

SLU Partnership Alnarp

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Project title

SLU Partnership Alnarp's project no.:

Project title in Swedish according to the project application: Fenolkemiska profiler och växtrespons av europeisk aska (*Fraxinus* spp) på askadödande (*Hymenoscyphus fraxineus*) och smaragdaska (*Agrilus planipennis*)

Project title in English according to the project application: Chemical profiles and plant response of European ash (*Fraxinus* spp) to ash borer (*Hymenoscyphus fraxineus*) and emerald ash borer (*Agrilus planipennis*)

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Department:SSFRC

Project duration:2 years

Project partners: Skogforsk

Project summary

European ash trees are increasingly threatened by both emerald ash borer (EAB) and ash dieback (ADB). In this project, we examined how three European ash species (*Fraxinus excelsior*, *F. ornus* and *F. angustifolia*) respond chemically when exposed to EAB, ADB, or both stresses together. The results show that all three species share many metabolites, but their chemical responses differ clearly depending on the species, the type of stress, and where in the tree the sample is taken. Importantly, both EAB and ADB triggered measurable changes not only near the attack or infection site but also in more distant tissues, indicating that ash trees mount both local and systemic responses.

Our main output is that ash in decline from a pathogen and insect. Their effects may overlap and influence tree condition in different ways than either stress alone. The study highlights that different ash species may vary in their response, which is relevant for monitoring, planting, and future resistance screening. Several metabolites were identified as potential indicators of stress, suggesting that chemical markers may in future help support earlier detection or selection of more tolerant trees. However, compounds need to be further validated before they can be used diagnostics or tested against prevent damaged by these pests in the trees.

Swedish:

Europeiska askar hotas i allt högre grad av både smaragdpraktbagge (emerald ash borer, EAB) och askskottsjuka (ash dieback, ADB). I detta projekt undersökte vi hur tre europeiska askarter (*Fraxinus excelsior*, *F. ornus* och *F. angustifolia*) reagerar kemiskt när de utsätts för EAB, ADB eller båda

stressfaktorerna samtidigt. Resultaten visar att alla tre arterna delar många metaboliter, men att deras kemiska responser tydligt skiljer sig beroende på art, typ av stress och var i trädet provet tas. Viktigt är att både EAB och ADB gav upphov till mätbara förändringar inte bara nära angrepps- eller infektionsstället, utan även i mer avlägsna vävnader. Detta tyder på att askar utvecklar både lokala och systemiska försvarsreaktioner.

Vår viktigaste slutsats är att askar som försvagas av både en patogen och en insekt kan påverkas på mer komplexa sätt än vid en enskild stressfaktor. Effekterna kan överlappa och påverka trädens vitalitet på andra sätt än om de utsätts för endast ett angrepp. Studien visar också att olika askarter kan skilja sig åt i sin respons, vilket är relevant för övervakning, plantering och framtida screening av resistens eller tolerans. Flera metaboliter identifierades som potentiella indikatorer på stress, vilket tyder på att kemiska markörer i framtiden kan bidra till tidigare upptäckt av angrepp eller till urval av mer toleranta träd. Samtidigt behöver dessa ämnen valideras ytterligare innan de kan användas i diagnostik eller testas som grund för metoder att förebygga skador orsakade av dessa skadeorganismer.

Background:

European ash (*Fraxinus excelsior*) is currently threatened by two invasive alien species; the first is an aggressive fungal pathogen (*Hymenoscyphus fraxineus*) causing ash dieback (ADB) that during the last 20 or more years has caused a slow, steady decline in the ash population and the second pest is emerald ash borer (EAB, *Agrilus planipennis*), a buprestid beetle that is killing ash in Eastern Europe and is on the fringe of invading the rest of the European continent. Buprestids are a family of beetles that attack and kill stressed or dying host plants (Evans et al. 2007). Recently however, several buprestids have been introduced around the world and they have become primary pests on evolutionary naïve host plants, pests that kill healthy host plants. EAB is probably the most well-known buprestid; it was accidentally introduced to North America in the late 1980s (Siegert et al. 2014) and has become the most costly biological invasion by an invasive forest pest in USA history (Aukema et al. 2011). This beetle has killed hundreds of millions of ash trees (*Fraxinus* spp.) at more than a 99% mortality rate (Herms and McCullough 2014) and as a result six North American *Fraxinus* species are now red-listed. With recently finding its way into Eastern Europe, where it established in European Russia around 2003 (Baranchikov et al. 2008). EAB has since spread, having recently invaded Ukraine (Drogvalenko et al. 2019) and is expected to continue to invade the range of European ash species unhindered from natural dispersion and the unintentional movement of wood products (Herms and McCullough 2014), putting the fate of ash across the rest of Europe at risk, particularly if no measures are taken to prevent or minimize eventual impact. In addition, there is a strong likelihood that the expanding EAB population will heavily impact more *Fraxinus* species as well as other Oleaceae species, including olive trees (*Olea europaea*) and related ornamental landscape trees (Cipollini et al. 2017, Peterson and Cipollini 2020) in southern parts of Europe. The impact is particularly important since ash is an economically valuable tree species in the wood products industry and plays a vital role as a 'keystone' species in entire ecosystems. In fact, the loss of ash populations in some countries, due only to ADB, has already instigated an extinction cascade with other associated species (Hultberg et al. 2020).

