Slutrapportens över projektet

Askskottsjuka. Skötselalternativ och säkrande av resistent odlingsmaterial

Huvudsökande med kontaktuppgifter

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Projektets löptid

2013 01 01 – 2015 12 31

2. Sammanfattning: En populärvetenskaplig sammanfattning av de huvudsakliga resultaten. (max ½ A4-sida)

Askskottsjukan har funnits i Sverige sedan 2001 och de flesta askar i Sverige har utsatts för angrepp. En stor del av träden har dött och asken är upptagen på rödlistan över hotade arter. Vi har tidigare visat att askskottsjukan inte är systemisk utan består av ett stort antal individuella infektioner i kronan på träden. En del askskottsjukeinfektioner i kronan på askar dör av med tiden men att en del fortsätter vidare in mot huvudstammen. I projektet studerade vi i detalj infektioner i grenar och slutsatsen är att svampen växer inne i grenen framför synlig skada. På ett avstånd av 3 dm innanför synlig skada fann vi ingen svamp. Rekommendationen är därför att om man kvistar ask i syfte att befria träden från askskottsjukeinfektion bör man kapa kvistar/grenar minst 3 dm innanför synligt angrepp. Denna åtgärd är arbetskrävande men bör kunna tillämpas i parker och alléer.

Vi har inventerat asksjukeangrepp över askens utbredningsområden i södra Sverige. Markägare och myndigheter som noterat träd med god överlevnad har rapporterat in detta till oss. Vi har sedan besökt dessa bestånd. I bestånd med kraftiga angrepp av asksjuka har drygt 500 träd med frisk krona identifierats och märkts upp. I samarbete med Skogforsk har ympmaterial från de mest motståndskraftiga individerna sparats i ett arkiv och också planterats i fält för framtida fröproduktion. Träden ingår även i en genetisk screening av molekylära markörer för resistens som utförs i ett annat projekt.

Vi har också undersökt avkomman från träd som i tidigare studier visat hög tolerans för askskottsjuka. Avkomman från sådana träd var mycket mer motståndskraftig än avkomman från träd med kraftiga symptom av askskottsjuka.

3. Resultat: En redovisning av de resultat som projektet har lett till samt en redogörelse för hur projektet har löpt i förhållande till projektplanen

1) I ansökan planerades en utförlig test av kvistning och hamling av ask. Under projektets gång insåg vi att liknande arbeten också utförts av andra forskare i Sverige och vi koncentrerade arbetet på att undersöka hur långt svampen spridit sig i grenar framför synliga symptom av nekros. Vi studerade i detalj 138 infektioner i grenar och konklusionen är att svampen finns i hög frekvens något tiotal cm

framför synlig skada. På ett avstånd av 3 dm innanför synlig skada fann vi ingen svamp. Rekommendationen är därför att om man kvistar ask i syfte att befria träden från askskottsjukeinfektion bör man kapa kvistar/grenar minst 3 dm innanför synligt angrepp. Denna åtgärd är arbetskrävande men bör kunna tillämpas i parker och alléer.

Vi har tidigare visat att askskottsjukan inte är systemisk utan består av ett stort antal individuella infektioner i kronan på träden. En del askskottsjukeinfektioner i kronan på askar dör av med tiden men att en del fortsätter vidare in mot huvudstammen. Andra forskares resultat i Sverige har visat att veteranträd som hamlats kontinuerligt fått något bättre asksjukestatus medan träd som tidigare inte hamlats snarare blivit mer angripna av att hamlas.

2) Vi ansökte om att studera möjligheten att reducera sporspridning av sjukdomen genom att samla ihop och förstöra löv i bestånd med ask. Sådana försök är arbetskrävande och behöver göras noggrant i stor skala för att kunna ge pålitliga resultat. Efter noggranna överväganden insåg vi att det inte är möjligt att utföra experimentet inom ramen för forskningsprojektet. Vi har istället fokuserat resurser runt att så gott som är möjligt utföra delprojekt 3 om att samla in resistent/tolerant material till framtida askfröplantager.

3) I klonarkiv i Sverige och Danmark har man konstaterat att en del genotyper av ask är mer resistenta mot sjukdomen än andra. För framtidens askplanteringar är det således viktigt att vi tar vara på ympmaterial och frö från resistenta genotyper från askens utbredningsområde i Sverige. Tidigare ympmaterial insamlat av Lars-Göran Stener, Skogforsk, kommer att testas med infektionsförsök för att bekräfta den relativa resistensen.

