ISEFOR

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FINAL REPORT

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Executive summary

The aim of ISEFOR was to generate robust evidence to inform policy development for forest management against risks from alien invasive pests and pathogens in relation to climate change, by increasing knowledge on alien species that may enter Europe, and to generate improved methods for phytosanitary authorities to detect and diagnose pests and pathogens in plant consignments entering Europe.

Databases on alien pests and pathogens invasive in Europe were updated and used to determine hosts most vulnerable to attack, prepare an ISEFOR database and to list forest pests and pathogens at risk of introduction to Europe based on available information and sentinel trees in Asia. Novel techniques used to establish lists of potentially damaging organisms not yet present in Europe included surveys of arboreta in Asia containing European plants (sentinel trees) and examination of plants widely traded in Europe in sentinel nurseries.

Distribution of over 1000 pests of woody plants was determined by hierarchical cluster analysis, and the likelihood of pest and pathogen establishment in Europe classified. Records for European pests and pathogens were compared with other databases for verification. Finally, interception data for woody plant pests in Europe in the last 15 years were added to databases of exotic woody plant pests in Europe.

Forest pests and pathogens collected from infected trees, spore traps, sentinel trees/nursery stocks were characterized at the molecular level. Either general Eukaryotic primers or group specific primers were used to analyse forest soils, spore traps, and swabs from birds using next generation sequencing (NGS), detecting numerous unknown species. The NGS approach was validated for detection and diagnostic purposes. These tools were efficient in tracking alien species in pathways, and adaptable for use in national diagnostic laboratories.

The plants for planting pathway was analysed for forest pests, by examining commercial movements through national inspection points. A dataset of almost 380,000 entries based on information from NPPOs of 14 countries was collated. Analysis revealed the main importing countries, main species and genera imported, main countries of origin, and will provide valuable support to pest risk analyses. A list of alien insect pests and pathogens attacking ornamental and forest tree species and established in EU countries was prepared and the organisms posing the greatest threat to EU forests highlighted using these data. Alien pests and pathogens intercepted but not established were listed, with their origin and their points of entry. Finally, lists of alien invasive species already established in EU countries and those regulated or recommended for regulation compared. A critique of ISPM 36 was prepared by referring to phytosanitary regulations on importing live plants and findings from ISEFOR. Two 'sentinel nurseries' were established in China (Beijing and Fuyang) each with five tree species commonly exported to Europe. Five pest species (three fungi and two insects) found in these sentinel nurseries were submitted to PRAs and the species, amongst those identified in the sentinel nurseries, presenting the greatest risks and requiring full PRAs identified.

Sustainability functioning of European forest ecosystems is threatened by global climate change, one aspect of which is increased movement of species beyond their native ranges through increased global trade. Predictions of the impacts of introduced species become more complicated because of uncertainties associated with the complexity of forest ecosystem processes in relation to climate change. Faced with the complexity of forest pest risk assessments, modelling tools for risk mapping and analysis were developed and validated, which can be used for accurate prediction of the establishment and spread of alien pests and pathogens. We developed, applied and validated various models for the assessment and mapping of the climatic suitability, forest susceptibility and the spread of selected potentially invasive pests and pathogens under past, present and projected climate conditions in Europe.

This work led to the publication of over 100 scientific papers, over 100 oral and poster presentations to conferences and 41 articles in the popular press. Partners in ISEFOR also gave numerous press interviews for TV and radio and provided workshops to end users to showcase project results.

Summary description of project context and main objectives

European forests are of enormous environmental and societal importance, providing a multitude of tangible and non-tangible benefits including timber and non-timber products, biodiversity, water catchment protection, landscape and protection against avalanche, flooding and landslides. There is also increasing demand on forests for recreational purposes, leading to much greater political recognition of the multiple functions of forest ecosystems, over and above the production of timber (FAO 2005).

Forests, however, are now under unprecedented threat from two principal inter-related and interacting factors:

1. Rapid climate change places ecosystems under stress, as the plant species comprising the ecosystems have little time to adapt to changes occurring over a few decades. Long-lived tree species cannot adapt rapidly to environmental change, particularly to the predicted higher frequency of extreme events (e.g. floods, storms, frosts at atypical times, abrupt changes between warm and cold weather conditions, drought) coupled with gradually increasing average temperatures and changes in rainfall patterns (Easterling et al. 2000). These factors also seriously impact host-parasite interactions at all levels;
2. Increased global trade, mobility and tourism have led to an escalation in the numbers of alien pests and pathogens intercepted at ports of entry (Brasier 2008); escapes into natural and plantation forest ecosystems are occurring, along with establishment and enormous damage in some ecosystems. Climate change will increase the probability of permanent establishment of many damaging alien pathogens and pests in Europe.

The interactions between climate change, including increases in both mean temperatures and precipitation, and pests and pathogens (indigenous or alien) will seriously impact on host susceptibility to attack and a large number of novel, unprecedented forest health problems are likely to occur in the near future, leading to reductions in forest primary production, with consequent losses in timber yields, biodiversity, increased risk of flooding, avalanches and landslides, and marked reductions in water catchment efficiencies. Local extinctions of highly susceptible plant species may result, following attacks by pests and pathogens with which the plants have had no recent evolutionary contact. Additional potential impacts include loss in recreational potential of affected areas, changes to landscapes, loss of cultural heritage (e.g. cypress and plane in Italy and Greece; sweet chestnut in Southern Europe), and risk to public health through use of pesticides and, sometimes, from the pests themselves. Particularly significant is the reduction in carbon sink capacity of the forest ecosystems resulting from damage.

Numerous pathways of introduction of non-native forest pests and pathogens are known. Imports of live plants for plant nurseries (incl. bonsai) probably represent one of the most problematic pathways since the widespread adoption of ISPM 15, controlling invasives in woody packing material from 2005. Casual imports by tourists and other travellers, and transport systems (aircraft, ships, cross-border road haulage) doubtless contribute further to the load of alien invasive pests and pathogens with which Europe is now faced. In addition, a search on the World-Wide Web will present a large number of live organisms available for order with a simple credit card transaction. Entry into the EU for these exotics is via mail, which is most unlikely to be inspected as closely as other modes of delivery (Everett 2000). As a result of these changes in trade activities, the number of exotic forest insect species newly recorded per year in Europe is increasing at an enormous rate (Roques 2008; Santini et al. 2013).

Climate change is also causing shifts in pest populations as they respond to changing climatic patterns, enabling migration and establishment in new locations and possibly onto new hosts.

Amongst the numerous examples of alien pests and pathogens invading previously uncolonized regions and causing major problems is the genus *Phytophthora*, many species of which have spread in international trade. *P. cinnamomi*, a particularly virulent pathogen with a recorded host range of over 950 plant species, is possibly the most widespread invasive organism (Hardham 2005). *Phytophthora cinnamomi* invasions have resulted in ecological devastation, as illustrated by severe eucalypt diebacks in the forests of Western Australian and Victoria. *P. cinnamomi* is also widespread in European chestnut forests and in the evergreen oak forests of the Iberian Peninsula characterized by a warm, dry climate. *Phytophthora lateralis* emerged in Oregon in the early-mid 20th Century, probably entering on infected material from the Far East, totally altering forest productivity in the Pacific North West (Hansen et al. 2000); this pathogen is now established in France (Hansen and Delatour 1999) and the UK, and is recognised by EPPO as a quarantine pathogen. Other examples in Europe include *P. quercina*, frequently a primary factor in the recently emerging problems of oak decline (Jung et al. 1999), *P. alni* (and sub-species thereof) attacking riparian alders in northern Europe (Brasier et al. 2004) and *P. ramorum,* first characterised in a Dutch-German collaboration in 2000 (Werres et al. 2001), but particularly noted for the widespread disease it causes on a range of tree species, for example 'Sudden Oak Death' in California and Oregon (Rizzo et al. 2005). During the course of the ISEFOR project, *P. ramorum* 'jumped' hosts' and now is killing Japanese larch in the UK and Ireland. Another aerial Phytophthora, *P. kernoviae* was described in the UK in 2005 (Brasier et al. 2005); later it was discovered that the same species, then incompletely characterised, had been found in native forests of New Zealand in the 1950s, where it is probably native. A number of invasive *Phytophthora* species are associated with serious beech decline in Europe (Jung et al. 2005). Widespread deaths of *Juniperus communis* on the rare juniper heaths of Northern Britain are caused by *P. austrocedrae* which is otherwise known only in Patagonia. In 2007 it was confirmed that a needle disease defoliating *Pinus radiata* in Chile is caused by a previously unrecognised species, *P. pinifolia* (Durán et al. 2008). *Phytophthora alni* highlights a serious additional risk associated with biological invasion: hybridization events between species and subspecies of pathogens (Brasier 2008).

Many other important groups of alien pathogen threats were targeted in ISEFOR, including *Ceratocystis* spp., *Dothistroma* (*Mycosphaerella*) spp., rust and bacterial pathogens. The genus *Ceratocystis* includes a range of cryptic species with very similar morphologies (Wingfield et al. 1997; Harrington and Wingfield 1998; Johnson et al. 2005; van Wyk et al. 2006; Marin et al. 2005; Wingfield et al. 2006), many of which pose considerable threats to a wide range of European plant species. Recorded hosts include tropical crop plants (e.g. sweet potato, cocoa, *Colocasia, Ficus*, mango, coffee) and temperate trees (*Populus*, *Quercus*, *Platanus*, *Prunus* and *Carya*), as well as many Pinaceae (Kile et al. 1993; Harrington and Wingfield 1998). *Ceratocystis platani* is notable in causing wilt/canker stain of *Platanus* spp., and is spreading rapidly in Italy, France, Switzerland and more recently in the native range of *Platanus orientalis* in Greece. The genus *Mycosphaerella* spp. is extremely large, including some 10,000 species, many of which are serious pathogens on a range of agricultural and horticultural crops, and on trees. *M. pini* (*Dothistroma septosporum*), cause of Dothistroma needle blight of pine, for example, has become particularly problematic in an extended range within Europe in the last 20 years. In eastern, central and northern Europe *Hymenoscyphus pseudoalbidus*, a species native in the Far East is causing a serious decline and dieback disease of European ash species (Kowalski 2006; Queloz et al. 2011).

Exotic invertebrates, particularly insects, also pose enormous threats to forestry (Kenis et al. 2009). Dramatic effects have occurred in North America with invasions of many European and Asian forest pests. For example the balsam woolly adelgid, *Adelges piceae*, and the hemlock woolly adelgid, *Adelges tsugae*, threaten unique forest ecosystems in eastern North America by killing respectively fir (*Abies* spp.) and hemlock (*Tsuga* spp.) on a large scale, altering normal succession (Jenkins 2003; Weckel et al. 2006). The gypsy moth, *Lymantria dispar,* deliberately introduced from Europe in the 19th century, rapidly became a major pest of broadleaved trees, particularly oak, in Eastern North America (Liebhold et al. 2005). Other examples of European and Asian insects causing serious concern for North American tree species include the European spruce aphid, *Elatobium abietinum* (Lynch 2004), the Asian long-horned beetle, *Anoplophora glabripennis* (Herard et al. 2009), and Chinese emerald ash borer, *Agrilus planipennis*. Several of these organisms threaten European trees.

Although some alien forest insects are considered minor pests, certain species have a tremendous impact on forestry by transmitting or facilitating diseases. For example, the European elm bark beetle, *Scolytus multistriatus* is the vector of the infamous Dutch elm disease, *Ophiostoma ulmi* and *O. novo-ulmi* in North America. European beech scale, *Cryptococcus fagisuga,* in association with the fungus, *Neonectria faginata,* causes beech bark disease on American beech (*Fagus grandifolia*). Organisms other than insects and diseases can be extremely harmful to forests. The pine wood nematode, *Bursaphelenchus xylophilus*, an American species vectored by long-horned beetles of the genus *Monochamus*, has had a devastating effect on native and planted pine species in East Asia and, more recently, Portugal.

Until recently, damage by exotic forest invertebrates was relatively limited compared in Europe to North America. Of over 450 exotic species associated with woody plants already established in Europe, 35% came from temperate Asia (Roques 2008). Many alien species remain confined to parks and gardens or roadside trees (Roques et al. 2008), although several destructive pests are now causing serious problems in European forestry. The pine wood nematode (PWN) is established in parts of Portugal and spread into Spain despite the implementation of a strict quarantine programme (Peňas et al. 2004). PWN is vectored by native long-horned beetles (*Monochamus* spp.), but Asian *Monochamus* vectors are also frequently intercepted by European quarantine authorities. Populations of both *Anoplophora glabripennis* and *A. chinensis* have been found several times in Europe, introduced from Asia in wood packaging material (*A. glabripennis*), and on nursery stock (*A. chinensis*). At least one population of *A. chinensis* in Venetia, Italy is now considered out of control, having killed thousands of trees and shrubs (Herard et al. 2009). Chestnut gall wasp, *Dryocosmus kuriphilus*, a Chinese species already present in Japan and North America, has spread throughout Italy and to neighbouring countries, despite strict quarantine measures. *Agrilus planipennis* is spreading from the established area in Moscow, where damage on a similar scale to that in North America (Baranchikov et al., 2008), and is now close to the Byelorussian border. There is no doubt that *A. planipennis* will soon reach the EU border, threatening European ash species and ash-dominated forest ecosystems. Several Siberian pest species, such as the Siberian moth (*Dendrolimus sibiricus*) and the larch gall midge (*Dasineura rozhkovi*), have already crossed the Ural mountains aided by climate change, and are likely to expand westwards where susceptible hosts are present and climatic conditions favourable.

