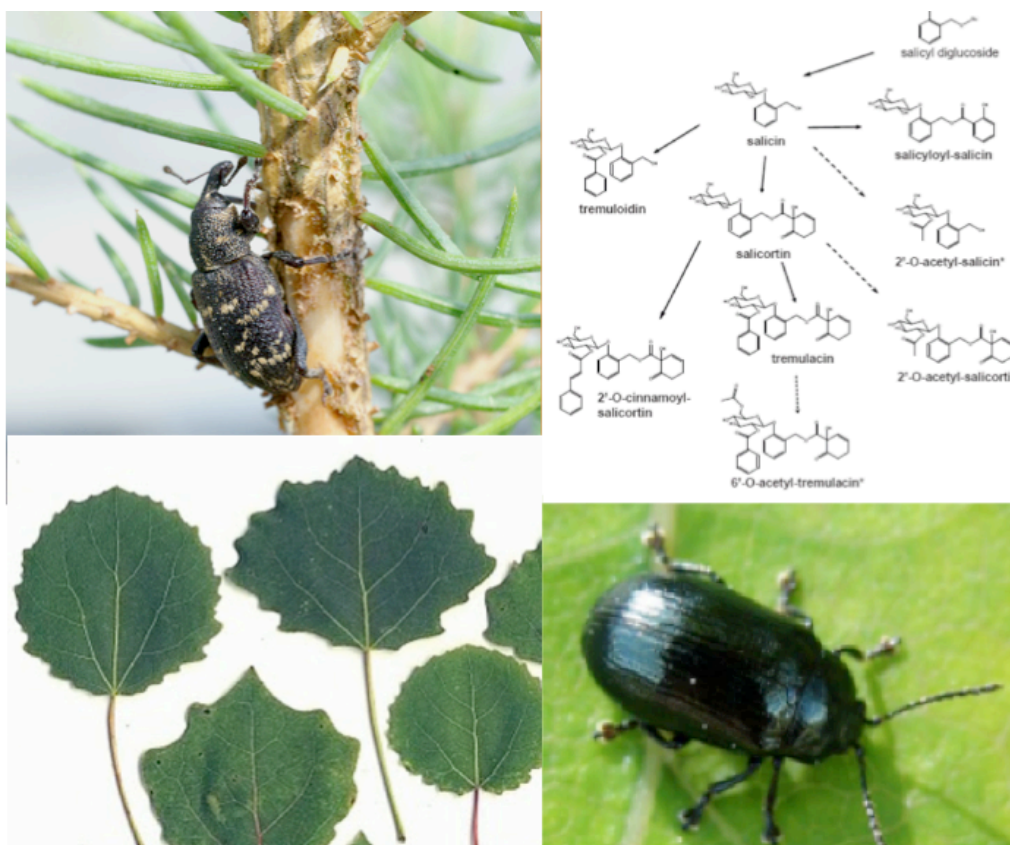


# Parasite resistant trees

## *Final report*

**November 2015** (revision april 2016)



Umeå Plant Science Centre  
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## Summary

The project “Parasite resistant trees” (PaReTree) started in January 2010. The long-term goal of PaReTree is to find durable and sustainable ways to produce conifer seedlings that are not – or at least somewhat less – attacked by pine weevils (*Hylobius abietis*), but also in a general sense to understand and demonstrate how modern genetic and genomic tools can be used to develop forest tree populations that are healthier. The vision was to combine the expertise of The Swedish *Hylobius* Research Program (Snytbaggeprogrammet), which is run by SLU (Sveriges lantbruksuniversitet) in Uppsala, with the expertise and research infrastructure at UPSC (Umeå Plant Science Centre) and at KTH (Kungliga Tekniska Högskolan). The question of forest tree health is of paramount importance for Swedish forestry, as pine weevils cause yearly losses estimated at several 100 million SEK, and the current protection regime using insecticides has to be phased out by the forest owners if FSC-certification should be maintained.

PaReTree had two parts, one utilize the toolbox available for *Populus* research to study the genetic and environmental factors behind herbivore resistance in aspen. The second part focused on research that may lead to ways to protect conifer seedlings against pine weevils, taking the current conifer genetic material for granted. We assumed that the genomic resources for conifers would advance rapidly so the same approaches that we have developed for *Populus* should be available for application in conifers in the second half of this decade. With the launching and very rapid advance of the Spruce genome sequencing project, we could already within the PaReTree project, use genomic tools to enable more efficient conifer breeding for less susceptibility towards pine weevils.

In the *Populus* part, the main resource used has been the SwAsp collection, 116 natural aspen genotypes collected throughout Sweden, cloned and planted in 4 replicates in two common gardens. In a parallel project, we generated a reference genome of aspen. This took longer than expected but now we are finally done. The resequencing of the genomes of all clones in the collection has been more straightforward, but the full utilization of this, for example in performing GWAS (genome-wide association studies) has not been realistic until recently. Within PaReTree we have developed methods to quantify the diverse kinds of phenolic compounds in aspen, and we have scored the SwAsp collection for variation in flavonoid and salicinoid composition as well as for condensed tannins. The collection has also been scored for variation in susceptibility against different insect herbivores. Aspens with differences in phenolic composition have been investigated for herbivore susceptibility and we have also been able to draw conclusions about the evolution of herbivore traits and the co-evolution between trees and their parasites. We have also established fields trial with transgenic aspens, and studied the relationship between growth and susceptibility towards herbivores.

In the conifer part, we have studied the feeding behaviour of pine weevil and the susceptibility of conifer seedlings to pine weevils. We have mainly focused on induced defences, i.e. those that are activated after biotic damage. The induced defences have been triggered both naturally, through insect feeding, and artificially, by exogenous application of Methyl jasmonate (MeJa). The effect of these treatments on the volatile chemicals emitted from the seedlings, and their associated influence on the feeding behaviour of the pine weevil, have been investigated in detail. A large scale field experiment showed that elicitation of inducible defences in seedlings in the nursery appears to be an attractive alternative to protect conifer seedlings against pine weevils.

Finally we have found in a large screening experiment that there appears to be a useful genetic variation for pine weevil susceptibility in the Swedish spruce breeding population.

3 PhD students and 2 postdocs has been employed in the project, and additional PhD students and senior scientists are affiliated to the project, 18 original papers have been published, 7 ms are submitted or in preparation, one PhD thesis has been presented and two are on their way.

## 1 The objectives and history of the project

### 1.1 The conception of the project, the background, motivation and original vision.

Forest trees – that dominate most terrestrial ecosystems - are characterized by large size, robustness, longevity, long generation times and an enormous genetic variation. Since these traits all strongly influence the interactions between forest trees and their parasites and pests, pest management in forestry is different than in agricultural systems. The objective of PaReTree was to fully combine the **research infrastructures at Umeå Plant Science Centre (UPSC)** with the **know-how of The Swedish Hylobius Research Program** to address the question of parasite resistance in trees. We wanted to apply novel approaches and create and utilize **synergy between the two project parts** and the three universities involved, to increase the knowledge-base necessary to develop **novel ways of managing the pine weevil (*Hylobius abietis*)** in Swedish forestry. The research foci of the participating groups should be used in a complementary way to study parasite resistance in *Populus* – where we can capitalize on infrastructure already developed - to get a deeper basic understanding of the genetics of parasite resistance and demonstrate how the underlying polymorphisms can be identified. Superior *Populus* genotypes for potential use in Swedish forestry may also be developed. In addition, we will with equal emphasis study the attack of the pine weevil on conifers. We wanted, in the short time perspective, try to develop a pine weevil management strategy based on natural or synthetic chemical compounds and in the longer perspective generate tools for breeding.

*Populus* is only of moderate economic importance for Swedish forestry but is **one of the most advanced model systems for plant biology**, to a large extent due to the research of the groups in this project. Aspen - the only *Populus* species native to Sweden – is a good model for forest genetics since it, like pine and spruce, is outbreeding, has a very long generation time, a very large population size and, as a consequence, high levels of genetic diversity, heterozygosity and linkage disequilibrium that extends only a few hundred base pairs. Ecologically or economically important traits tend to be quantitative and hence governed by a large number of genes, where each gene contributes only a small portion to the trait on interest. By using aspen as a model we can therefore, with high efficiency, study the genetic architecture of parasite resistance in a tree species and how this architecture is most effectively dissected using modern genetic tools.

In contrast to *Populus*, Scots pine and Norway spruce are not well-developed model systems for studies of basic plant biology but they are of **paramount importance for Swedish forestry**. By damaging newly planted conifer seedlings the pine weevil is the most economically important insect pest affecting Swedish forestry, causing yearly losses estimated at **several 100 million SEK**. In southern Sweden, mortality typically exceeds 20% among insecticide treated seedlings, and in the absence of protective measures more than **80% are killed**. Worryingly, the rate of seedling mortality is also broadly increasing in northern Sweden. The seriousness of the pine weevil problem will further increase because the use of effective insecticides is currently being phased out by FSC-certified forest owners (i.e. most of the major companies). Research aimed at decreasing the damage caused by pine weevils has made considerable progress in finding environmentally friendly alternatives to insecticides but commercially available alternatives are still relatively costly.

The interplay between the pine weevil and its host is intricate and represents a **very special case of plant-parasite interaction**. The pine weevil feeds on the bark of young conifers causing severe damage or death of seedlings of both Scots pine and Norway spruce. The seedling's ability to defend against the pest depends on the rate at which it can establish on the planted site and divert resources for the production of defence compounds. Given the devastation frequently caused by pine weevils to new conifer plantings, new ways to decrease the impact of this pest are urgently needed.

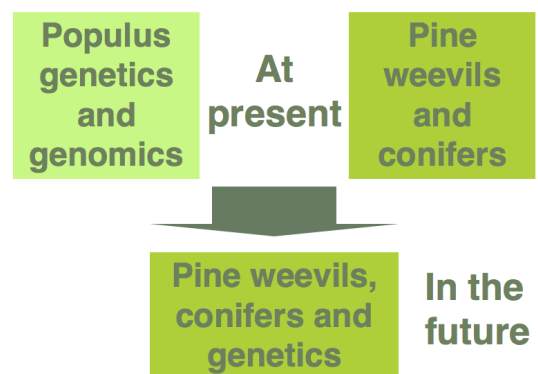


Fig 1 The long-term relation between the project parts

## 1.2 Basic organization, relation to other grants etc.

Senior researchers in PaReTree was **Stefan Jansson (SJ)** Coordinator. Expert on photosynthesis, *Populus* physiology, genetics and genomics and natural variation. **Benedicte Albrechtsen (BA)** Expert on plant-pest interactions, insect herbivores, profiling of resistance compounds and defence traits. **Pär Ingvarsson (PI)** Expert on genetics, in particular population genetics, on *Populus*. **Göran Nordlander (GN)** Expert on forest entomology, in particular biology and management of the pine weevil. Manager of the Swedish *Hylobius* Research Programme. **Anna-Karin Borg-Karlson (AKBK)** Expert on ecological chemistry, particularly the interactions between insects and compounds produced by coniferous trees. Works, among other things, with feeding deterrents of the pine weevil. **Vaughan Hurry (VH)** Expert on plant physiology, photosynthesis, respiration and abiotic stress and **Torgny Näsholm (TN)** Expert on tree nutrition and metabolism.

