Recommendations of the External Advisory Board of EXTEMIT-K 2020

The External Advisory Board (EAB) consisted of (in alphabetic order): Bill S Hansson (Chair), Sigrid Netherer, Martin Schröder and Johanna Witzell.

Preamble

The EAB and the leadership of EXTEMIT-K (E-K) met at Kostelec Castle east of Prague during February 26-28, 2020. The meeting included a site visit to observe E-K activities and experiments in the Kostelec forest. The meeting started by presentations from the organisational leader Prof Marek Turçani and the scientific leader Prof Fredrik Schlyter.
Following these initial lectures each Level leader (Ewald Große-Wilde (G); Anna Jirosová (T); Rasto Jakus (L)) presented a talk on the progress for each Level. Thereafter, group discussions according to the "café style" including all E-K representatives and the complete EAB took place during two hours. During the second day 7 PhD students presented talks about their ongoing research. In addition, a number of students presented posters during extensive poster sessions. In a final session the main conclusions of the EAB were presented to the E-K leaders.

The general impression of the EAB was very positive. A clear profile of the E-K program is now visible and clear scientific progress has been made. In addition, significant work has been invested to improve the social cohesion in the group. This has resulted in a much stronger impression that the group work together and collaborate. Important for this success is the scientific leadership by Prof Fredrik Schlyter, the administrative leadership by Radek Rinn and the overall leadership and support by Prof Marek Turçani.

In the 2018-19 report we stated one specific point that we saw as outstandingly important for the program to function in a cohesive way:

The team should construct a conceptual framework that clearly illustrates the novelties with respect to fundamental and applied research

We now see such a physical map, which helps everyone to gain a clear impression of how the different parts of the extensive E-K program are connected and feed into each other. In appended maps it is also possible to see which outcomes can be expected and which collaborators will be involved in the different levels and sublevels. We find that these scientific maps have helped in creating an informed and cohesive environment.

The EAB also asked for a clear publication list from the complete E-K program. A list was now presented.

THE SCIENCE

The EAB was generally impressed by the progress in E-K science. The presentations by the Level leaders showed that work at all levels is progressing and publications are produced. In general, the EAB was pleased regarding the direction of the science being performed and planned. We do, however, have some general and some more specific remarks.

General remarks

The E-K program has matured scientifically. We see that all three Levels now make significant progress and have well-functioning PhD students and postdocs working in the laboratories and in the field. Again, the overviews created have helped everyone to understand how their part feeds into the overall picture. It will be important for the leaders to judge where the highest chance for impact resides. When such areas have been identified, the project can be concentrated in such directions. More about this below.

Gene Level (G)

The E-K crew is now approaching the goal of characterizing the complete *Ips typograhus* genome. A high-quality genome, which surpasses many other available insect genomes, will be a very important hallmark of the project. Here, we see the interactions with Lund University in annotating the genome as very important and the presentation from this laboratory during the evaluation was highly appreciated. In this exercise it will be important to have very clear agreements regarding future publications and data use as we see potential conflicts on the horizon. We urge the scientists involved to have very clear discussions with the Lund team regarding genome use, annotation, receptor deorphanization and future publication strategies.

The deorphanization of olfactory receptors is still a key task within E-K. We urge the scientists involved to push this project with urgency as other labs are pursuing similar paths. Here, again, the Lund laboratory is already publishing a couple of deorphanized receptors and if E-K wants to stay at the frontline a major thrust has to happen very soon. Luckily, the E-K laboratory has now got the permission to work with transgenic *Drosophila melanogaster* flies. This means that the empty neuron system can be used to perform the deorphanization. The method does, however, require time, as flies have to be made for each individual receptor to be investigated. It is therefore, also here, of essence that the laboratory activity runs with full speed.

We are very happy to see that the GC-SSR setup is up and running. This will provide the team with the unique opportunity not only to test single substances on all receptors expressed, but also to test extracts from different relevant odour sources run through the gas chromatograph. In general, we see great progress and are confident that the G-Level will produce excellent results in the coming year.

