Final Report for PA 1167 & 1195: “Test- och screeningsresistens i europeisk lärk (*Larix decidua*) mot lärkkräfta"

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In Sweden, there is a renewed interest in the use of larch (*Larix*) during the last decade as evidenced by the increase in sales of larch seedlings from commercial growers. This is mainly attributable to a desire to find an alternative to spruce especially after the severe windstorms in 2005 and 2006, but also because of the uncertainty associated with climate change and the need to spread the risks through increasing species diversity in forest stands.

At present, the research related to larch in southern Sweden has mainly been concentrated on hybrid larch (*Larix decidua* x *L. kaempferi*) because of its good production and notable tolerance to the larch canker disease (*Lachnellula willkommii*). This ascomycete fungus is apparently native to Japan, but established in Europe, where it became well known due to its damage to plantations of exotic and native *Larix* species, beginning in the nineteenth century. Local spread of the fungus between trees appears to depend on dissemination and survival of airborne ascospores. Climatic conditions of humidity and temperature appear to limit natural spread from regions of establishment (Ostaff, 1985).

Damage caused by the fungus appears as swellings on twigs and branches, and sunken cankers on larger stems (Figure 1). The first circular or elliptical depressions often form around dwarf shoots. Resin is commonly exuded from lesions. The bark cracks and is loosened. A ridge of wood develops around enlarging cankers on stems and trunks as the tree grows. Needles above the canker shrivel up and die or turn yellow early. If the stem or trunk is girdled, branches and young trees will die.

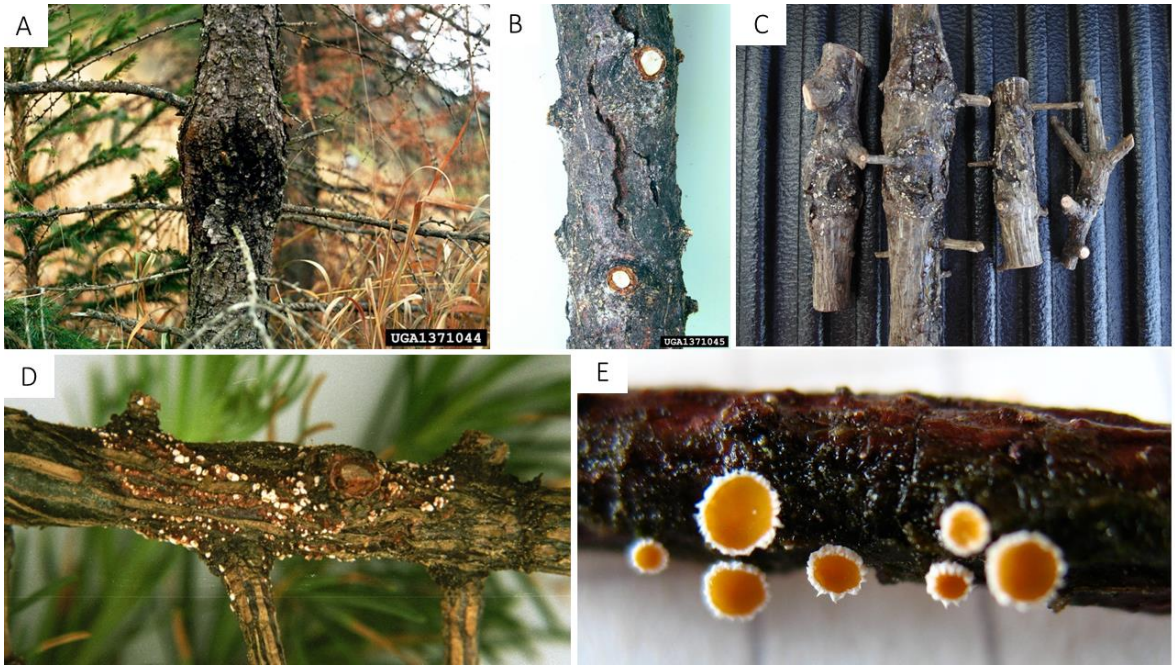

Figure 1. A) Resinous stem canker on European larch infected with *Lachnellula willkommii*; B) Swelling and cracking of bark on infected stem; C) branch swelling and resinosus around lesions; D) fruiting of L. willkommii on diseased branch; E) apothecia (reproductive fruiting body) of *L. willkommii* on European larch