**Umeå Plant Science Centre (UPSC)** is the leading centre for plant research in northern Europe. It hosted, when PaReTree started the *Metabolomics facility*, the *Populus* transgenic facility, the *genomics facility*, the *Populus* genome program and the *database/website PopGenIE* ([www.popgenie.org](http://www.popgenie.org)) generally regarded as the “gold standard” for tree genome databases. PaReTree was also built on the large project to obtain the reference sequence of aspen (*Populus tremula*) and the work to characterize the *SwAsp* and *UmAsp* collections including resequencing of their genomes. The huge *Populus* reverse genetics efforts, perform in collaboration between UPSC and the spin-off company SweTree Technologies ([www.swetree.com](http://www.swetree.com)) is also an invaluable resource for PaReTree. Moreover, the ongoing activities in the *Spruce genome program* ([www.congenie.org](http://www.congenie.org)) and, in particular, those that are still in the start-up phase (the Spruce functional genomics project utilizing the spruce transformation facility of the *UPSC Berzelii Centre of Tree Biotechnology* and the Spruce association mapping efforts. The research groups of Stefan Jansson, Pär Ingvarsson and Benedicte Albrechtsen consisted of in total ca 15 persons funded by external grants, working on e.g. genetics, genomics, natural variation, biotechnology and herbivore interactions of aspen. The groups of Torgny Näsholm and Vaughan Hurry deal with carbon and nitrogen relations in plants consisted of additionally ca 10 persons funded by external grants.

The research group of Professor Göran Nordlander at the Department of Ecology, SLU, Uppsala, was primarily studying the ecology and pest management of the pine weevil, *Hylobius abietis* ([www.snytbagge.se](http://www.snytbagge.se)). It included at the start of the project 7 people that are all almost entirely funded by external grants. The largest and most diverse project of this group is the Swedish *Hylobius* Research Program, “**Snytbaggeprogrammet**” funded by the Swedish forestry sector (3 million SEK per year 2010-2014).

The research group of Professor Anna-Karin Borg-Karlson, **Department of Chemistry, KTH**, Stockholm, are identifying chemical signals in various insect-plant-microbe interactions with focus on conifer-associated organisms. The group consisted over 10 persons and had a project funded by Formas and another by Södra skogsägarna focus on identifying chemical resistant markers for (1) the spruce bark beetle and (2) the *Heterobasidion* complex.

## 1.2 Changes of the project during its period.

The short-time perspective part of the project (development of a pine weevil management strategy based on natural or synthetic chemical compounds) was modified during the project. We performed the planned study on the bark metabolome of spruce aiming at identifying compounds that could deter pine weevils. We encountered more methodological problems than expected, but reached after ca 2 years the conclusion that this was not a good way forward. Therefore, this subproject was terminated. Instead, we explored effects on pine weevil feeding by induced defences elicited by pretreatment of conifer seedlings with a plant hormone (methyl jasmonate). This strategy showed a lot of promise, and we put much emphasis to this in the latter part of the project, in collaboration with a group in Spain (which was also developed as a part of PaReTree).

The long-term objective of this project was to find **durable and sustainable ways to produce conifer seedlings that are not – or at least somewhat less – attacked by pine weevils** but also in a general sense understand and demonstrate how **modern genetic and genomic tools** can be used to develop forest tree populations that are healthier. When this five-year project was formulated (in

2009), the only forest tree model system where genomic tools were available was *Populus*, and therefore we decided to embark on a two-way strategy. One half of the project should utilize the toolbox available for *Populus* research to study the genetic and environmental factors behind herbivore resistance in aspen aiming at identifying the metabolites, genes/alleles and treatments that influence the preferences of different insect herbivores feeding on aspen leaves. The second half of the project should focus on research that may lead to ways to protect conifer seedlings against pine weevils, taking the current conifer genetic material for granted. We envisioned that the genomic resources for conifers were likely to advance rapidly and that - in a somewhat longer time frame - the same basic approaches that we will develop for *Populus* in this project should be available for application in conifers (Figure 1). At that point, we believed that the knowledge we have gained from working with *Populus* may help developing pine and spruce material which are more resistance against pine weevils and other pests in a ca 10-year perspective.

During PaReTree, the **roadmap for conifer genetic and genomic research has changed**. Two of the PIs of this project (Stefan Jansson and Pär Ingvarsson) were deeply involved in the **sequencing the Norway spruce (*Picea abies*) genome, the first conifer genome to be characterized and at the time of publication (May 2013) was the largest genome, all categories, which had been sequenced**. This project, funded by KAW, also ending now, has in the end focused on bioinformatics and genetic resources development to enable use in the Swedish spruce breeding program (led by Skogforsk), association mapping etc. Other conifer genomes have also been completed or are in the pipeline, for example is a European project (where Pär Ingvarsson is a partner) embarked on the project to sequence Scots pine (*Pinus sylvestris*). In PaRe Tree, we reallocated resources to, in collaboration with Skogforsk, investigate if there existed variation in susceptibility to pine weevil feeding in the Swedish spruce breeding population.

## 2 Scientific results of the project

### 2.2 Scientific approach and results

#### 2.2.1 The genetics and genomics of parasite resistance in aspen

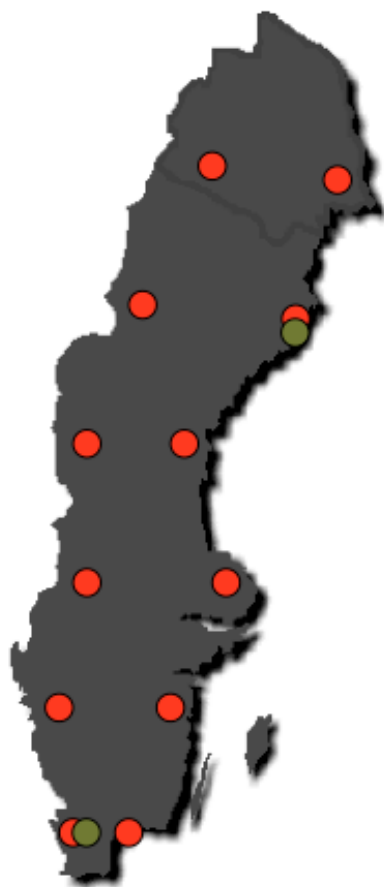


Fig 2 The SwAsp collection sites (red) and common gardens (green)

Our strategy to study the genetics and genomics of parasite resistance in aspen is tripartite. First, we score the enormous genetic variation within aspen for herbivore susceptibility and defence traits, including the composition of e.g. phenolic glucosides. This work is combined with the aspen genome project. Secondly, we also use subsets of the clone collections to do “focused studies” and thirdly, we have established a field trial with transgenic aspens to be used in PaReTree.

Growth, leaf metabolism and susceptibility and tolerance to herbivory were assayed in the SwAsp collection that consists of 116 natural aspen genotypes, collected throughout Sweden in 2003. The collection was planted in 2004 in two common gardens (Fig 2, four replicates of each clone in each garden. Luquez et al. 2008) and has been extensively phenotyped for many traits.

A large number of genes have been sequenced from the whole or a subset of the collection, and many SNPs and SSRs have been scored in the whole collection. Most (102 genotypes) of the collection is now available in tissue culture and *Agrobacterium*-mediated transformation works relatively efficiently for a large fraction of the genotypes. A complementary collection, the UmAsp collection, consisting almost 300 genotypes sampled from the Umeå area, has been created (Fracheboud et al. 2009). It has been cloned, some ramets have been planted in two common gardens and in 2012 the gardens were completed (comprising one to six replicates of each genotype in each garden). Together, these

natural genotypes represent a unique resource for many types of studies on natural variation of important traits in aspen.

##### 2.2.1.1 The metabolite composition of aspen leaves (Participating researchers Vicki Huizu Guo, Ken Keefover-Ring, Kathryn Robinson), PIs Benedicte Albrechtsen and Stefan Jansson)

The leaf metabolite composition of aspen was determined using different methods. We identified a wide variety of salicinoid compounds (Keefover-Ring et al. 2014a, Figure 3), including a novel compound 2'-(Z)-cinnamoylsalicortin (Keefover-Ring et al. 2014b) using the methods that we have developed (Abreu et al. 2011). We were able to separate the genotypes into different chemotypes and determine their geographic distribution (Keefover-Ring et al. 2014a, Figure 4)

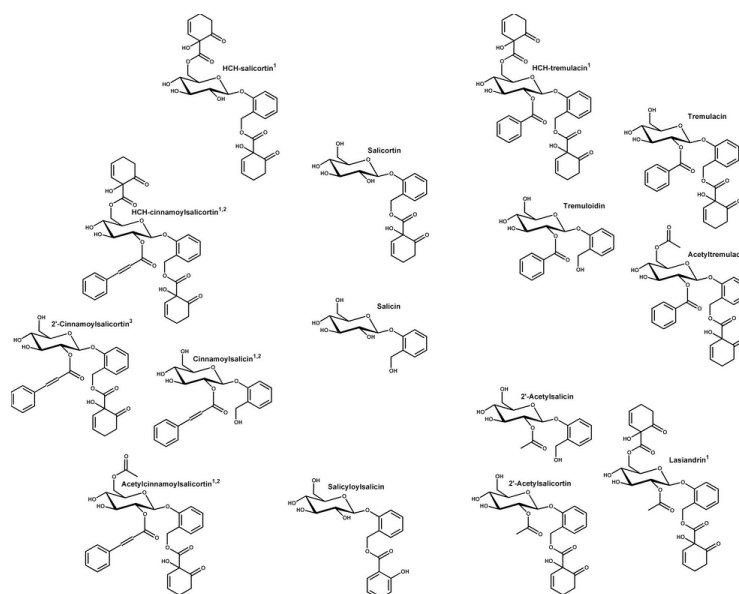


Fig 3. The salicinoids of *Populus tremula*



Total phenolics and condensed tannins were analysed in the field collection of SwAsp (Robinson et al. 2012) and the influence of fertilization of growth and tannin chemical composition has been investigated (Bandau et al. 2015). From these data we have selected extreme genotypes to carry on more detailed experiments with, most of which we have brought into tissue culture.

Analyses of the gene activity (QPCR) of key genes in the phenylpropanoid pathway relevant for tannin biosynthesis have been performed to study the effect of soil fertility on the activity of defence associated genes that code for various phenolic compounds in the anthocyanin and tannin related branch of the phenylpropanoid pathway (Figure 5).

We have also studied the expression of key genes in the metabolism of defence compounds in different genotypes of aspen under different growth regimes (Guo et al, in preparation).

#### 2.2.1.2 Resistance against herbivory (Participating researchers Vicki Huizu Guo, Ken Keefover-Ring, Kathryn Robinson, Pls Benedicte Albrechtsen and Stefan Jansson)

This subproject focussed on susceptibility towards and resistance against several insect herbivores, including leaf beetles (*Phratora vitellinae* and *Chrysomela tremulae*) and aphids (*Chaitophorous*) aiming at identifying metabolites and volatiles that may explain (part of) the herbivore preferences. Growth, morphology and phenology traits are also included in the analysis. The patterns are often complex, but some traits (like the number of leaf galls) could associate with salicinoid diversity (Figure 6).