Amenity trees are particularly vulnerable to invasive species, both climatically and by proximity to the points of import into most countries. Recent examples include horse chestnut leaf miner, *Cameraria ohridella*, damaging *Aesculus hippocastanum* throughout Europe (Gilbert et al. 2004). North-American black locust gall midge, *Obolodiplosis robiniae*, is also rapidly expanding all over Europe (Glavendekic et al. 2009). Exotic seed bugs (e.g. *Leptoglossus occidentalis*) and seed chalcids

(*Megastigmus*) have serious consequences for availability of tree seed and natural regeneration of tree stands. The North American *L. occidentalis* colonized most of Europe within a few years (Dussoulier et al. 2008); 8 of 21 species of *Megastigmus* recorded in Europe are of exotic origin (Roques and Skrzypczynska 2003).

The potentially destructive nature of alien invasive pests and pathogens emphasises the need to maintain and improve high vigilance in plant quarantine and at potential points of entry. Early detection of the threats and improved abilities to identify the invasive organisms is of paramount importance in the rapid deployment of efficient and effective management and control measures against these organisms.

Reflecting these concerns, ISEFOR focused on several highly significant quarantine pests and pathogens from the EPPO A1 and A2 lists, representing different ecological groups of invasive species. For insects, choices were based on feeding guild (wood borer, phloem-feeder, defoliator, seed feeder, leaf miner and gall-formers), host type and family (trees vs. shrubs; conifers vs. broad-leaved species), and taxonomy. For pathogens, a wide range of biological attributes was used, including parallels with 'feeding guilds' (pathogens causing root, shoot, canker, foliage and wilt diseases) and including bacteria, fungi, oomycetes and nematodes.

Spread of invasive species between countries and on an intercontinental scale results from human activities. Trade in plant materials, including live plants in the horticulture trade, and plant parts, such as timber, flowers, fruit and tourist items, is the most likely route for long-distance transmission between affected and unaffected areas. Hence, the 'plants for planting' pathway was a particular focus of the work in ISEFOR.

In order to order advance the state of the art in this area and address the problems that will arise from (1) climate change impacts on forest ecosystem vitality, (2) increasing threats from alien invasive pests and pathogens and (3) changing threats from indigenous pests and pathogens, or alien species already established in Europe, the objectives of ISEFOR were:

- To define the alien invasive threats likely to impact on European forest ecosystems, based on current knowledge of the pest and pathogen organisms known as potentially invasive, and the host genera attacked by these organisms (**Workpackage 2**);
- to develop state-of-the-art molecular methods for the detection and diagnosis of potentially problematic alien organisms at ports of entry, and in pathways of dispersal (**Workpackage 3**);
- To critically analyse the plant nursery trade, the major pathway for dispersal of alien pests and pathogens, in order to (1) provide a quantified approach for use in preparing generic pest risk assessments for this pathway, and (2) set up sentinel nurseries in China to monitor woody plants commonly exported to Europe for the presence of pests and pathogens (**Workpackage 4**);
- Using the data obtained in the work outlined above to develop modelling software allowing accurate prediction of the spread and impacts of alien pests and pathogens which may become (more) invasive under climate change conditions (**Workpackage 5**).

Each Workpackage in ISEFOR was focused on one of the individual objectives listed above.

ISEFOR also included substantial dissemination plans for the transfer of knowledge and techniques developed to the end-user community (**Workpackage 6**).

Description of the main S & T results

Workpackage 2: Defining the Threats

Research in workpackage 2 was divided into 3 tasks. **Task 2.1 Collation of existing information on alien threat organisms** involved the interrogation of existing databases on alien invasive pests and pathogens present in Europe to obtain information relevant to the needs of ISEFOR. Distribution data of pests and pathogens of woody plants in Europe and worldwide were obtained from sources including the CABI Crop Protection Compendium and Plantwise knowledge bank. Interception databases compiled by phytosanitary services from EPPO countries were obtained from the EPPO website and via personal contacts. Data collected during the EU-funded projects ALARM and PRATIQUE on Asian pests attacking European woody plants in Russian arboreta and Asian sentinel tree plantations were collated in collaboration with ISEFOR partners INRA, IOZ-CAS, and Russian and Chinese partners. The DAISIE database was analysed by INRA to extract relevant information on insect pests established on woody plants, particularly related to the pathway analyses planned in WP4. The FORTHREATS database was used by IPP-CNR to obtain information on forest pathogens.

These databases were updated throughout the ISEFOR project.

In **Task 2.2 Collation of information on host taxa, t**he objective was to compare host species susceptibility to alien pests and pathogens, in order to better inform the research in WPs 3 and 4. Data on interceptions by phytosanitary inspectors at import were obtained from EPPO for 1995-2010. Interceptions of pests and diseases on woody plants for planting and timber during this period were analysed for the EU and Switzerland.

Data on woody plant hosts of the alien invertebrates and pathogens established in Europe was compiled from the DAISIE and FORTHREATS databases. These databases were analysed with regard to putative pathways (plants for planting vs. wood and wood products), plant taxonomy, invaded environment and time period in order to develop an understanding of possible changes in tree species susceptibility to invasions with time. Data on woody plant hosts of alien forest pathogens established in Europe was obtained from the FORTHREATS database. The main pathway for this group of invasive species is **plants for planting**.

Interception data showed that the majority of intercepted organisms were nematodes and insects and that most intercepted organisms originated in Asia or other regions of Europe. Of many host plants affected by invasives, the most frequent genera were *Rhododendron*, *Euphorbia* and *Acer*. The families and origins of the intercepted and established insects were similar, but the actual species were very different. These analyses were integrated into the tree susceptibility analyses presented in deliverable D2.1.

Analysis of the alien invertebrates established on woody plants in Europe indicated that the plants for planting pathway was the predominant source of invasive species during recent years. Large differences were found in the colonization of plant species by alien invertebrates, with *Citrus*, various Palmae, *Prunus, Picea, Acer* and *Eucalyptus* the species with most alien insects. However, the trend appeared to change with time. Whereas the number of new species colonizing native broadleaved, palm trees, tropical legumes and eucalypts largely increased during 2000-2008, colonisations on conifers and fruit trees appeared to decrease over the same period. More precisely, the recent increase in broadleaved colonization was due to higher numbers of alien species on *Fraxinus, Salix* and *Castanea*.

Alien pathogens established on woody plant species in Europe also arrived mainly through the plants for planting pathway, and spread through Europe both by plants for planting or as air-borne spores.

Pinus, *Rhododendron*, *Quercus*, *Alnus* and *Fagus* were the genera showing greater susceptibility to attack by invasive pathogens regardless of the time of arrival of the pathogens. The time series analyses demonstrated a decreasing trend in numbers of pathogens arriving that attacked conifers. In contrast, there was an exponential increase in newly recognised pathogens of broadleaved trees and shrubs pathogens. *Acer, Fagus, Quercus* and *Alnus*, and *Rhododendron* and *Syringa* were the broadleaved trees and shrubs particularly vulnerable to attack in the last 30 years. These data were kept updated throughout ISEFOR.

The objective in **TASK 2.3 Database of Forest Pathogens and Pests Posing an Immediate Risk of Invasion in Europe** was to compile a list of potential forest pest and pathogens at risk of introduction in Europe based on literature reviews, available databases and sentinel trees located in Russia and China. Presence data for 351 regions of eight invertebrate and four microorganism groups (ca. 2000 taxa) were obtained from CABI. Invertebrate species distributions in Europe were updated with records in *Fauna Europaea* and the DAISIE database. Only species with woody hosts (including bananas and palms) were included. Countries and regions with similar pest species assemblages were identified for each of the organism groups using hierarchical cluster analysis and the number of clusters was determined using the Davies-Bouldin's Index. Invasion risk was calculated as the fraction of countries within a cluster containing EU countries where the species occurs.

A list of the potentially most harmful Asian pests and pathogens of European wood plants was extracted from the database collected during PRATIQUE by CABI, INRA and IOZ-CAS, in collaboration with Russian and Chinese partners.

The FORTHREATS and DAISIE databases were updated and integrated, in order to produce an ISEFOR database to meet the needs of this project. Concerning invasive pathogens, the database has been implemented by adding data from Serbia, a non-partner country that voluntarily contributed data to this task which was not present previously. Literature was also integrated and organized in the database. Based on these data, the classes of organisms at greater risk introduction were identified.

A vast literature on Russian forest pests and pathogens was assessed. Preliminary results were presented and critically discussed at two Russian national forest entomology and forest protection workshops.

For each EU country and organism group (except Oomycetes for which no good clusters were formed), a list of potential future invasive species was assembled. The results showed that most species in this dataset were already present in at least some EU countries.

A list of 30 insect species having frequently (> 5 times) colonized the sentinel trees planted in China (PRATIQUE) was made available as an early-warning measure. Approximately 50% of the pests were identified to the species level by IOZ-CAS, whereas the others, mostly lepidopteran larvae, were characterized by molecular markers (barcoding), and tentatively referred to the closest species at a phylogenetic level. Five of these species were retained as potential invaders likely to cause substantial damage, and were proposed for pest risk analysis. One species (a defoliator psychid moth) was reared under quarantine conditions in France and its ability to cause damage to a large range of broadleaved European trees confirmed.

A highly significant result was the discovery of *Cydalima perspectalis*, a new invasive pest attacking *Buxus* spp. in Europe, on sentinel trees in China. This finding supports the hypothesis that sentinel trees provide a reliable technique for forecasting potentially damaging pests and pathogens that could be introduced into Europe and cause serious damage.

Time series analysis of pathogen flows revealed that species more recently established in Europe are mainly Oomycetes and Ascomycota. In contrast, the trend in Basidiomycota arrivals had not changed; an increasing phenomenon to be considered in particular is the possibility of hybridization between endemic and invasive species (e.g. *Heterobasidion annosum* and *H. irregulare* in Italy).

A preliminary list of the 25 most important invasive Russian forest pest insects, nematodes and fungi potentially threatening European forests was compiled.

Ongoing changes in the data management of CABI's Plantwise Knowledge Bank meant that data were acquired only at the start of 2012. Although this problem caused some delay in the production of the relevant deliverable, it also resulted in a more up-to-date database. The dataset was divided into taxonomic groups, which added greatly to the level of detail in the results.

Since prediction of which pests and pathogens may become invasive in the future is difficult and the available sources of information were fragmented amongst a myriad of documents, some of which were difficult to access, a range of methods was used by the teams involved in WP2 to produce data for Deliverable 2.2. At present, in order to perform a risk assessment, the main issue is to rationalize and render available the information already known about the organism, and possibly to combine it with experimental evidence from case studies. A possible strategy for forecasting the risk of introduction of pests that have not yet arrived in Europe is to use data available from countries where the pests have already spread and, based on similarities with the pest profiles of European countries, try to evaluate the risk of introduction and spread of each pest to Europe. In addition, sentinel trees of European species can be planted in the countries from which new invasive waves are expected. The possible risk of further spread of alien forest pathogens already present in Europe also needs to be addressed.

Initially an improved version of the FORTHREATS database of established forest pathogens in Europe was used to develop indicators of the potential invasiveness of the alien fungal forest pathogens established in Europe (Figures 1, 2). To establish lists of potentially harmful organisms not yet present in Europe, other approaches were used. Firstly, we presented a novel method to detect new potential pests in their regions of origin, before introduction into a new continent. This method, based on work in previous EU projects and completed in ISEFOR, involves the planting of sentinel European plants and surveys of botanic gardens and arboreta in Asia (Figure 3). Then, we analysed distribution data for over 1000 pests of woody plants using a hierarchical cluster analysis and classified, for single European countries, the likelihood of establishment of pests. Data were extracted from the CABI Plantwise Knowledge Bank database in January 2012. This database primarily contains data on the distribution of economic invertebrate pests and diseases and their host plants. The records for European invertebrate taxa were compared with the *Fauna Europaea* for native species and the DAISIE database for invasive species (http://www.faunaeur.org/, accessed in March 2012; Roques et al., 2010), and records of presence added to the Knowledge Bank dataset. Indigenous species explicitly recorded as absent in *Fauna Europea* were removed. The records for fungi and oomycetes were compared with the FORTHREATS database (Santini et al., 2013). Records were updated to January 2013. Finally, interception data for woody plant pests in Europe were analysed for the last fifteen years as well as the lists of quarantine pests from RPPOs and NPPOs of other temperate regions and assessed their usefulness for developing databases of exotic woody plant pests in Europe.

The literature database was compiled in Bento 4.0.1 (File Maker), simple software for compiling and consulting databases that can export the dataset in Excel format. The database includes approximately 900 accessions and is available on request from **Partner 5 IPP-CNR**.