In Sweden, growing European larch (*L. decidua*) has been problematic due to its high susceptibility to the larch canker disease, and it is the main reason why people avoid cultivating and planting it today. Forest tree breeding for larch in South Sweden is now based largely on research started in 1950 's and 1960 's. The European clones from hybrid larch seed plantation Maglehem likely originated from the alpine region which incidentally is considered one of the worse provenances. Despite this, the production is reasonable on good sites in southern Sweden. However, new prospects for growing European larch have resurfaced – with material sourced from Poland and Czech Republic. In addition to high yield (similar in scope to hybrid larch), European larch in this region have exhibited remarkable resistance against the pathogen *L. willkommii*. This can provide potentially a new species option for southern Swedish forests!

In 2017, seeds were picked from approximately 85 half-sib families of European larch from the area of Sudety mountains in southern Poland in order to establish cultivation tests to study growth, stem quality and resistance to *L. willkommii* in southern Sweden. This material was propagated at Skogforsk and it is now planted out in several field trials for testing. In the Partnerskap Alnarp project we aimed to develop a screening assay for testing the resistance of this material that will allow for the best families to be selected for further propagation and establishment of seed orchards for commercial production.

In the project we sought out and ollected local isolates of *L. willkommii* from diseased European larch from known locations in Sävar and Kättnas. Branch cankers exhibiting characteristic symptoms (branch swellings, necrotic lesions, resin flow) and signs (i.e. fruiting bodies) of larch canker. In order to confirm the identity of *L. willkommii* on samples, and to exclude the possibility of having collected and isolated the similar species *L. occidentalis*, isolations were made from the apothecia and DNA analyses performed to confirm fungal species identification (Figure 2).

Figure 2. Left: Canker with resin outflow; Middle: Removal of apothecia from infested branch; Right: pure fungal culture of *L. willkommii* (Photos: Erik Kügler)

A greenhouse inoculation experiment was performed on 185 1-year-old European larch seedlings from a single half-sib family from Sudetes in Poland. Inoculations were performed by peeling back the seedling's bark to expose the cambium using a sterile scalpel, and placing either an agar plug of mycelia or a colonized woody segment (Figure 3). Different fungal isolates (from Sweden and Czech Republic) were used with each treatment also to test whether there was any variability in the fungal virulence among isolates.

Figure 3. Greenhouse inoculation trial with European larch, inoculum substrate and isolate treatments applied to plants (photos: Michelle Cleary).

After three months, all seedlings showed necrosis around and inside the artificial wound, and callus formation and resin outflow was observed. In testing different inoculum substrates, the agar plug seems to have higher inoculum potential (resulting in significantly longer lesion lengths) than the woody inoculum (Figure 4). In addition, there were no significant differences in lesion length among the different fungal isolates used in the study (Figure 5).