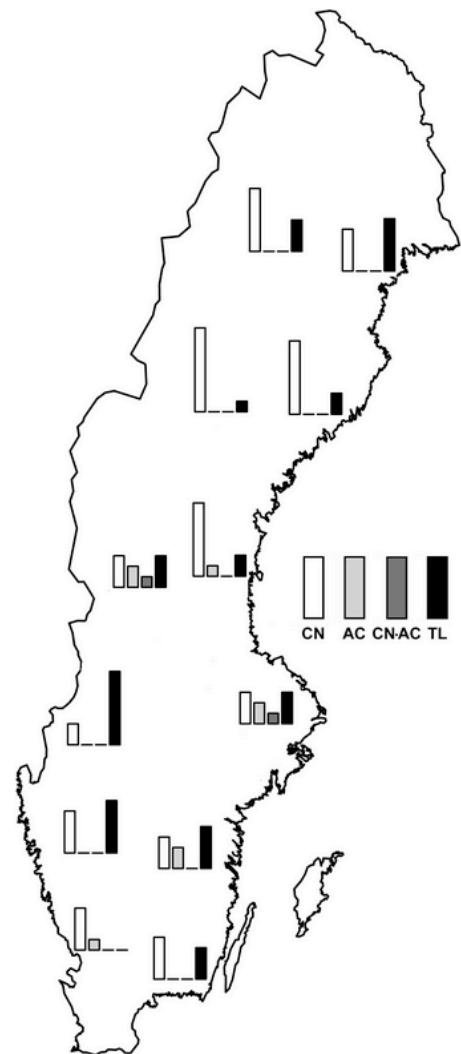


Fig 4. The genographic distribution of salicinoid chemotypes of *Populus tremula*

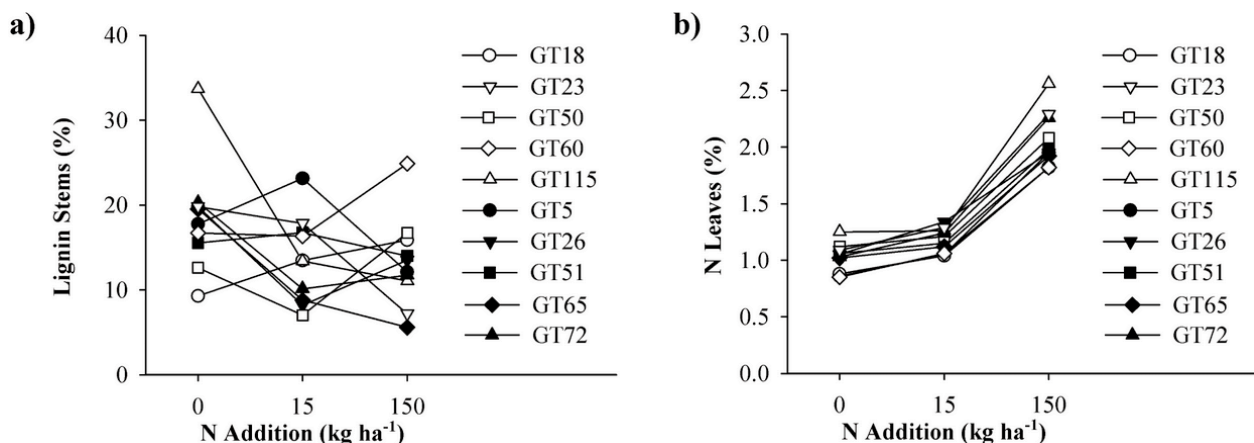


Fig 5. Tannins in aspen genotypes under different fertilization regimes

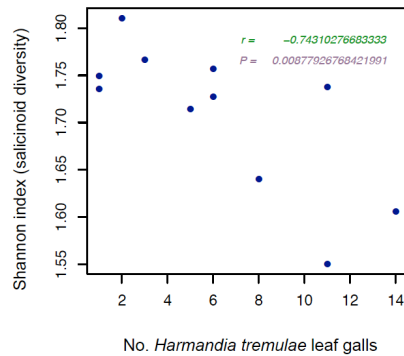


Fig 6. Relation between salicinoid diversity and leaf galls

clinal patterns we have compared the natural prevalence of two parasites on *Populus tremula* and our data supported the assumption of the GMTC model that clines exist in the border between hot and cold spots and that they may be less persistent for parasites with an elevated gene flow, and/or for parasites that cover relatively larger hot spots surrounded by fewer cold spots (Albrechtsen et al. 2010)

After an accidental episode of hare feeding on the trees, we found that the damage of feeding correlated with the amount of a salicyloisalicin (Figure 7)

According to the geographic mosaic theory of coevolution (GMTC), clines of traits reflecting local co-adaptation (including resistance genes) should be common between a host and its parasite and should persist across time. We tested the GMTC-assumption of persistent

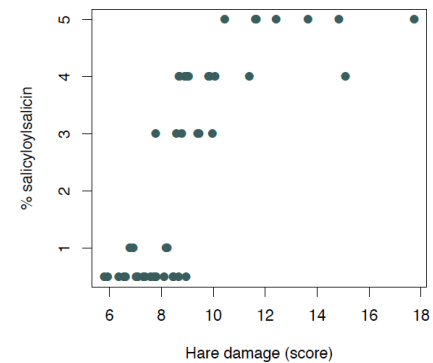


Fig 7. Relation between salicyloisalicin content and hare feeding

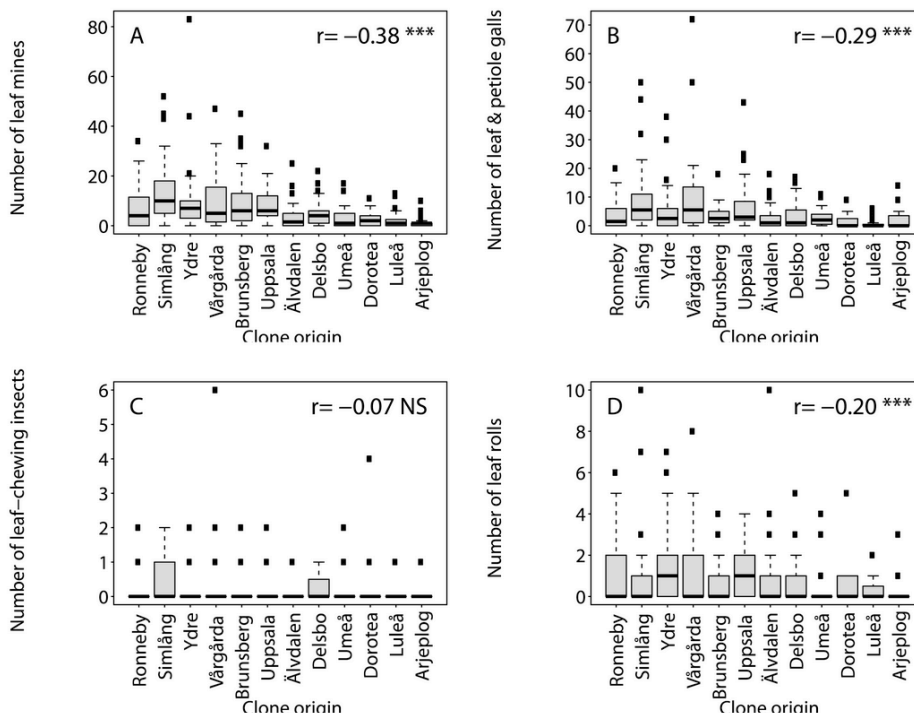


Fig 6. Numbers of arthropods categorized by feeding guilds recorded in SwAsp clones grouped by population

We have also studied the relation between functional leaf traits and herbivory in the SwAsp collection (Robinson et al. 2012). In surveys of phytophagous arthropods over two years, we found the aspen canopy supports nearly 100 morphospecies. We identified significant broad-sense heritability of plant functional traits, basic plant defence chemistry, and arthropod community traits. The influence of natural variation in plant traits on the arthropod community indicates the importance of maintaining genetic variation in wild trees as keystone species for biodiversity.

We have analyzed antagonistic plant–herbivore bipartite networks using common gardens in two contrasting environments comprised of aspen trees with differing evolutionary histories of defence against herbivores. These networks were tightly connected owing to a high level of specialization of arthropod herbivores that spend a large proportion of the life cycle on aspen. Variation in plant vulnerability, measured as the frequency of herbivore specialization in the aspen population, was significantly partitioned by environment (common garden) but not by evolutionary origin of the aspens. Significant values of modularity were observed in all network matrices. Trait-matching indicated that



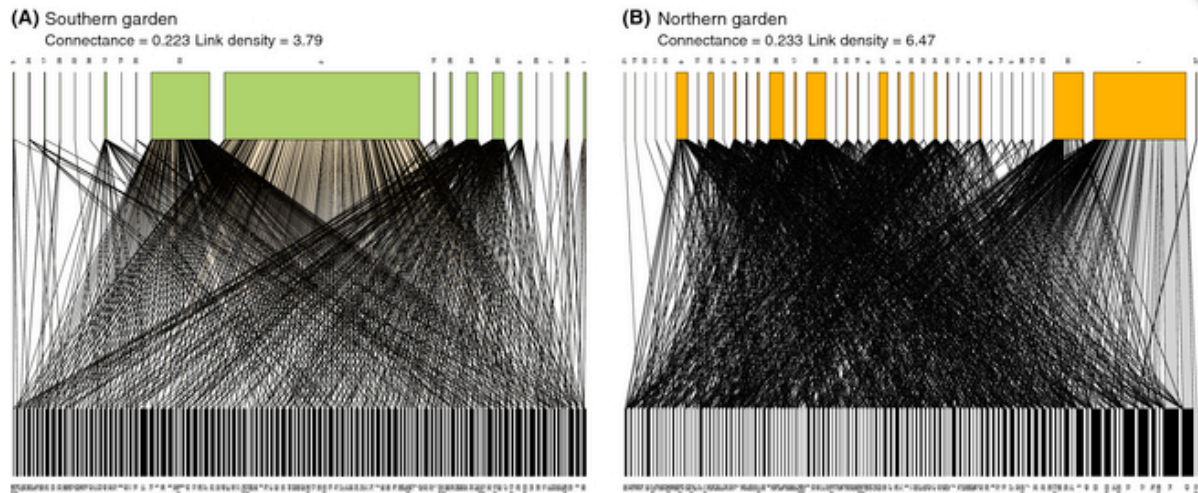


Figure 3. Bipartite graphs for all genotypes in (A) Southern garden and (B) Northern garden. The upper and lower boxes represent the higher trophic (arthropod morphospecies, detailed in Table 2) and lower trophic (aspen genotypes, each assigned a unique number) levels, respectively. Lines connecting upper and lower boxes represent interactions between morphospecies and aspen genotypes, and line thickness is scaled to the number of interactions.

Fig 7. Networks of plant-herbivore interactions in aspen

growth traits, leaf morphology, and phenolic metabolites affected modular structure in both the garden and evolutionary groups, whereas extra-floral nectaries had little influence.

We have used the leaf beetle *Chrysomela tremulae* as a model herbivore for our work with leaf chewing insects and optimised protocols for laboratory culture and maintenance. This enabled us to conduct beetle experiments on selected SwAsp clones in controlled conditions to assess both plant and beetle responses. We examined beetle feeding and oviposition preferences, showing that the beetles have distinct preferences for some clones and not for others. We have also scored fungal endophytes (Albrechtsen et al. 2010, Albrechtsen et al. 2012b) and parasitoids (Albrechtsen et al. 2012a) in the collection.

