytoalexins was found in the cultivars with different sensitivity to this pathogen.

Do you have any possible explanation for inhibition of the progressive spreading of infection in resistant cultivars (if it was not caused by phytoalexins themselves)? Do you have idea about the distribution of the individual phytoalexins over the area of infected leaves?

A formal comment: Within the text the full names of vine cultivars are used, but their abbreviations in Figs. (without explanation in Legend). Either explanation of the abbreviations in Fig. legend or in the list of abbreviations would be helpful to increase clarity.

Chapter 3.1.4

In introduction you have mentioned: „A dual UV-excitation method has been proven as technique for screening phenolic compounds in leaves (Bilger et al., 1997; Ounis et al., 2001; Cerovic et al., 2002) ....." According to my opinion this statement is not precise (moreover according to my opinion in British English "has been proved" is better in this case):

a, a mentioned technique is not based on the comparison of excitation of chlorophyll a fluoresence by the two UV lights (dual UV excitation) but on the ratio of Chl fluorescence excited by visible (blue or red) radiation and UV radiation (UV-A or UV-B).

b, this technique was originally designed as the measure (in the mentioned paper by Bilger et al in 1997) of UV-shielding efficiency by phenolics (flavonoids) rather than content of flavonoids itself. Although both Bilger et al. (2001) and Cerovic et al. (eg. 2002) have proved a correlation between UV-shielding and amount of phenolics later on and Dualex was developed on this platform, this correlation is valid only if the phenolics are located preferentially in the leaf epidermis. For the leaves this is not always the case. **Can you comment on possible localization of the flavonoids in the leaf/berry tissue?** This paragraph should be rephrased with caution.

General comment to all the three unpublished manuscripts: Figures (including axes descriptions etc.) are in many cases too small to be easily "readable".

Final evaluation

I think that set of published papers and/or prepared manuscripts represents a nice piece of work on the topic of presented theses that has been done with a considerable contribution of the candidate. During the defence I expect response to questions (or comments) highlighted by bold italics.

Despite of the mentioned criticism I recommend presented disertation thesis for defence. Further on, after successful defence, I recommend to award Ing. David Šebela the title Ph.D. in Biophysics.

In Ostrava 13. 09. 2016

Assoc. Prof. Dr. Vladimír Špunda, CSc.
Department of Physics
Faculty of Science
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Subject: Opponent´s assessment of the dissertation thesis by Ing. David Šebela

Title: The development of methodological and technological platform for non-invasive estimation of phenolic compounds in leaves and berries

Dissertation thesis by Ing. David Šebela presents a particularly interesting and innovative topic on how to use non-invasive methods of optic signals for detection of secondary phenolic compounds in leaves and berries of grapevine (*Vitis vinifera* L.) and rice (*Oryza sativa* L.) respectively.

The dissertation thesis is, apart from minor typing errors, free of grammatical errors and with the standard of English being at a very high level.

The author has aptly structured the paper into three chapters. Chapter One outlines a considerably extensive subject of phenolic compounds in floral kingdom. What I particularly value here is the author´s ability to describe important facts without focusing too much attention on smaller details, which would unnecessarily increase the volume of theoretical part. The chapter finishes with a theoretical basis of used detection methods.

Chapter Two presents a fluid continuation from Chapter One and describes used plant material, detection methods and laboratory equipment for pieces of individual presented work. What I would object to here for consistency, are the absence of used programmes for data analysis, as well as the discrepancy in using apostrophes when referring to cultivars.

Chapter Three represents the fundamental part of the dissertation. Here, the author demonstrates his scientific credentials with provided publications (3.1.1. and 3.2.3.) and prepared manuscripts (3.1.2., 3.2.1. and 3.2.2.). The chapter is divided into two sections – the work with leaves and the work with berries. The experiments on the surface of the leaves show interesting results with the infection of *Plasmopara viticola* decreasing the photosynthetic activity and pigment concentration and ,conversely, increasing the production of *trans-resveratol and 2,4,6, trihydroxyfenantren-2-0-glucoside.* What I particularly appraise here is the systematic link to resistant cultivars in subchapter 3.1.2. where, at the same time, I would recommend to change the title of the manuscript. In relation to subchapter 3.1.2., I would suggest consistency in writing *Plasmopara viticola* or *P.viticola* and, above all, also avoiding the choice of interspecific varieties that are sensitive to the studied pathogen. Undoubtedly, an interesting thought is presented by outlining a possibility of testing genetic properties of individual cultivars. In terms of further research, I would be rather cautious when preparing field conditions and the purity of used plant material. The experiments with vine berries (*Vitis vinifera L.)* indicate the author´s effort, and the effort of the research team respectively, in finding the answers for complex questions of oenological practice concerning phenolic ripeness and an optimum timeline for harvest which I regard highly (3.2.1. and 3.2.2.). What I appreciate in this section is a high demonstration of conformity of *in vivo* laboratory measurements and field trials including the "WinePen" prototype, which could be used in future as a foundation basis in industries. However, the author seemed to have neglected the visualisation of the key picture 8 on page 79 (3.2.2.) and it therefore unnecessarily downgrades the standard of the dissertation and navigation in results of such interesting experiments.

As a whole, the dissertation thesis demonstrates a comprehensive piece of work which is dedicated to innovative topics. The author has proven an extreme level of expertise in his field and an ability to

present the acquired results appropriately. I evaluate the overall dissertation extremely positively with the standard conforming not only to a dissertation but to a habilitation thesis.

I recommend the paper to be admitted to candidacy.

8 September 2016, Lednice

Doc. Ing. Mojmír Baroň, Ph.D.

Subject: Questions for the defence of the dissertation thesis by Ing. Davida Šebela titled as:

The development of methodological and technological platform for non-invasive estimation of phenolic compounds in leaves and berries

Question 1:

In your dissertation thesis you outline the potential of optic methods for testing genetic diversity between resistant and sensitive cultivars of grapevine (*Vitis vinifera* L.). How would the author envisage the referential material (the same location, age, condition of bushes and so on) for such a complicated experiment?

Question 2:

Does the author think that the used optic methods would be suitable for the testing of spray mixtures in protection against the *Plasmopara viticola* pathogen?

8 September 2016, Lednice

Doc. Ing. Mojmír Baroň, Ph.D.