Vi har inventerat asksjukeangrepp över askens utbredningsområden i södra Sverige och på Åland. Vi började med en press-release via Skogstyrelsen om askskottsjuka (http://www.skogsstyrelsen.se/Myndigheten/Press-och-information/Pressmeddelanden/Ny-kunskap-kan-radda-asken---Sveriges-kungstrad/) som beskrev projektet. Ett stort antal artiklar skrevs i olika tidningar tidskrifter (t.ex. Land, Natur and Trädgård). Markägare som noterat träd med god överlevnad har rapporterat in detta till oss. Vi har sedan besökt dessa bestånd tillsammans med askbestånd identifierade hos länsstyrelser. I bestånd med kraftiga angrepp av asksjuka har drygt 500 träd med frisk krona identifierats och märkts upp. Från närmare 300 av dessa träd har, i samarbete med Skogforsk, ympmateral tagits som ympats på rotmaterial under 2014-2015. Dessa genotyper har sparats i ett arkiv och också planterats i Snogeholm för framtida fröproduktion. Träden ingår även i en genetisk screening av molekylära markörer för resistens som utförs i ett projekt finansierat av FORMAS.

Vi har också undersökt avkomman från träd som i tidigare arbete utfört av Lars Göran Stener vid Skogforsk visat hög tolerans för askskottsjuka. Avkomman från sådana träd visade sig vara mycket mer motståndskraftig än avkomman från träd med kraftiga symptom av askskottsjuka.

Mer detaljerad redogörelse finns i bilaga 1.

4. Kommunikation: En redogörelse för hur resultaten och projektet har kommunicerats och om ytterligare kommunikationsåtgärder finns planerade.

Projektet har beskrivits i ett antal artiklar i fackpress. Vi har beskrivit resultaten i manuskript till internationella tidskrifter. Resultaten har kommunicerats vid flera mörten organiserade av det europeiska samarbetsprojektet FRAXBACK, och i Almedalen 2016 tillsammans med Future Forests.

Vi har också intervjuats vid ett antal tillfällen av radio och tidningsjournalister.

Manuscripts:

Marčiulynienė, D., Davydenko, K., Vasaitis, R., **Stenlid, J.,** and **Cleary, M.R.** 2016. Pruning as a method to maintain high-valued ash trees in urban landscapes? Urban Forestry and Urban Greening. [in preparation]

Cleary, M., Nguyen, D., Stener, L-G., **Stenlid, J.,** and Skovsgaard, J-P. 2016. Ash and ash dieback in Sweden: A review of disease history, current status, pathogen and host dynamics, host tolerance and management options in forests and landscapes. In: *Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management (FRAXBACK) (Eds: Vasaitis, R., Enderle, R., Cleary, M.). Synthesis paper [submitted]

Direct communications in oral presentations:

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Hosted field excursions:

"Clonal differences in susceptibility to the dieback of Fraxinus excelsior in southern Sweden; controlled inoculations of susceptible and resistant genotypes; future plans for collection and propagation of a more resistant ash population in Sweden". EU Cost Action FRAXBACK. Malmö, Sweden. September 5, 2013. 100+ participants

Masters Thesis Project:

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Lövträdtidningen. Svenska Lövträdföreningens tidskrift. Nr. 13, Maj 2014. p. 11-13.

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Stenlid, J. "Handla närproducerade växter!". Intervju av Ulla Ahlgren för artikel i Miljötrender, sid. 30-31, augusti, 2014.

Stenlid, J. "Skogens skadegörare kostar miljarder". Intervju för artikel i Forskarbladet, 8 juni, 2014.

Bilaga 1.

ASKSKOTTSJUKA

Alternative skötsel och säkrande av resistent odlingsmaterial

Rapport sammanställd av Michelle Cleary, Sydsvensk skogsforskning. Michelle.Cleary@slu.se och Jan Stenlid, Skoglig mykologi och växtpatologi, Jan.Stenlid@slu.se

Summary:

A new invasive fungal disease is killing common ash (*Fraxinus excelsior*) in Sweden. The disease, commonly known as ash dieback, was first observed in the mid 1990´s in Poland and is now found in more than 25 countries in Europe. The disease spread very rapidly in Sweden; in 2001 it was known only in a few places, and within a few years its occurrence was reported on trees of all age classes throughout the whole natural range of ash. The causal agent has been identified as a new fungal pathogen *Hymenoscyphus fraxineus* which has originated from Eastern Asia. Symptoms include leaf death, shoot dieback and bark canker formation, ultimately leading to tree death. The situation in Sweden is critical since large populations of ash are rapidly disappearing from our forests. Intensive research has shown important aspects of the infection biology and resistance conditions in the host which now need to be converted into practice to ensure that ash has a future in Swedish forests, cities and landscapes. In this project we investigated various management techniques aimed at limiting disease spread and genetic control of the disease. The project has been instrumental in developing a more resistant ash population for future planting in Sweden.