### 2.2.1.3 Genetic differentiation of “herbivore genes” (Participating researchers Carolina Bernhardsson and Kathryn Robinson, Pls Pär Ingvarsson, Benedicte Albrechtsen and Stefan Jansson)

We have examined the evolution of eight genes that are all associated with defensive responses against pests and/or pathogens, and have earlier been shown to become strongly up-regulated as a response to wounding and insect herbivory (Bernhardsson and Ingvarsson, 2011). The majority of these genes show patterns of intraspecific polymorphism and site-frequency spectra that are consistent with a neutral model of evolution but two genes showed multiple deviations from the neutral model. We have also studied 71 single nucleotide polymorphisms (SNPs) from these seven genes in the SwAsp collection. Four of them show substantial population differentiation, and a principal component analyses conducted on the defence SNPs divides the Swedish population into three distinct clusters (Bernhardsson and Ingvarsson, 2012). Five SNPs (located within T14 and T15) show strong longitudinal clines that remain significant after multiple test correction.

In addition, we studied geographic patterns of abundance and diversity of herbivorous insects, the untargeted metabolome of the foliage and genetic variation in a set of wound-induced genes and show that the geographic structure co-occurs in all three data sets. In response to this structure, we observe local maladaptation of herbivores, with fewer herbivores on local trees than on trees

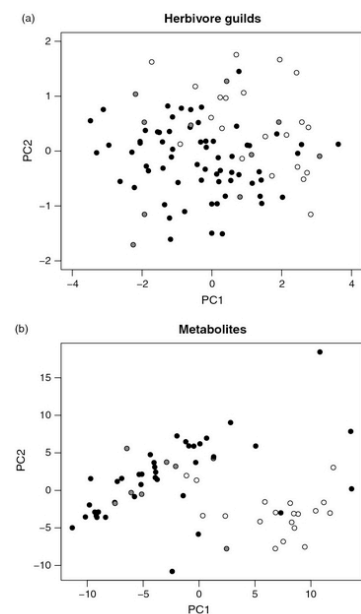


Fig 8. Phenotypic distances between population clusters

originated from more distant localities. Finally, we also identify 28 significant associations between single nucleotide polymorphisms SNPs from defence genes and a number of the herbivore traits and metabolic profiles (Bernhardsson et al. 2013, Figure 8)

We have also found that there exists no sexual heterogeneity in these traits in aspen (Robinson et al. 2014).

#### 2.2.1.4 GWAS of metabolite and herbivore traits (Pls Pär Ingvarsson and Stefan Jansson)

As mentioned above, we hoped to be able to associate all the traits measured with all polymorphisms in the aspen genome, to get more information of the genes behind the traits. This part has been significantly delayed as the work to establish the reference sequence of aspen has been delayed more than we hoped and expected. This analysis, however, is being performed at the moment and we expect to have the results ready in the beginning of 2016. RNA has also been prepared from SwAsp individuals of varying chemotypes, and subjected to deep RNAsequencing using Illumina.

#### 2.2.1.5 Field experiments with transgenic trees

We have started the biggest – at least in terms of number of genotypes – field experiment with transgenic trees in Europe. The expression levels of over 1000 aspen genes have been modified with RNAi or overexpression, in collaborations between UPSC and SweTree Technologies, and out of these, 16 constructs that show significant increase in growth (in the greenhouse) have been selected. These were planted in 2010 in two common gardens along with wild-type controls. Twelve SwAsp genotypes were also planted, in 10 replicates each, at the same site to relate the variation in traits scored to the natural variation in the same traits. For all “non-target” traits measured (for example herbivore and pathogen susceptibility), the variation among the SwAsp genotypes were larger than of the transgenic lines

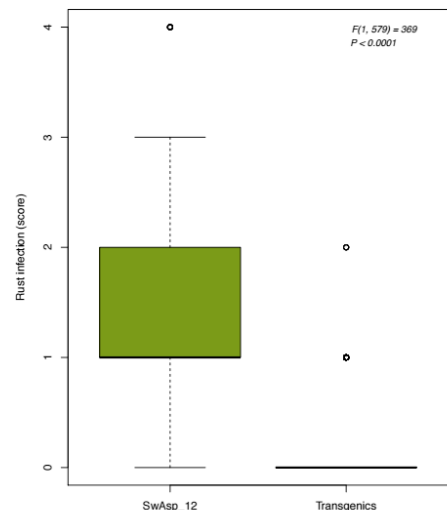


Fig 9. Phenotypic variation in foliar rust infection score between SwAsp genotypes and transgenic lines

### 2.2.2. Conifers and pine weevils

The defence system of plants consists of constitutive defences that are permanently expressed and induced defences that are only expressed in response to various stimuli, such as damages by insect pests. The advantage of induced defences is that their costs are only incurred when they are needed. The induced defence consists of physiological changes, including increased concentration of chemical defenses (terpenoids and phenolics), lignification, traumatic resin ducts in the xylem, increased biomass allocation to fine roots, reduced shoot growth and shifts in nutrient allocation from roots to shoots. All these changes can be triggered by exogenous application of Methyl jasmonate (MeJA). MeJA is a substance that also is produced naturally when a plant is attacked.



Fig 10. The experimental arena for pine weevil odour experiments

Here we combine new developments within analytical chemistry and biochemistry with improved physiological understanding of the host plant in its environment to open new areas for research to decrease the damage caused by the pine weevil. This new knowledge about the seedling's chemistry and physiology is coupled to in-depth studies of pine weevil feeding behaviour and bioassay platforms to study how various chemical cues are used by pine weevils in different phases of the process of finding and consuming food (Fig. 4 and x). Central to these studies is the capacity for testing how pine weevils sense and react to an altered physiology/biochemistry of the seedlings, e.g. as a response to induction by a chemical elicitor such as MeJA.

#### 2.2.2.1 Diel behaviour, time budget and structure of feeding behaviour of the pine weevil (Participating researchers Frauke Fedderwitz and Niklas Björklund, PI Göran Nordlander)

The diel behaviour, time budget and the detailed feeding behaviour on Norway spruce seedlings were assessed in the laboratory (Fig. 11; Fedderwitz et al. 2014, 2015a).

The locomotion pattern showed a distinct maximum during the beginning of the dark phase, whereas most feeding occurred during the second half of the dark phase and the first hours of the subsequent light phase. On average, weevils spent 34% of their time in locomotion and 6% on feeding. Analysis of the feeding behaviour of animals using such a high temporal resolution that meals can be defined may improve our understanding of the mechanisms regulating feeding. Meals can be distinguished in an ethologically meaningful manner by using the 'meal criterion', the shortest non-feeding interval between feeding bouts recognized as meals. However, such a criterion has only been determined for a few insect species. Applying a recent method developed for assessing meal criteria for vertebrates, we determined the meal criterion for the pine weevil. Weevils had 4–5 meals per day (Fig. 12). Each meal lasted about 24 min during which about 13 mm<sup>2</sup> of bark per meal were removed. The determined meal criterion was later used to evaluate the influence of chemical defences on the feeding damage caused by pine weevils (see below).

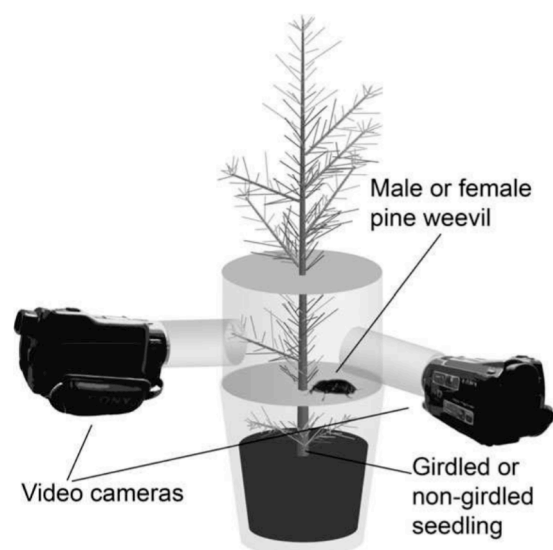


Fig 11. Setup for monitoring of pine weevil feeding behaviour

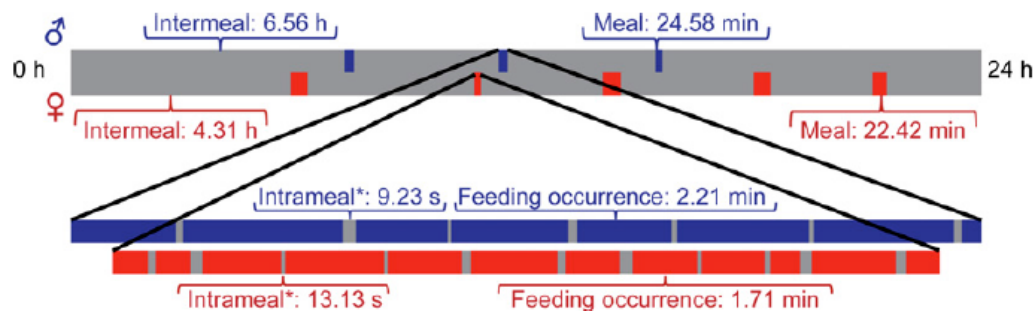


Fig 12. Schematic feeding pattern of male and female pine weevils during 24 h; medians based on mean values for individuals (\* $P < 0.05$  between sexes; linearmixed-effectsmodels)

#### 2.2.2.2 Methyl jasmonate for field protection of conifer seedlings against pine weevils (Participating researcher Niklas Björklund, PI Göran Nordlander)

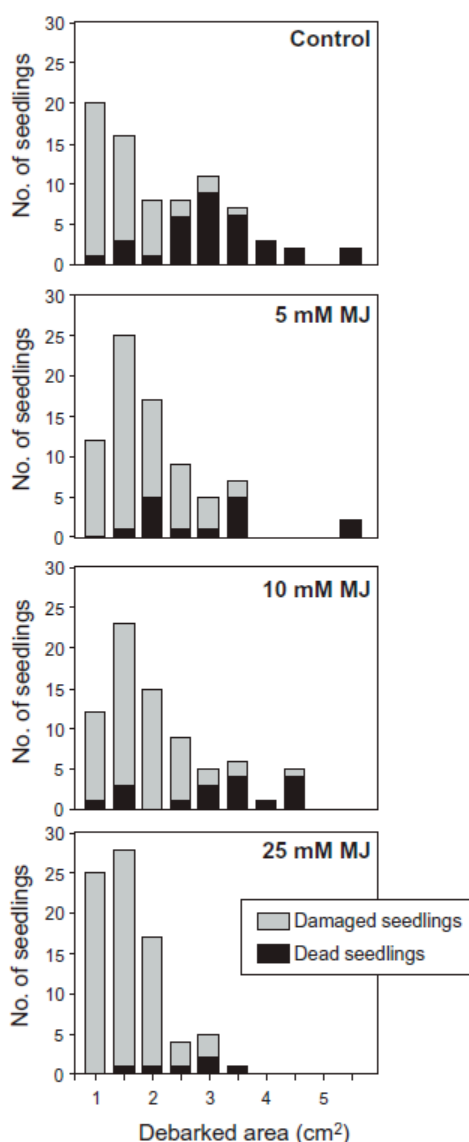


Fig. 13. Effect of methyl jasmonate application (0, 5, 10 or 25 mM MJ) on the number of attacked and killed *P. sylvestris* seedlings in relation to the amount of debarked area caused by the pine weevil (*H. abietis*) during two

Together with our Spanish collaborators we conducted two twin field experiments, one in Sweden and other in Spain, to test whether activation of defences with MeJa a few weeks before planting could help to protect conifer seedlings when planted in a highly risk site (Zas et al. 2014). One year old conifer seedlings of *Pinus sylvestris* and *Picea abies* (in Sweden) and *Pinus pinaster* and *Pinus radiata* (in Spain) were sprayed at the nursery with either 0, 5, 10, or 25 mM solution of MeJa. Four weeks after the application of the induction treatments the seedlings (560 plants of each species in each site) were planted out on a clear-cut infested by pine weevils. Weevil damage, chemical defences, and seedling growth were studied during the two growing periods following planting. In general, treated plants showed increased quantitative defences, and were less attacked, less wounded, less girdled and showed lower mortality rates than their untreated counterparts (Fig. 13). Effects were mostly dose dependent, although some interactive effects with tree species were observed. The treatment initially caused a growth reduction but it was later compensated by the benefit, in terms of growth, of being less damaged. Thus, elicitation of inducible defences in seedlings in the nursery appears to be an attractive alternative to protect conifer seedlings against pine weevils.