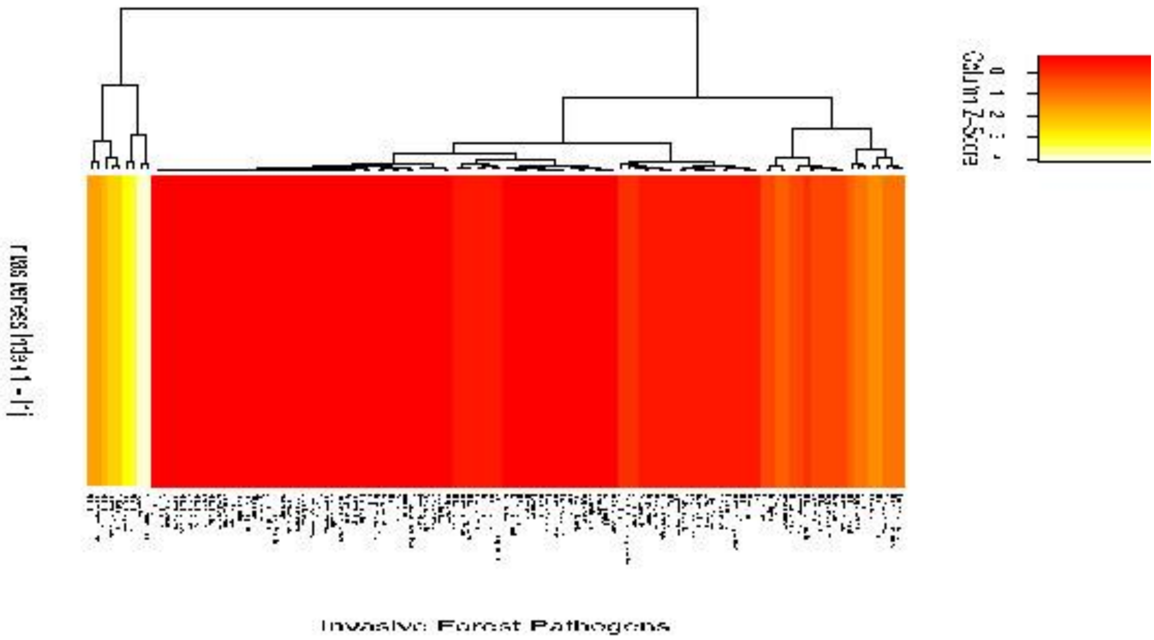

Figure 1. *Invasiveness index-1* ($I1_j = d_j/n_i$) is an estimate of the yearly rate of linear diffusion of the invasive pathogen *j* from the area first invaded to adjacent countries. This index describes invasiveness as the linear distance in kilometres covered by each pathogen between the extremes of the invaded range of countries.

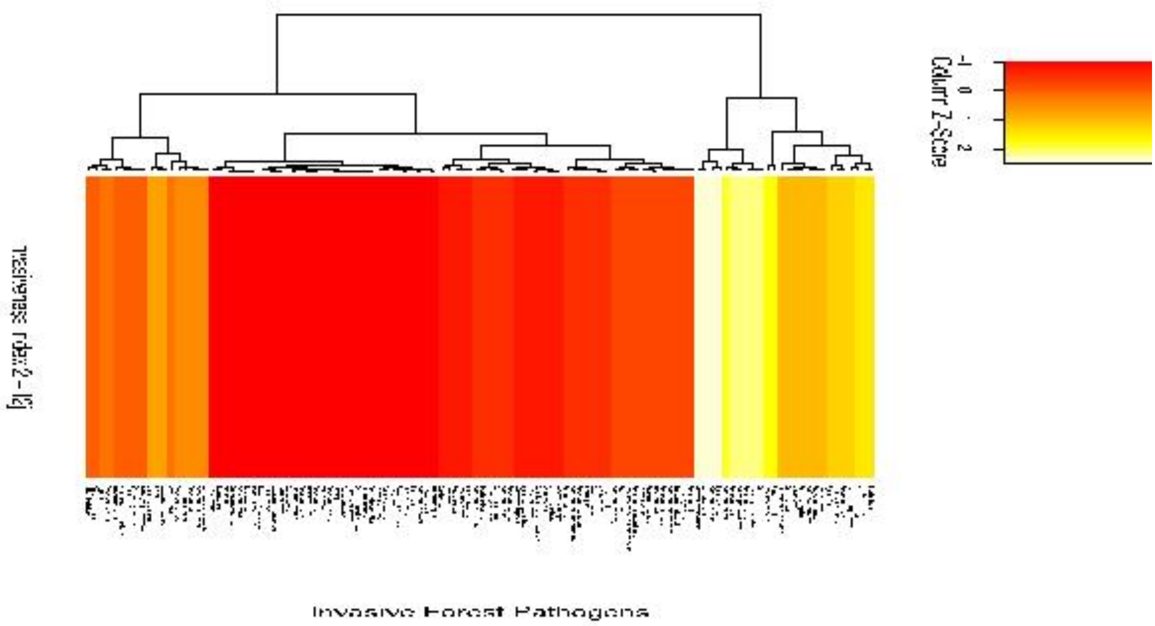

Figure 2. *Invasiveness index-2* ($I2_j = F_j/maxF_j$) measures the invasiveness of fungal pathogen *j* as the fraction of the total forest area of all the countries where the pathogen is present. This index is based on the hypothesis that a wider forest area corresponds to larger number of potential hosts for the pathogens.

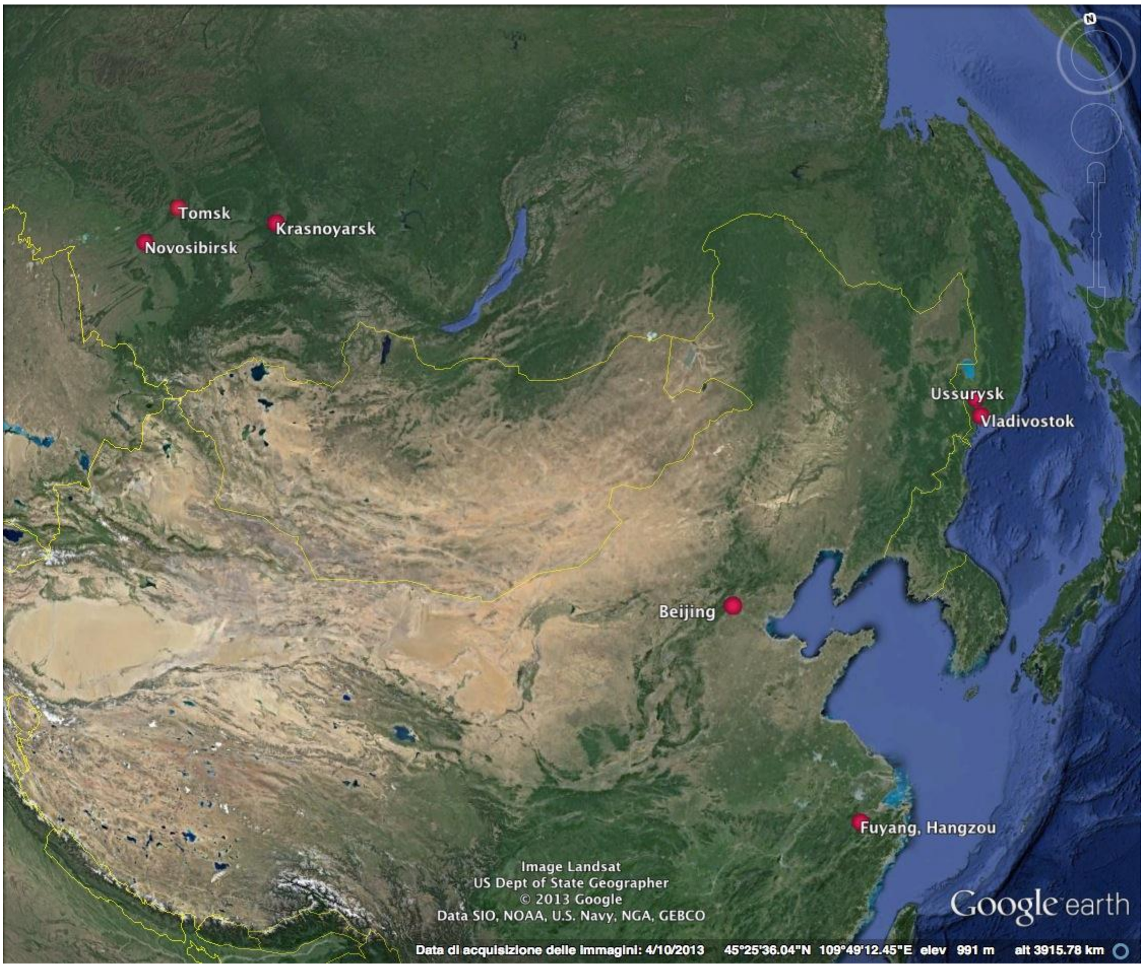

Figure 3. Locations of arboreta (Tomsk, Novobirsk, Krasnoyarsk, Ussurysk, Vladivostok in Russia) and Sentinel tree experimental fields (Beijing, Fuyang, CN) in Asia from which additional data on potential invasive pests and pathogens were obtained.

Work Package 3 Detection and development of diagnostic methods

WP3 within ISEFOR comprised the development, in conjunction with other EU-funded projects on parallel themes (e.g. PRATIQUE; QBOL), of state-of-the-art molecular systems for the detection and diagnosis of potentially problematic organisms at ports of entry, and along pathways of dispersal. The aim of the work was to improve the abilities of phytosanitary authorities to detect and diagnose alien invasive pests and pathogens at points of entry, at strategic points along pathways of invasion and in affected trees, using the most up-to-date molecular techniques. The specific objectives were to:

i) use DNA signatures to design methods for rapid and specific detection of the most relevant invasive organisms;
ii) develop methods for multi-locus sequence typing (MLST) and multi-locus microsatellite typing (MLMT) as fingerprinting/barcoding methods for these organisms;
iii) compile all sequence and other information for each invasive pathogen into existing databases; and
iv) apply state-of-the-art emerging massive high throughput sequencing techniques to diagnostics (mainly 454 FLX/Roche Solexa/Illumina).

ISEFOR WP3 initially focused mainly on two tasks:

i) **Improving abilities to diagnose and detect quarantine pests targeted in the project**. In this task, poorly known quarantine and other relevant forest pests were thoroughly characterized at the genetic level. For example, *Ceratocystis* spp., *Phytophthora* spp. and numerous insect species collected from affected trees, spore traps and/or sentinel trees were investigated. Two novel approaches investigated and applied in this part of the work contributed greatly to attaining the aims of ISEFOR. The use of spore traps for the detection of *Ceratocystis platani* on plane trees (and *Ophiostoma novo-ulmi*, pathogenic on elm trees and other putative invasive forest pathogens), and the use of sentinel European trees planted in 2008 in two areas of China and monitored at regular intervals to survey colonization by indigenous arthropods and pathogens.

ii) **Applying emerging massive sequencing technologies for detection of invasive pests and pathogens**. Using either Eukaryotic general primers and/or group specific primers forest soil samples, spores collected by spore traps and other environmental samples were subjected to a 454 FLX/Roche sequencing approach. This strategy resulted in the identification of numerous new species not yet described and revealed the diversity of organisms truly present in environmental samples.

The final and major outcome of ISEFOR WP3 is, alongside work carried out in other EU-funded projects, the development of new methods for diagnostics based on next generation sequencing technologies. These cutting edge sequencing technologies were adapted in ISEFOR to the detection of putative threats to European forests, aspects relevant EU state health authorities and to regional diagnostic laboratories. The best example of how this technology can be used in such systems was the work carried out in ISEFOR on canker stain (wilt) disease of *Platanus* species, considered the most damaging problem threatening plane forests and urban trees in Europe and beyond. Native forests dominated by *Platanus orientalis* in Greece are highly impacted by this disease and we are facing another major ecological disaster in the south-eastern Mediterranean Basin. The tools developed within ISEFOR will help to manage the disease. Another positive aspect in this project was the generation of data to identify the invasive pest or pathogen either before its entry into European Union and/or before becoming established in Europe. This process was made possible by the use of sentinel trees and spore traps.

During ISEFOR, changes were made to Deliverables D3.2 and D3.3 due to advances in molecular technologies between proposal submission and the start of the project. Deliverable D3.2 was changed to read: '*Practical evaluation of usefulness of pyrosequencing*' instead of '*Practical evaluation of usefulness of the array*' and D3.3 to '*Development of tools for identification of putative pests from pyrosequencing'* instead of '*A guide to use and troubleshooting for the array*'. This change was necessary given technological advances in pyrosequencing, making it the best strategy available to detect and diagnose alien and invasive pests and pathogens.