Pruning to reduce dieback damage on ash

Silvicultural control of ash dieback is primarily aimed at minimizing disease occurrence and/or limiting the spread of *H. fraxineus.* One possible way of achieving this is by frequent pruning of infected branches. In Sweden, pollarding (=hamling) has been used extensively to manage ash trees in the traditional rural landscape for foddering of livestock. Though pollarding is a less common practice these days, it still exists and many pollarded trees can be found in wooded forests, wooded meadows, open meadows, small wooded ‘islands’ in fields, and along roads (Höök 1998). Pruning of branches has proved to be an effective technique to reduce the incidence of cankers caused by other fungal diseases (e.g. white pine blister rust). Since the disease is not systemic but rather is the sum of several parallel infections, it is reasonable that regular maintenance by pruning may mitigate disease levels by removing infected branches before *H. fraxineus* becomes established in the main stem.

A deviation in the initial project plan was caused by new awareness of some focussed research studies on ash dieback and pollarding in Sweden (Bengtsson et al. 2012, 2013; Bengtsson 2014). To avoid any overlap in our research efforts, we decided to focus our study on examining the internal spread rate of infections on ash shoots. The main premise behind pruning in this case is that spread of *H. fraxineus* can be limited and tree mortality avoided if infected branches in the crown are cut before the fungus reaches the main stem. However, inadequate information exists on the extent of fungal necrosis in stems ahead of the actual visible necrosis in the bark. This information is critical to be able to provide management guidelines for effective pruning to achieve better disease control.

We initiated a study whereby 76 *Fraxinus excelsior* trees were selected along an alley in Alnarp, in southern Sweden. All trees were infected with *H. fraxineus*, but to varying degrees and most lesions were confined to outer branches (i.e. no visible stem infections). The crowns of all trees were assessed and assigned a disease severity score based on the extent of visual dieback according Kirisits and Freinschlag (2012). Up to 38 trees were selected for pruning and 38 trees

(every second tree in the alley) were left as control. The lower branches of each tree (2-3 branches per tree) were pruned at a height of approximately 2 m. Bark lesions on all cut branches were mapped and the lesion lengths measured (Figure 1). For all lesions, tissue samples, including bark and wood, were collected from the margin of visible necrotic tissue on the outer bark, and distally at 5 cm increments (up to 30 cm where possible) in order to determine the extent of necrosis. In the lab, DNA was extracted from all samples and the presence of *H. fraxineus* detected via PCR using specific ITS primers (Johansson et al. 2010).

Figure 1. Pruning of ash trees in Alnarp alley; field sampling procedures, and collection of tissue samples showing necrosis for testing the presence of the pathogen.

We examined a total of 60 pruned branches from 38 trees, and examined and mapped 138 lesions. A total 841 tissue samples were collected on branches up to 30 cm distal to visible bark cankers to analyze for presence of *H. fraxineus*. 126 out of 138 (91.3%) necrotic lesions in the bark were confirmed to be infected by *H. fraxineus*. At the margin of the lesions (0 cm) the number of positive samples was largest and represented more than 90% (n=126) of all lesions examined. At 5 cm from the margin of lesions, *H. fraxineus* was confirmed in more than 80% of the samples (n=111). The pathogen could still be detected at 20 and 25 cm distal to the margin of lesions, though the frequency at which this occurred was much less and represented only 8.5 % (n=10) and 4 % (n=4) of the lesions investigated, respectively. *H. fraxineus* was not detected from any lesions collected at 30 cm distal to the margin of lesions.

From this work we can recommend that pruning of at least 30 cm distal from a bark lesion is needed in order to ensure that the branch would then be free of the disease. Though pruning may be an effective way to reduce infections to the main stem by removing the pathogen, this practice is only feasible if conducted early enough, i.e. when the infections are confined to the outer branches and have not yet reached the main stem, to prevent eventual tree death via girdling. Incomplete removal of diseased branched can otherwise negate the effect of this treatment. Pruning activities must however be thoroughly conducted in order to be efficient and for trees to remain vital. While pruning is labour intensive and therefore expensive, it is not feasible to do in forest stands, and should be considered only for amenity trees in urban landscapes or those with high genetic value.