#### 2.2.2.3 Effects of methyl jasmonate-induced resistance in conifer plants on the feeding behaviour of pine weevils (Participating researcher Frauke Fedderwitz, Pls Niklas Björklund and Göran Nordlander)

The experiment described above showed that methyl jasmonate-induced resistance in conifer plants can protect seedlings against pine weevils. However, the insect behavioural mechanisms involved are poorly understood. Thus, effects of artificially induced plant defences on feeding behaviour of the pine weevil were examined to explore mechanisms involved in decision-making processes connected to feeding (Fedderwitz et al. 2015b). The weevils' feeding

behaviour on plants with and without MeJA treatment was studied in both a no-choice and a choice laboratory experiment. MeJA treatment did not affect the initiation of feeding, but it affected the weevils' subsequent feeding patterns. In the no-choice experiment, the only observed effect of its treatment was that it reduced the size of the initial feeding scars. In the choice experiment, it reduced both the numbers and sizes of the feeding scars and hence the total debarked area. Thus, the MeJA-induced resistance did not deter the pine weevils from attacking the spruce plants, but reduced the amounts they consumed at one place, which would reduce risks of girdling and plant death. This may be the behavioural mechanism behind the previously recorded increases in survival rates of MeJA-treated plants in the field.

#### 2.2.2.4 Chemicals produced and emitted by conifer seedlings

Considerable time has been allocated to start up our onsite built two-dimensional GC-MS. This is the key instruments used for the chiral analysis during in the project. The conifer constituents that the seedlings produce and emit to the environment have been analyzed using gas chromatography and mass spectrometry (GC-MS) with the aim to identify chemical resistance markers against various threats especially the pine weevil. To increase the knowledge of the biochemical pathways we are investigating possible activation of specific monoterpene synthases using chiral analysis: e.g. separation of the two enantiomers of monoterpenoids were made on the two-dimensional GC-MS.

Collection and extraction of needles and bark (phloem) samples have been made from *Pinus sylvestris* and *Picea abies* (Figure 15). Chemical analyses of terpenes from *P. sylvestris* treated with methyl jasmonate at two occasions during the growing period in spring 2011 resulted in a significant increase of the monoterpenes (-)- $\beta$ -pinene and 1,8-cineol (the latter a well-known pine weevil antifeedant) in the top shoots and the phloems (Lundborg et al, submitted to J Chem Ecol). We found that the MeJA treatment increased contents of the monoterpene (-)- $\beta$ -pinene in phloem (the weevil's main target tissue) of both tree species, however, the (-)- $\beta$ -pinene/(-)- $\alpha$ -pinene ratio increased more in the phloem of *P. sylvestris*. We also found an increase of (-)- $\beta$ -pinene in the phloem of *Pinus radiata* and *P. pinaster* where the pine weevil or the processionaria moth (*Thaumetopoea processionaria*) larvae had eaten (Moreira et al 2013).

Diterpene acids in the phloem of MeJA treated and control plants of the Spanish pines *P. radiata* and *P. pinaster* were methylated and separated by GC-MS. Mainly quantitative differences were observed between the MeJA treated and control plants with higher amounts in MeJA treated plants.

We also tested the attractiveness of individual monoterpenes found in conifer tissues (needles and phloem) for pine weevils using an arena with traps baited with single-substance dispensers and pine twigs. Trap catches were reduced when the pine material was combined with a dispenser releasing (-)- $\beta$ -pinene, (+)-3-carene, (-)-bornyl acetate or 1,8-cineole. However, (-)- $\alpha$ -pinene did not have this effect. Thus, the greater field protection of MeJA-treated *P. sylvestris* seedlings may be due to the selective induction of the deterrent (-)- $\beta$ -pinene, in contrast to strong increases in both non-deterrent (-)- $\alpha$ -pinene and the deterrent (-)- $\beta$ -pinene in *P. abies* seedlings.



Fig 14. Collection of volatiles

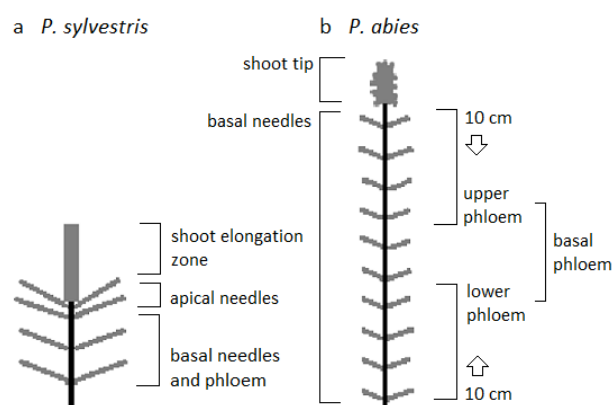
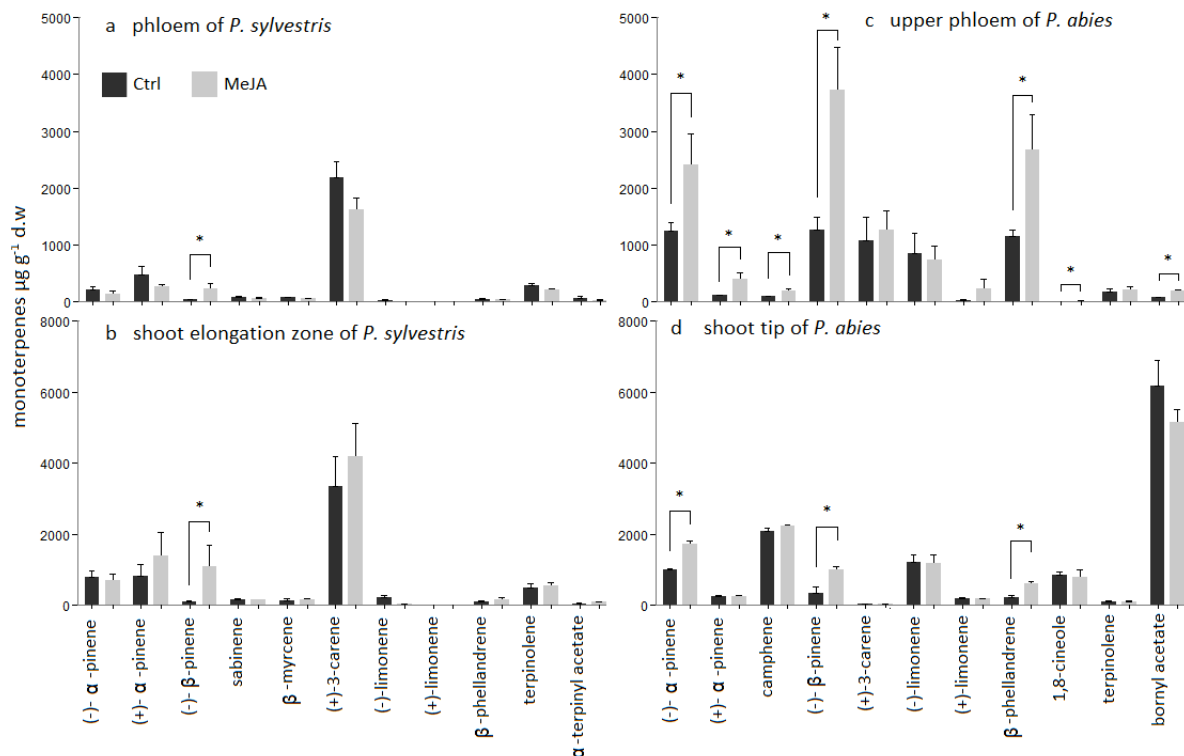
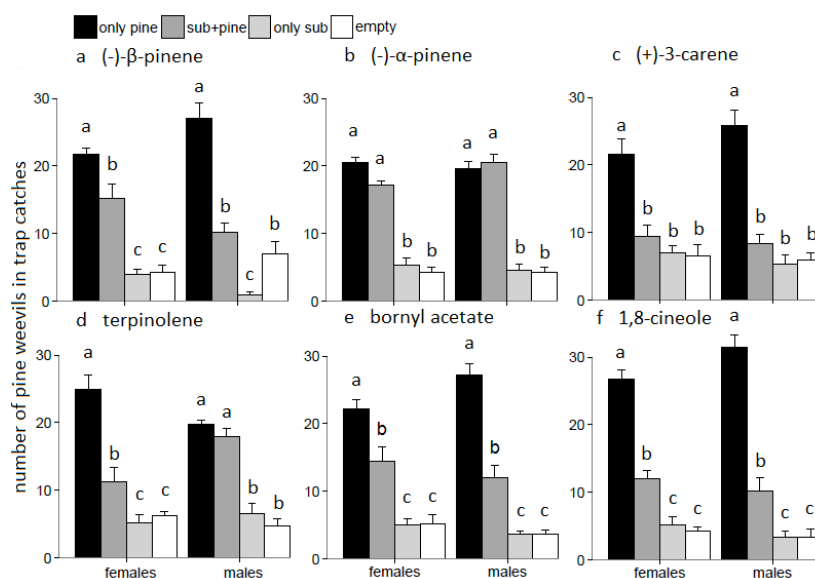


Fig 15 Scheme of chemical sampling sites of tissues of (a) *Pinus sylvestris* and (b) *Picea abies*





**Fig 16.** Effect of MeJA treatment of the monoterpenoids produced in the seedling tissues. Contents of total monoterpenes in tissues of (a, b) *P. sylvestris* and (c, d) *P. abies* seedlings, both basal (a, c) and apical (b, d), expressed in absolute amounts of pentadecane equivalents ( $\mu\text{g g}^{-1}$  d.w.) + SE. The asterisks between bars indicate significant differences according to *t*-tests at  $P < 0.05$  ( $N = 16$ )



**Fig 17** Effect of induced terpenoids on the number of pine weevils trapped. Catches of pine weevils in traps baited with pine twigs (pine) selected volatiles (sub) alone and together, and empty controls, in a multi-choice arena bioassay. Error bars denote SE. Different letters indicate significant differences according to the Tukey range test at a 95% confidence level.

The effects of MeJA on conifer chemical defences of Scots pine seedlings (*Pinus sylvestris* (L.)) and its effects on feeding behaviour of the pine weevil (*Hylobius abietis* (L.)) on phloem and needles was investigated (Figure 16). To our knowledge, this is the first study to combine chemical analysis of artificially activated conifer defences with effects on short-term feeding behaviour of a phytophagous insect. Solvent extracts of phloem and needles were analysed by two dimensional GC-MS. In parallel, meal properties of pine weevils were analysed based on video-recordings of weevils feeding on seedlings from the same MeJA treatment batch. Weevil feeding activated production of the pine weevil attractant monoterpene (+)- $\alpha$ -pinene in the phloem of control seedlings but not in the MeJA-

treated seedlings. Treatment with MeJA also increased the antifeedant 2-phenylethanol in the tissues, which might influence pine weevils to feed shorter meals on MeJA-treated seedlings and female pine



weevils also decreased feeding rate on MeJA phloem. Seedlings treated with MeJA had more similar volatile content in phloem and needles. In accordance, meal duration on phloem and needles was more similar on MeJA treated seedlings than on control seedlings.

