Final Report
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Improvement of forest reproductive material for ash: characterizing the resistance against ash dieback (Askskottsjuka)

Project: 2016-015

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Popular Scientific Summary:

Over the last two decades common ash (*Fraxinus excelsior*) has been threatened by an alien invasive fungal pathogen (*Hymenoscyphus fraxineus*) introduced from Asia. The rapid spread and intensification of the disease has resulted in a serious population decline; since 2010 ash is a Red-listed species in Sweden and since 2015 its status has worsened to become 'critically endangered'. 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. Genetic resistance is an important tool for disease management to conserve the species from further population decline. Large variation in susceptibility to the pathogen has been observed within natural populations; with less than 5% of trees showing disease resistance. Studies have shown that suceptibility to damage is a heritable trait that is genetically controlled, and that considerable gain can be achieved through selection and breeding.

The aim of the work is to support the development of a more resistant ash population for planting in Sweden. To achieve this, we have initiated two projects that use both traditional selection and modern phenotyping techniques to characterize the resistance in ash.

Previously, during 2013-2015 we generated a large inventory of putatively resistant genotypes (showing high levels of natural resistance to the pathogen) across the whole range in Sweden, and propagated a first test population to screen for, and characterize, the resistance. The funded work consisted of two sub-projects: one aimed at classical selection and propagation for resistance breeding, and the other to test modern phenotyping technologies that can potentially identify resistant ash genotypes and thereby expedite the process of tree selection in practice.

In the first project, we established two addtional field trials at Snogeholm using propagated selections of wild genotypes. In the second project, we used a state-of-the-art chemical fingerprinting technique known as Fourier-transform infrared (FT-IR) spectroscopy and chemometric modelling on a unique collection of material acquired from genetic field experiments across Europe, to distinguish between resistant and susceptible ash genotypes. Using a soft independent modeling of class analogy model built with infrared spectra of phloem phenolic extracts, we discovered that spectra from the mid-infrared of phloem extracts correctly predicted the tree phenotype. This model was validated across large populations of ash in Europe.

These work in the first project was a criticial first step to enable targeted genotypes to be selected for further commercial propagation, breeding and possible future establishment of new seed orchards. The results from the second project suggest that modern phenotyping technologies can provide a promising approach for identifying disease resistance and can drastically advance the efficiency and timing of selecting genetically resistant ash trees, thus potentially, expediting our current selection and screening protocols for resistance breeding.

Collectively, the results of both projects will be extremely important for the restoration and sustainable management of this important noble broadleaved tree species in Swedish forests, cities and other urban and natural landscapes.

PROJECT 1:

During 2016 and 2018, three field trials were established at Snogeholm with 2-year-old ash tree clones (grafted plants propagated with scions) of healthy, vital, mature *F. excelsior* trees. The trees were previously selected based on extensive surveys conducted between the years 2013 and 2015 in forests and the natural landscape including key habitat areas for ash (nyckelbiotop) and known seed stands throughout the natural distribution range of ash in the southern half of Sweden. In those surveys, more than 500 vital ash trees were identified and marked for selection and further monitoring. The estimation of tree vitality was done based on the percentage of crown damage in relation to other damaged trees in heavily diseased areas. For a tree to be considered vital and marked for selection, at least 80 percent of the crown needed to be intact. Stem quality and growth characteristics were secondary to tree vitality (Fig. 1).

Figure 1. (LEFT) Example of a resistant ash tree showing high vitality with little to no dieback in the crown (red arrow) situated alongside severely diseased trees showing extensive dieback. (RIGHT) Scions collected from those resistant genotypes from the wild population grafted to root stock and grown in a controlled climate chamber for 1 year prior to establishment in the field trial.

Preparatory work for trial establishment included a deep plowing and harrowing of the soil, and herbicide treatment to help control competing vegetation. The trial perimeters were marked by a fence to prevent browsing of seedlings by rabbits and/or deer (Fig 2.). For this first test population, we selected 56 of those genotypes and up to 13 replicates were propagated for each selected genotype by grafting onto *F. excelsior* rootstock originating from a known resistant genotype from Denmark. We also included in the trial design four known susceptible mature *F. excelsior* tree clones and five Asian *Fraxinus* tree clones, to serve for comparison of genotype performance. The Asian *Fraxinus* selections belonging to the species *mandshurica*, *japonica* var. *stenocarpa*, *platypoda*, and *spaethiana*, were selected from known accessions at arboreta and botanical gardens and included to give a unique comparison of what is presumably a known host to *H. fraxineus* in its native origin of Asia. The trials at Snogeholm was established using a randomized block design. A total of 65 clones were planted in May 2016 at 1.5 m spacing divided among 12 plots where at least one graft per clone was randomly established in each plot. In May 2017 an additional 65 clones were planted at another location 300 m from the 2016 trial. Trees were planted at similar spacing and divided among 12 plots. In 2018, an additional 42 clones were planted and divided among 7 plots. Periodic health assessments were conducted on all trials.