In **TASK 3.1 Generation of molecular data from poorly characterized pathogens** the objective for this period was to generate molecular data for poorly characterized pests and pathogens of relevance to European forest ecosystems. From the pathogens listed in the Description of Work (Annex 1 Part B Table 1) as relevant for forest ecosystems in Europe, detailed data were developed for three groups:

i) **Ceratocystis species complex**: *Ceratocystis fimbriata* is a species complex that includes many undescribed taxa making morphological and/or physiological identification methods difficult and error prone. Members of the *C. fimbriata* complex have a wide host range and are widely distributed in the world, attacking forest, plantation and urban trees, as well as root crops and ornamental plants, causing wilt diseases, cankers and rot of storage organs. Fourteen plant families have been reported as hosts for *C. fimbriata*, including plane (*Platanus* spp.), which is attacked by *C. platani* resulting in canker stain (wilt). This fungal pathogen causes severe loss of plane trees in both urban and in natural ecosystems and is, therefore, highly relevant to work in ISEFOR.

ii) ***Phytophthora*** **and** ***Pythium*** **species:** Oomycetes (Stramenopiles) form a diverse group of the Eukarya, and are essential components in terrestrial and aquatic ecosystems as decomposers, mutualists and pathogens. Numerous plant-pathogenic oomycetes cause devastating diseases in most crop, ornamental and native plant communities. Over 120 species are characterized in the genus *Phytophthora* and are arguably the most devastating pathogens of dicotyledonous plants. Despite the agricultural and ecological importance of oomycetes, and the abundance of alien invasive species in this Kingdom, their ecology, genetics, phylogenies and even basic biologies and classification remain poorly understood. The recent application of molecular biology for characterization of oomycetes mainly the genus *Phytophthora* has led to important advances in our understanding of their phylogeny, genetics and evolution. Unfortunately progress remains slow, as indicated by the few studies in the literature compared to other groups, such as the true fungi, arguably because of limited knowledge of oomycete diversity in natural ecosystems and a lack of clear phylogenetic relationships between different genera, as well as an absence stringent molecular markers, such as genus- or taxon-specific primers. Extensive work on the extent of environmental diversity in the oomycetes is warranted, with the prerequisite that a sensitive high-throughput technology is developed to overcome this bottleneck.

iii) **Invasive insect species**: morphologically-based identification of many closely-related insect species is particularly challenging. In most cases, taxonomic keys are only available for a certain life stage, essentially adults. Insecta is one of the most diverse classes of organisms on earth with approximately one million species of which 75% are described, but it is likely that many more species remain to be discovered. Amongst insects, the Coleoptera is currently accepted as the most species-rich group with approximately 360,000 described species. Sentinel European trees planted in 2008 in two areas of China were monitored at regular intervals to survey colonization by indigenous arthropods (and pathogens).

In the first 18 month period of ISEFOR, considerable progress was made towards the objectives. The three pest and pathogen groups were characterized in detail at the molecular level; results were subsequently used to design more targeted and specific means for detecting these pests and pathogens (Deliverable 3.1).

MLST is an unambiguous procedure for characterising isolates of micro-organisms using sequences of internal fragments, approximately 450-500 base pairs in length, of (usually) seven housekeeping genes. The gene fragments can be accurately sequenced in both directions using automated DNA-sequencing. For each housekeeping gene, the different sequences present within a given species are assigned as distinct alleles and, for each isolate, the alleles at each of the seven loci define the allelic profile or sequence type (ST). A great advantage of MLST is that sequence data are unambiguous and allelic profiles of isolates are easily compared to those in large central databases via the Internet thus allowing data exchange across laboratories. In **Task 3.2 Development of Methods for MLST, MLMT and Fingerprinting / Barcoding Methods for Forest Pests and Pathogens**, *Heterobasidion* was chosen as a model forest pest species to develop an MLST scheme. *Heterobasidion* MLST was based on 8 genes, including nuclear and mitochondrial markers. The genes were translation elongation factor 1-alpha, glyceraldehyde 3-phosphate dehydrogenase (gpd) gene, ATP synthase subunit 6 (atp6), RNA polymerase II largest subunit (RPB1) gene, manganese superoxide dismutase (SOD1) gene, calmodulin (cam) gene, laccase gene and the hydrophobin 1 (Hah1) gene. The results provided a means for tracking *Heterobasidion* infection sources across Europe.

Using a large collection of *Heterobasidion* isolates held in the forest pathology laboratory at UNIABDN (Partner 1), considerable progress in MLST and MLMT analyses was made in the first 18 month period by UNINE, and the objectives were achieved. The method developed will enable phytosanitary authorities to track pathogen sources effectively.

UNINE and UNIABDN developed the entire dataset for the *Heterobasidion* collection. UNINE and UNIABDN made these resources available to all ISEFOR partners and in time it will be made publicly available. Their release in appropriate public databases will help to promote efforts towards developing similar datasets for other forestry relevant pathogens.

In order to take advantage of existing resources to develop specific means for diagnostics and detection of various relevant forest pests and pathogens, work in **Task 3.3 Use of DNA signatures to design methods for rapid and specific detection of the most relevant invasive organisms**, UNINE conducted studies on *Ceratocystis platani*, a pathogen invasive in Italy, France, Switzerland and Greece. In Switzerland, *C. platani* is infecting and killing plane trees in Geneva. IPP-CNR focused on both *Ceratocystis platani* and *Ophiostoma novo-ulmi* (causal agent of Dutch elm disease).

During ISEFOR, molecular techniques were used to differentiate the *Ceratocystis* genus at the levels of species and/or isolate. Based on PCR amplification with purpose-designed taxon-specific primers, all *C. platani* isolates could be specifically diagnosed avoiding cross reactions with closely related species. The use of a nested PCR approach enabled enhanced sensitivity of detection. The use of these taxon-specific primers in real time PCR experiments facilitated efficient, sensitive and reproducible detection of *C. platani*. Accurate DNA detection was optimum when 1ng target-DNA was used, irrespective the presence or absence of non-target DNA. The assay was accurate over a range covering at least three orders of magnitude, relevant to environmental conditions where such an assay would be used. Based on these results, the power of the molecular technologies for detecting the presence of *C. platani* in various biological matrices was clearly demonstrated.

It is interesting to note that, with the use of the newly designed *C. platani*-specific PCR primers, a reliable and reproducible distinction between *C. platani* and closely related species or species complex was obtained. PCR-RFLP diagnostics, as suggested by EPPO (2003), undoubtedly offers precise diagnosis of *C. platani*, but the procedure is time consuming and expensive. Two restriction digestions are required to identify *C. platani* isolates. Taxon-specific PCR primers developed in the course of this work, in contrast to their use either directly or in a nested approach in a classical or real time PCR format, enabled rapid and unequivocal identification of *C. platani* isolates. This technique will thus facilitate routine work in plant pathogen diagnostics. In any laboratory providing plant pathogen detection services, it is essential to have access to these kinds of techniques to promote accurate and rapid identification of the agents causing the diseases, and to provide the crop producers with a rapid response so that they can take the necessary steps towards the control of these diseases and reducing damage to urban or natural ecosystems.

In collaboration with Partner 5 (IPP-CNR), the utility of the methods developed for *C. platani* were tested in the urban setting of Firenze, Italy. Bait traps, comprising Petri dishes containing moist filter paper discs, were hung in trees at various distances from trees which the City Council were pruning or felling because of *C. platani* infections. The filter paper discs were directly analysed for *C. platani* DNA using the species-specific primers. The method proved extremely sensitive, and will prove useful in the detection of the pathogen during spread, enabling prophylactic measures to be taken.

One of the most important objectives of ISEFOR was to develop and apply emerging pyrosequencing technologies for forest pest and pathogen diagnostics and detection in to monitor both known and putative alien pest and pathogens. In **task 3.4 application of emerging massive high throughput sequencing techniques for diagnostics (mainly 454 flx/Roche and Solexa/Illumina)**, UNINE, UNITUS and SLU, using different approaches and primers, developed this state-of-the-art technology to make it available for end users and plant health authorities.

UNINE: The primer pair used targets all eukaryotic organisms making it possible in a single NGS run to identify known and unknown putative pathogens. The marker used is the 18S gene. This marker proved effective in amplifying a broad range of Eukaryotic groups including Oomycota, true fungi and insects (Lara & Belbahri, 2011). The work carried out by at UNINE targeted forest soils either from Europe or from other parts of the world. Some soils were collected from nurseries and forest ecosystems and examined for the presence of putative alien pests and pathogens.

UNIABDN: in collaboration with UNINE, demonstrated that NGS can be used to determine the biodiversity of eukaryotic micro-organisms present in environmental samples, including the detection of a very wide range of pathogens amongst a background of saprotrophs. DNA was extracted from swabs taken from song birds (principally in the genus Turdus) netted by The British Trust for Ornithology (BTO) during their routine annual ringing operations in the west of the UK. Analyses produced sequences from 3,345 eukaryotic organisms, including pathogens of humans (109 taxa), other animals (80 taxa) or plants (117 taxa). The plant pathogens detected included 4 Protista, 8 Nematoda, 67 genera of true fungi and 4 genera of Oomycota. The massive data set arising from this work is currently being analyzed for publication. There is little evidence that plant pathogens carried by birds are subsequently transmitted to susceptible hosts, but the work gives a strong indication of where future research should be carried out.

UNITUS: The ITS region was chosen as the target sequence, in particular ITS1. The UNITUS pyrosequencing strategy targeted species in two major Oomycota genera of forest pathogens: *Pythium* and *Phytophthora*. The strategy was validated using DNA from a known assemblage of 9 Pythiaceae, and could also detect the presence of Oomycota pathogens in forest and chestnut orchard soils. While limited to *Pythium* and *Phytophthora*, this strategy is effective for in depth characterization of these genera and detection in the plants for planting pathway and in forest ecosystems.

SLU: developed a novel strategy for the routine diagnostics of alien invasive species. Spore traps combined with 454 sequencing were used for monitoring invasive forest pathogens. This strategy is new for detecting plant pests and pathogens (see Deliverable 3.1). Pathogens targeted included *Dothistroma septosporum* along with various other pine needle pathogens such as species of *Lophodermium.* The techniques developed provided effective detection of these pathogens in pines in Sweden, and are able to assist in monitoring of introduction events and/or establishment of alien forest pest and pathogens.

INRA and BOKU: INRA and BOKU focused on their original strategy of analysing pests associated with sentinel European species planted in China, providing will a unique opportunity to recover putative alien and invasive pests and pathogens that are candidates for establishing in European forests.

Tasks 3.5 & 3.6 Development of Tools for Further Identification of Putative Pests from NGS and Addition of Sequences to Databases: provided additional characterization of pests detected by pyrosequencing; the work was necessary because the sequence reads recovered by pyrosequencing are short and further characterization is necessary to determine the taxonomic status of these pests and pathogens.

The specific objectives of the work included application of state-of-the-art emerging massive high throughput sequencing techniques for diagnostics (454 FLX/Roche Solexa/Illumina). The results of Task 3.4 demonstrated unambiguously that NGS approaches were very effective diagnostic tools, and also indicated the existence of hitherto unknown pathogen genotypes and species. Task 3.5, therefore, was undertaken to characterize the diversity newly revealed by NGS. The partners were successful in describing either unexpected species in European forests (UNINE, UNITUS, SLU and IPP-CNR) or previously unknown species (UNINE, UNIABDN, IPP-CNR). The data obtained in this work were added to international databases in Task 3.6; all sequences were collected and submitted to these international databases (mainly GenBank). Moreover, type cultures of newly recognised pathogens were submitted to international culture collections.

Workpackage 4 Pathways of Invasion

The great majority of current information available on pathways of invasion is based on statistics regarding imports and on interception data by national plant protection organisations. Interception data focus on specific quarantine pests and commodities and do not record negative observations. They thus provide incomplete information on pests transported in the various commodities, but they also highlight the importance of different pathways in the spread of alien pests and pathogens. WP4 addressed the plants for planting pathway for forest pests. Its main objectives were to:

- establish a database of all commodities in the plants for planting pathway and of associated risks in terms of exotic diseases or pests;
- analyse movements of these commodities into and, where possible, within Europe (origin, nature, volumes, routes and destinations of the goods);
- proceed to detailed samplings and analysis along the pathway of selected commodities, from the production area to the final destination;
- determine and quantify the pests and pathogens linked to these commodities;
- match these results with those of specific standard PRAs and with interception data from national plant protection organisations in order to assess the accuracy of the interception data;
- provide analysis and information to aid development of an ISPM for plants for planting;
- use the 'sentinel tree' technique for identifying Asian pests attacking local (Asian) tree species that are imported in large numbers into Europe.

1.3. Description of the main scientific and technical results

We merged the Commercial flow and Commodities databases for pathway, because the volumes, origins, destinations and nature of the imported commodities included information common to both databases.

For **analysing commercial flows of plants for planting**, data were collected from different sources (NPPOs, individual inspection points) in 14 countries (BE, CY, CZ, DE, DK, EE, FI, FR, GB, IT, LT, NL, PL, SK) on imports of live plants for planting both from outside or within the EU (Table 1). These data were gathered in a single dataset of ca 379,600 entries, containing the 52 fields: *Country; Year; Delivery.date; Delivery.month; Inspection.date; Inspection.month; Month; Order; Family.original; Family; Genus; Species; PlantGrowthForm; Woodiness; Quantity; Unit; Data.source; Description; Inspection.place; Import.company; Destination.country; adress.corrected; Destination.country.adress; Product.class; Origin.country; Iso.Code; Alien Alert; Commodity; Continent; Origin.continent; Origin.city.region; Entry.point; Entry.point.type; Expedition.country; Decision; Inspection.type; Order.RE; count; TREE/SHRUB; EPPO_NOI; INVASIVE; APHIS; EU-TOP1; EU-TOP2; AQUATIC; SOILRISK; BOOMKWEEK; EPPO-A1; EPPO-HOST; Indoor.outdoor; Deleted ; EU.filter*.

