



ForestSpeCs



Wood bark and peat based bioactive compounds, specialty chemicals, and remediation materials: from innovation to applications (FP7-KBBE-2008-2B-227239)

NewsLetter

Issue 1, March 2010

Welcome to the first issue of the ForestSpeCs Newsletter!

The purpose of this newsletter is to disseminate widely information about the **ForestSpeCs** project: its aims, working methods, and achievements. We plan to collate this newsletter four times during the project ('quarterly newsletter'); however, the most comprehensive and up-to-date information about our project can always be found at the project website:

<http://www.forestspecs.eu>

I would like to take this opportunity to thank all of you who have contributed to the proposal preparation, submission, and negotiation stages, and have supported the successful start of the project. **ForestSpeCs** started officially on 1 May 2009, and it will run for 3 years. In this Newsletter we will first present briefly the project concept, the consortium members, and some of the very first results.

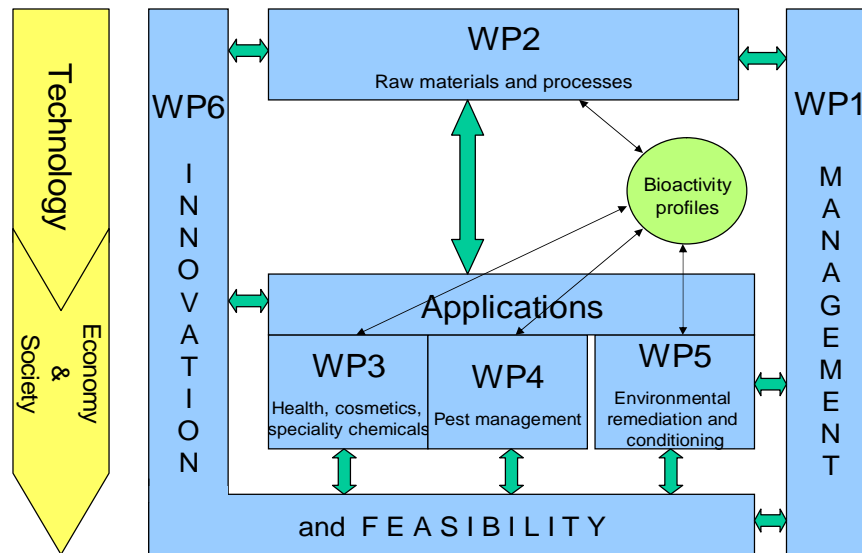
Heikki Hokkanen

ForestSpeCs coordinator

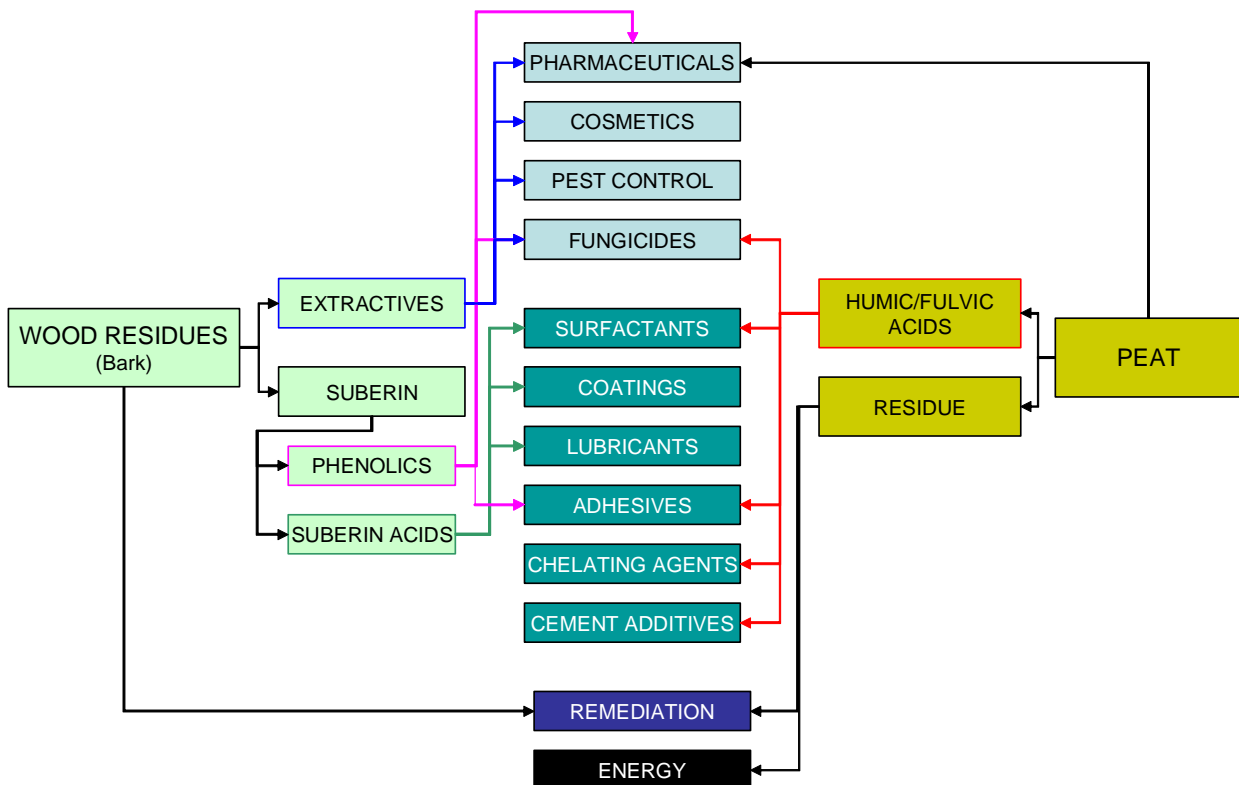
ForestSpeCs objectives and workplan in a nutshell