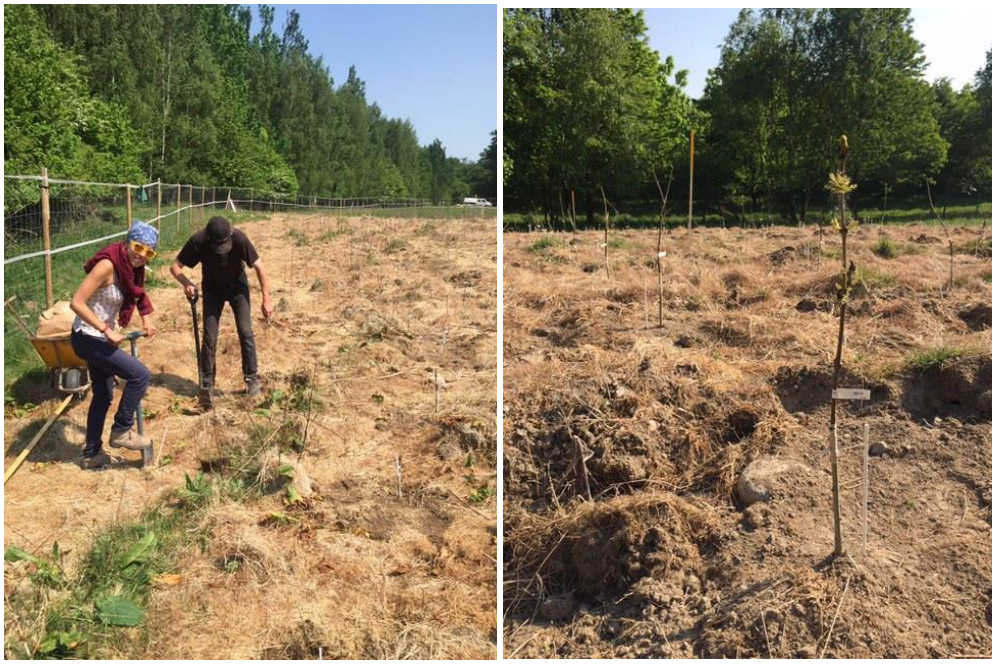

Figure 2. Site preparation and planting of the Snogeholm trial with selected genotypes from wild populations in spring 2016.

Funding support has permitted this important work to be started which will serve as the basis for developing a more resistant population of ash for planting in Sweden. Additional genotype assessments will occur during subsequent years (with support from Skogsforsk) and a final ranking of clone performance (health) will be made for further selection. A large number of deliverables related communications with stakeholders, the public and the scientific community have already been completed. Further information dissemination and extension activities centred around these established trials are planned in the coming years.

PROJECT 2:

Vibrational infrared (IR) spectroscopy is a highly sensitive, rapid and high-throughput chemical fingerprinting technique which can separate biological samples into functional groups on the basis of how samples absorb infrared radiation. Fourier Transform (FT-) IR spectroscopy shows great potential for non-invasive measurement of quality parameters that are important in disease resistance (Martin et al. 2005; Conrad et al. 2014; Conrad and Bonello 2015). In order to evaluate the usefulness of this technique for future use in practice, we conducted large scale testing on populations of European ash where the genetic, inheritable resistance of individuals against damage by *Hymenoscyphus fraxineus*, is known based on field evaluations conducted over several years. Consequently, our goal was to determine the feasibility and efficacy of FT-IR to phenotype European ash for resistance to ash dieback.

We collected phloem and leaf samples from *F. excelsior* trees with known susceptibility to *H. fraxineus* in six European countries: Austria, Denmark France, Germany, Lithuania, and Sweden, in collaboration with several colleagues (tree geneticists) in each of those countries. Source material originated from genetic trials established as either clonal seed orchards (Kirisits and Freinschlag 2012; Stener 2013; Enderle et al. 2015) or for testing ash provenance (McKinney et al. 2011) or progeny (Pliūra and Baliuckas 2007, Pliūra et al. 2011, 2014; Muñoz et al. 2016) (See Table 1).

