"*Förbättrat föryngringsmaterial för ask (selektion och test av resistens mot askskottsjuka)*" with project number PA1237.

RISE FROM THE ASHES – WORKING TOWARDS A RESISTANT POPULATION OF EUROPEAN ASH FOR SWEDEN.

Ash dieback, caused by the alien invasive fungus *Hymenoscyphus fraxineus*, is currently threatening the survival of common ash (*Fraxinus exclesior*) throughout most of Europe. Starting in the early 1990s, large-scale decline of common ash was reported from Poland and Lithuania (Juodvalkis and Vasiliauskas 2002; Przybył 2002). In Sweden, the disease was first noticed in 2001 but by 2005 it was widespread throughout the natural distribution range in the country. Though the exact origin of *H. fraxineus* is still unknown, evidence suggests the same fungus occurs in Japan, China, Korea, and Far East Russia (Zhao et al. 2013; Zheng and Zhuang 2014; Han et al. 2014; Cleary et al. 2016), and that most likely it was inadvertently introduced into the Baltic countries in the late-1980s on nursery stock of Asian *Fraxinus* species (Woodward and Boa 2013).

As with most introduced pests and pathogens where the host plant lacks a history of co-evolution, the damage is devastating. The situation is now critical in Sweden and elsewhere across Europe as large populations of ash are disappearing from our forests and the urban landscape (Figure 1). In 2010, ash was put on the Swedish Red-List but by 2015, its status worsened to be 'critically endangered' considered to be at risk of extinction in the wild. This is concerning not only for the loss of this important noble broadleaved tree species, but also for the biodiversity of species dependent on ash that are indirectly affected by the disease. In Sweden, it has been estimated that there are 483 species dependent on ash, 123 of which are threatened (Hultberg et al. 2020). Several of these species are already red-listed and a large proportion are either obligately- or highly associated to ash which means further loss of ash will, if it hasn't already, instigate an extinction cascade.

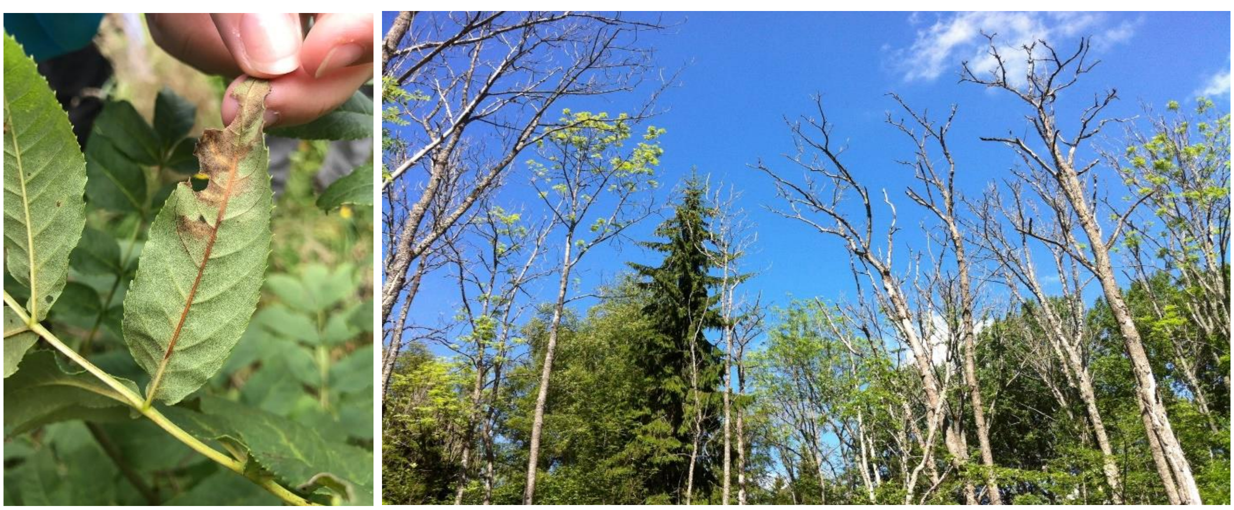

Figure 1. On right, the fungus *Hymenoscyphus fraxineus* infects the leaves and then woody shoots of trees annually. On left, a stand of European ash at a nature reserve in Säffle in 2014. Trees have either died or are moribund with severe dieback of the crown. Photo credit: Michelle Cleary

