

Recommendations of the External Advisory Board of EXTEMIT-K 2019

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

Preamble

The EAB and the leadership of EXTEMIT-K (E-K) met at Bäckaskog Castle in southern Sweden during May 26-28, 2019. The meeting included a site visit to study Swedish forestry and forest industry. The meeting was started by presentations by the organisational leader Prof Marek Turçani and the scientific leader Prof Fredrik Schlyter. Following these initial presentations each Level leader (Ewald Große-Wilde (G); Anna Jirosová (T); Rasto Jakus (L)) presented the progress for each Level. In addition, group discussions including all E-K representatives and the complete EAB took place during two hours. In a final session the main conclusions of the EAB were presented to the E-K leaders.

The E-K program is now half way. This means that conclusions can be drawn regarding which parts of the program are well under way and which still require substantial work to get to a stage of fruition. The general impression of the EAB was very positive. 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 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

Such a framework or map has still not been presented and the EAB hopes that such a step can be achieved as soon as possible. We would like to see this happen during 2019 and the result to be sent around as soon as possible. Beyond this general point, we provide input regarding both organisational and scientific issues below.

For the next report the EAB would like to see a list of the publications produced (1) since the beginning of E-K and (2) specifically during the reporting period. Each publication should be followed by an indication to which Level (e.g. T) and Sublevel (e.g. T2) it belongs. The list could also include manuscripts that are in an advanced stage of completion and can be expected to be published within the next year.

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

As the program is half way timewise it is now time to contemplate which goals are indeed realistic and crucial and which should receive less attention. The team still casts quite a wide net when it comes to the projects. Here we see that it is extremely important to be selective in which projects the E-K team decides to tackle and to critically revisit the reason behind each subproject (more in detail below). As stated above, we repeat our strong recommendation from our previous visit: *to make the informed choices needed it will be highly important to build a clear map over the complete project. The map should include both completely internal E-K components as well as those performed in collaboration with outside partners.* Only with such an overview available will it be possible to reach the overall project goals. This map should be imprinted in the minds of all co-workers. A comprehensive project map will also make it clear where strong synergies can be found between the different scientific disciplines represented within E-K. We are pleased to see that regular meetings between leaders at different levels suggested earlier indeed take place and in this way cohesion has increased. Below we provide specific input to the three scientific levels within E-K.

Gene Level (G)

The gene level is presently aiming at characterizing the complete *Ips typographus* genome. Such a result will indeed be extremely important to everyone working on this bark beetle and the team should be commended for taking on such a task. The annotated genome can provide the basis for further studies of e.g. olfactory receptors and their function, of biosynthetic pathways for pheromones and of beetle mechanisms to overcome tree defence. Again, it has to be pointed out that there is now three years left of the project and the endeavours should be framed in a realistic time perspective. Dr Ewald Große-Wilde has taken up the leadership of the G Level and is doing an excellent job in concentrating the work in the proper directions. To achieve the characterisation of olfactory receptors identified from the genome it is, however, of utmost

importance that he gets parallel support from the laboratory for single sensillum recordings (SSR) and for gas chromatography-SSR. According to the E-K leaders a new postdoc has been hired to be the major force in the electrophysiology laboratory.

Limiting factor to the present activities to deorphanise receptors is the lack of permission to use genetically modified flies. This must be remedied with the highest priority. No work with *Drosophila* flies expressing *Ips* receptors can be performed before this permission has been granted. However, even if this process still takes some time, the GC-SSR setup can be optimised and a high-throughput system to characterise single olfactory neurons of the *Ips* antenna can be brought into action. This can start tomorrow. Continuing with electroantennogram-based experiments is complementary to the GC-SSR work, but standing alone, is of quite limited value.

In conclusion, we are very happy with the activity and the progress at the G Level on the molecular biology side. We are, however, still worried regarding progress in installing a routinely functioning GC-SSR setup allowing high throughput characterisation of olfactory sensory neuron responses to extracts of and volatile collections from relevant substrates. A final recommendation would be to strongly contemplate where to concentrate the primary force at the G Level. As we see it, it is not realistic to go for both olfactory receptors, biosynthetic pathways and anti-tree-defence mechanisms in the limited time frame remaining.

Tree Level (T)

The one-page reports circulated by the E-K team before our EAB meeting provided information on:

T1A: Prediction of tree stress by physiology and dendroecology

General information on the experimental plots for the study of acute/chronic drought stress

Physiology measurements

T1B, newly L3A (and in T1A): Early warning from reflectance by remote sensing

T1C, newly L3B: Real-time attack detection by thermal emission under different crown and

stand geometry T2A: Field activity of new physiology active semiochemicals and blend function

T2B: Establishment of rapid attacked tree detection by sniffer dogs

As far as we understood from the presentations at the meeting and publications listed on the website, the E-K team has gathered first results from tree physiological measurements at the Kostelec plots and sniffer dog experiments, and is currently running field experiments to test for

antennally active compounds of *Ips typographus*. The set-up of different stress treatments at the Kostelec experimental sites was postponed to fall 2019 for a couple of reasons. Remote sensing and thermal emission experiments are still in planning status. We did not fully understand how study fields were moved between T and L level, yet this can be easily clarified by the desired conceptual map. Moreover, responsibilities for T and L level experiments seem to partly overlap now, which from our point of view can easily lead to conflicts between the involved scientists. In this respect we would like to point out the necessity of communication and supportive cooperation between the project leaders and the involved team leaders.