Table 1. Source material of resistant and susceptible genotypes used in the FT-IR study.

			Trial details							No. of samples collected per susceptibility class			
Country	**Location / Trial name**	**Type of genetic trial**	**Coordinates**	**Elevation (m asl.)**	**Est. year**	**Size (ha)**	**No. of clones**	**No. of ramets per clone**	**Spacing of trees (m)**	**suceptible**	**Inter-mediate**	**resistant**	**No. ramets per clone**
Austria	Feldkirchen an der Donau, Upper Austria	Seed orchard	48°19'12.5" N, 14°04'15.9" E	264	1993	1.36	51	2 to 4	7.5 x 8.6	7		7	2
Denmark	Tuse næs, Northern Sealand	Seed orchard	55° 45' 57.99" N, 11° 42' 47.48" E	22	1998	2	39	25	3 x 6	3	2	3	3
France	Devecey, East	Provenance + family comparison trial	47°19'31.5" N, 06°01'54.1" E	250	1995	1.36	788	1	4 x 4	7	7	7	1
Germany	Weisweil, Baden-Württemberg	Provenance trial	48°11'29.7" N, 7°42'02.5" E	173	2005	0.22	577	1	2.0 x 2.0	5	0	5	1
Lithuania	Sasnava, Marijampolė	Clonal archive	54°37'32.1" N, 23°33'55.5" E	100	2012	2.5	228	3 to 7	6.0 x 5.4	4	3	5	2-3
Sweden	Snogeholm	Seed orchard	55°32'33.8" N, 13°42'22.7" E	50	1992	4.4	100	40-60	3.5 x 3.5	4	8	7	1-3

Sample collection was performed between May and June. The timing for sample collection was critical to ensure that samples were collected prior to the normal sporulation period for *H. fraxineus* which typically occurs between June and September, with peak sporulation between mid-July and mid-August.

At each site, a minimum of three and up to eight genotypes, were selected per susceptibility class (low, intermediate and high susceptibility) based on a relative measurement of dieback intensity as determined in previous assessments. In the case of clonal trials, between two and three ramets per clone were sampled. From each individual, the current year's shoots were harvested. Some shoots that were not reachable by ground were taken at a higher height (up to 5 m) with a pole scissor. On susceptible genotypes, most if not all sampled shoots were from epicormic shoots. Leaves collected in the field were labelled according to country, trial, family, ramet and susceptibility status, placed in a plastic bag and immediately stored on dry ice. At the base of each leaf, sections of phloem consisting of outer bark, cortex and some cambium were dissected from the stem with a sterile razor blade, and similarly labelled and stored on dry ice in the field. All samples were then transported cold to SLU laboratory at Alnarp for further processing.

In the lab, phloem and leaf tissues were finely ground in liquid nitrogen and stored at -80˚C. 200 ±1 mg aliquots of either tissue type were placed in individual 2 mL microcentrifuge tubes. Samples were kept cold during this process with liquid nitrogen so as to avoid any warming and oxidation of tissue, and then stored at -20˚C until chemical extraction. Chemical extracts of samples were obtained by adding 700 µl of 70% acetone, 30% water to each tube. Samples were subjected to sonication for 30 min under room temperature, followed by centrifuging at 1600 rcf for 8 min. The supernatant was transferred to a new 2 mL tube and twice the volume of chloroform added. Samples were then centrifuged at 10000 rcf for 2 minutes at 10˚C and the supernatant collected and transferred to a new 2 mL screw-cap tube with O-ring seal. Samples were then lyophilized and stored at room temperature. Crude sample extracts purified on a C18 column using HPLC-grade methanol and collected in new microcentrifuge tubes. Samples were stored in -20˚C until further analysis.

Samples representing the extreme susceptibility groupings of *F. excelsior* genotypes, resistant and susceptible, were analyzed on a Cary 630 FT-IR spectrometer. Spectra were collected over a range of 4000-7000 cm-1 at 4 cm-1 resolution and an interferogram of 64 scans was co-added for each sample. Spectral data were displayed in terms of absorbance and viewed using Win-IR Pro Software (Aglient Tehcnologics Inc. Santa Clara, CA, USA), and then analyzed using a multivariate classification software for the selective differentiation and identification of the target classes (susceptible vs resistant). Soft independent modeling of class analogy (SIMCA), a well-developed and accepted pattern recognition method in IR spectroscopic analysis (De Maesschalck et al. 1999) was be used to identify variables important to discriminate between susceptible or resistant individuals.