We compared these data with those from Eurostat and detected discrepancies between the two datasets. Our dataset is not complete and obviously does not represent the total trade volume of plants in the EU. However, it comprised representative components from a series of major importing/exporting countries (NL, BE, FR, IT ...) and allowed relevant analyses. Analysis of trade volumes showed that the majority of plants imported plants into the EU are non-woody herbs. The Netherlands is by far the major importer of live plants for planting and also imports large numbers of non-woody genera, mainly from Asia, whilst other European countries import preferentially woody plants. The origins and types of imported plants also differ greatly from one European country to another, which could lead to different risks of pest introduction.

There was a general increase in imports of live plants for planting and in volumes of plants exchanged between European countries. This change can be contrasted with temporal changes in numbers of interceptions, which fluctuate substantially in the short term (3-4 years) but were constant on average over the last fifteen years. Surprisingly, the number of interceptions did not increase with increasing imports of live plants for planting. Further analysis will determine whether this is due to the nature of the goods or that the inspection effort remained constant in recent years and thus inspections were unable to detect more pests. In addition, it will be interesting to link the import and interceptions data and the results of the evaluation questionnaires of inspections to determine whether the effectiveness of inspection is related to the nature and the volume of goods imported.

The data also show that the most harmful consignments of plants are not necessarily the biggest ones: relatively small consignments were found with significant numbers of pests. Moreover, consignments do not present the same risks of pest introductions based of the area of origin. Consignments coming from Asia and North America present by far the greatest risks (Figures 4, 5). In addition to the analysis of the genera and origins known to generate many interceptions, we investigated the combination of new genera and origins. For The Netherlands, we identified ten newly imported genera coming from Asia. Amongst these genera, three already presented interceptions of harmful Asian organisms.

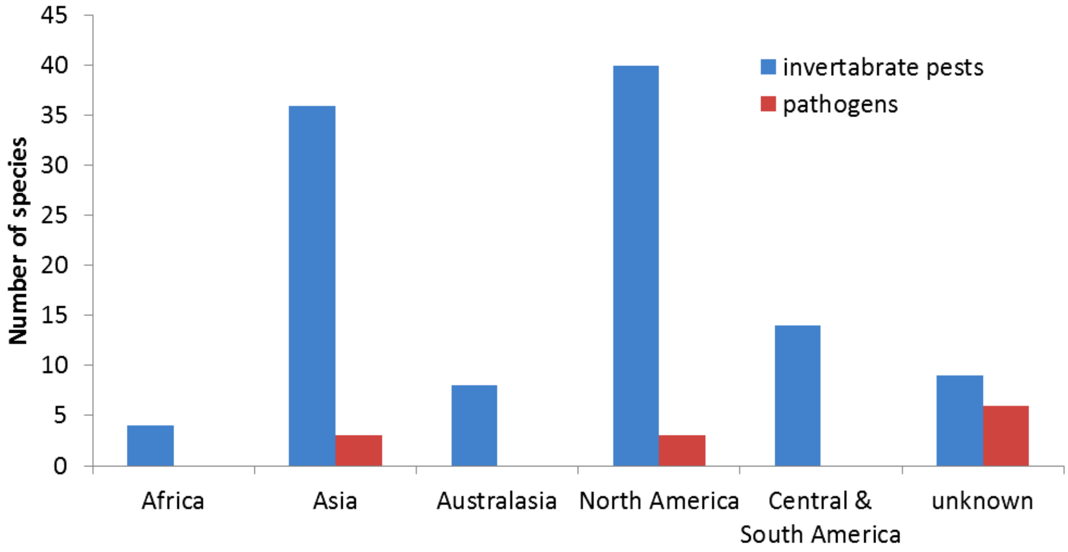

Figure 4 - Origin of alien invertebrate pests and pathogens established in EU countries

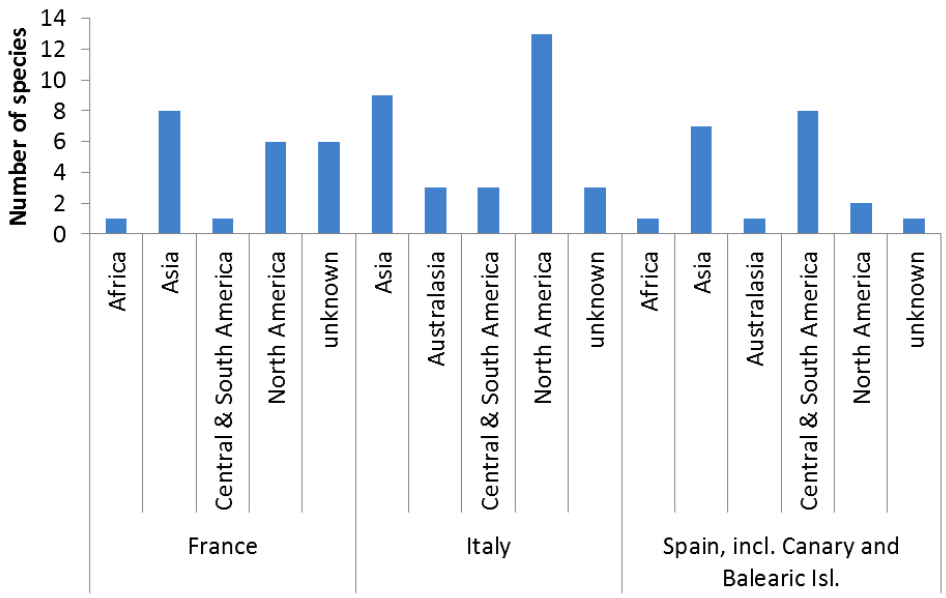

Figure 5 - Origin and number of established alien invertebrate pests and pathogens recorded for the first time in France, Italy, and Spain

Table 1 - Isefor commodity database for plants for planting

Country	Period	Provider of the data	Entry points	Date of entry	Origin	Final destination	Level of identification of the commodities	Quantity
Belgium	IV.2009 – XII.2011	NPPO at local entry point (Antwerp harbor)	Antwerp harbour only	Day	Country	Country	Species	pieces
Cyprus	2010	Central Services of the NPPO	2 entry points (Limassol port, Larnaca airport)	Not mentioned	Country	Not mentioned	Not always mentioned, genus if mentioned	pieces
Czech Republic	2010 – II.2012	Central Services of the NPPO	1 entry point (Ruzyne airport)	Day	Country	Not mentioned	Genus and categories of plant	pieces
Denmark	2011	Central Services of the NPPO	Not mentioned	Not mentioned	Country	country	categories of plant	pieces
Estonia	2000 - 2010	Central Services of the NPPO	All points of entry, mentioned	Day	Country, region, sometimes city	City	Genus or species	pieces
Finland	2010 - 2011	Central Services of the NPPO	Not mentioned	Not mentioned	Country	country	categories of plant	pieces
France	2005 - 2010	Central Services of the NPPO	All points of entry, mentioned	Day	Country	Importing company	Genus	pieces
Italy	2009 - 2010	Regional services of the NPPO	6 airports and 7 harbours, mentioned	Day or year depending of entry point	Country	Not mentioned	Genus or species, depending of the entry point	pieces
Lithuania	2012	Central Services of the NPPO	1 entry point	Day	Country	Not mentioned	Genus, species	pieces
Poland	2007 - 2011	Central Services of the NPPO	All points of entry, mentioned	Day	Country	Not mentioned	Genus or species	pieces
Slovakia	2005 - 2011	Central Services of the NPPO	1 airport and 1 road entry point, mentioned	Day	Country	Country	Not always mentioned, genus if mentioned	pieces
The Netherlands	2000 - 2010	Central Services of the NPPO	All points of entry, not mentioned	Day	Country	Not mentioned	Genus	pieces

Alternative pathways were explored by consulting a series of agencies, organizations and companies in 2011 in order to obtain the data necessary for our analyses, namely Eurostat; the national customs of the EU member states; National Plant Protection Organizations (NPPOs); EPPO; Cost Action PERMIT (Pathway and Evaluation and Pest Risk Management in Transport); 144 nurseries (mostly in Belgium, some in The Netherlands and Italy); ministries (the Belgian Ministry of Public Health, the Belgian Ministry of Agriculture, The Flemish and Walloon Ministry of Environment, The

French Ministry of Agriculture, The German Ministry of Agriculture and The Dutch Ministry of Agriculture); airport custom offices in Brussels (BE), Charleroi (BE), Schiphol (NL), Paris Charles de Gaulle (FR), Heathrow (GB) and Frankfurt (DE); major express courier delivery companies (DHL, TNT and UPS); the Belgian postal service.

Overall, it proved extremely difficult to retrieve precise and complete data regarding the import of nursery stock for most EU member states. The Eurostat data were too aggregated (for example the code 06029041 is used for “forest trees and shrubs” and therefore could be used for risk analyses bearing on particular taxa. The most detailed data used in this study (gross import of plants for planting) were provided by some NPPOs (e.g. The Netherlands, France, Belgium and Poland). The customs and postal services were not able to send any data, and the private companies contacted (express delivery companies, nurseries) mostly did not reply, or sent very little poor quality data.

It should also be noted that the data collected by each NPPO differed in quality (the level of detail was not the same) and did not cover the same period. They thus do not form a homogeneous dataset.

Another general remark is that we were unable so far to find reliable data on the volume, nature and paths of goods imported by travelers or via the postal parcel pathways, because of the negative or incomplete information from the customs, postal services and express delivery companies. However, finding no information does not imply that these pathways do not exist but, instead, highlights the need for further original investigation along these lines.

And last but not least, an important collateral result of this particular study is that it demonstrated a serious shortage of detailed and homogeneous information regarding the flows of plants for planting into, from or within the EU. This obviously constitutes a serious weakness in the European plant health regime.

Exotic pests and diseases in pathway; comparative assessment of pest risk analyses. We provide a series of studies: a) *Comparative analysis of the historical establishment of insect pests of woody plants in Europe and North America through various pathways* [e.g. Figure 6]; b) *Interception of pests on five commodities along a pathway of introduction*; c) *Invasive species established in EU countries*; d) *Alien organisms intercepted but not established – their origin and points of entry*; e) *Comparison of list of alien species already established in EU with the list of intercepted species*. Although these were not directly linked to pathway analyses, the studies provided data or information enabling further understanding of pathways of introduction into Europe or issues related to them and potential solutions.

Pest risk analyses for targeted alien organisms. Five species found in the Chinese sentinel nurseries (see below) were selected: three fungi (*Phaeosphaeria oryzae*; *Pestalotiopsis mangiferae*; *Phytophthora citrophthora*) and two insects (*Cydalima perspectalis*; *Ceroplastes rubens*), and express PRAs for Europe carried out. In addition, a system was developed to quickly assess which species, amongst those identified in the sentinel nurseries, present the highest risks and should be targeted in full PRAs.

Comments on ISPM for plants for planting. The International Standard for Phytosanitary Measures (ISPM) 36, *Integrated measures for plants for planting*, was adopted in 2012 (FAO 2012). [This was during the course of the ISEFOR project and the original title/objective of this Deliverable ("*Comments on draft ISPM for plants for planting*") was changed accordingly]. Acceptance of the ISPM also changed the scope of the present Deliverable. ISPMs are adopted by consensus by the highest organ of the International Plant Protection Convention, the Commission on Phytosanitary Measures, which comprises representatives of all 151 signatory countries and contracting parties (such as the EU). Standards are regularly reviewed, but this is unlikely to lead to major changes in the text. Hence, rather than finding flaws in, or suggesting improvements to ISPM 36, we commented on the ISPM by referring to phytosanitary regulations on the import of live plants in the EU and the findings of the ISEFOR project, in particular D2.2 and D4.4.

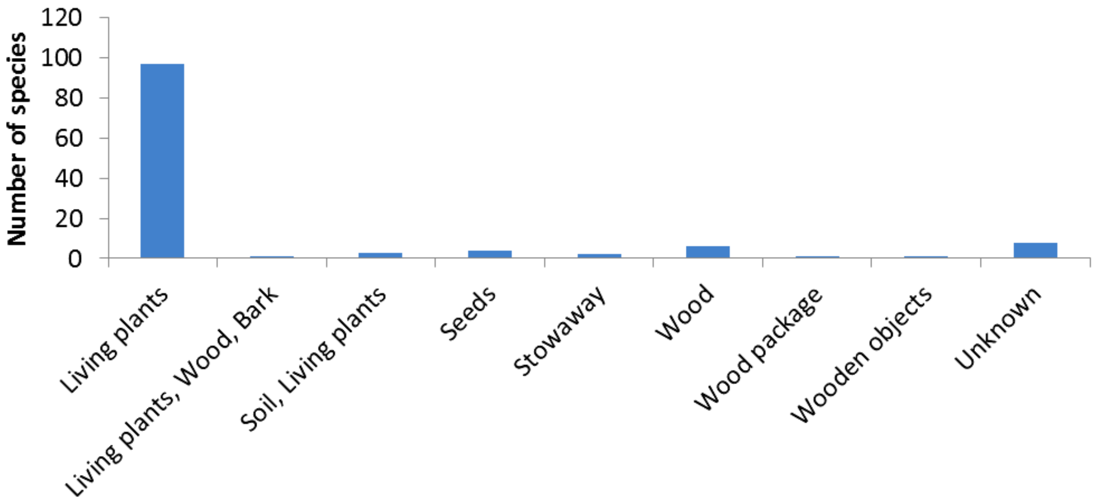

Figure 6 - Most likely pathways of introduction of alien invertebrate pests and pathogens in Europe

Sentinel nurseries. In a previous EU FP7 project, PRATIQUE, several of ISEFOR partners successfully used the "sentinel tree" method in Asia to identify Asian pests and diseases that may damage European woody plant species. For this purpose, European species were planted in Asia and subsequently monitored very carefully for Asian pests. In ISEFOR, we used the "sentinel nursery" technique for identifying Asian pests attacking Asian tree species that are imported into Europe. Five commonly imported Asian plant species in the region of export were planted to obtain lists of potential pests that may be expected on these imported commodities. This method will allow EU member states and EPPO to carry out new pest and pathway risk analyses, and compare the information gathered with that from literature reviews. Furthermore, knowing which pests and diseases are associated with particular plant commodities would also allow the EU and member states to alert inspectors to the possible presence of specific pests and diseases and their symptoms, and to develop new surveillance and detection techniques accordingly.