The overall objective of the **ForestSpeCs** project is to develop and encourage sustainable, environmentally responsible, and economically attractive management of natural resources based on side products from the forest and forest-based industries. The ultimate target of the project is to replace certain large-volume, oil-based chemical materials with bio-renewable and innovative products based on wood-related residues and humic substances. The aim is to find feasible ways to produce high value added, bioactive compounds such as pharmaceuticals and biological plant protection products, as well as to develop new environmentally benign industrial chemicals and polymers. Furthermore, one of the main targets is to create economically attractive options for the total usage of processed wood and peat residues either as a whole, or after extraction of the main bioactive fractions, for example in soil remediation.

ForestSpeCs utilizes different types of wood residues from the wood processing industry, such as bark from economically important tree species including *Betula spp.*, *Pinus sylvestris*, *Picea abies*, *P. ajanensis*, *Larix sibirica*, *L. decidua*, *L. gmelinii*, *Abies nephrolepis* and *Populus tremula*. Peat is used as a source of humic substances. These rapidly renewable raw materials are available in large quantities throughout the year, and are currently used as a low-value source of energy. Our work includes innovative natural products chemistry, extraction and process technology, as well as applications in the pharmaceutical and medical sectors, cosmetics, bioremediation, plant protection products including pest antifeedants, insecticides, herbicides, antifungal and antibiotic products. Linking interactive and innovative research, selected unmodified or modified fractions and/or compounds from the source materials are subjected first to screening of bioactivity and safety, followed by assessments of technical and economic feasibility.



Structure of the **ForestSpeCs** project



The strategy and value chain for upgrading of wood residues and substances of humic origin to value-added chemicals and materials in **ForestSpeCs**. The chart describes the two main types of basic raw materials as the starting points (bark on the left and peat on the right hand sides of the chart), the value-added secondary raw materials (towards the middle), and their possible application areas (middle of the chart) as bioactive substances (upper part, light blue), speciality chemicals (middle part, turquoise), as core substances in remediation materials (lower part, dark blue), or as sources of energy (bottom; not a topic for **ForestSpeCs**).

The ForestSpeCs consortium

Partner Number	Partner name	Partner short name	Country
1	University of Helsinki (coordinator)	UHEL	Finland
2	Technical Research Centre of Finland	VTT	Finland
3	St Petersburg State Medical Academy	SPSMA	Russia
4	University of Surrey	UNIS	United Kingdom
5	Northern Research Institute of Forestry	NRIF	Russia
6	FGU Far East Forestry Research Institute	FEFRI	Russia
7	Research Institute for Organic Agriculture	FiBL	Switzerland
8	Trifolio-M	TrfiM	Germany
9	Oy Granula Ab Ltd	GRAN	Finland

European forest based industry has a period of adjustments ahead of it. The industry has no other alternative than to keep optimising its activities, and to search for solutions from new products in order to improve profitability. In this time of globalisation and societal change the forest industry has to participate in supply chains from cost competitive regions, and has to use the available resources in an innovative and sustainable way. The mission of **ForestSpeCs** and the composition of our consortium reflect these needs.