To be practically useful, chemotypes should be associated with constitutive composition and levels of specialized phytochemicals. Therefore, it was critical to ensure that *H. fraxineus* was not present in plant tissues. All samples from all locations were confirmed free of the pathogen via PCR. We processed FT-IR chemical fingerprints using a SIMCA chemometric approach to discriminate between resistant and susceptible trees. In order to resolve overlapping peaks, minimize background and improve the model predictions, spectral data were pre-processed via the standard normal variate function, and smoothed and transformed into their second derivative using a Savitzky-Golay polynomial filter. We initially analyzed both leaf and twig phloem tissues, for a total of 108 and 89 samples, respectively. Preliminary observations of the SIMCA 3D class projection plots showed that geographic location of the trees strongly affected the chemistry of

the leaves (Fig. 3). This difference may be attributable to a higher sensitivity of foliage to solar irradiation, temperature and nutrient or water availability associated with the different latitudes, geographic locations, and microclimates. However, this effect was not evident in twig phloem tissues (Fig. 3), therefore, we built the chemometric model using only the FT-IR spectra of the twig phloem samples. We do not exclude the possibility that resistance is expressed at the leaf level; rather, we suggest that environmental variation masked any possible chemical signature in the leaf tissues.

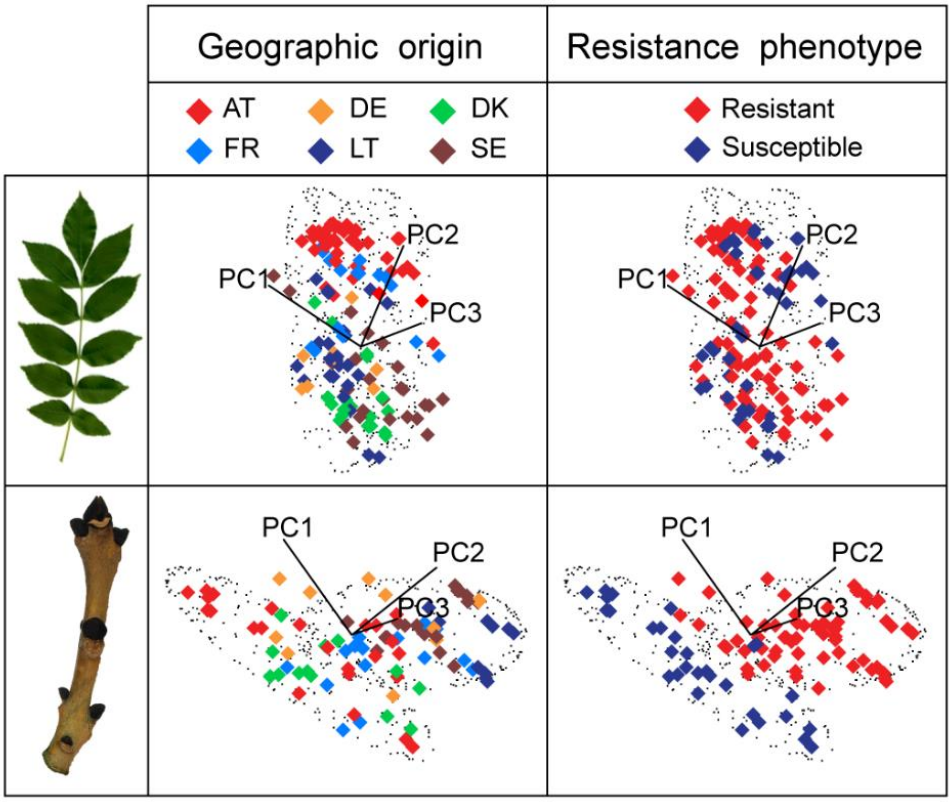

Figure. 3. SIMCA 3D class projection plots for spectral data of *Fraxinus excelsior* leaf and twig phloem tissue phenolic extracts visualized either as a function of the sample geographic location or its resistance phenotype. Spectral data were pre-processed using the standard normal variate function and then smoothed and transformed into their second derivative. Two technical replicates were analyzed separately. Clouds of black points indicate the 95% confidence interval for each group.