T-level:

The core experiment of the T-level, aimed at a better understanding of tree stress and bark beetle attack, critically relies on the maintenance and protection of the six study plots. The fact that all originally selected study trees are still alive and being monitoring for sap flow, changes in diameter and bark temperature is obviously owed to the enormous efforts of forest protection in the Kostelec area, involving timely detection of attacked trees by sniffer dogs. E-K now definitely profits from development and successful testing of methods in the field T2B to train canines for identification of infested trees early in the project phase. The study area is also monitored by the remote sensing team, which indicates that the interaction between project levels has much improved.

T1A Prediction of stress by physiology and dendro-ecology

Tree physiological and soil water measurements

We consider the presented combination of data from intra-annual dynamics of stem water and transpiration (sap flow and changes in tree diameter) to compare "normal" and drought years as highly relevant. However, as physiological indicators of drought stress are already well established, and to advance knowledge on thresholds indicating increased predisposition of bark beetle attack, we strongly encourage the team to perform bark beetle attack experiments as originally planned. The comprehensive dataset based on measurements at a large number of target trees and over the period of at least two consecutive years, is of course highly valuable *per se* to examine the variability of individual tree responses within a forest stand to stress conditions. We are looking forward to the article in preparation! Some more considerations:

- Soil water potential is only one measure to assess site water supply but does not provide the full picture. A range of drought stress indicators are based on actual plant water availability that strongly depends on the combination of soil and tree physiological parameters. We recommend comparing different stress indices (e.g. transpiration deficit, tree water status, relative proportion of plant available water) to check whether they tell the same story.
- The Kostelec sites differ slightly from phyto-sanitary point of view and there are variations regarding soil and growth conditions, but all are lowland sites with similar soil type and thick organic matter. Assessment of soil parameters, in particular soil texture (sand, loam, clay), soil depth and stone content, can therefore be done easily and will definitely add to a better understanding of site water supply.

Experimental setup to test for tree defence status and bark beetle attack

As discussed above, tree water supply has been recorded since 2018, providing an excellent overview on tree individual and seasonal variations (*status quo*). Although we understand that the setup of treatments is challenged by the outbreak situation in the study area as well as by

objections of the funding agency, we strongly recommend starting the experiments at least at some of the plots this spring. Remaining project time is just too short for a further delay of activities. We think that

- Irrigation will supposedly pose the least risk to study trees and should be favoured over roofing if possibilities are limited. Completely cutting trees from water supply by the roofs at already xeric sites creates conditions far from natural occurrence and might tell more about drought-induced rather than bark beetle-induced tree mortality. Yet, soil water supplies seem to be replenished this winter season.
- Studying bark beetle attack at newly created stand edges can indeed bring novel and important results, yet, is most risky with regard to the probability of natural attack. Moreover, natural attacks hinder the observation of earliest bark beetle-tree interactions and will force a removal of all trees within the treatment. The use of passive landing traps to document bark beetle activity at subplot treatment trees, which was started last year, is interesting but makes sense only when traps are controlled daily and not too few beetles are caught in the early phase before pheromone release starts. If there is a risk of too low catches, as in earlier experiences, more traps should be used per tree.
- The presented methodological approach of B. Stribirska's PhD thesis involving field bio-assays by use of *attack boxes* is well-thought-out and promising. It might, however, be reasonable to have an alternative plan at hand in case trees at the newly created forest edge are attacked early in the season (switch to irrigation plots?).
- Measurement of composition and quantity of bark secondary compounds and VOCs at a large number of differently treated trees (MeJa applications, trees of different treatments) as started in 2019 is one core task of T1A and will for sure deliver novel insights into constitutive and induced defence of Norway spruce in response to stress.

<u>T2A Field activity of new physiological active semio-chemicals and blend function</u>

We appreciate the established cooperation with Jena (D. Kandasamy) and consider a continuation of experiments in 2020 promising. According to the presented 1p plan, activities in this research field seem to be well planned.