Two nursery fields were established in China, near Beijing (temperate region) and Fuyang near Hangzhou (sub-tropical region). Five woody plants were selected from the plants for planting presently commonly imported from China to Europe: *Acer palmatum, Ilex cornuta, Buxus microphyllus, Fraxinus chinensis* and *Zelkovia schneideriana*. The two nurseries were surveyed every two weeks from April to October, in 2012 and 2013 (= 2 seasons of observations), and every month in winter. For all the plant species included in this study, a literature review was carried out and their known pests and diseases in China were listed and compared with the lists of species observed on the sentinel plants. At the end of the project, another literature survey was carried out to search for all organisms identified at species level, the known distribution in China and Europe, their invasion status in the two regions, and their known host range in China. Each species found on a sentinel plant was assessed for its likelihood of being exported with plants, taking into account the period of presence on the plant and its coincidence with the period of export, the conspicuousness of the organism or damage. Insects were classified as low likelihood when they were unlikely to be transported because the organism is usually not on the tree when it is exported.

A total of 109 insect species x hosts combinations and 15 pathogen species were found on sentinel plants in Beijing compared to 114 and 25, respectively at Fuyang. Of the insects, 34.9% and 25.3% were identified to species level at Beijing and Fuyang, respectively. For pathogens, 13% were identified to species level at Beijing and 52% at Fuyang. Molecular analyses (barcoding) were more efficient for the identification of Lepidoptera than species from other orders because the former group is significantly more represented in the genetic databases such as Bold and GenBank. However, many other species were barcoded and all sequences were archived for future comparisons. At the two sites, many more insect species were found on the broadleaved species *Zelkova* and *Fraxinus* than on the two evergreen species, *Buxus* and *Ilex*, whereas *Acer* was highly colonized in Beijing but less so in Fuyang. General foliar damage levels showed a similar pattern. About 40 families of insects were recovered from the five host trees in Fuyang and Beijing, most belonging to Coleoptera, Lepidoptera and Hemiptera.

Amongst the 96 combinations of insect species x hosts x region identified to species level, 92.7% insect x host associations were not found in a general literature survey of pests of the five plants, which included many common, damaging species. All species were known from China. From a later Chinese literature survey focusing on the 36 insects identified to the species level at Fuyang, it appeared that all were known from China but 78% of the plant-insect interaction records were new for the country. Of the 223 insect records, 9% were considered to present a high likelihood of introduction, 8% a moderate likelihood and 83% a low likelihood. The latter category comprised mainly insect species found only on foliage of deciduous trees which are exported only in winter without foliage. Pathogens were considered to present a higher risk of introduction, with 42.5%, 50% and 7.5% scoring high, medium and low, respectively (n=40).

The general conclusions were that protocols to identify exotic pests and pathogens potentially carried by imported plants for planting in the framework of a pathway risk analysis could include a combination of classical literature surveys (preferably including national literature) with sentinel plantings. These latter should:

- Be installed for at least two years since the first season, as very few organisms were found, at least in Beijing.
- Be installed in the regions and environments where they are grown and stored before export, considering that nurseries in different regions and environments will provide different insects and pathogens. Ideally, exporting nurseries should install untreated sentinel plantations in the vicinity of the nursery, and exported plants should not be stored elsewhere before being shipping.
- Be inspected particularly in the season of export, and on tissues that are exported. For example, broadleaved trees exported in winter without foliage should have the stems, branches and roots carefully examined in winter. Evergreen trees should have all the plant tissues examined throughout the year.

When possible, insects should also be identified using barcoding, taking into account that some groups are better represented in the genetic databases than others.

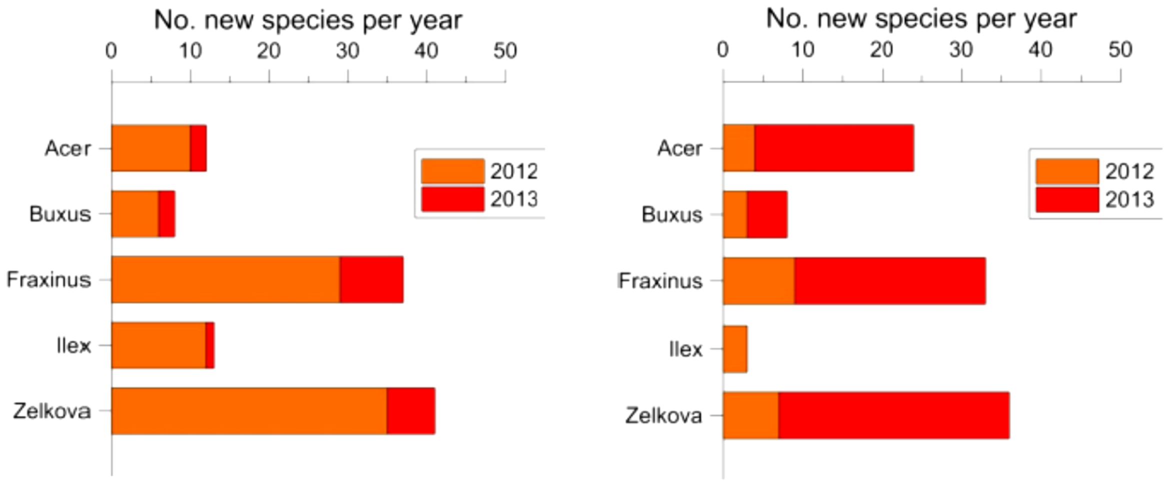

Figure 7 - Number of insect species found on the five tree species in Fuyang (left) and Beijing (right). The red bars indicate the additional species found in 2013.

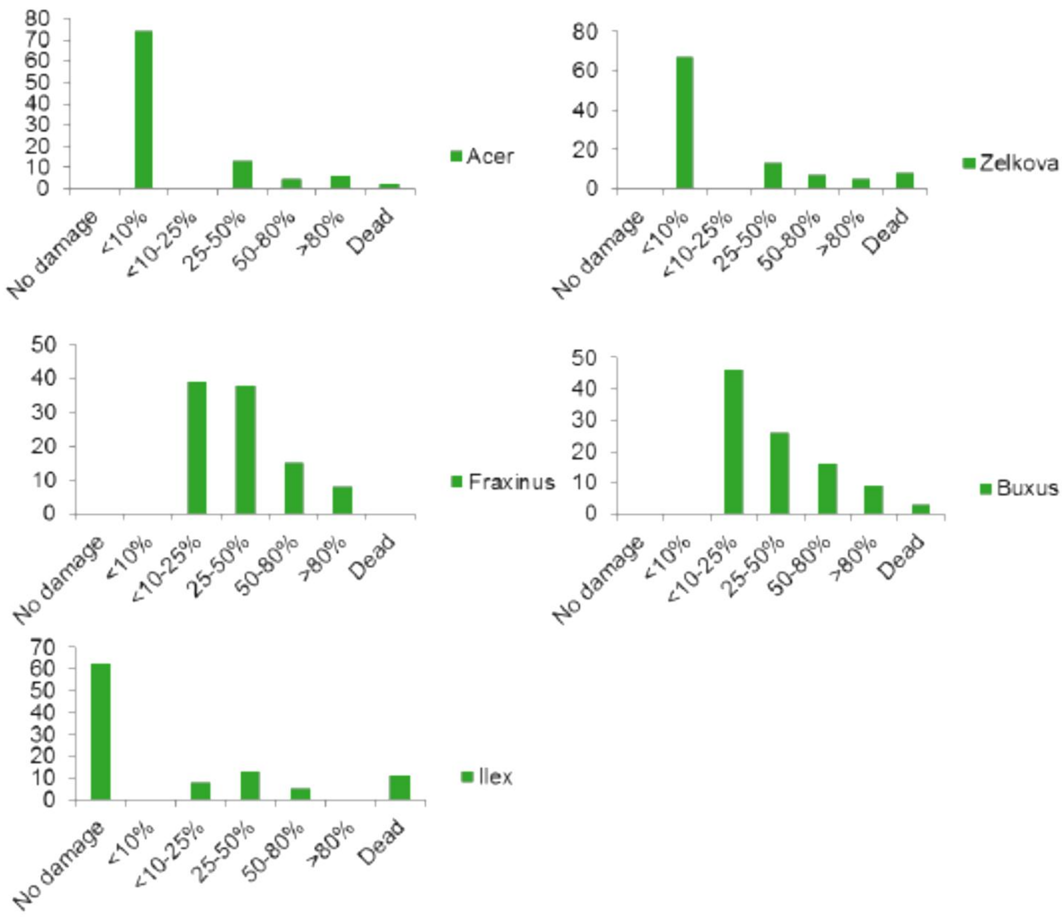

Figure 8 - Damage level on the five tree species in Beijing in 2013.

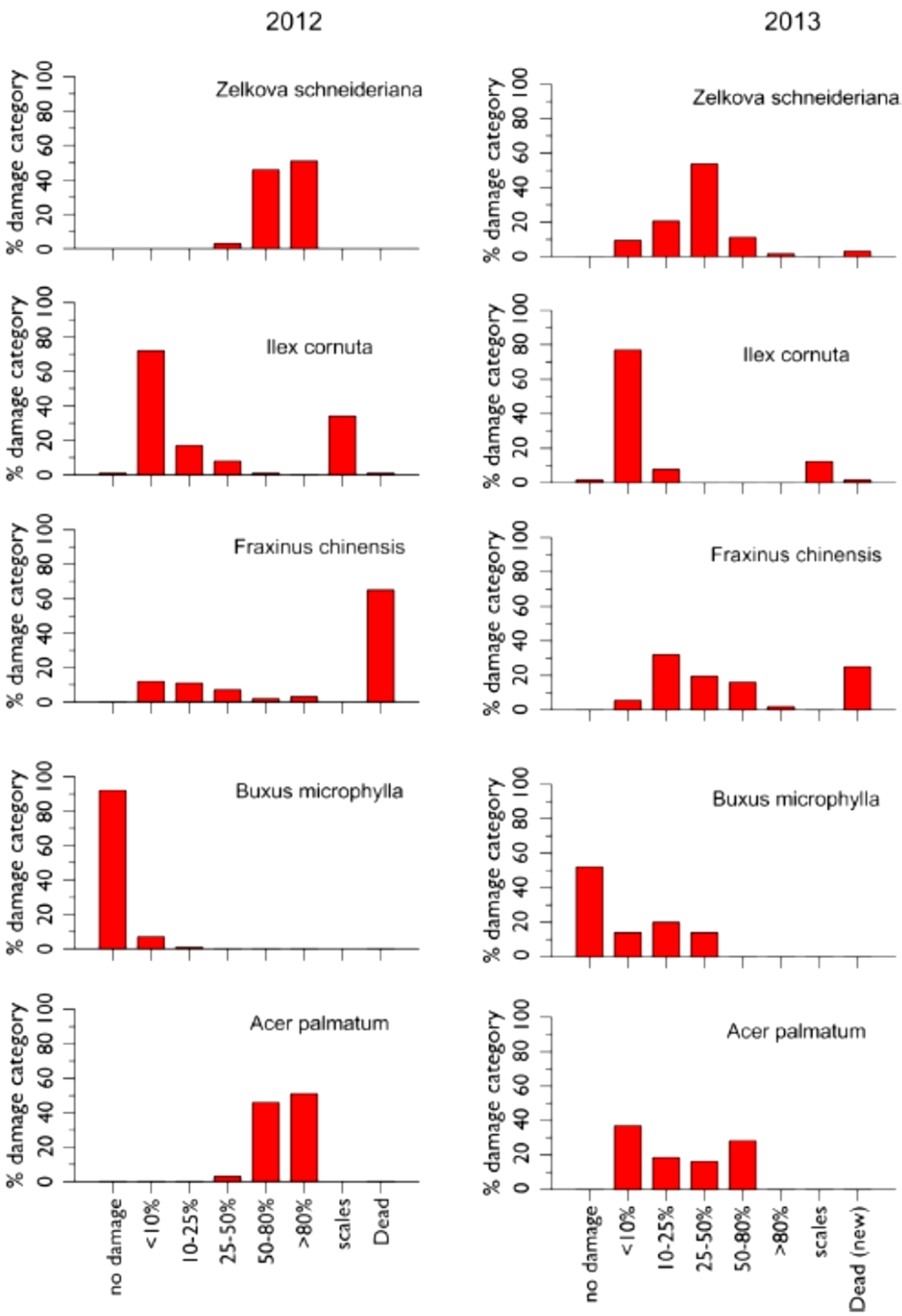

Figure 9 - Damage level on the five tree species in Fuyang in 2012, 2013

Workpackage 5 Modelling of invasion potentials in relation to climate change

The cellular automation (CA) model-type utilized in WP5 incorporates both temporal and spatial aspects, and is applicable for the assessment of spread of alien species at different geographical scales. The results consist of stochastic spatial models, implemented by the CA-technique, for seven potentially invasive species:

- *Bursaphelenchus xylophilus* (pine wood nematode)
- *Hymenoscyphus pseudoalbidus* (ash dieback)
- *Phytophthora alni* (alder Phytophthora)
- *Fusarium circinatum* (pine pitch canker)
- *Dothistroma septosporum* and *D. pini* (Dothistroma needle blight)
- *Agrilus planipennis* (emerald ash borer)
- *Dendrolimus sibiricus* (Siberian moth)

The effect of climate was incorporated in each CA-model using CLIMEX, allowing the analyst to predict the vigour and spread potential of the invasive species in a changed climate. Running the model many times produced a map of the probability of spread. These maps help managers and policy makers to identify areas and regions where risks are high and where special preventive measures may be required.