We used Coomans plots and 3D class projection plots also to identify outliers, which were removed from the model. After trimming the data in this way, the complete data set comprised a minimum of three and up to seven genotypes per susceptibility class per country, and up to two ramets per clone, for a total of 64 samples (42 resistant and 22 susceptible). Of these, 75% of samples within each geographic location were selected for the training data set to build our calibration model (Fig. 4a), while the remaining 25% were used as the testing data set for model validation (Fig. 4b).

The SIMCA calibration model that best discriminated between resistant and susceptible ash trees was a 3-factor model obtained by including spectral regions from ~ 748 to 798 cm^{-1} and from ~879 to 947 cm^{-1} wavenumber (Fig 4c), which primarily correspond to the C−H wagging of substituted benzenes of aromatic compounds. The highest discriminating power peak (discriminating power of 47.7) corresponded to ~ 895 cm^{-1} wavenumber, which may also correspond to the wagging of the hydrogen on the C-1 position of the cellulose glucose ring.

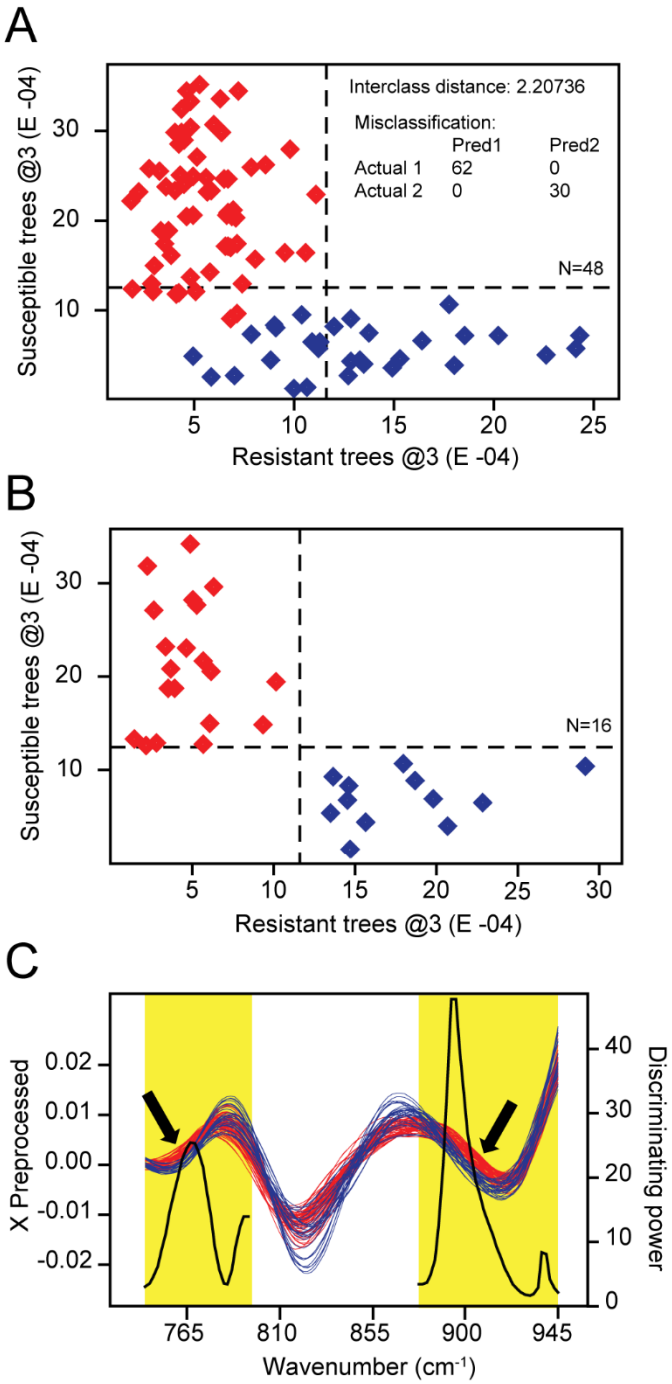

Figure 4. A, SIMCA Coomans plot showing the relative, dimension-free distance between the samples of the training data set used to build the 3-factor (@3) calibration model designed to discriminate between ash dieback resistant (red diamonds) and susceptible (blue diamonds) *Fraxinus excelsior* trees. X-axis represents the distance from the resistant class, while y-axis represents the distance from the susceptible class. Two technical replicates were analyzed separately, for a total of 92 spectra, corresponding to 48 biological replicates. Dashed lines indicate critical sample residual thresholds. B, SIMCA Coomans plot showing the relative, dimension-free distance between the samples of the testing data set used to validate the 3-factor (@3) model. Two technical replicates were analyzed separately, for a total of 32 spectral data, corresponding to 16 biological replicates randomly selected from each of the six European countries. Dashed lines indicate critical sample residual thresholds. C, SIMCA discriminating power plot of the 3-factor calibration model. The disseminating power (black lines) is overlaid on the second derivative, smoothed and standard normal variate transformed spectra. The SIMCA calibration model that best discriminated between resistant (red lines) and susceptible (blue lines) ash trees included spectral regions from ~ 748 to 798 cm^{-1} and from ~879 to 947 cm^{-1} wavenumber (highlighted in yellow). The black arrows point to regions of the spectra where the discrimination between the two resistance phenotypes is evident by visual inspection.

However, since the extraction protocol we adopted is highly specific for phenolic compounds, the presence of polysaccharides in the analyzed extracts is very unlikely, and the ~ 895 cm^{-1} wavenumber almost certainly corresponds to the C−H wagging of the aromatic hydrocarbon groups of phenols. The second highest discriminating power peak (discriminating power of 25.4) corresponded to ~ 770 cm^{-1} wavenumber. Interclass distance between the groups was 2.2074, indicating good separation between the phenotypes, and 100% of extracts from both resistant and susceptible ash trees were correctly classified, showing high specificity of the model. The low number of factors included in the model (i.e., three) argues against model overfitting.