Purpose

Novel information on defense strategies in congeneric species of *Fraxinus* against EAB, specifically, knowledge on critical defense traits associated with resistance in different European *Fraxinus* species which can be used for tree improvement programs

Method

WP1-Inculation of ash trees with EAB eggs

Laboratory experiment will be established in the Biotron facility at SLU, Alnarp, Sweden. Two-year-old plants (N= 120, n=10/treatment/species) of evolutionarily naïve hosts, i.e. European ash (*F. excelsior*), narrow-leafed ash (*F. angustifolia*), manna ash (*F. ornus*) will be either inoculated with EAB larvae, dually inoculated with *H. fraxineus* and EAB, or left as control. The eggs of EAB will be sourced from USDA ARS, Beneficial insect laboratory, Newark, Delaware, USA from Dr. Jian Duan. The US facility produces EAB

eggs for the use of biological control agents. Young eggs, six-seven day old eggs attached to coffee filters will be shipped to the Biotron in Alnarp. Upon arrival, the EAG eggs will be immediately moved and quarantined in the Biotron and only removed from the package once they are in withing the quarantine room.

Eggs come on coffee filters since it is a substrate that female adults will oviposit on and it allows the eggs to be easily be separated for experiment. Once the eggs arrive, they will be transferred into the quarantine laboratory space and attached to the stems of the ash trees. The eggs will be individually cut off of coffee filters and attached to ash tree stems (n=4 eggs/stem). Parafilm will be wrapped over the eggs and around the stem to securely attach the eggs. Two weeks after egg attachment the parafilm and eggs will be removed from the trees and hatch rate will be assessed. Hatch rate is determined by checking eggs for sawdust in the leftover egg shell and checking for neonate exit holes. All eggs and parafilm used will be destroyed by placing everything into a plastic bags and incinerated.

WP1.1. Phloem chemical defenses of naïve and co-evolved ash species

Thirty days after egg hatch the outer bark layers were removed and trees assessed for larval size and gallery width (24). Phloem samples were collected by removing sections of tissue, freezing on dry ice, and transporting them for storage at -80°C. In control treatments, phloem samples were collected at least 10 cm apart, while in inoculation treatments phloem samples was collected adjacent to larval galleries. Chemical analyses: Secondary metabolites were extracted from frozen, ground phloem tissue with laboratory grade methanol at 0.5 mg/mL and stored until further processing. High-performance liquid chromatography with mass spectrometry (HPLC-MS) was used to quantify and identify phenolic compounds consistent with previous methods by sending them to the Swedish Metabolomics Centre, Umea.

Results

Chemical analyses showed that all three ash species (*Fraxinus excelsior*, *F. ornus* and *F. angustifolia*) responded to emerald ash borer (EAB), ash dieback (ADB), and the combination of both stresses. Using untargeted LC-MS, a total of 1,563 chemical features were detected. Around 54% of these were shared among the three species, showing that they have a large common chemical background. At the same time, the analyses showed clear differences in how the species responded to the different treatments.

Multivariate analyses (PCA, PERMANOVA and PLS-DA) showed that the chemical profiles differed depending on both species and treatment. Within each species, a large number of metabolites were associated with treatment effects, indicating that both insect attack and disease infection caused substantial changes in tree chemistry. Several of the most important compounds were further annotated, although many still need additional confirmation.

The targeted LC-MS analyses supported these results and identified 11 compounds that differed significantly depending on the combination of species and treatment. This confirms that the chemical response to EAB and ADB is not the same in all ash species. Overall, the results show that both EAB and ADB trigger measurable changes in ash chemistry, and that these responses vary among species and between single and combined stress. These findings provide a useful basis for future work on early detection, resistance screening, and improved management of ash under pest and disease pressure.

Discussion

The chemical analyses show that both emerald ash borer (EAB) and ash dieback (ADB) cause changes in ash chemistry, and that these responses differ among the three ash species studied. This suggests that European ash species do not respond uniformly to insect and pathogen stress, which is important when assessing their vulnerability and potential tolerance. The results also indicate that combined stress from EAB and ADB may influence trees differently than either stress alone, highlighting the need to consider multiple threats together rather than separately.

Although many metabolites were shared among species and treatments, the multivariate analyses showed clear differences in the overall chemical profiles. This indicates that the main response is not the appearance

of completely unique compounds, but rather changes in the levels of existing metabolites. Such shifts are typical of plant stress and defence responses and suggest that both local and systemic changes occur in the tree following attack or infection.

The targeted analyses further supported these patterns by identifying compounds that differed significantly depending on species and treatment. This provides an important first step towards identifying chemicals that are connected to stress responses in ash. However, most candidate compounds still require further validation before they can be used in practical diagnostics or resistance screening or testing against their biological relevance against pathogen or EAB tolerance.

Overall, the study provides new insight into how European ash species respond chemically to two major threats. The findings strengthen the basis for future work on early detection, improved monitoring, and the identification of ash material with greater tolerance to pest and disease pressure.

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