Currently, there are no effective means to control the spread of ash dieback and the majority of trees in all age classes appear to be affected. The most promising outlook to ensure that ash has a future in forests, cities and landscapes is to find resistant individuals and cultivate more resistant ash genotypes for planting. Some evidence of natural resistance has been observed even in heavily damaged ash stands whereby some trees appear quite healthy, displaying very few bark cankers and dieback symptoms. In Denmark, susceptibility was found to be under strong genetic control and genotypic variation was substantial in two seed orchards (McKinney et al. 2011). In a progeny trial in Lithuania, ten Lithuanian and 14 other European populations of ash showed high heritability in tolerance to *H. fraxineus* infection (Pliura et al. 2011). Here in Sweden, surveys of 106 plus-tree ash clones in 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 several years of heavy infection pressure (Stener 2013) (Figure 2). This genetic variation in the ash population demonstrated by these studies appears to be substantial enough to suggest considerable gain through selection and long-term breeding.

Figure 2. Susceptible and tolerant European ash genotypes at a seed orchard trial located in Snogeholm. Photo credit: Michelle Cleary

Since 2013, concerted efforts have been made through the project "Save the Ash" (Rädda Asken) to develop a resistant population of ash in Sweden. Initial work between 2013-2015 generated a large inventory of more than 500 putatively resistant genotypes (showing high levels of natural resistance to the pathogen) across the whole range of the distribution area in Sweden (Figure 3).

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Figure 3. Selection of putatively resistant ash from key habitat (nykelbiotop) areas and landscapes heavily affected by ash dieback disease in the southern half of Sweden. Selection of 'resistant' trees is based on trees that characteristically show very little evidence of crown or shoot dieback, minimal stem and/or branch necrosis, and minimal evidence of adventitious shooting (which is a typical stress response to the disease), such that 80% or more of the trees' crown is intact compared to other heavily diseased neighbouring trees.

During 2016-2017, two field trials were established at Snogeholm using propagated selections of select genotypes from the wild to screen for, and characterize, the resistance. In those trials, 130 genotypes were selected from our resistant inventory database, were propagated and planted. Funding from Partnerskap Alnarp allowed the continuance of this work. In 2018, a third field trial was established at Snogeholm with an additional 42 clones to screen for resistance against the ash dieback pathogen *H. fraxineus* (Figure 4).

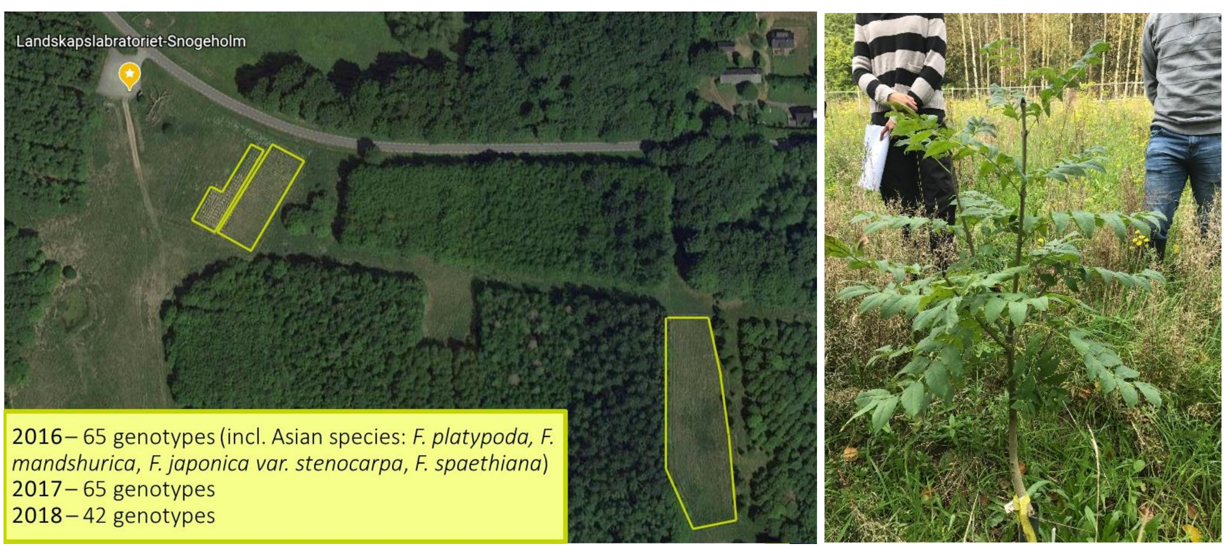

Figure 4. Ash screening trials located at the Landscape laboratory in Snogeholm (right) and a tolerant genotype of European ash (right).