Validation of the chemical marker-based statistical model is a crucial step to ensure accurate predictions and confirm the applicability of the model at a larger scale. We validated our SIMCA model with the testing data set, and 100% of the ten resistant and six susceptible ash ramets randomly selected from each of the six European countries were correctly identified as belonging solely to their phenotype group (Fig. 4b). Such accurate model performance is impressive given the vast heterogeneity of host genotype, developmental stage, and environmental conditions represented by the tested ash population.

Thus, these results show that European ash possesses readily exploitable levels of resistance that can be detected using FT-IR spectroscopy. This work represents a major advancement in the application of marker-assisted technology for tree breeding and offers a novel solution in the fight against ash dieback. Compared to labor-intensive and time-consuming traditional tree phenotyping techniques based on artificial inoculations or natural infection assays, or even nascent nucleic acid-based phenotyping, FT-IR spectroscopy can significantly accelerate the process of selecting resistant phenotypes and limit the need for growing out large segregating progenies as in conventional breeding programs. This technique can be applicable at a landscape level for screening large naïve populations for disease resistance, at dramatically reduced costs than traditional selection. These findings also opens the possibility for the development/refinement of advanced instrumentation for chemical metabolite profiling to be used in practice (e.g. a portable device that would allow for *in situ* analysis of specific plant traits in standing forest trees).

A manuscript for this project has been written and is now submitted and under review at the journal Nature: Scientific Reports. Several deliverables and communications have already been met during the project period (see below), however furher information dissemination and extension activities are planned also in near future including popular science articles after publication of our manuscript.

Relevant communications during the project period (2016-2018):

Manuscripts:

1. Villari, C., Dowkiw, A., Enderle, R., Ghasemkhani, M., Kirisits, T., Kjaer, E., Marčiulynienė, D., McKinney, L., Metzler, B., Rostgaard Nielsen, L., Pliūra, A., **Stener, L-G.**, Suchockas, V., Rodriguez-Saona, L., Bonello, P., and **Cleary, M.** 2017. Advanced spectroscopy-based phenotyping offers solutions to the ash dieback epidemic. Nature Scientific Reports [*submitted*]
2. **Stener, L-G.** 2018. Genetic evaluation of damage caused by ash dieback with emphasis on selection stability over time. Forest Ecology and Management. 409: 584-592
3. Skovsgaard, J-P., Wilhelm, G-J., Thomsen, I.M., Metzler, B., Kirisits, T., Havrdová, L., Enderle, R., Dobrowolska, D., **Cleary, M.**, Clark, J. 2017. Silvicultural strategies for *Fraxinus*

excelsior in response to dieback caused by *Hymenoscyphus fraxineus*. Forestry 2017; 90, 455–472, doi:10.1093/forestry/cpx012