The CA models were developed in co-operation between the UEF team and specialists from the other partners. Geographical information about the occurrence of the host species was acquired from several sources. Population density information from the whole of Europe was also obtained, along with maps detailing rivers for the modelling of alder *Phytophthora*, and elevation for both pinewood nematode and alder *Phytophthora* modelling. The CLIMEX software was used to develop maps of ecoclimatic index (EI) for emerald ash borer, Siberian moth, pitch canker, and Dothistroma needle blight. The EI maps were developed for the current climate and that of 2100, based on climate scenario A1B. Interpolation was used to derive the climatic conditions of a certain location in given simulation year. Other methods were used to integrate climate change in the other CA models. Since ash dieback, pinewood nematode and alder *Phytophthora* were not included in the CLIMEX model, different approaches were used to describe their specific climatic requirements and the impacts of climate change in the spread model. Climate was not included in the model for alder *Phytophthora* since the climatic requirements for this disease are poorly understood.

Some models (pinewood nematode, ash dieback, Dothistroma needle blight) were validated by comparisons between the simulated and observed spread. This process was not possible for other species, as they are either not established or have not spread a great deal in Europe to date. Models for these species, therefore, were validated by comparing the simulated spread rate and the simulated amount of damage to observations from other parts of the world. The models developed are considered mechanistic, since the mechanisms affecting spread are simulated to match what is known to occur in nature. Spread rates depend on a limited number of biological parameters such as flight distances for spores or of insects, parasitism, or predation. Values for several of these parameters can be found in the literature. The remaining the parameters were controllable (e.g. number of entries, transport of infected seedlings or contaminated cargos) and there are no correct or incorrect values. Therefore, the models can be regarded as valid if the biological parameters are correct and the simulated mechanisms correspond closely enough to the true mechanisms occurring in nature. The model structure, i.e. the mechanisms included in the models and the ways in which they are simulated, were evaluated by the experts in ISEFOR, and further evaluated by reviewers for the scientific journals to which the model descriptions are submitted.

Risk maps were produced with all the developed models. For example, Figure 10 shows the model output for pine pitch canker (host distribution, ecoclimatic indices for present and future climate, and probability of spread). In addition, many other outputs were produced, such as the year of invasion and effect of control measures. Systematic and extensive sensitivity analyses were conducted with each model, by analysing separately the effect of each biological and controllable parameter on the risk and extent of invasion.

CA models can be used in very high numbers of varied analyses by changing the degree of control, modifying host coverage (creating spread breaks by clear-cutting all host trees around infested areas), or modifying the length of the simulation period. Therefore, the main outputs of WP5 are the models, not model runs. It can be expected that these models will be used in the future for analyses related to risk and risk management for the modelled species.

For the assessment of climate suitability and host tree susceptibility to potentially invasive insect pests, GIS-based spatio-temporal models were developed using species-specific climatic thresholds and constraints. Modelling was performed for past and current climate and under climate change conditions. Modelling and GIS-based mapping of climatic suitability and forest susceptibility to the mountain pine beetle (*Dendroctonus ponderosae*) and Siberian moth (*Dendrolimus sibiricus*) was based on the basic eco-physiological requirements and climatic constraints of these insects. Climate change effects on climatic suitability and forest susceptibility were assessed using the climate projections of different regional climate models from ENSEMBLES. The results of GIS-based mapping of spatio-temporal changing climatic suitability and susceptibility to the selected invasive pests are accessible on the World-wide web (http://ifff-server.boku.ac.at/Services/ISEFOR/). Mapping of climatic constraints and susceptibility of forests generated essential basic data and provided various relevant informations and thematic maps for pest management.

Spatio-temporal modelling of the climatic suitability revealed different suitability of present-day climate and specific responses of the two species to projected climate change in Europe (Figure 11). For the mountain pine beetle, the projected rise in temperature will increase the likelihood of winter survival of hibernating larvae. Elevated temperatures in future will shift the potential suitable habitats for univoltine development of the mountain pine beetle northwards and to higher elevations. The phenotypic plasticity and variation in development time of mountain pine beetle populations may contribute to the maintenance of a synchronized lifecycle in a warmer climate. Depending on locally different projected changes in precipitation, vulnerability of susceptible *Pinus sylvestris*-stands to mountain pine beetle will vary considerably.

Larvae of the Siberian moth require continuous winters of a continental type with a low frequency of autumn thaws for successful hibernation in the forest litter. Current mild autumn and winter conditions in Europe will therefore limit the potential invasion and range expansion of the moth. The projected increase in temperature, especially in Northern Europe, will restrict range expansion of the moth in the future.

The different responses to climate change apparently depend on the basic biological and eco-physiological requirements of the respective insect species. Detailed knowledge of key parameters which define climatic constraints of potentially invasive pests is essential for assessing the likelihood of establishment and spread of invasive pests and for estimating responses to climate change.

The use of different regionalized climate models may contribute to the reduction of uncertainties of climate projections. Comprehensive risk analysis of invasive (and native) forest pests and confident predictions of future responses to climate change depend not only on the availability of detailed (daily) unbiased climatic data and climate projections at regional scale; climate also affects insect pests through its influence on their host species (i.e. host resistance, changes in host distribution) and through effects on the interactions with other insects/pathogens (competitors, predators, and mutualists). These factors complicate attempts to predict present and future impacts of invasive pests. Modelling of climate suitability and forest susceptibility using GIS is a valuable contribution to the management, prevention and control of invasive forest insects. Confident prediction of future impacts of invasive forest insects, however, is challenging and requires a highly detailed understanding of the complex system.

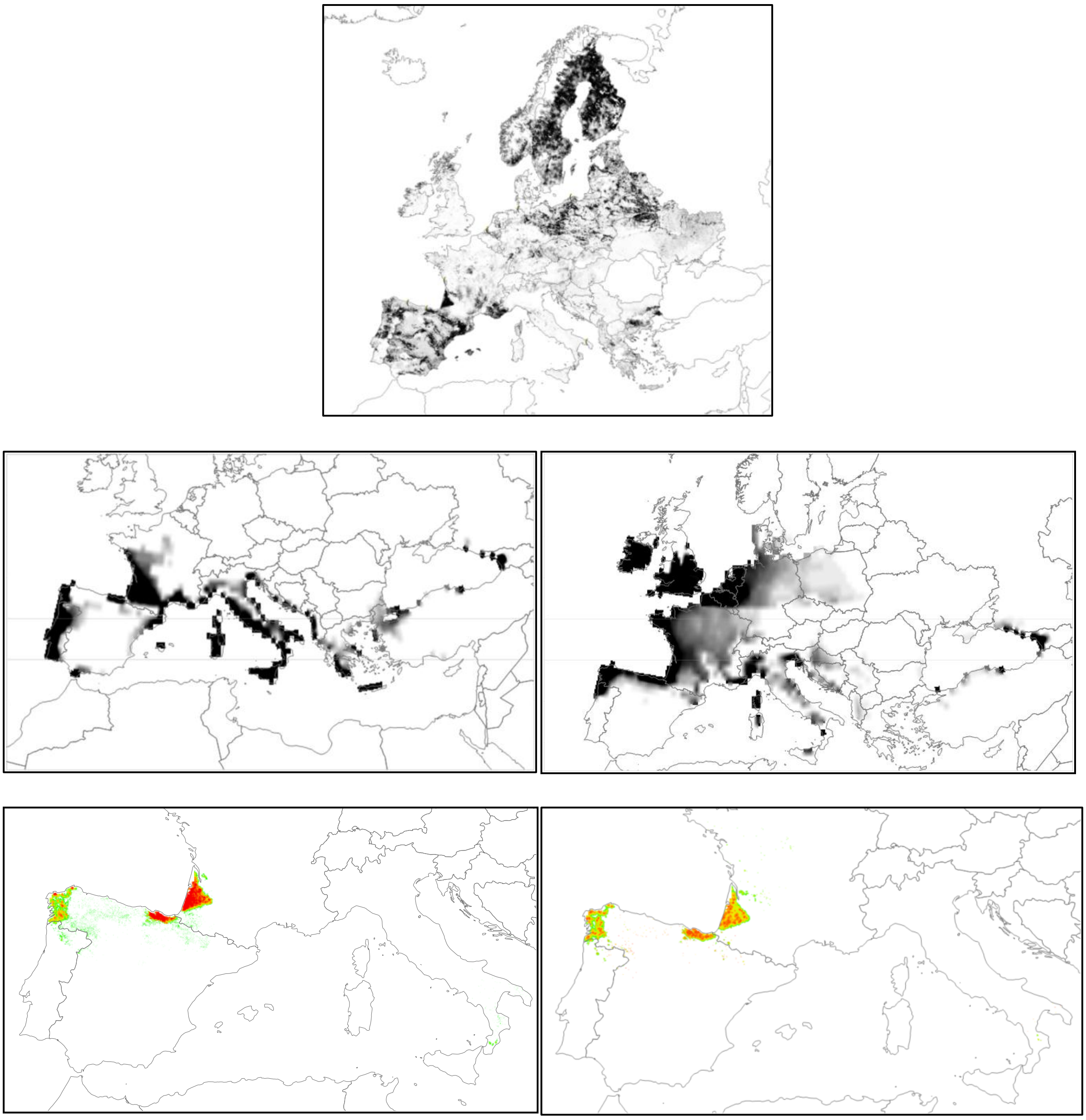

Figure 10: Coverage of the host species of pitch canker (*Pinus* spp. and *Pseudotsuga menziesii*) in Europe and the entry points used in model runs, ecoclimatic index of pitch canker for current climate and for the climate of year 2100 (black = suitable, white = unsuitable), and probability of pitch canker spread in 20 years from the three current entry points (red = high, green = low). The lower right map shows the year of entry in a single 20-year simulation (red = early, green = late).

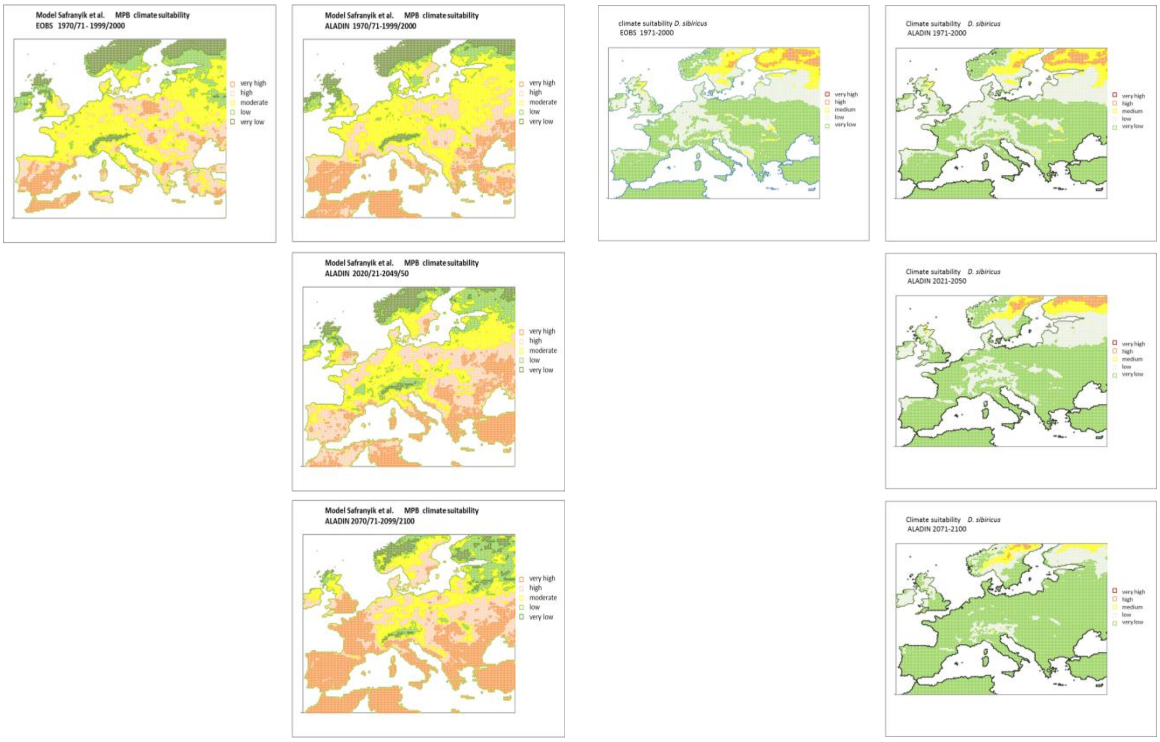

Figure 11: Classified assessments of climatic suitability for the mountain pine beetle (left) and for the Siberian larch moth (right) based on climate projections of the RCM Aladin compared with baseline climate RCM Aladin (1971-2000) and observed climate normal (EOBS 1971-2000).

