Parasite resistant trees

1. Introduction

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. Our aim is to fully utilize the research infrastructures at Umeå Plant Science Centre (UPSC) to address the question of parasite resistance in trees. We will 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 in Swedish forestry. The research foci of the participating groups will 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 can also be developed. In addition, we will with equal emphasis study the attack of the pine weevil (*Hylobius abietis)* on conifers. The pine weevil is the economically most important pest for forest regeneration in Sweden. We will, in the short time perspective, try to develop a pine weevil management strategy based on natural or synthetic chemical compounds.

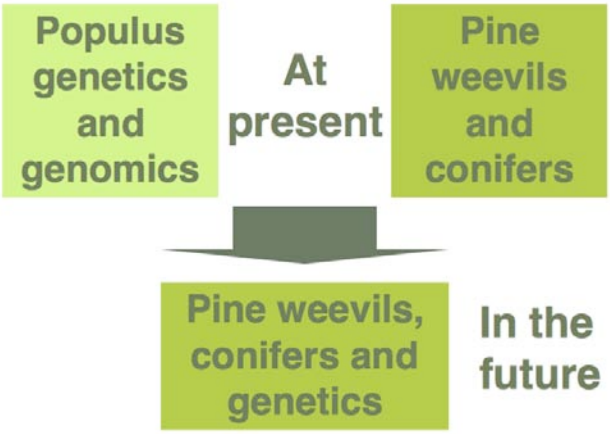

Fig 1 The long-term relation between the two legs of the project

As the genomic resources for conifers are likely to advance rapidly over the next five years we believe that in a somewhat longer time frame (2015-2020), the same basic approaches that we will develop for *Populus* in this project should readily be available for application in conifers (Figure 1). At this point, the knowledge we have gained from working with *Populus* will allow for the development of pine and spruce material which are more resistance against pine weevils and other pests.

2. The genetics and genomics of parasite resistance in Populus – (Leader Stefan Jansson)

This part of the project is not presented here.

3. Conifers and pine weevils – (Leader Göran Nordlander)

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 (*Hylobius abietis*) 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 after 2010 because the use of currently effective insecticides will be 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 largely lacking.

The interplay between this pest and its host is intricate and represents a very specific 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. We will 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 seedlings chemistry and physiology will be coupled to a specific bioassay platform that has have been developed at the Department of Ecology, SLU, to study how various chemical cues are used by the pine weevil in different phases of the process of finding and consuming food. A circular arena with the possibility to trap weevils at up to 16 discrete odour sources is used to measure how individual compounds, or more complex odour blends, affect orientation to a food source. A two-choice test with single individuals is used for measuring the extent to which chemical compounds inhibit the initiation of feeding or decrease the amount of feeding on a highly attractive food source. The strength of the effects can be compared by use of several parameters: concentration of the compound, amount of feeding, time until feeding is initiated, and consumption per time unit.

As a validation of this approach, recent studies have identified a range of substances that have a feeding-deterrent effect on the pine weevil, and patterns in the relationships between molecular structure and biological activity have been determined. Some of these deterrents have been shown to occur in the bark of conifer seedlings whereas others are found as metabolites in the gut of bark-feeding pine weevils. However, the extent to which these are present in the bark of conifer seedlings is currently unknown. The company SweTreeTechnologies AB is currently, and with financial support from forest companies, establishing a high-throughput technology for characterization of the bark metabolome of Scots pine, Norway spruce, and for comparison Lodgepole pine, to screen for potential defence substances. Preliminary results from the SweTreeTechnologies project suggest that a number of these substances with known activities against pine weevils can be detected using our metabolomics technique. However, these substances are present in concentrations appreciably lower than those found to be effective in deterring the weevil and one of the key objectives of this research will be to establish cultural methods for increasing the concentrations of desirable deterrent compounds in seedlings produced in nurseries.

3.1 Sub-projects

The research on pine weevil and conifer seedlings will be carried out in three closely associated sub-projects sharing the same biological material. Central to these studies is the capacity for testing how pine weevils sense and react to an altered physiology/biochemistry of the seedlings and therefore, a specific bioassay platform for such tests has been developed. The three sub-projects will target the physiology of planted seedlings related to their emissions of volatile cues for the weevil and their quality as food sources for the weevil. The combined research efforts within these three projects will further our basic knowledge of the biology and chemistry of the conifer-weevil interaction and by that enable development of new methods for decreasing weevil impact on planted seedlings.

3.1.1 Enhancing the defence of conifer seedlings – (Torgny Näsholm and Vaughan Hurry)

Within this project we will target the physiology of planted conifer seedlings during cultivation in nurseries and during field establishment and we will specifically study the development of defence against weevil feeding during these phases. Resource capture and the fraction of acquired resources diverted to defence compounds in the bark will be studied during nursery production of seedlings and from the point of plantation and over several growth seasons. In parallel with the characterization of the bark metabolome and cell wall analysis and their dynamics during various growth phases, we will investigate techniques and treatments that can increase allocation of resources to production of defence compounds. We will also investigate possible treatments for increasing the content of these defence substances in conifer bark tissue. We will add various precursors of known defence substances to seedlings during cultivation and follow the effects of these on the metabolome of conifer bark. The choice of precursors will be based on results from the SweTreeTechnologies project where the occurrence of endogenous defence compounds will be characterized. Tests with additions of precursors to defence substances to seedlings of Scots pine, Lodgepole pine and Norway spruce will be carried out. The extent to which the added precursors are absorbed and their metabolism will be studied using ^{13}C-labelled compounds. Results will be evaluated and the most efficient substances for boosting endogenous levels of defence compounds in conifer bark identified.

3.1.2 Emission of volatiles from conifer seedlings – (Anna-Karin Borg-Karlson)

The main goal is to chemically analyze the emissions of Scots pine, Norway spruce and Lodgepole pine during chemical, biotic and abiotic stress. The aim is to identify attractants and repellents for the pine weevil. The plants will be treated by labelled precursors (see 4.2.1) as amino acids, sugars and hormones, to follow the biochemical pathways of the volatiles. Spruce clones will be used to follow the differences in volatile production between treatments. Both dynamic and static collection of volatiles will be made, preferably by SPME (solid phase micro extraction) with special effort to separate the enantiomers of chiral compounds using a multi-dimensional GC technique. Unknown volatiles will be isolated by preparative GC and identified by GC-MS and non volatile precursors by MPLC and NMR.

3.1.3 Pine weevil feeding in relation to plant properties – (Göran Nordlander)

As a basis for the further experimental studies the natural feeding pattern of individual pine weevils will be investigated. In particular, more detailed knowledge is needed regarding temporal feeding patterns and factors influencing the initiation and termination of feeding. Factors of primary interest here are the chemical and physical properties of the host plant. Experimentally treated conifer seedlings with known differences in metabolome composition will be tested in the bioassays with regard to attractiveness and feeding preferences of the pine weevil. This will be an iterative process where the results of the biotests lead to new plant treatments, which are again tested and so on. The performance of seedlings with properties making them less attractive as food in the laboratory will be further evaluated in field tests scoring the frequency and severity of pine weevil attack

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