#### **2.3.2.5 Introducing new analytical techniques: Analysis of monosaccharides and amino acids from conifer seedlings using CE**

The pine weevil has antennal and taste receptors for both sugars and amino acids (A. Wibe, unpublished). Therefore the content and distribution of both sugars and free amino acids should be measured in the conifer seedlings to fully understand the feeding preferences of the pine weevil.

The number of compounds produced in the conifer tissues is much higher than for *Populus*. It is therefore of pivotal importance to separate the compounds as they have different properties, different taste and thus affect the overall preference and performance of the feeding pine weevil.

At KTH we have developed a miniaturized method based on capillary electrophoresis (CE) for separating primary and secondary metabolites that only need small amounts of material (Emmer et al.). Both sugars and amino acids have been analysed in samples from spruce seedlings treated and non-treated with MeJA and a clear qualitative difference between the treatments was observed (KTH, Diploma works 2013,2014).

In Lundborg et al (manuscript in preparation) the difference in phenolics and amino acids in the bark were determined by CE-MS in high and low susceptible spruce seedlings infested by the bark beetle associated fungi *Ceratocystis polonica*. These results in combination with the terpene analyses in the bark, differences in induced and constitutive chemical composition in low and high susceptible spruce clones were found.

#### **2.3.2.6 Is there a genetic variation for pine weevil resistance in the Swedish spruce population? (Participating researcher Niklas Björklund, Pls Göran Nordlander and Stefan Jansson) Financed by the “Nyttiggörande”-allocation 600 kkr.**

The rapid progress of the Spruce genome project has opened up new avenues to study parasite resistance in conifers. Genome-wide association mapping studies in spruce – only a dream three years ago – will soon become a reality as the *Spruce genome project* attempt to perform high-throughput genotyping of at least 1000 individual genotypes from the Swedish spruce breeding program, which is aimed to become “THE Swedish spruce association mapping population”. However, in order to use this technique to identify genes behind and genetic markers for resistance towards pine weevils, there must exist a heritable and measureable genetic variation in pine weevil resistance in the population. So far, no research has been performed in Sweden that directly have aimed at assessing this, but studies performed by our Spanish collaborators have indicated that at least in Spanish conifer breeding populations, significant variation exist.

PaReTree initiated a screening project to study this. 52 parental trees with appropriate provenance from the Swedish breeding program were selected, and 200 offsprings from each of them (i.e. about 10 000 seedlings) were planted in 2012 on a very uniform clear-cutting area with expected moderate pressure of pine weevils. Feeding damage by pine weevil (and by bark beetles of the genus *Hylastes*) on these seedlings was monitored during two seasons. The proportions of seedlings attacked and killed by pine weevil as well as feeding scar area for each seedlings were assessed. In a follow-up experiment we selected 24 out of the 52 families that were included in the first experiment with the aim to cover the whole range in resistance. The selection was based on results from the first season of the first experiment. One hundred seedlings from each family were planted during the spring 2015 and damage was recorded in the autumn. We found a significant family effect for debarked area and there was a lack of relevant GxE interaction. These encouraging results indicate that the exploitation of variation in genetic resistance can contribute to the integrated pest management methods against this pest.

#### **2.3.2.8 Sequencing the pine weevil genome**

Within the Spruce genome project, world-leading competence in sequencing of complex genomes using Next-Generation Sequencing techniques and all downstream analysis has been developed, as

the spruce genome is the largest genome – all categories – that have been sequenced to date. This infrastructure and excellence is planned to be used in the studies of other plant genomes, but could also be used to study much smaller and less complex genomes as insect genomes, the genomes of Coleopterans – to which pine weevils belong – are typically only 10-20 % of the size of the human genome, i.e. less than 1/50 of the spruce genome. We argued that if PaReTree would provide the “biological competence”, including DNA preparations from pine weevils, the Spruce genome project has expressed willingness to generate the sequence data and use to competence of the assembly and bioinformatics team for help with the initial analysis. We have also established contacts with Martin N Andersson at Lund university who would like to carry on the analysis. We prepared pine weevil DNA with a quality sufficient for Illumina sequencing, but as we decided it would be better to use the PacBio technology – which yields much longer reads – the DNA preparations were not good enough; longer reads requires longer DNA. We are currently redoing the DNA preparations, hoping for better quality, this will have to be financed by other grants.

### **3. The “graduates” of the program**

PaReTree financed **three PhD students**: Vicki Huizu Guo, (supervisors BA and SJ), Frauke Fedderwitz (GN and NB) and Lina Lundborg, (AKB-K) and had **three associated PhD students** Carolina Bernhardsson (SJ, BA and PI), Karolin Axelsson (AKB-K), and Franziska Bandau (BA) which utilize the resources of PaReTree. All of them are still in academia, but none have established themselves as independent researchs (yet).

An important purpose with workshop and excursion activities (see below) was to provide a productive environment for PaReTrees PhD students (and post doctoral fellows). At every event the junior members of the project have presented their research. Expert scientists have been invited to the workshops in an effort to bring the activities up to highest international standard. The PhD students are also encouraged to apply for research schools together and most students (FB, FF, VH, LL) participated in a relevant course in Alnarp in May 2011. The students also participated in a workshop in Spain in September 2012 in connection with our excursion. Common and specific PhD training is given locally, for example in scientific writing, metabolomics, Q-PCR etc according to need. The program itself has not developed “formal courses”, but external PhD students have participated in some of the workshops and excursions. A joint meeting/excursion with the SSF “sister project” **“Understanding and preventing root rot of conifers”** has also been held.

#### **4. Impact of the project – to industry and society**

Through several years of work within the Swedish *Hylobius* Research Program (Snytbaggeprogrammet) we have built up a **rich network of contacts with the Swedish forestry sector**, i.e. forest companies, forest owner's organisations, forest plant nurseries, certification organisations (FSC), The Swedish Forest Agency, The Forest Research Institute of Sweden, etc. Our expertise regarding the pine weevil problem is frequently asked for and used by industry, organisations, and media. We thus feel that our information and advice has considerable impact. Information is primarily mediated through the website [www.snytbagge.se](http://www.snytbagge.se) but also through other media and participation in various seminars and meetings for the forestry sector.

Every year we are also conducting several studies on commission by different forest companies and by The Swedish Forest Agency. Generally, we have been free to publish the results of these studies although made on commission. This cooperation has given us deeper insights in current and forthcoming trends in Swedish forest management, and has thereby been of strategic help for us.

As Skogforsk now are being integrated in the activities of PaReTree – through participation in our workshops and common projects – full transfer of knowledge to the tree breeders is ensured.

A major presentation and discussion of the results from this project with stakeholders was arranged at the meeting "Plantans dagar" held at Gysinge 26-27 November 2014. "Pine weevil" was the main theme of this meeting, which was attended by about 100 participants, mainly people involved with production of forest plants and with forest regeneration. Speakers included Göran Nordlander, Niklas Björklund, Stefan Jansson and also Jan Stenlid and Bo Karlsson from the companion SSF project on root rot. We feel that this meeting provided a very efficient platform for communicating results of the SSF projects and for discussing possible future implementation with stakeholders.

## **5. Impact of the project – to the academic system**

### **5.1 Collaborations between different disciplines and departments**

As obvious from the research plan - and the publication list - almost all research in PaReTree is conducted in collaboration between two or more of the involved research groups/departments involved. XX of the XX publications has authors from more than one department. All the groups working at UPSC meet frequently and have sometimes joint group meetings. To strengthen the links between the sites, lab visits of individuals or groups took place. PaReTree has organised 3 workshops, 3 excursions (one together with “Understanding and preventing root rot of conifers”) and one “Sequence meeting”.

### **5.2 Collaborations between universities**

Likewise, as the departments involved belong to four universities, the same holds true here. The administrative issues were handled smoothly by the four universities.

### **5.3 Collaborations with other SSF projects**

As mentioned above, we had close contacts and one joint excursion with “Understanding and preventing root rot of conifers”.

### **5.4 International collaborations**

*Prof. Joerg Bohlmann* **University of British Colombia (Canada)** visited one WS and was associated with PaReTree – and also the Spruce genome project –, and is expert on terpenes, phenolics and defense biology of both *Populus* and conifers. *Prof. Paal Krokene* **Norwegian Forest and Landscape Institute** participated in one WS and one excursion, he is an expert in conifer defence mechanisms. Main collaboration partners in the aspen part include *Prof. Richard Lindroth's group* at **University of Wisconsin, USA**. KKR worked fulltime for two years within this collaboration, and was at the end of the project partially employed in the US; partially in Sweden. For chemical analyses there was a collaboration with *Dr. Yuxin Pei* in **China**. The effort to identify the genes behind biosynthesis of phenolic glycosides was coordinated with *Professor C.J. Tsai* (**University of Georgia, USA**), (who visited one WS), world-leader in this field. Herbivore community modelling collaboration is performed in collaboration with *Dr. Nicolas Loeuille* at the **Pierre and Marie Curie University in Paris, France**. Extended studies of phenolic profiling are being performed together with *Dr. Christian Lexer*, **University of Fribourg, Switzerland**.

In the sub-project: Conifers and pine weevil most research was conducted in close cooperation with the leading research team on defence in conifers at **CSIC**, Pontevedra, Spain (*Rafael Zas, Luis Sampedro, Xoaquín Moreira*; see <http://genecolpines.weebly.com/>). They participated in most meetings an arranged one excursion, and several shorter visits took place (in both directions).

Several PaReTree researchers have visited these labs – and vice versa – during the project.

Pär Ingvarsson participates in the EU project “ProCoGen” on conifer – in particularly pine – genomics.

### **5.5 Contribution to mobility of students and researchers**

Three researchers (one professor, one postdoc and one PhD student/postdoc) involved changed university affiliation (between the participating universities) during the course of the project. In addition, many research visits in other labs (including the international collaborators) during the project.

### **5.6 Contribution to academic research**

The project has been an opportunity for researchers to interact interdisciplinary and to build networks within and out side Sweden. Connections of aspen work has included collaborations mainly with the group pf Professor Rick Lindroth and a uni-lateral exchange between UmU and UW established, with students visiting UmU from UW. This exchange has been active for three years 2013-2015. Another example of network strengthening is that BA was invited as a co-responsible and representative at the

meeting of the chemical ecological society in Stockholm by AKB-K, secretary of the chemical ecological society, and contributed to the meeting financing of a forest biotic stress symposium. Two of the postdocs of the project have now long-term academic positions, Kathryn Robinson is at UPSC and Ken Keefover-Ring in Madison University of Wisconsin.

**5.7 What has it meant to the researchers in the project?**

The project has meant freedom to do meaningful research under responsibility, a freedom to interact, develop new ideas and create networks.

**5.8 Relations between host university and participating universities**

Very good

**5.9 What has the project meant for the universities locally?**

There is no doubt that the project has contributed to better contacts between the involved departments/universities, and better research in this area.