4. **Cleary, M.**, Nguyen, D., **Stener, L-G.**, Stenlid, J., and Skovsgaard, J-P. 2017. 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: "Dieback of European Ash (Fraxinus spp.) - Consequences and Guidelines for Sustainable Management. *Eds*: Vasaitis R, & Enderle R pp. 195-208.
5. Marčiulynienė, D., Davydenko, K., Stenlid, J., and **Cleary, M.** 2017. Can pruning help maintain vitality of ash trees affected by ash dieback in urban landscapes? Urban Forestry and Urban Greening. 27: 69-75 https://doi.org/10.1016/j.ufug.2017.06.017
6. Marčiulynienė, D., Davydenko, K., Stenlid, J., Shabunin, D., and **Cleary, M.** 2017. *Fraxinus excelsior* seed is not a probable introduction pathway for *Hymenoscyphus fraxineus*. Forest Pathology. 2017;e12392. https://doi.org/10.1111/efp.12392
7. Stenlid, J., Elfstrand, M., **Cleary, M.**, Ihrmark, K., Karlsson, M., Davydenko, K., Brandström-Durling, M. 2017. Genomes of *Hymenoscyphus fraxineus* and *Hymenoscyphus albidus* encode surprisingly large cell wall degrading potential, balancing saprotrophic and necrotrophic signatures. Baltic Forestry. 23: 41-51 https://www.balticforestry.mi.lt/bf/PDF_Articles/2017-23%5B1%5D/Baltic%20Forestry%202017.1_041-051.pdf
8. **Cleary M.**, Nguyen D., Marčiulynienė D., Berlin A., Vasaitis R., Stenlid J. 2016. Friend or foe? Biological and ecological traits of the European ash dieback pathogen *Hymenoscyphus fraxineus* in its native environment. Nature: Scientific Reports. 6, 21895; doi:10-1038/srep21895 (2016)
9. Nguyen D, **Cleary MR**, Enderle R, Stenlid J. 2016. Analyses of the ash dieback pathogen *Hymenoscyphus fraxineus*, suggest role of tree species diversity on colonization and population structure differentiation. Forest Pathol 46: 82-84

Direct communications in oral presentations:

1. Cleary, M. "Phoenix from the ashes: exploiting host resistance to conserve and restore populations of European ash". **6th International Workshop on the Genetics of Tree-Parasite Interactions: Resistance to Insects and Diseases: Putting Promise into Practice.** Ohio, USA, 5-10 August, 2018.

2. Cleary, M. "Ash dieback and modern techniques for resistance phenotyping". **IUFRO 125th Anniversary Congress, All Division 7 – Forest Health meeting** in the session entitled 'Are we doing the right things to deal with invasive forest pest and pathogens? Lessons from history and current strategies'. Freiburg, Germany, 19-22 September, 2017.

3. Cleary, M. "Advanced phenotyping using FT-IR distinguishes disease resistance in Fraxinus excelsior against Hymenoscyphus fraxineus". **HealGenCarWorkshop: Fighting ash dieback with new and old tools.** 23-25 August 2017. Skovskolen, Denmark.

4. Stener, L-G. "Ash breeding projects in Sweden". **HealGenCarWorkshop: Fighting ash dieback with new and old tools**. 23-25 August 2017. Skovskolen, Denmark.

5. Cleary, M. "Ashes to Ashes: The demise of European ash (*Fraxinus excelsior*) and opportunities to exploit host resistance to conserve populations". **Invited talk** at the Canadian Forest Service, Pacific Forestry Research Centre, Victoria, British Columbia, Canada. 17 May, 2017.

6. Cleary, M. "Ashes to Ashes: The demise of European ash (*Fraxinus excelsior*) and opportunities to exploit host resistance to conserve populations". **Invited talk** at Kalamalka

Forestry Centre, British Columbia Ministry of Forests, Lands and Natural Resource Operations, Vernon, British Columbia, Canada. 25 May, 2017.

7. Cleary, M. "Selection and testing Fraxinus excelsior genotypes for resistance against *Hymenoscyphus fraxineus* in Sweden." **IUFRO Foliage and Stem Disease meeting**. Niagara Falls, Canada, 7-11 May 2017.

8. Cleary M. "Advanced phenotyping using FT-IR distinguishes disease resistance in *Fraxinus excelsior* against *Hymenoscyphus fraxineus*." **IUFRO Foliage and Stem Disease meeting**. Niagara Falls, Canada, 7-11 May 2017.

