



**Centre for
Population Biology**

IMPERIAL COLLEGE LONDON AND
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Dear Doc. Gaš,

I am writing to you to submit my referee report on the thesis of Mgr. Zuzana Lhotakova titled 'Study Of Coniferous Needles In Relation To Environmental Factors Using Approaches Of Quantitative Anatomy And Laboratory Spectroscopy.' The thesis consists of an introduction, a review of the topic at hand and of a collection of five academic papers, one of which is in submission stage. The thesis in its entirety is an original contribution and is a result of Mgr. Lhotakova's work during her PhD studies.

The thesis forms a compact unit, detailing the progress of Mgr. Lhotakova from method development in the field of stereology and confocal microscopy, through method validation, to finally arriving at using the said methodology for the evaluation of the influence of environmental factors on coniferous trees' tissue characteristics. In addition, the candidate has made use of recent developments in the field of spectral reflectance index analysis and attempted to link foliage reflectance data to forest floor nutrient status. Developments of both lines of enquiry are, in my opinion, timely and form a substantial contribution to our knowledge in the area.

Being party to information detailing Mgr. Lhotakova's contribution to the published output, I am satisfied that the value of the presented work is sufficient for the successful completion of her PhD.

Please find attached further comments and some points which need clarification in order to improve the quality of the thesis.

Faithfully yours,



Martin Lukac

Referee report:

The Introduction and Critical Review sections are well researched and presented in a concise and easy-to-follow manner. However, some subsections are rather long and tend to go off topic. For example, section 3.3 does not appear to have a lot in common with the main thrust of the thesis and should be re-focused.

Section 4 is where the thesis really comes to life, I think the background information about the current state of the art is rather well interwoven with illustrations of the author's contribution to the field; however some material is then repeated in the PAPERS section leading to needless repetition.

Section 4.2.2.1 – I think the principles of SURS needs to be better explained here, especially for a lay person it might be difficult to grasp how an experimental approach can be systematic and random at the same time. Further, how can the application of SURS significantly improve needle morphology characterisation if this is uniform along 80% of its length? Is the additional analytical effort worth the improvement in precision? Fig.6 in PAPER I shows the dependence of section area (and hence of volume) on the distance from the tip, while Fig.3 in PAPER II shows that there is no influence of this distance on internal surface or % of intercellular spaces. This means that all information about the internal surfaces of a needle can be gained from just 1 central transverse cut and external observation of the needle.

Section 6.4, a direct relationship between leaf N content and soil dissolved organic nitrogen is rather well defined in aerated soils, I am not quite sure this would hold true in soils where the decomposition of organic matter is impeded. This needs qualification in the text.

PAPER III – this is an interesting topic, there aren't many observations of this sort on conifers. However, I have two problems with the data analysis in this draft. (A) The more serious issue relates to the whole experiment, it seems to me that there is only one

replicate dome for ambient and one for elevated CO₂. This would mean that all presented analyses are based on pseudoreplication. (B) Why did you analyse the results with repeated measures? Do you have needle anatomy and chemical composition observations prior to the initiation of the experiment that are not presented here?

PAPER IV – there is no mention, as far as I can see, as to why would the azimuth orientation of a sunlit branch affect needle properties. Can you expand on this to justify your study?

PAPER V – I find that an important mechanism is not mentioned in the paper, most trees (if not all) tend to strip the foliage off its most valuable nutrients during leaf (needle) senescence (e.g. Torgny Näsholm, *Oecologia* **99**: 290-296). This process would surely have an impact on the relationship between crown foliage N content and DON in the soil, could you comment on this.

Editorial comments:

The thesis should have a uniform page numbering; it is difficult to find one's way through the thesis without it.

General introduction needs bigger line spacing.

The hypotheses and the papers (pages IV and V) relate to each other, sort of. They could be better linked so they correspond and are easily compared.

Please remove the (blue) hyperlinks from the text.