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Annually, health class rating and growth traits are assessed on all trees in all trials. Early results show that a small proportion of tested clones are showing good tolerance (in green) even after several years of heavy infection pressure (Figure 5). With expanded surveys in recent years, we have increased our inventory database to have now more than 900 resistant trees selected from 290 locations.

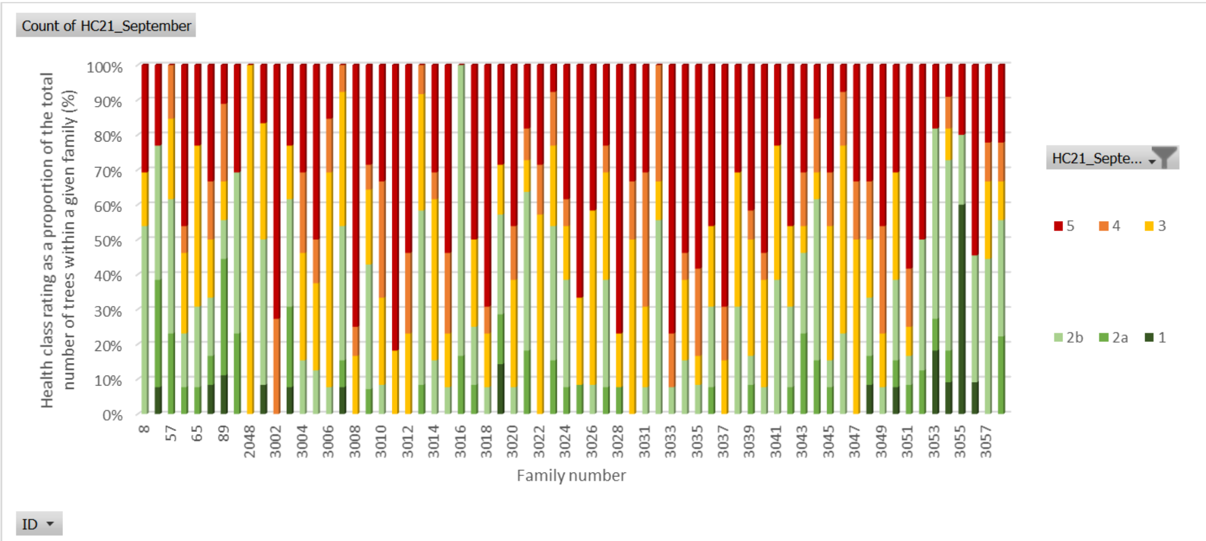

Figure 5. Health class (disease severity) rating of clones in the 2016 trial, where 1 is healthy, 2a and 2b are considered tolerant (less than 1/3 of the crown affected), 3 and 4 are more severely diseased and 5 is dead.

Assessments were also made to a progeny trial established in 2014 in the landscape laboratory at Alnarp. The trial is set up as a randomized block design with a total of ten plots (blocks), 30 plants per plot for a total of 300 seedlings and at least one meter spacing between trees. Seedlings originate from open-pollinated families from 13 mother clones of varying susceptibility to *H. fraxineus,* according to earlier evaluations from Stener (2007), and thereafter assessed to determine disease severity and growth performance. In general, tolerant families show higher survival and lower mean health class rating compared to susceptible families; but a lot of variation was evident among and within families. Nevertheless, early indications show at least a few genotypes with relatively high disease tolerance (low disease severity), especially families 14, 18 and 21 (Figure 6). Tolerant families tend to have higher diameter growth and are growing taller than susceptible families. Each growing season, trees are infected by the fungus, and after the fourth growing season, we see a strong effect of the disease with reduced tree growth in susceptible families.

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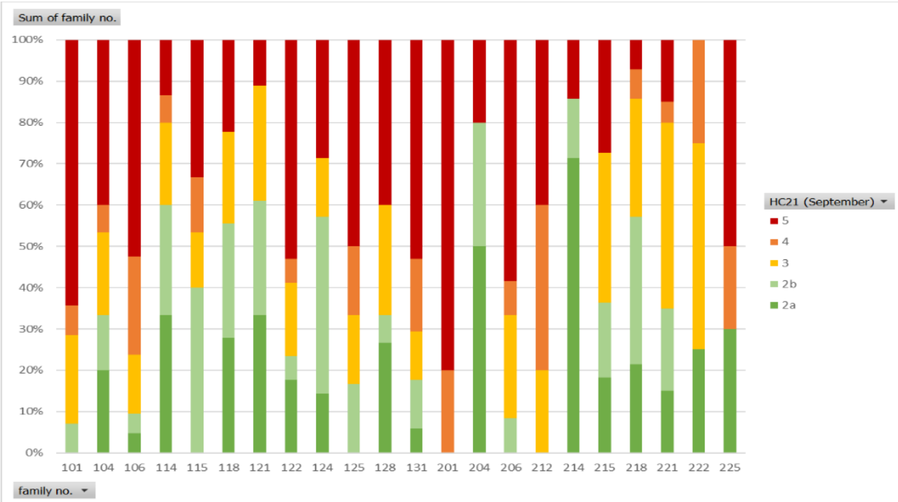