Specifically with respect to pollarded ash trees in Sweden, restoration of old pollards should generally be avoided. Since the disease has been present in Sweden now for more than a decade, it is likely that on old pollard trees the fungus has already progressed to the main stem and any new shoots sprouting from the trunk will subsequently wilt and die as a result of the fungus spreading from the trunk to the shoot. However, on trees where the fungus has not progressed to the main stem, infected branches with lesions (cankers) can be pruned. As before, this may be

feasible only for highly valued trees of genetic importance or in urban environments. Careful maintenance of pruning (pollarding) on those trees can promote better tree health. The interval of pollarding should be no longer than 3-5 years, since on young shoots the disease can be rapid (as also indicated in a recent report by Bengtsson et al. 2012), and the fungus is capable of spreading to the main stem within just a few years.

Genetic resistance and initiation of tree improvement program for ash

Genetic resistance is an important tool for disease management. Evidence of natural resistance, albeit at low-levels, has been observed in heavily damaged ash stands whereby some trees appear quite healthy, displaying very few bark cankers and dieback symptoms. In Denmark, McKinney et al. (2011) showed that susceptibility was under strong genetic control and that the genotypic variation was substantial. In southern Sweden, surveys of 106 plus-tree ash clones in two seed orchards indicated strong genotypic variation in their level of susceptibility to *H. fraxineus* (Stener 2007). Though no individual appears to be fully resistant, some individuals show dramatically lower susceptibility to the disease and continue to express this resistance after at least six years of heavy infection pressure (Stener 2013). This genetic variation in the ash population appears to be substantial enough to hope for considerable gain through selection and long-term breeding. However this requires large-scale collection of seed or scion material for further propagation and testing.

We initiated a field trial to test the inheritable resistance of seedlings originating from open-pollinated seeds from 13 clones of varying susceptibility to *H. fraxineus*; source material from Trolleholm seed orchard where the relative susceptibility to *H. fraxineus* has been previously characterized (Stener 2013). The test material includes nine clones which were more notably tolerant under field conditions and four with high susceptibility to the fungus – to be were used as a comparable control. The progeny trial is located at the Landscape laboratory at SLU Alnarp and was established during spring 2014. The trial has required continued maintenance of machine and manual weeding to ensure good establishment. Periodic assessments and inventories were conducted during the growth seasons in 2014, 2015, and is now continuing during 2016 under a new Masters thesis project (main supervisor M. Cleary).

To date, cumulative mortality ranges between 0-29% (Figure 2). Progenies from three of the ash clones (no. 114, 118, and 121) showed no mortality after two growing seasons. Similarly, the level of damage on these three clones was substantially less compared to others (Figure 3). Continued monitoring of this trial and other test populations will be important in order to make complementary selections of material to support the development of a more resistant ash population in Sweden.

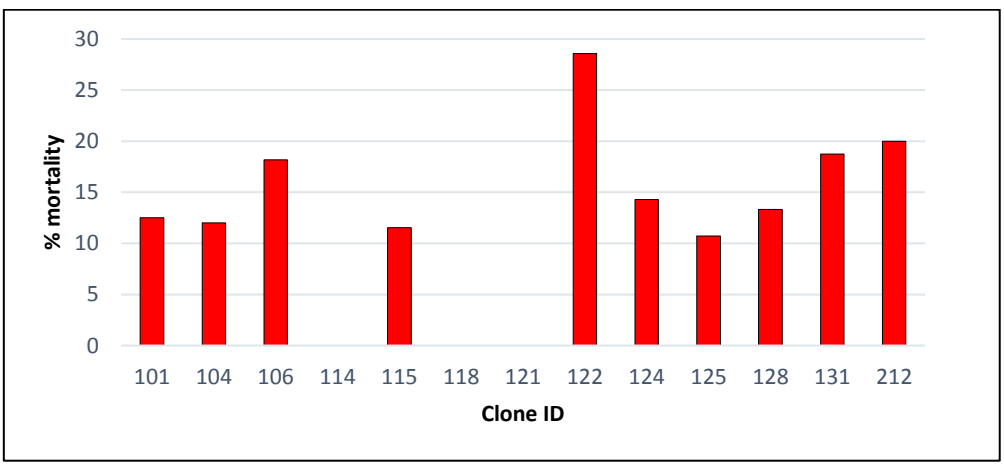

Figure 2. Cumulative mortality of progenies from 13 ash clones after two years in the Alnarp trial.