**5.10 Contribution to handling of immaterial rights**

None

**5.11 Changes in the university system induced by the project**

None



## 6. Lessons from the project

As we see it, the main lessons to be learned from the project are:

- Identifying defence chemicals affecting feeding preferences is even more complex in trees than in most other plants due to the enormous phenotypic and genotypic variation in tree populations. Yet, we think this is a way forward, in particular if it can be combined with genomic studies
- Defence chemistry in trees is a driver of differentiation and can influence the whole ecosystem - the “Extended phenotype” concept realized
- There may be no genetic variation in pine weevil resistance in the Swedish spruce breeding population, at least no that for the moment can be utilized in breeding
- MeJA pre-treatment may be a way forward to reduce seedling susceptibility toward pine weevils

## 7. Outlook

As obvious from the background and the concrete goals, much of the research will be continued after the project is finished and applications are expected to be realized during that phase.

- The SwAsp and UmAsp common gardens will be maintained and scored for herbivore damage, similar to what we do within this project; there is a plan for continued funding for these activities.
- All these data will be used as traits for genome-wide association mapping.
- Likewise, as the amount of data from these gardens accumulate, improved correlation and co-variation analyses will be possible
- If – or rather when - *Populus* species or hybrids will become more used as commercial trees in Sweden, the markers developed in this program could be used in breeding programs
- The understanding of the pine weevil feeding behaviour will be used to better score for weevil susceptibility
- Methodology to trigger the defence of conifer seedlings by MeJA pre-treatment in the nursery that make the seedlings less susceptible for pine weevil attack is currently developed with the aim of use under standard commercial conditions
- It is possible, even likely, that the Swedish tree breeding programs will start to adopt methods like genomic selection, and then projects like this that could develop markers for beneficial traits will be important (although there may be no good marker for weevil resistance)
- The project to sequence the pine weevil genome will hopefully be completed. This will lead to many new opportunities for the pine weevil research and may result in a new wave of research projects that capitalize on this resource

We think and hope that in ten years from now, pine weevil will be less of a problem for Swedish forestry, due to better knowledge on pre-treatment of seedlings, better forestry practices and, hopefully, also better genetic material, in particular will the combination of these strategies be the way forward .

## 8. Economic report

	2010	2011	2012	2013	2014	Total
	Year 1	Year 2	Year 3	Year 4	Year 5	Sum
PI Salary	242 592	252 470	252 092	260 941	0	1 008 095
Seniors Salary	504 301	1 541 424	543 800	443 759	491 328	3 524 612
Postdoc salary	210 017		1 102 173	532 810	768 135	2 613 135
PhD Salary	210 574	996 473	891 769	1 154 398	1 265 633	4 518 847
Equipment						0
Materials/travels	511 112	713 184	878 555	437 736	226 918	2 767 505
Exploitation of results						0
Administration						0
Information						0
Other costs	3 640					3 640
<b>Sum costs</b>	<b>1 682 236</b>	<b>3 503 551</b>	<b>3 668 389</b>	<b>2 829 644</b>	<b>2 752 014</b>	<b>14 435 834</b>
Universitets OH	509 186	982 940	1 444 848	854 776	1 172 416	4 964 166
% OH	30,27%	28,06%	39,39%	30,21%	42,60%	34,39%
<b>Sum incl VAT</b>	<b>2 191 422</b>	<b>4 486 491</b>	<b>5 113 237</b>	<b>3 684 420</b>	<b>3 924 430</b>	<b>19 400 000</b>
Stefan Jansson	1 286 647	2 139 562	2 913 261	1 713 520	2 050 236	10 103 226
A-K Borg Karlsson, KTH	315 540	763 645	634 482	766 911	599 422	3 080 000
Vaughan Hurry	301 021	733 053	675 956	241 744	0	1 951 774
Göran Nordlander, SLU	288 214	850 231	889 538	962 245	1 274 772	4 265 000
	<b>2 191 422</b>	<b>4 486 491</b>	<b>5 113 237</b>	<b>3 684 420</b>	<b>3 924 430</b>	<b>19 400 000</b>

## **A Appendices**

### **A.1 A list of everybody who has at any time been a member of the project steering group including affiliation and their period.**

The steering group has through the whole project consisted of

Stefan Jansson (Umu)  
Benedicte Albrechtsen (Umu)  
Pär Ingvarsson (Umu)  
Göran Nordlander (SLU)  
Anna-Karin Borg-Karlson (KTH)  
Vaughan Hurry (UMU, later SLU)  
Torgny Näsholm (SLU)

**A.2 A list of the activities and responsibilities of the steering group (if any)**

No

**A.3 A list of the researchers (senior researchers, postdocs, ...) including university and department, type of position, project, year-of-birth and gender.**

Stefan Jansson, Umu, UPSC, Department of Plant Physiology, professor. born 1959, male

Benedicte Albrechtsen, Umu, UPSC, Department of Plant Physiology, Researcher. born 1960, female

Pär Ingvarsson (Umu). Umu, UPSC, Department of Ecology and Environmental sciences. professor. born 1969, male

Göran Nordlander, SLU, Department of Ecology, professor, born 1952, male.

Anna-Karin Borg-Karlson, KTH, Department of Chemistry, professor, born 1949, female.

Vaughan Hurry Umu, UPSC, Department of Plant Physiology, professor. born 1960, male (Later SLU, Department of Forest Ecology and Management)

Torgny Näsholm, SLU, Department of Forest Ecology and Management, professor, born 1959, male

Kathryn Robinson (Umu later SLU). UPSC, Department of Plant Physiology, researcher. born 1975, female

Ken Keefover-Ring (Umu). UPSC, Department of Plant Physiology, postdoc. born 1965, male

Niklas Björklund, SLU, Department of Ecology, researcher (docent), born 1974, male.

Jeanette Nilsson (Umu). UPSC, Department of Plant Physiology, researcher. born 1974, female

**A.4 A list of selected publications (books, articles in refereed journals, papers presented at conferences, reviews, other publications). Indicate clearly publications with international and/or industrial co-authors.**

What is the cross-national share? 33 %  
The cross-university share? 56 %  
The crossdepartmental share? 61 %  
The cross-project share? 28 %

Only publications where SSF funding is relevant and thus duly acknowledged should be included.

Authors funded by the SSF project are in **bold**.

**Articles in refereed journals (\*=with international co-author):**

Abreu IN, Ahnlund M, Moritz T, **Albrechtsen BR** (2011) UHPLC-ESI/TOFMS determination of salicylate-like phenolic glycosides in *Populus tremula* leaves. Journal of Chemical Ecology: 37:857-870

\*Azeem, M., Rajarao, G. K., Terenius, O., **Nordlander, G.**, Nordenhem, H., Nagahama, K., Norin, E., & **Borg-Karlson, A. K.** 2015. A fungal metabolite masks the host plant odor for the pine weevil (*Hylobius abietis*). Fungal Ecology 13: 103-111. <http://dx.doi.org/10.1016/j.funeco.2014.08.009>

Bandau F, **Decker VHG**, Gundale MJ, **Albrechtsen BR** (2015) Genotypic Tannin Levels in *Populus tremula* Impact the Way Nitrogen Enrichment Affects Growth and Allocation Responses for Some Traits and Not for Others. PLoS ONE 10(10): e0140971

**Bernhardsson C, Ingvarsson PK** (2011) Molecular Population Genetics of Elicitor-Induced Resistance Genes in European Aspen (*Populus tremula* L., Salicaceae). PLoS One.6(9):e24867..

**Bernhardsson C, Ingvarsson PK** (2012) Geographical structure and adaptive population differentiation in herbivore defence genes in European aspen (*Populus tremula* L., Salicaceae). Mol Ecol. 2012 Mar 15

**Bernhardsson C, Robinson KM, Abreu IN, Jansson S, Albrechtsen BR, Ingvarsson PK** (2013) Geographic structure and adaptive population differentiation in herbivore defence genes in European aspen (*Populus tremula* L., Salicaceae). Molecular Ecology 21(9), 2197–2207. doi: 10.1111/j.1365-294X.2012.05524.x.

**Fedderwitz, F., Björklund, N., Ninkovic, V. & Nordlander, G.** 2014. Diel behaviour and time budget of the adult pine weevil, *Hylobius abietis*. Physiological Entomology 39: 103-110. <http://dx.doi.org/10.1111/phen.12053>

**Fedderwitz, F., Björklund, N., Ninkovic, V. & Nordlander, G.** 2015. The structure of feeding behavior in a phytophagous insect (*Hylobius abietis*). Entomologia



- Experimentalis et Applicata 155: 229-239.  
<http://onlinelibrary.wiley.com/doi/10.1111/eea.12302/abstract>
- Fedderwitz, F., Nordlander, G., Ninkovic, V. & Björklund, N.** 2015. Effects of jasmonate-induced resistance of conifer plants on the feeding behaviour of a bark-chewing insect, *Hylobius abietis*. Journal of Pest Science  
<http://dx.doi.org/10.1007/s10340-015-0684-9>
- Gruffman A., Ishida T., Nordin A, **Näsholm T.** 2012. Cultivation of Norway spruce and Scots pine on organic nitrogen improves seedling morphology and field performance. Forest Ecology and Management 276: 118–124.  
<http://dx.doi.org/10.1016/j.foreco.2012.03.030>
- Keefover-Ring K,** Carlsson M, **Albrechtsen BR** (2014). 2'-(Z)-Cinnamoylsalicortin: A novel salicinoid isolated from *Populus tremula*. Phytochemistry Letters, 7:212–216
- Keefover-Ring K,** Ahnlund M, Abreu IN, **Jansson S,** Moritz T, **Albrechtsen BR** (2014) No Evidence of Geographical Structure of Salicinoid Chemotypes within *Populus Tremula*. PLoS ONE 9(10):e107189
- \*Moreira, X., **Lundborg, L.,** Zas, R., Carrilo-Gavilán, A., **Borg-Karlson, A.-K. &** Sampedro, L. 2013. Inducibility of chemical defences by two chewing insect herbivores in pine trees is specific to targeted plant tissue, particular herbivore and defensive trait. Phytochemistry 94: 113-122.  
<http://dx.doi.org/10.1016/j.phytochem.2013.05.008>
- \*Nystedt B, Street NR, Wetterbom A, Zuccolo A, Lin Y, Scofield DG, Vezzi F, Delhomme N, Giacomello S, Alexeyenko A, Vicedomini R, Sahlin K, Sherwood E, Elfstrand M, Gramzow L, Holmberg K, Hallman J, Keech O, Klasson L, Koriabine M, Kucukoglu M, Kaller M, Luthman J, Lysholm F, Niittyla T, Olson A, Rilakovic N, Ritland C, Rossello JA, Sena J, Svensson T, Talavera-Lopez C, Theiszen G, Tuominen H, Vanneste K, Wu Z, Zhang B, Zerbe P, Arvestad L, Bhalerao R, Bohlmann J, Bousquet J, Garcia Gil R, Hvidsten TR, de Jong P, MacKay J, Morgante M, Ritland K, Sundberg B, Lee Thompson S, Van de Peer Y, Andersson B, Nilsson O, **Ingvarsson PK,** Lundeberg J, **Jansson S** (2013) The Norway spruce genome sequence and conifer genome evolution. Nature 497(7451):579-584 (No funding acknowledgment in this kind of article)
- Robinson KM, Ingvarsson PK, Jansson S, Albrechtsen BR** (2012) Genetic Variation in Functional Traits Influences Arthropod Community Composition in Aspen (*Populus tremula* L.). PLoS ONE 2012; 7(5):e37679
- \***Robinson KM,** Delhomme N, Mähler N, Schiffthaler B, Onskog J, **Albrechtsen BR, Ingvarsson PK,** Hvidsten TR, **Jansson S,** Street NR (2014). *Populus tremula* (European aspen) shows no evidence of sexual dimorphism BMC Plant Biol. 14(1):276
- \***Robinson KM,** Hauzy C, Loeuille N, **Albrechtsen BR** (2015). Relative impacts of environmental variation and evolutionary history on the nestedness and modularity of tree–herbivore networks. Ecology and Evolution 2015; 5(14): 2898–2915
- \*Zas, R., **Björklund, N., Nordlander, G.,** Cendán, C., Hellqvist, C. & Sampedro, L. 2014. Exploiting jasmonate-induced responses for field protection of conifer

seedlings against a major forest pest, *Hylobius abietis*. Forest Ecology and Management 313: 212-223. <http://dx.doi.org/10.1016/j.foreco.2013.11.014>