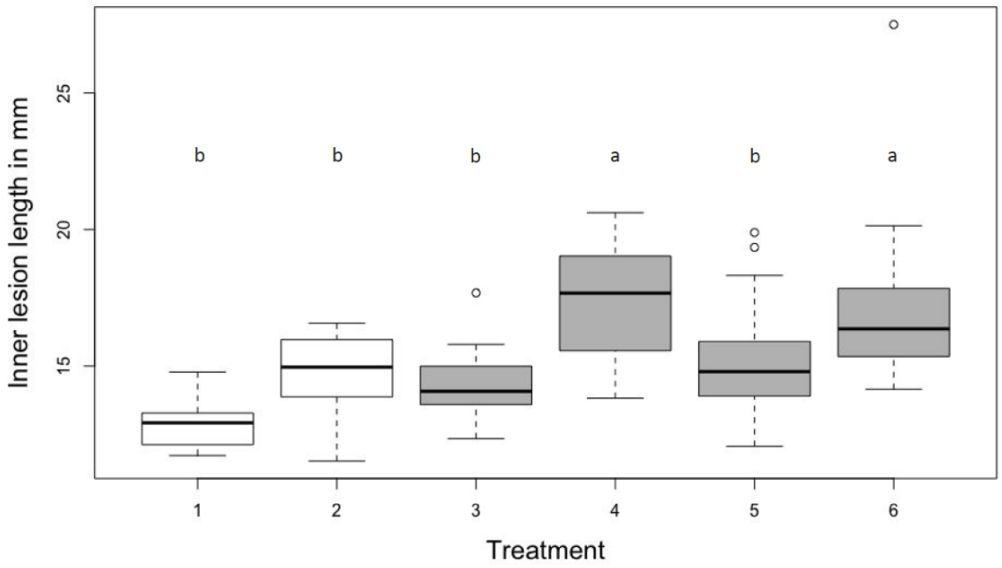

Figure 4. Boxplots of inner lesion lengths from Experiment 1 comparing six treatments. Letters denote significant differences in lesion lengths as determined by Tukey HSD at the $\alpha = 0{,}05$ level; Treatments: 1 = Wood control, 2 = Agar control, 3 = Wood IS 329 R1, 4 = Agar IS 329 R1, 5 = Wood IS 61 R1, 6 = Agar IS 61 R1

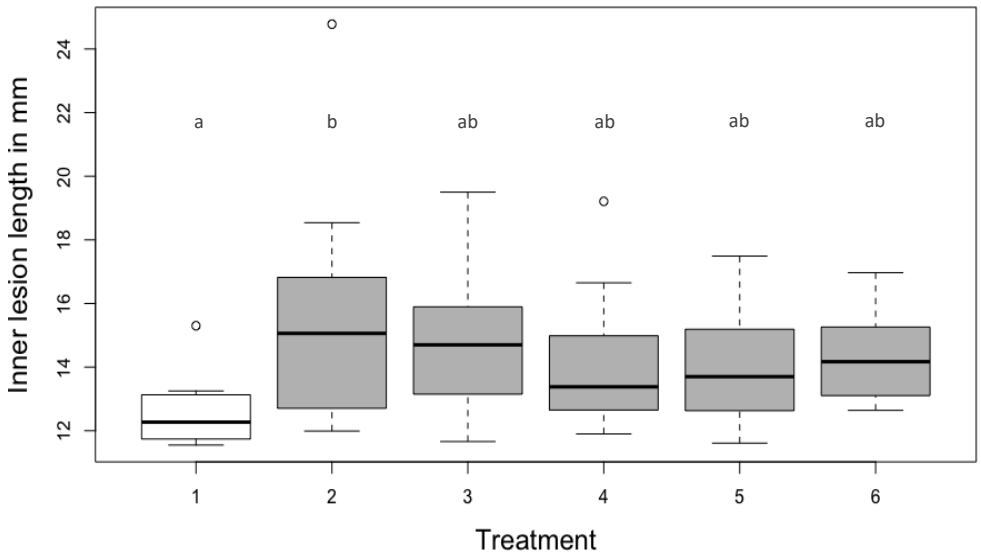

Figure 5. Boxplots of inner lesion lengths from Experiment 2. Letters denote significant differences in lesions lengths as determined by Tukey HSD at the $\alpha = 0{,}05$ level: 1 = Control, 2 = IS 329 R1, 3 = IS 61 R1, 4 = S.053.15, 5 = S.054.15, 6 = S.055.15

Based on the results of this work, neither geographic location nor individual differences of isolates from the same geographic location seem to influence disease severity. Only IS 329 R1 (a Swedish isolate) showed significantly longer lesions than the control treatment. This would be a good candidate isolate for use in future screening trials. Climatic factors and site conditions play an important role in influencing Larch canker disease host-pathogen interactions (Cech, 2013). The future breeding experiments should therefore take the intensified symptoms in cold, moist sites with little air drainage such as valley bottoms or hollows into account (Cech, 2013; Schober, 1977).

To improve future inoculation experiments for screening disease resistance, more research should be invested in host-pathogen interactions to understanding the mechanisms affecting disease tolerance. Standardizing methods are critical for this purpose the results of this work will help proceed with screening efforts that will lead to tree-improvement programmes for resistant European larch in Sweden. In the long-term, if a breeding program finds suitable resistant genotypes of European larch, this would give new or renewed possibilities for reintroducing a valuable tree species alternative for Sweden and northern Europe.

References

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