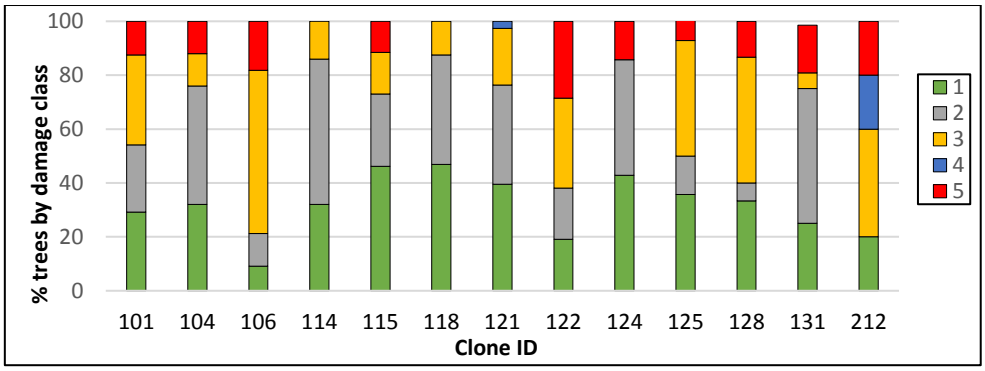

Figure 3. Damages on progenies from 13 ash clones two years after initial establishment in the Alnarp trial. Damage class ratings defined as **1,** symptomless; **2,** minor symptoms on shoots or leaves, AND/OR a single necrotic lesion on the shoot; **3,** moderately damage; necrotic leader shoot AND 2 or 3 necrotic lateral shoots; resprouting from below the damaged shoots; **4,** severely damaged; top shoot AND half of the main stem AND/OR most shoots are necrotic; trees not resprouting from below damaged shoots or resprouting from the root collar; and **5,** dead. Damage class ratings were adopted from Pluria et al. (2014).

A substantial portion of the larger project focussed on the identification and collection of resistant genotypes from wild populations for the purpose of propagation and testing. During the project we engaged stakeholders (e.g. foresters, private land owners) and the general public to identify and report on the location of seemingly resistant ash in diseased stands. Following a press-release by Skogstyrelsen on ash dieback (http://www.skogsstyrelsen.se/Myndigheten/Press-och-information/Pressmeddelanden/Ny-kunskap-kan-radda-asken---Sveriges-kungstrad/) which discussed the intent of the collaborative work between SLU and Skogforsk to select vital ash trees, a large number of articles were written in several newspapers throughout Sweden as well as forestry and nature magazines (e.g. Land, Natur and Trädgård). In addition, a number of articles were posted to websites including Skogstyrelsen, Länstyrelsen, Skogforsk, Jordbruksverket, SLU, Växtforum, and ATL Lantbrukets affärstidning, encouraging participants to help "Save the Ash". The result of this public awareness campaign prompted broad responses from the public reporting the location of resistant trees. We acquired through our stakeholder collaborators, locations of ash stands and areas where ash is recorded as key habitat and worthy of protection, which was used as our initial basis for the inventories in 2014 and 2015. Extensive work was conducted with reconnaissance surveys in more than 100 sites across 12 counties in southern Sweden. At each location, trees were assessed and prioritization was assigned for scion collection. As a result of these efforts, selection of more than 500 vital ashes (i.e. trees without extensive crown dieback symptoms) was performed in stands seriously damaged by *H. fraxineus*, across the entire natural distribution range of ash in Sweden (see example Figure 4). All data is being managed within a GIS database (Figure 5). During two winter seasons contractors revisited sites and made collections of scion material from a sub-selection of trees, which were grafted to rootstock in the spring 2015 and 2016 and maintained at SLU and Skogforsk facilities.

Figure 4. Vital, disease-tolerant trees (shown with arrows) marked for selection. On left, near Trosa; on right near Alnarp.

Figure 5. Grafting of scions to root stock at Skogforsk (on left). Maintenance of grafted plants during one season in controlled climate chamber at SLU Alnarp prior to establishment in field trial.