All the consortium partners share a common motivation and a common understanding of the mission of the project. To create a competitive, knowledge-based industry, research and development on the extended use of renewable forest resources - including wood waste and peat - is of utmost importance. The consortium members strive to ensure their societal contribution by deepening the scientific basis for the utilization of forest biomass waste products.

The consortium interlinks different groups to ensure the multiplicity of competence and experience to carry out efficient R&D. We cover a vast geographical area reaching from the Russian Far East to westernmost Europe, giving us the advantage to tap onto the extraordinary biodiversity of basic raw materials

available in the whole Eurasian sphere. Furthermore, the **ForestSpeCs** consortium offers access to an enormous variety of ecologically diverse testing sites, ranging from continental tundra (extremely important in view of the anticipated climate change and the opportunities for improved productivity) to cool temperate (most of northern and central Europe), up to hot and dry ecosystems (Russian Far East).

The **ForestSpeCs** consortium consists of a perfectly balanced mix of research institutions with leading experts in all of its four core activity areas: (i) advanced process technology [VTT, UNIS, UHEL, SPSMA]; (ii) medical and pharmaceutical applications [SPSMA, UHEL, UNIS, GRAN]; (iii) plant protection applications [FiBL, UHEL, FEFRI, TrifM]; (iv) environmental remediation and conditioning [NRIF, UNIS, FiBL]. It should be noted that for the main application areas the **ForestSpeCs** consortium has an industrial/commercial SME-partner to link our activities into emerging business opportunities, and to contribute to the feasibility studies described in our work programme. This wide variety of profound expertise available in our consortium is the best guarantee for arriving at innovative solutions to the problems tackled in this project.

Highlights of research carried out during the first quarter

Extraction

Bark material has been obtained from all but two species included in the **ForestSpeCs** study, and two different types of peat. The material originates from different parts of Finland, Arkhangelsk Region in Northern Russia, and from Khabarovsk Region in Russian Far East. It has been air-dried at room

temperature and shredded for further processing. At UNIS ten samples from five plant species have been processed so far. Each sample was milled and five samples have been extracted to date using:

(1) Steam distillation, (2) Microwave assisted extraction using dichloromethane, (3) Microwave assisted extraction using ethyl acetate, and (4) Microwave assisted extraction using methanol.

GC-MS analysis for fingerprinting purposes has been undertaken for the steam distillates of the five species. Components of steam distillates are being identified

using the NIST library. The microwave extraction of remaining five samples and GC-MS fingerprinting of remaining extracts are in progress.

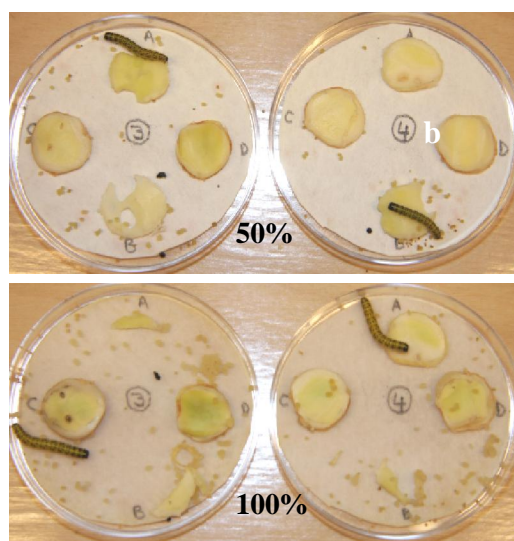
Insect bioassays

At UHEL the shredded bark samples were extracted for their total soluble components using water (30/60°C) and absolute ethanol (30°C) as solvents. These extracts were subjected to standard insect antifeedant choice-bioassays using *Pieris brassicae* (cabbage white butterfly) at the Department of Agricultural Sciences,

University of Helsinki. Additional assays are being carried out with *Spodoptera littoralis* (African cotton leafworm) and *Phaedon cochleriae* (mustard beetle). Some of the extracts show a high feeding deterrence: three samples up to 70 % .



