

**IDENTIFYING AND TESTING DIEBACK-TOLERANT
ASH (*FRAXINUS EXCELSIOR*) ON GOTLAND ISLAND,
BALTIC SEA, SWEDEN**

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**ПОЛЕВЫЕ ИССЛЕДОВАНИЯ ЯСЕНЯ (*FRAXINUS EXCELSIOR*)
ТОЛЕРАНТНОГО К УСЫХАНИЮ ОТ ГРИБА
HYMENOSCYPHUS FRAXINEUS НА ОСТРОВЕ ГОТЛАНД,
БАЛТИЙСКОЕ МОРЕ, ШВЕЦИЯ**

В рамках проекта ЕС программы ЛИФЕ+ЕЛМИАС на острове Готланд (Балтийское море, Швеция) была выполнена следующая работа:

1) инвентаризация и GPS-мэппинг здоровых деревьев ясеня в насаждениях подвергнутых массовому усыханию вызываемым грибом *Hymenoscyphus fraxineus*;

2) собрание семян с этих деревьев;

3) идентификация, выкопка и рассаживание в испытательной плантации более 1000 саженцев возрастом 2–3 года, а также посев там же собранных семян;

4) последующий мониторинг состояния здоровья семянных деревьев, саженцев и сеянцев;

5) сравнение эффективности молекулярного маркера (предложенного британскими и датскими коллегами) с нашими данными полевых работ по достоверности определения устойчивых генотипов ясеня.

Ash dieback (ADB) caused by the invasive fungal pathogen *Hymenoscyphus fraxineus* results in massive mortality of *Fraxinus* spp. trees in Europe. However, it has been noted that a certain proportion of ash individuals remain seemingly healthy during prolonged periods of time, indicating their potential tolerance to the disease.

The work has been done within the framework of EU LIFE+ Nature project “Saving wooded Natura 2000 habitats from invasive alien fungi on the Island of Gotland, Sweden” (ELMIAS, NAT/SE/001139). The project was co-ordinated by Swedish Forestry Agency with Swedish University of Agricultural Sciences as one of the partners, responsible (among several others) for the Action 5: “Identification of dieback-resistant *F. excelsior* genotypes to create a database of at least 100 presumably dieback-resistant *F. excelsior* genotypes native to Gotland”. Aims: i) search for visually healthy trees in severely damaged ash stands focusing on Natura 2000 sites; ii) select, measure, photograph and GPS record at least 100 of such trees; iii) collect seeds from those and sow them on former agricultural land; iv) search for at least 1000 visually healthy 2-3 years-old saplings of ash in neighbouring disease-affected stands; v) collect and out-plant those in the designated area; vi) continuously monitor health status of selected, sawn and planted ash trees.

Preliminary results of the health status of the established plantation (aims iii, iv, v and vi) after first two vegetation seasons demonstrated that 64% of planted saplings remained symptomless, 25% showed dieback symptoms, and 11% were dead (Menkis et al. 2018). Notably, apart of apparent fungal infections, there was a severe draught during the 2nd vegetation season of year 2018, with presumably negative influence on survival rates. Corresponding results after the 3rd and 4th vegetation seasons (years 2019 and 2020), as well as the data on numbers and health status of germinated seedlings will be presented during the lecture.

Moreover, as pointed out above, while achieving the aims (i) and (ii), we searched for, GPS-mapped, and during 6 years monitored 135 visually healthy *F. excelsior* in heavily ADB-infested areas. This (apart of collecting seeds from those and sowing, see above) has been done in order to explore the results of their apparent tolerance to the disease for eventual long-term future use in resistance breeding and propagation. Monitoring of healthy-looking *F. excelsior* showed that after 3–4 years since mapping 99.3% of trees had 0–10% crown damage, thus remaining in a similar health condition as when mapped. After 5–6 years, there was 94.7% of such trees.

Recently, a molecular marker based on cDNA single nucleotide difference between three paralogous genes was recently identified as a potential moderately good predictor of reduced susceptibility to ADB, thus presumably allowing to select resistant trees based on DNA analysis directly from the plant material, thus avoiding long-term field monitoring (Harper

et al. 2016). Therefore, the second part of the presented study comprised checking for the presence of that marker in our mapped healthy-looking *F. excelsior* trees, and to compare the occurrence of the marker in trees exhibiting severe ADB symptoms.

Molecular analysis of leaf tissues from 40 of those showed the presence of the molecular marker for disease tolerance in 34 (85.0%) trees, while it was absent in 6 (15.0%) trees. Analysis of leaf tissues from severely ADB-diseased trees showed the presence of the molecular marker in 17 (42.5%) trees, but its absence in 23 (57.5%) trees (in chi-square test the difference significant at $p < 0.0001$) (Menkis et al. 2020). Thus notably, almost half of checked ADB-susceptible (severely diseased) trees showed the presence of molecular marker suggested in fact to be the reliable indicator for disease resistance.

In conclusion, the cDNA-based molecular marker (despite labour-consuming and expensive testing for it) revealed moderate (or limited) capacity on its own to discriminate between presumably ADB-tolerant and susceptible *F. excelsior* genotypes. For an efficient marker assisted selection, a larger set of markers will be needed, or, alternatively, focus should be directed towards simpler and more straightforward (although time consuming) approach, as the search for, mapping and monitoring of healthy-looking *F. excelsior*. Indeed, our study demonstrated that such work (the second option) has the potential to provide a valuable ash material for future breeding.

References

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