Figure 4. Proportion of half-sib families rated according to health class (disease severity) where 1 is healthy, 2a and 2b are less than 1/3 of the crown damaged, 3 and 4 are increasing damage (more than 2/3, and 5 is dead.

Tolerant trees have better stem forms than susceptible trees. Over the years the proportion of trees with multiple stems increases mainly as a result of annual infections killing and, essentially knocking down, the leading shoots. The inherent response of the tree to the killing of shoots is sprouting from the base or epicormics shoot regeneration at the junction of the main stem. Health class rating (disease severity) influences the trees' stem form: trees belonging to a good health category tend to show better stem form with a higher proportion of trees having single stems as opposed to multiple stems sprouting from the base.

So far, the field experiments that have been established are a critical first step to enable targeted genotypes to be selected for further commercial propagation, breeding and possible future establishment of new seed orchards. In order to support the development of a more resistant ash population for planting in Swedish forests, cities and landscapes, we need continued support for an operational tree improvement program at Skogfork to ensure a future for this important noble broadleaved tree species. For more information, visit our website "Save the Ash" www.raddaasken.nu.

References

Cleary M, Nguyen D, Marčiulynienė D, Berlin A, Vasaitis R, Stenlid J. 2015. Friend or foe? Biological and ecological traits of the European ash dieback pathogen *Hymenoscyphus fraxineus* in its native environment. Scientific Reports 6, 21895; doi:10-1038/srep21895 (2016)

Han, J-G., Shrestha, B., Hosoya, T., Lee, K-H., Sung, G-H., & Shin, H-D. 2014. First report of the ash dieback pathogen *Hymensocyphus fraxineus* in Korea. *Mycobiol.* **42**:391-396

Hultberg, T., Sandström, J., Felton, A., Öhman, K., Rönnberg, J., Witzell, J., & Cleary, M. (2020). Ash dieback risks an extinction cascade. Biological Conservation, 244. https://doi.org/10.1016/j.biocon.2020.108516

"*Förbättrat föryngringsmaterial för ask (selektion och test av resistens mot askskottsjuka)*" with project number PA1237.

Juodvalkis A., Vasiliauskas A., The extent and possible causes of dieback of ash stands in Lithuania. LŽŪU Mokslo Darbai, Biomedicinos Mokslai. **56**:17-22 (2013). (In Lithuanian with English summary.)

McKinney, L.V., Nielsen, L.R., Hansen, J.K., Kjær, E.D. Presence of natural genetic resistance in *Fraxinus excelsior* (Oleaceae) to *Chalara fraxinea* (Ascomycota): an emerging infectious disease. Heredity, **106**: 788–797 (2011)

Pliura A, Lygis V, Suchockas Y, Bartevicius E. Performance of twenty four European *Fraxinus excelsior* populations in three Lithuanian progeny trials with a special emphasis on resistance to *Chalara fraxinea.* Balt For. **17**:17–33. (2011)

Przybył K., Fungi associated with necrotic apical parts of *Fraxinus excelsior* shoots. For. Pathol, 2002. **32**:387-394

Stener L-G., Studie av klonskillnader i känslighet för askskottsjuka. *In:* Arbetsrapport från Skogforsk nr 648, 2007.14 pp (2007)

Stener L-G., 2013. Study of clonal differences in susceptibility to the dieback of *Fraxinus excelsior* L. in southern Sweden. Scandinavian Journal of Forest Research. Scand J For Res. **28:** p. 205-216

Woodward S, Boa E. 2013. Ash dieback in the UK: a wake-up call. Mol Plant Pathol **14**:856-60

Zhao, Y–J., Hosoya, T., & Baral, H-O., Hosaka, K., & Kakishima, M. *Hymenoscyphus pseudoalbidus*, the correct name for *Lambertella albida* reported from Japan. *MycoTaxon* **122**:25-41 (2013)

Zheng, H-D., Zhuang, W-Y. *Hymenoscyphus albidoides* sp. nov. and *H. pseudoalbidus* from China. *Mycol. Progress*. doi, 10.1007/s11557-013-0945-z (2014)