

PROJECT I.D.
1.1. Acronym: FORPLAG
1.2. Project title: Development of strategies for the integrated control of insect pests associated with <i>Pinus radiata</i> plantations in the Basque Country.
1.3. Financial backers: National Institute for Agricultural Research (INIA), Basque Government Dept. of Agriculture, Fisheries and Food (DAPA) and the Basque Government Dept. of education, Universities and Research.
1.4. Participating bodies: Neiker, the University of Hamburg, the University of the Basque Country
NEIKER DETAILS
1.5. Lead researcher: Arturo Goldarazena Lafuente (agoldarazena@neiker.net)
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1.6. Start date: November 2007
1.7. End date: November 2008

Summary:

Pitch canker caused by the *Fusarium circinatum* fungus is a serious disease that endangers the continuity of crops of different species of conifers in Northern Spain, as in the medium term it causes the death of the infected tree and is causing major phytosanitary problems along the Cantabrian coast (Galicia, Asturias, Cantabria, Basque Country, Navarre). There are no measures that provide effective guarantees for controlling the disease. Its epidemiology appears to be at least partly related to pine boring insects (coleoptera scolytidae and curculionidae) and pineapple boring insects (anobid coleoptera). The project was designed to identify the insect species that played a significant role as fungus carriers, test the effect of three promising natural compounds for the integrated control of the pheromonal aggregation of the vectors, develop a mathematical model for predicting the risk of propagation of the disease on the basis of data regarding the frequencies of association and space-time-meteorology dynamics of the insects involved and develop an interactive computer tool for identifying potential vectors of the disease. The project aims to develop strategies for the integrated control of scolytidae of the *Pityophthorus* genus in the Basque Country, as our research group has shown that the insect is associated with the transmission of *Fusarium circinatum*, the pitch canker fungus. The first part of the project carries out a taxonomic study of the *Pityophthorus* genus in the Basque Country by analysing the morphological and molecular characters. We have also studied the biological cycle of the most abundant species in both controlled laboratory conditions and in the field. Finally, we have studied the frequencies of association of *Fusarium circinatum* with said insect in order to create a predictive model. The project is designed to study the chemical ecology of the insect in the field, testing a number of semiochemicals and seeing what role they play as repellents of *P. pubescens*.

Overall Aim: The overall aim of the project during 2008-2012 is to study the biology, chemical ecology and systematics of the *Pityophthorus* genus on the Iberian Peninsula.

Specific Aims:

- To study the chemical ecology of *Pityophthorus* in *Pinus radiata* forests: To find repellents and attractants based on blends of semiochemical compounds.
- To develop tools for identifying European species of *Pityophthorus* spp.
- To study the biological cycle of *Pityophthorus pubescens* in laboratory conditions.
- The morphological and molecular identification of the fungi vectorised by *Pityophthorus* spp.
- To develop a predictive model of the risk of propagation of *Fusarium circinatum* by insect vectors.

Results:

The most significant results are:

The morphological and molecular identification of the species making up the *Pityophthorus* genus in Spain

We studied the morphological characters of the following species: *Pityophthorus pityographus* (Ratzeburg), *P. lichstensteinii* (Ratzeburg), *P. glabratus* Eichhoff, *P. pubescens* (Marsham) and *P. buysonii* Reitter, all present on the Iberian Peninsula, as well as other species present in Eastern and Central Europe: *P. micrographus* L., *P. tragardhi* Spessivtseff, *P. balcanicus* Pfeffer, *P. carniolicus* Wichmann, *P. henscheli* Seitner, *P. exsculptus* (Ratzeburg). Due to the presence of a single species, we have been able to show that the morphological characters are enough to allow us to differentiate *P. pubescens* from the other European species, meaning that it is not necessary to use molecular tools to differentiate it quickly and effectively.

Study of the chemical ecology of these species in order to evaluate different volatile repellent substances derived from non-host plant species

2.a) The effect of farnesene on the pheromonal attraction of *Pityophthorus pubescens* (Marsham, 1802) in 4 *Pinus radiata* D. Don plantations in the Basque Country. 51,082 *Pityophthorus* were captured. Apparently, most of the examples correspond to *Pityophthorus pubescens*, with the males being more abundant than the females (40,773 against 10,309). We found over 800 slightly different individuals (all males). They seem to have lost pilosity at the back of the abdomen (the lacked chetotaxia on the elytral slope), and showed piliferous granules with short or highly broken hairs. We found significant differences between the attractants ($P < 0.05$ for Pityol (+) and the racemic pityol in comparison with the conophthorin and the mixture of pityol (+)-conophthorin). However, geographically speaking there were no significant differences in the control trap captures. It is interesting to underline the little effect that conophthorin has as an attractant, as can be seen from the control traps.

2.b) Effect of limonene on pheromonal attraction of *Pityophthorus pubescens* (Marsham, 1802) in 4 *Pinus radiata* D. Don plantations in the Basque Country. We captured 16,371 individuals. Gorosika was the location where most *P. pubescens* were captured, with 51.60% of the total. The statistical analysis carried out showed us that there is a dose-dependent relationship between the limonene emission rate and captures of male and female *P. pubescens*, showing a significant fall in the same as the emission rate of the compound increased. The results obtained show that limonene is a product with potential as an effective anti-aggregant for *P. pubescens*.

Study of the association of *Pityophthorus pubescens* with the *Fusarium circinatum* fungi

During the last year we isolated numerous fungi associated with different populations of scolytidae, especially the species *Pityophthorus pubescens*. The fungi were isolated from adult individuals of the scolytidae taken from the terminal branches of *P. radiata* (in the case of *Pityophthorus pubescens*) and from divided sections in Morga and Muxika (Bizkaia). *Pityophthorus pubescens* was the scolytidae showing the highest frequency of association with the pitch canker fungi.

Impact

The project generated innovative results, as it is the first time that a Spanish research group has dealt with the subject of forest scolytidae infestations from the holistic, multidisciplinary points of view. Particularly interesting are the results obtained for the epidemiology of the disease, for the part corresponding to the insects, as for the first time we have identified the frequencies of association of the fungal pathogen with each of the species of scolytidae infestation. Finally, we explored the chemical ecology of the infestation beetles and studied natural substances such as verbenone, limonene, farnesene and conophthorin for future use as repellent substances that favour the protection of cut timber. Here, the results are particularly useful for developing integrated woodland control strategies, where the use of traditional chemical pesticides is banned.