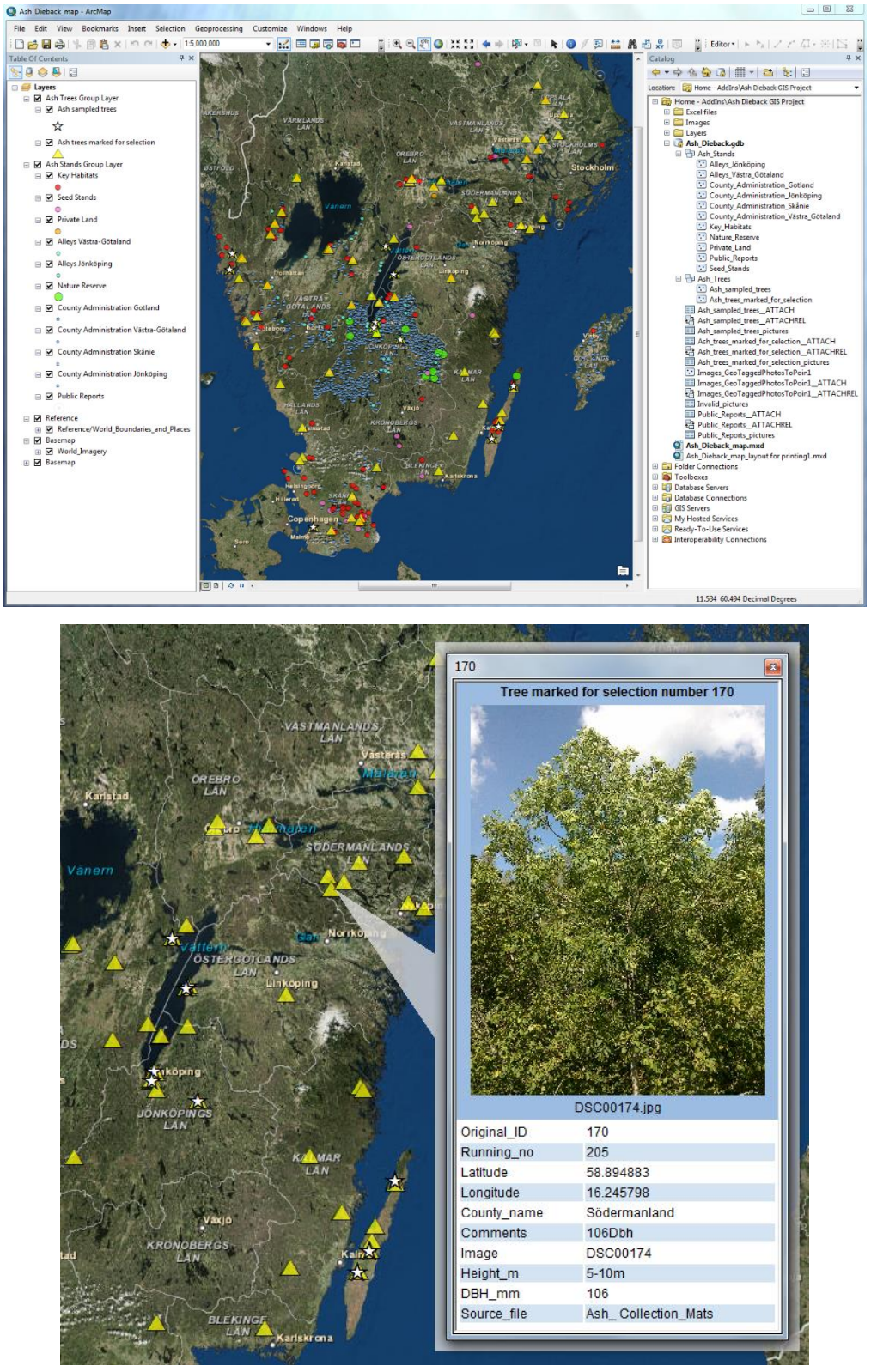

Figure 6. The Arc GIS inventory database of resistant ash showing locations of surveyed sites (top) and individual trees marked for selection, and associated tree attributes.

The work we have completed under this project has now enabled specific ash populations with high resistance to *H. fraxineus* to be established, which can help form the foundation of a future breeding programme for large-scale reintroduction of ash trees into forests, cities and landscapes. Resistance testing of ash genotypes are now being conducted under a separate project funded to Michelle Cleary and Lars-Göran Stener on improving the regeneration material of ash in Sweden with emphasis on tolerance to the ash dieback disease and establishing clonal archives for gene

conservation. The new test populations were established in a long-term field trial at Snogeholm (southern Sweden) during the spring 2016.

Key deliverables under this project:

Manuscripts:

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