(a) The bark extracts based on water (30/60°C) and absolute ethanol (30°C) solvents, and a choice bioassay in progress in the **ForestSpeCs** lab at the Department of Agricultural Sciences, University of Helsinki, Finland



(b) The choice bioassay (A, B = controls) with *Pieris brassicae* shows a high feeding deterrence. 50% = half of the assay time, 100% = full assay time, when almost all of the cabbage leaf disc controls have been eaten by the larvae, and treated discs are untouched.

Medicinal chemistry

The medicinal chemistry group at University of Helsinki aims at the development of new antibiotic, antiviral, and antiparasitic compounds based on the abundant terpenes, such as betulin from the birch bark, and abietanes from the coniferous resin, respectively.

At this stage of the project, our work has been focused to (i) the preparation of pure betulin starting material for further modifications and (ii) to the synthesis of common intermediates for further elaboration, such as betulonic acid, betulinic acid, and allobetulin.

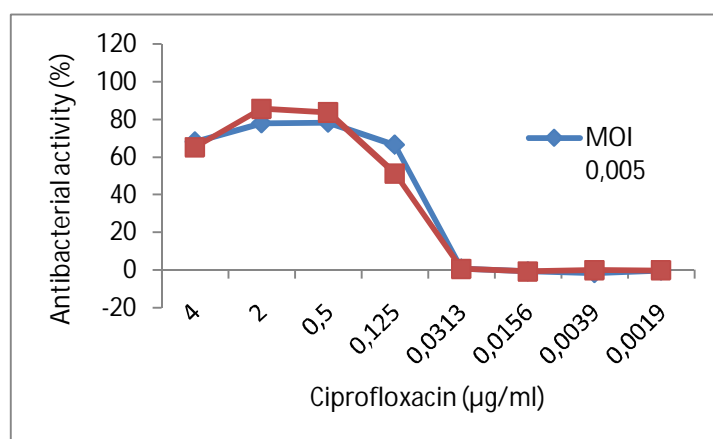
The purification has been achieved by recrystallizing the crude betulin from a mixture of 2-propanol and water to give >95% pure betulin, pure enough for the synthesis of its derivatives. Betulonic acid has been synthesized by the classical Jones procedure, betulinic acid has been obtained from betulonic acid by the stereoselective reduction of its keto carbonyl group of the A ring and allobetulin has been prepared from betulin by the acid-catalyzed isomerisation reaction.

Bioactivity screening

At the Centre for Drug Research (UHEL-PHA) screening assays for testing the antibacterial activity of ForestSpeCs samples are currently in final stages of validation. The assay set-up is based on standards set by the NCCLS/CLSI for antibacterial testing. These standards define, for example standard strains and inoculum size to be used as well as reference antibiotics (incl. concentration). The work has so far involved the use of *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Assays are carried out in 96-well microplates and the activity of test compounds on bacterial growth is followed for 24 h by turbidometric measurement. Next, after finalising the reproducibility analyses, the assay will be used for testing bark extracts and betulin derivatives originating from the **ForestSpeCs** consortium.

For studying simultaneously the efficacy and tolerability of antibacterial compounds, an assay based on co-culturing of secondary human cell line (HL,

derived originally from human lung) and a pathogen has been established. This system mimics the natural bacterial infection, and gives thus more information on the antibacterial potential of test compounds. In the assay, host cells are incubated in the presence of the test sample and are infected with bacteria (carried out on 96-well microplates). After incubation of 2-3 days, the host cell survival is determined by using fluorescein diacetate to measure the viability of the host cell. As outputs, the assay provides both half-maximal inhibitory concentrations (IC₅₀) and half-maximal cytotoxic concentrations (CC₅₀) for the test compound. During assay validation, both the host cell and the pathogen culturing have been optimised (number of cells used, inoculum size, incubation time, etc.). In addition, the assay has been determined to tolerate DMSO (dimethyl sulphoxide) up to 2%. The Figure below presents results obtained with a reference compound, ciprofloxacin. Further on, this assay will be used for studying in more detail compounds that are found active in the primary antibacterial screen.



Dose-response for ciprofloxacin determined with a co-culture of HL cell and *Staphylococcus aureus* (MOI= multiplicity of infection).



ForestSpeCs Partners from the FGU Far East Forestry Research Institute (FEFRI), Khabarovsk, Russia.

For more photographs, and information, please go to the **ForestSpeCs** website: <http://www.forestspecs.eu>