### Manuscripts to be submitted:

- Axelsson, K.**, Kruse, V., Rajarao, G. K., Seriot, L., Nordenhem, H., **Nordlander, G.** and **Borg-Karlson, A.-K.** Antifeedants produced by bacteria associated to the gut of the pine weevil (*Hylobius abietis*).
- Fedderwitz, F.**, **Björklund, N.**, Ninkovic, V. & **Nordlander, G.** Does the pine weevil (*Hylobius abietis*) prefer conifer seedlings over other utilized food sources?]
- Keefover-Ring K.**, Martin N, Robert S, and **Albrechtsen B.** Use of aspen host compounds by poplar leaf beetle for its own defense
- Keefover-Ring K.**, Ericsson S, Cole CT, and **Albrechtsen B.** Salicinoid diversity in Swedish Salix
- Lundborg, L.**, **Fedderwitz, F.**, **Björklund, N.**, **Nordlander, G.**, Ohlsson, A. & **Borg-Karlson, A.-K.** Conifer chemical defenses influence meal properties of the pine weevil.
- Lundborg, L.**, **Nordlander, G.**, **Björklund, N.**, Nordenhem, H. & **Borg-Karlson, A.-K.** Methyl jasmonate induced volatiles in Scots pine and Norway spruce tissues affect pine weevil orientation.(submitted)
- Robinson KM**, **Möller L** .**Hertzberg M**, **Jansson S.** Variation in non-target traits in genetically modified aspen is smaller than the natural variation)

### Conference papers/abstracts

- Björklund, N.**, **Nordlander, G.**, **Fedderwitz, F.**, Ninkovic, V. **Lundborg, L.**, Sampedro, L. & Zas, R. 2015. Improved forest regeneration by triggering the induced defence of conifer seedlings against bark-feeding insects. P 163 in: ISCE2015, International Society of Chemical Ecology, Stockholm, Sweden 29<sup>th</sup> June – 3<sup>rd</sup> July, 2015.
- Fedderwitz, F.**, **Björklund, N.**, Ninkovic, V. & **Nordlander, G.** 2012. Pine weevil (*Hylobius abietis*) feeding pattern on conifer seedlings. Pp. 333-337 in: Proceedings of Measuring Behavior 2012 (Utrecht, The Netherlands, August 28-31, 2012) 524 pp.
- Lundborg, L.**, Moreira, X., Zas, R., Sampedro, L., **Björklund, N.**, Hellqvist, C., **Nordlander, G.**, & Borg-Karlson A.-K. 2012. Chemical analysis of methyl jasmonate treated Scots pine *Pinus sylvestris* using GC-MS and LC-MS. Oral presentation, ISCE, Vilnius, 2012.
- Zas, R., **Björklund, N.**, **Nordlander, G.**, Cendán, C., Hellqvist, C. & Sampedro, L. 2013. Moving to the field: effective protection of conifer seedlings against a forest pest by eliciting jasmonate-induced resistance. Proceedings: Induced resistance in plants against insects and diseases, Avignon, France, 10-13 June 2013. IOBC-WPRS Bulletin 89: 277-281.

## **A.5 A full list of events organised by the project (conferences, work shops, summer schools, industry meetings, ...)**

**WS 1, February 2011 (Umeå) Soil fertility and experimental studies of plant defence.** Participants: BA, CB, VH, PKI, SJ, VH, KKR, JN, TN, KMR, NB, FF, GN, AKBK and LL. External participants from SLU Umeå: Michael Gundale, University of Galicia: Luis Sampedro, Rafael Zas, Turkey: Ceren Saglem.

**Excursion 1, August 2011 (Uppsala).** Experiments with *Hylobius abietis*, field excursions, seminars, discussions and networking. Participants: BA, VH, SJ, KMR, NB, FF, GN, AKBK, LL, Tao Zhao from KTH and Norisha Kusumoto from Japan.

**WS 2, February 2012 (Vindeln, Västerbotten) Association mapping and defence genes.** Participants: BA, CB, FB, VH, PKI, SJ, VH, KK-R, JN, TN, KMR, NB, FF, GN, AKBK, LL. External participants included from University of Wisconsin: Richard Lindroth, Liza Holeski, University of Georgia: CJ Tsai. University of British Columbia: Jörg Bohlmann. Umeå University: Nathaniel Street, SLU, Umeå: Michael Gundale, University of Galicia: Luis Sampedro, Rafael Zas, KTH: Tao Zhao; KTH and University Akita University, Japan: Norisha Kusumoto, Skogforsk: Bo Karlsson (Ekebo) and Bengt Andersson (Sävar), SLU, Uppsala: Jan Stenlid (leader of the SSF “sister project” Understanding and preventing root rot of conifers), SSF-representative; Christer Larsson.

**Excursion 2 September 2012 (Madrid, Spain), Pine forest diversity and resistance properties.** Participants: NB, FF, GN, AKBK, LL, BA, VH, SJ, KKR, JN, KR. External participants: Luis Sampedro, Rafael Zas (CSIC, Pontevedra, Spain), Jose Climent, Santiago Gonzalez-Martinez, Katharina Budde, Luis Santos, Alejandro Solla (CIFOR-INIA, Madrid, Spain), Marta Silva, Marta W Vasconcelos (Escola Superior de Biotecnología, Portugal), Paal Krokene (NBIO, Norway).

**WS3, Feb 2013 (Stockholm) Method meeting, including chemical, behavioral and practical breeding methods.** Participants: AKBK, LL, TZ, MD, ÅE (KTH); NB, FF, GN, (SLU); SJ, TN, BA, VH (UPSC). External participants: Aileen Berasategui (Max Planck Inst., Jena); Rafael Zas, Luis Sampedro (CSIC, Spain) Paal Krokene (NIBIO, Norway).

**Excursion 3 September 2013 (Ekebo and surroundings, Skåne),** Joint with “Understanding and preventing root rot of conifers”

**“Hylobius Sequencing meeting” February 2015 (Uppsala). Discussions about Hylobius genomics.** Participants: NB, GN, SJ, External participants: Nathaniel Street, Umu, Martin N Anderson (LU), Fredrik Schlyter (SLU Alnarp), Krystyna Nadachowska-Brzyska (UU), Anna Lundhagen (SLU), Paal Krokene and Adam Vivian-Smith (NBIO, Norway)

**A.6 A full list of all graduate/post-graduate courses developed within the project.**

None

**A.7 PhD exams. Enclose an updated list of students who have completed their PhD. Include at least year of birth, gender, thesis title, supervisor(s), university department, year of degree, university of basic academic training, total amount of Foundation funding received, and employer six months (or at a later time if available) after exam.**

**Carolina Bernhardsson**, female, born 1982. Thesis title: Molecular population genetics of inducible defense genes in *Populus tremula*. ISBN 978-91-7459-415-7. Department of Ecology and Environmental Science, Umeå University. Supervisor Prof. Stefan Jansson, Department of Plant Physiology, Umu (assistant supervisors: Docent Folmer Bokma, Department of Ecology and Environmental Science, Umu, and Docent Benedicte Albrechtsen, Department of Plant Physiology, Umu). Dissertation 2012-05-16. Full time funded by Umeå University. Thereafter employed as senior research engineer for 6 months at the same department and financed by SSF, followed by a 2 year Postdoc at the Department of Forest Genetics and Plant Physiology, SLU, funded by another project.

**Frauke Fedderwitz**, female, born 1986. Thesis title: Pine weevil feeding behaviour in relation to conifer plant properties. Doctoral Thesis No 2014:106, Faculty of Forest Sciences, SLU. Supervisor Prof. Göran Nordlander (assistant supervisors: Docent Niklas Björklund and Docent Velemir Ninkovic), Department of Ecology, SLU. FD, 2014-12-17. Full time funded by the SSF grant for 4 years and 3 months. Thereafter employed as researcher for 8 months at the same department but financed by two other projects (5+3 months).



**A.8 Lic exams. Ditto for students who have completed a licentiate exam.**

None

**A.9 Future exams. Enclose a similar updated list of students who have been at any time financed by the Foundation, but who have not yet completed their exam. Specify also the expected time for exam and the reason why they have not completed their exam yet.**

**Lina Lundborg**, female, born 1986. Started Ph D studies 1 of April 2011. Preliminary thesis title: Methyl jasmonate, weevil herbivory and blue-stain fungi infestation changes chemical defences of pine and spruce seedlings. Planned defence March 29, 2016, Supervisor Prof. Anna-Karin Borg-Karlson (assistant supervisor Prof. Åsa Emmer) Department of Chemistry, School of Chemical Science and Engineering. In part (70%) funded by the SSF-project, additional part (30%) by the Department of Chemistry, KTH.

**Karolin Axelsson**, female, born 1979, Preliminary thesis title: Chemical signals in [conifer-insect](#)-microbe interactions Supervisor Prof. Anna-Karin Borg-Karlson. Thesis defence planned to March 2016. In part funded by the SSF project, and additional parts by Formas and Department of Chemistry, KTH.

**Vicki Huizu Guo Decker**, female. Born in 1982. Preliminary thesis title: Defence compounds in Aspen, their biosynthesis and responses to nitrogen and biotic stress. Supervisor: Benedicte Albrechtsen. Thesis defence planned in the second half of 2016. The project is funded by the SSF project.

**Franziska Bandau**, female. Born in 1982. Preliminary thesis title: Does genotypic variability of foliar tannins allow *Populus* to occupy a range of environmental conditions? Supervisor: Benedicte Albrechtsen. Thesis defence planned in May 2016. The project is mostly funded by the centre of environmental research, Umeå. However the SSF project has covered some running costs and participation in conferences of FB. All of the biological material could not have been provided without SwAsp and the SSF project. The propagation of the plants and experimental gardens have been supported by SSF.

**A.10 No exams. Enclose a similar updated list of students who have been at any time financed by the Foundation, but who are no longer expected to complete their exam.**

None

**A.11 A list of innovations and prototypes that have been produced, spin-off companies founded or being contemplated, etc**

None

**A.12 A list of patents awarded or pending. Specify any exploitations or plans for exploitation, etc.**

None

### **A.13 A list of awards to participating researchers, etc.**

Stefan Jansson

2013 Roséns Linne award, Kungl Fysiografiska Sällskapet

2015 “Baltics samverkanspris med populärvetenskaplig inriktning”

2015 SPPS popularization prize

Torgny Näsholm

2015 The Physiologia Plantarum Award