Poster presentations:

1. Villari, C. Dowkiw, A., Enderle, R., Kirisits, T., Muñoz, F., Nielsen, L., Pliūra, A., Stener, L-G., Suchockas, V., Rodriguez-Saona, L., Bonello, P., **Cleary, M**., 2018. Advanced phenotyping using FT-IR distinguishes disease resistance in *Fraxinus excelsior* against *Hymenoscyphus fraxineus*. 6th International Workshop on the Genetics of Tree-Parasite Interactions: Resistance to Insects and Diseases: Putting Promise into Practice. Ohio, USA, 5-10 August, 2018.

Hosted field excursions:

Jubelexkursionen in southern Sweden with representatives from the forest sector. 20-21 June.

excursion 2017.pdf

Masters Thesis Project:

Jonsson, A. 2017. The genetic variation in susceptibility of juvenile *Fraxinus excelsior* to the invasive pathogen causing ash dieback. Southern Swedish Forest Research Centre, SLU Alnarp. https://stud.epsilon.slu.se/10448/11/jonsson_a_170711.pdf

Popular Science Articles:

"Asken kan räddas genom förädling" https://www.skogforsk.se/kunskap/kunskapsbanken/2018/asken-kan-raddas-genom-foradling/

P4-Kristianstad Radio. "Hoppas på snabbare hjälp för askträden" http://sverigesradio.se/sida/artikel.aspx?programid=101&artikel=6803897

"Fingeravtryck" hjälper asken. Nyhetsbrev från Institutionen för sydsvensk skogsvetenskap/Sveriges Lantbruksuniversitet. September 2017. https://www.slu.se/globalassets/ew/org/inst/ssv/nyheter/nyhetsbrev-ask.pdf

"Forskarnätverk ska skydda tallen" https://www.skogen.se/nyheter/forskarnatverk-ska-skydda-tallen

"Hot mot tallen växer i Europa" http://www.landskogsbruk.se/skog/hot-mot-tallen-vaxer-i-europa/

"Fingeravtryck" hjälper asken. Nyhetsbrev från Institutionen för sydsvensk skogsvetenskap/Sveriges Lantbruksuniversitet. September 2017.

"Ny metod kan rädda askar". http://www.atl.nu/skog/ny-metod-kan-radda-askar/

"Värdefulla askar kan beskäras för att räddas från askskottssjukan". SkogsSverige

"Hjälp att få för akut hotad ask". Skogsaktuellt http://www.skogsaktuellt.se/artikel/54929/hjalp-att-fa-for-akut-hotad-ask.html

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Enderle, R., Nakou, A., Thomas, K. and Metzler, B. 2015. Susceptibility of autochthonous German *Fraxinus excelsior* clones to *Hymenoscyphus pseudoalbidus* is genetically determined. Annals of Forest Science 72, 183-193.

Kirisits, T., and Freinschlag, C. 2012. Ash dieback caused by *Hymenoscyphus pseudoalbidus* in a seed plantation of *Fraxinus excelsior* in Austria. Journal of Agricultural Extension and Rural Development Vol. 4(9), pp. 184-191, DOI: 10.5897/JAERD12.046

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

Muñoz, F., Marçais, B., Dufour, J. and Dowkiw, A. 2016. Rising out of the ashes: additive genetic variation for susceptibility to *Hymenoscyphus fraxineus* in *Fraxinus excelsior*. bioRxiv, doi: http://dx.doi.org/10.1101/031393

Pliūra, A. and Baliuckas, V. 2007. Genetic variation in adaptive traits of progenies of Lithuanian and Western European populations of *Fraxinus excelsior* clones. Baltic Forestry 13, 28-38.

Pliūra, A., Lygis, V., Suchockas, V. and Bartkevičius, E. 2011. Performance of twenty four European *Fraxinus excelsior* populations in three Lithuanian progeny trials with a special emphasis on resistance to *Chalara fraxinea*. Baltic Forestry 17, 17-34.

Pliūra, A., Lygis, V., Marčiulynienė, D., Bakys, R. and Suchockas, V. 2014. Dynamics of genetic resistance to *Hymenoscyphus pseudoalbidus* in juvenile *Fraxinus excelsior* clones. Baltic Forestry 20, 10-27.

Stener, L. G. 2013. Clonal differences in susceptibility to the dieback of *Fraxinus excelsior* in southern Sweden. Scandinavian Journal of Forest Research 28:205-216.