<u>T3 Genetic understanding and long-term improvement of Norway spruce</u>

<u>T3A</u>: The goals with work on ecotypes are now clearer. An overview of all the ongoing and planned studies, including their specific hypotheses (e.g., the transcriptome study that was presented in a poster) could further clarify how the research in this task is structured, how it implements the concept of resilience, and how it provides input to EXTEMIT's goal "mitigation of pest hazards to forest stands made more vulnerable by climatic change".

<u>T3B</u> – Modification of terpene precursor enantiomers of insect pheromone production:

It has been demonstrated that there is a correlation in pheromone production with the proportion/amounts of (-)-alpha-pinene in the tree. There are a few earlier studies about this variation in spruce trees and we encourage the team to continue with new experiments. The feeding assays are a good start, but need to be refined with regard to dosage (what about the range of "natural" concentrations in trees?). We approve all quests to increase knowledge on how bark beetles detoxify MTs and, in the best case, to identify detoxifying genes. This link between G-level (G2) and T3B is great (and not delineated in the BASE map).

L-level:

Since the last meeting in May 2019, the L-level has made efforts to increase internal collaboration and to develop the science related to landscape level phenomena and processes. New studies have been initiated or are on the way, addressing various L-level aspects, such as tree resilience (last tree standing) and forest disturbance ecology, and new technological solutions have been applied for monitoring and prognosis purposes. The level has been strengthened by the new NAZV projects. This is all very positive. Continued attention on the L-level activities is needed during the remaining project time, in order to "provide fundamental answers to forest management" (citation from the E-K web page). We therefore strongly encourage the involved researchers to continue to interact and jointly develop the L-level towards the goals. Given that the T- and L-levels are intertwined, it is also important to maintain a close and constant dialogue between the L- and T-levels. A concrete measure to organize the interactions could be to have a workshop where the plans are updated and revised, and an action plan made for the remaining project time (with regular follow-ups). The base map and the envisioned outcomes will provide a guideline.

Progress has already been made with L1A, L2A and L3A. In L2A, field tests will be conducted in 2020 based on results from T2A experiments. Especially the activities in L3A are timely, considering the increasing interest in monitoring and early warning solutions. It is also a strength that this task is developed in collaboration with SLU, Umeå. The idea of L2B (manipulation of bark & beetle landing) is based on a published (small-scale, N-American) study, and its feasibility remains to be tested. If successful, the method may be used for protecting especially valuable trees. It is also still unclear whether it is possible to utilize thermal sensing as envisioned in L3B. Thus, these two tasks may deserve extra attention during the next period.
Specific attention could also be given to establishing the external collaborations tagged with a question mark in the map. Especially important would be to establish the needed contacts to regulatory actors, to ensure that the research outputs will be compatible with the existing regulations.

SUPERVISION AND MENTORSHIP

The E-K project has now reached so far that a number of PhD students and postdocs are working in the different Level teams. It is thus important to think about how supervision and mentorship works in a modern perspective. We strongly suggest that each student has a Thesis Advisory Committee (TAC) consisting of the Level supervisor, a second supervisor from within E-K and a third member from outside the project. This committee should meet once per year to discuss both the scientific progress and the situation of the PhD student. For postdocs, a clear discussion regarding career development and opportunities should be organised in the second year within the project.

As a general feature, it is also highly important to create clear reporting paths for potential cases of both scientific and non-scientific misconduct. Where does anyone working within E-K go in case of being bullied or harassed? Does CZU have clear routines also at the university level? These are question, which should also be put on the agenda in a well-functioning project like E-K.

THE ORGANISATION

In our previous reports (2017, 18 & 19) we have provided quite substantial input regarding the internal organisation of the project. Many of our comments have been taken to heart and we see much stronger communication and cohesion within the project. The leaders should be applauded for achieving an environment much more conducive to science. It is, however, always important to remember the quote from George Bernard Shaw: “The problem with communication is that it never stops….”

We thank the whole E-K Team for again organising a very interesting meeting and look forward to be informed regarding progress during next year’s meeting. Meanwhile, the complete EAB stays at your disposal for input and comments in the inter-meeting interval.