Comments on T1A and T1B (newly L3A): We regard the adaptation made to the experimental design at Kostelec to the changed situation after the severe drought in 2018 as reasonable. While this year's (2019) activities are still aimed to document the *status quo* (tree physiology, soil moisture) at the sites, water supply of the study trees is going to be manipulated for the coming season 2020. At each of the six study sites, treatments will include one large roof/rainout shelter covering 12 sample trees, an experimentally exposed forest edge with 10 sample trees, one irrigation variant with 10 sample trees, as well as 10 control trees. This is highly ambitious, yet feasible when properly planned and organised. At this point of the study we therefore regard it of utmost importance to at least complete the structure for the roof in the autumn of 2019, seeing the risk of potential bark beetle damage and the few years remaining for experimental work and writing up publications. Furthermore, we strongly recommend that you reconsider the definition of chronic and acute drought stress with regard to the degree and duration of drought. In our opinion, all trees at the Kostelec sites are subject to chronic drought stress, but the quality and degree of the stress will differ between the treatments.

We understood that plot establishment could be realised only due to the strong commitment of certain team members. Roman Modlinger supervised the choice of sites and trees. A core part of tree physiological measurements was done in 2018 by Ivana Tomášková, including recording of sap flow and alterations in stem diameter by dendrometers. These data turned out to be of great interest as they were recorded in an unprecedented warm and dry period. We expect these results to add to a better understanding of how trees respond to extreme drought stress, and therefore recommend to critically examine the data for possible publication.

With regard to the ongoing project work and change of plans since the start of E-K, we recommend that you stay focused on your original hypotheses and study questions. In particular, do not lose your aim to examine the link between tree physiology, defence capability, attractiveness (although elusive), and bark beetle attack. The experimental sites and equipment installed offer a unique opportunity to learn about host tree-bark beetle interactions under extreme conditions, and this opportunity should not be missed out. What lacked in your presentations, and is now unclear to us, is which methods you will use to observe attraction /host acceptance/attack success of *I. typographus* to trees of differential stress status. Passive trapping

of the natural population might be a suitable method in view of the present high beetle abundance. However, discarding the attack box experiments (as originally planned) should only be done following the agreement of all scientists involved in the experiment.

Comments on T2A: The testing of fungal compounds in combination with pheromones for biological activity in *I. typographus*, as currently done by Anna Jirošová and her master student, is novel and worth following up. Anna and Jaromír Hradecký have also started to treat trees at the Kostelec site with methyl jasmonate, with subsequent sampling of bark and volatile substances for the measurement of secondary compounds. We missed a description of these activities, which are in our opinion central to the E-K project, in the one page documentations and presentations. Anna and Jaromir are skilled experts in chemical ecology and analytics, run a well organised lab, and will definitely contribute core results to the E-K project.

Comments on T3A: In the 2018 EAB report we asked about the rationale for characterizing different ecotypes in Norway spruce. The 2-page report we received in May 2019 provides more details about the background and practicalities of the ecotype project, but we encourage the team members to further develop and clarify the scientific hypothesis of the study. What are the scientific and applied rationales behind the efforts to characterize the genetics and biochemistry of the three different ecotypes?

Landscape Level (L)

Landscape Level continues to be of crucial importance for the impact of the E-K project on practical management to mitigate bark beetle damage. It is therefore important to maintain the focus on the planned studies on landscape level processes and phenomena, and to translate information from the T- (and G-) level to the L-level wherever possible. New theoretical elements (such as e.g. different plant defence hypotheses) should only be introduced into E-K if the team can contribute with novel aspects that add to the existing theories. It is important not to use project time and resources on reinventing the wheel.

L1A: The written summary and presentation indicate that the L1A sub-project is proceeding in good order and is productive in terms of publications. However, this sub-project appears to run parallel to E-K and we recommend that the team members continue to work on the integration between this sub-project and the other parts of E-K.

L2A and L2B: The presented study plans are preliminary and more information would be needed to allow for detailed comments. For instance, we would have expected to receive a more detailed description of the background and hypothesis of L2B, even if the experimental work has not yet started. We recommend the team members to start thinking about the challenges and possibilities related to practical application of new compounds for bark beetle management already early in the process (e.g. related to cost-effectiveness, environmental effects, patenting, involvement of companies, etc.). This may save some work later on and help to set effective research priorities.

THE ORGANISATION

In our previous reports (2017 and 2018) we provided quite substantial input regarding the internal organisation of the project. Some of our suggestions have been realized, but we see that the E-K team to some extent still suffers from lacking communication and information. We will not repeat all the comments from earlier reports but rather urge the leaders of EXTEMIT-K to further increase their efforts to keep all team members informed and included. Clear on-boarding mechanisms for new group members are vital.