Workpackage 6 Dissemination

Work in **Workpackage 6** was established to expedite the dissemination of project results at all levels, including publicity, training events and publications. The aim was to take ISEFOR results to the relevant end-user communities, through interfacing with national plant protection organisations both in the EU and internationally, and with forest managers, through the inclusion of forest management stakeholder partners in the consortium. Results were also disseminated to the scientific community and, in various non-technical publications and other media, to the general public.

The objectives of Work Package 6 were to:

- interface with plant health and quarantine organisations at the national, EU-wide and international levels, in order to provide the stakeholder community with data, techniques and technologies arising from the work
- provide forest health organisations and policy makers with recommendations on approaches to tackling problems from alien invasive species, particularly in terms of climate change
- disseminate project results to the scientific community
- communicate results to the general public in a suitably non-technical manner.

Achievements in WP6

Task 6.1 The Project Web Site (http://www.isefor.com/) was launched in April 2011 and maintained and updated as required, adding new and relevant information when it became available. The discussion forum (http://www.isefor.com/forum/index.php) was utilized at times, although most of the time, partners communicated via email, telephone contacts and skype.

TASK 6.2 Organisation of Project Workshops: Two workshops were held over the time of ISEFOR. The first was held in Brussels on 10th February 2012. Forty two delegates attended, representing a range of European plant protection organisations and researchers from four continents. Discussions focused on new pathways of invasion and ways to reduce risks.

The second workshop took place in Joensuu in February 2014, and was attended by a range of people from plant health and forest health organisations in various EU and non-EU near-neighbour countries. The workshop schedule over two days enabled delegates to work with the various models produced in workpackage 5, adjusting input parameters to examine how well the models might predict pest and disease outbreaks and spread. Tutorial guidance was provided by the Finnish partners and the delegates all reported positively on the meeting outcomes.

TASK 6.4 Popular Press: ISEFOR partners continued to ensure wide publicity and dissemination of project objectives and results. Full details of all activities are listed on the Participant Portal. Dissemination was successful in over 22 countries. Articles in the popular press reached 41 in total, with substantial contributions from the coordinator and the partners at BOKU. In addition, several partners were interviewed for radio and television programmes. The coordinator was also interviewed for numerous newspaper articles, mainly in the UK.

TASK 6.5 Scientific Conferences: Numerous oral presentations were made by ISEFOR partners to scientific audiences and public venues were made by partners, bringing the total over the ISEFOR project period to 103 (83 in the second reporting period). The partners at FTA in St. Petersburg were very active in organising conferences and presenting data to them.

TASK 6.6 Peer Reviewed Journals: ISEFOR researchers published 100 journal articles throughout the project; further peer reviewed publications will arise when ready. In addition, 246 proceedings papers, posters and popular press articles were published, along with 3 book chapters, 6 monographs and 6 theses which were completed under the aegis of ISEFOR. The complete list of publications with full details of the papers is entered on the Participant Portal.

Description of potential impact of ISEFOR

European forests are of enormous environmental, economic and societal importance, providing a multitude of tangible and non-tangible benefits including timber and non-timber products, biodiversity, water catchment protection, landscape and protection against avalanche, flooding and landslides. There is also increasing demand on forests for recreational purposes. Combined, these benefits have led to much greater political recognition of the multiple functions of forest ecosystems, over and above the production of timber.

Second to habitat fragmentation, invasions by alien species have become major drivers of biodiversity loss in ecosystems worldwide. The project DAISIE, funded in EU FP6, clearly highlighted that Europe is particularly affected by invasives, with over 10,000 alien species recorded, including plants, animals and micro-organisms. Over and above these effects, the climate is changing rapidly, particularly in the more northern latitudes of Europe, although increases in mean temperatures and changes in rainfall patterns are not limited to that region. Climate change is likely to increase latitudinal amplitude of tolerance of pest and pathogen species, leading to increased damage further north than current limits to the life cycles of these species.

The scientific information arising from work in ISEFOR has huge potential impact in many respects, in increasing knowledge of invasive pests and pathogens damaging forest ecosystems, both those species already present and species that may invade in the near to mid-term future. The research has improved understanding of how alien invasive pests and pathogens enter Europe, along with increasing the ability of phytosanitary authorities to detect and diagnose these problematic organisms in pathways of entry. The development, adaptation and validation of state-of-the-art molecular biology technologies for use in detection and diagnosis is of immense value to phytosanitary authorities, providing tools for finding numerous potential problems in plant consignments and plant products rapidly and cost effectively. The databases generated in the work are available to NPPOs and to RPPOs, providing up to date information on threats to phytosanitary security of Europe in particular, although the data are equally important to other continents and individual states.

Of particular value in terms of predicting alien invasive pests and pathogens was the adaptation of the sentinel tree technique to plant nurseries. The Plants for Planting Pathway brings millions of plants into Europe each year from all over the world. ISPM36, drafted and approved during the time in which ISEFOR was running, stresses that the onus for ensuring clean plants are exported rests with the producer, in conjunction with the relevant NPPO. The widespread adoption of a sentinel nurseries scheme, along with the improvements recommended in ISEFOR for preparation of Rapid (accelerated) Pest Risk Analyses (PRAs) would ensure that phytosanitary authorities around the world have as much information as is possible to scrutinize on the imported plants prior to setting up and approving the trade route.

Modelling and mapping of climatic suitability and forest susceptibility to invasive forest pests is a further valuable contribution to pest risk analysis. Although only a restricted number of pests and pathogens were analysed in this manner in ISEFOR, these models can be adapted rather simply for a wide range of organisms. Essential data for accuracy in models are the climatic parameters suitable for development and reproduction of the target organisms. In knowing the organisms, for example from information gathered in sentinel nurseries and further work on climate requirements for any poorly known organisms, the adaptation of the models already developed will be a relatively simple task.

Detailed impacts of ISEFOR results

Workpackage2: Scientific impacts of work in WP2 are already notable, partly based on the publication arising from the amalgamation of the database on pathogens from this workpackage with that obtained in the previous EU-funded project FORTHREATS (Santini et al. 2013), which has already been cited numerous times in subsequent work. Other scientific benefits are accruing, however, particularly the availability of databases on the alien invasive pests and pathogens already present in Europe, from which distribution for over 1000 pests of woody plants was derived, and the information on other pests and pathogens with potential to become invasive. Interception data for woody plant pests for the last fifteen years were gathered from National Plant Protection Organizations (NPPOs) in Europe, and collated in a database with lists of quarantine pests from Regional Plant Protection Organizations (RPPOs) and NPPOs in other temperate regions providing EPPO and NPPOs in Europe with direct access to these data. These databases are available for further interrogation by other interested parties. The addition of a list of forest insect pests indigenous in Russia, based on the collaboration with Russian partners in ISEFOR added greatly to the significance of these results.

The validation of the process using established sentinel trees in arboreta and botanic gardens and younger plants in sentinel nurseries to determine the pests and pathogens indigenous in a region, and which may become problematic if the plants are moved to Europe, is highly significant . An excellent illustration of this fact was the discovery on sentinel nursery plants in China, of *Cydalima perspectalis* (box moth), a new invasive pest attacking *Buxus* spp. in Europe. Box (*Buxus sempervirens*) is a significant understory component in many forests in Europe, already threatened by box blight caused by the fungal pathogens *Cylindrocladium buxicola*, and further damage may result in the woody species becoming locally extinct. Box is also widely used for hedging and topiary in ornamental plantings and hence is a valuable plant in the horticulture industry.

Pest risk analyses (PRAs) are the tools used widely in trying to prevent the introduction of non-native invasive pests and diseases. Defining which pests and pathogens should be targeted in PRAs, therefore, is a crucial, complex issue, requiring as much data as possible on the pest organism(s). the environmental conditions suitable for the organism(s) to develop and the probability of inclusion and survival of the organism in a given trade pathway. In many cases PRAs have focused either on:

1. species that have already invaded the region where the analysis is conducted or in a neighbouring region, in which case little can be done in reality to limit the ingress and spread, or on
2. species that are highly damaging in another continent but without considering *a priori* the likelihood of introduction before the PRA is conducted.

The two mathematical indicators developed to calculate the risk of further spread of already established pathogens in Europe can be used directly as objective criteria not biased by the expertise of each specialist, to rank alien forest pests and pathogens in relative to the risk of their becoming invasive in other regions. Hierarchical cluster analysis also contributed to these calculations, in order to calculate the frequency of a given invasive pest or pathogens species invading countries within the cluster containing EU countries. These data, indicators and methods can be used to target PRAs against organisms most likely to become problems on entry, rather than trying to prepare PRAs for every exotic organism known and, therefore, saving time, money and effort.

Workpackage 3: Work in WP3 is a perfect example of linking fundamental and applied research. The development of generic and specific primers for the organisms targeted in WP3 are of great interest scientifically, and will be valuable to groups world-wide to facilitate investigations into these species and other organisms in other work. The development and validation of next generation sequencing approaches for detection and diagnostic purposes is also highly significant in both scientific and end-user terms. The *Phytophthora*-specific primers, for example, will be used by the numerous research groups focused on these intractable organisms throughout the world. Using these various primers, WP3 partners detected not only known highly damaging alien invasive pathogens in European forest ecosystems and in other systems, but also revealed several alien species unknown in Europe. The primers, coupled with the NGS protocols and training provided will provide end users and diagnostic laboratories with highly efficient methods to detect known pests and pathogens and to diagnose putative alien species in different substrates.

The numerous pest and pathogen species detected in the NGS work which proved to be unknown genotypes or species hitherto unknown to science, highlight the success of WP3 in detecting new diversity that old tools failed to detect. These results have both scientific and socio-economic impacts, the latter due to the greater ease of finding potentially invasive species in the plants for planting pathway, for example.

Results achieved in WP3 are highly relevant to the forest industry, phytosanitary organizations and to the public. For the forest industry WP3 provided validation of Next Generation Sequencing strategies as diagnostic tools. Numerous invasive and alien pests and pathogens were detected effectively and efficiently, mainly in soil samples, which proved an important source of dissemination of alien invasive pathogens. Moreover, work conducted in WP3 by UNINE and UNIABDN clearly identified a hitherto unknown pathway of dissemination of Eukaryotic alien invasives: migratory birds, a finding potentially of immense relevance to the forest industry (and to agriculture and horticulture) which is applicable to the search for measures that help to prevent dissemination of alien invasive pests and pathogens in European ecosystems.

Phytosanitary organizations benefit directly from the NGS protocols developed in ISEFOR, as elaborated in WP3. These protocols are immediately applicable to environmental samples and goods that need to be checked for the presence/absence of alien invasives. A clear benefit to the public is the information on pathways of dissemination of alien invasives, which will help to reduce/prevent introductions of highly damaging pathogens and preserve the forests to deliver their role and functions. The potential socio-economic benefits arising from ease of detection of invasive organisms are extremely high (see below).

Workpackage 4: The in depth analysis of risks associated with the plants for planting pathway conducted in WP4, along with the consideration of alternative pathways for import of invasive organisms massively strengthened the case for regulation of pathways for quarantine regulation, rather than the 'species by species' approach used previously. Originally, an objective of WP4 in ISEFOR was to develop a draft for an ISPM on plants for planting, but in fact, a draft ISPM (36) emerged from elsewhere early during the project. There were doubts amongst IPPC officials, and certainly in the plant import/export trade, whether such rules and regulations were needed. The quantitative results obtained in ISEFOR underlined the need to regulate. This result is highly significant at the levels of policy and socio-economics considerations.

A particularly significant result in WP4 was the discovery that size of a consignment of plants is not the most important factor in terms of risk; it is the origin of the plants, the region in which they were propagated and/or grown on prior to export that determines the probability of potentially damaging organisms being transported. Clearly, this finding has repercussions for the entire plant import/export trade, in that the purchasing nursery in Europe now can choose to import plants from a region in the world where the likelihood of bringing in damaging pests or pathogens is lower. Of course, consideration of economic costs will be a factor too, but to prevent the types of environmental catastrophes witnessed with pine wood nematode, Dutch elm disease or ash dieback, to name a few, receiving nurseries in the EU must make these choices.

Another major outcome of activities in WP4 was the methodological development and first implementation of sentinel nurseries which it is hoped will be now established on a more generalized basis. In these nursery plantings of six woody plant species commonly exported to the EU, over 200 insect species in 40 families, and some 40 pathogens were discovered in a period of just two years. In PRAs conducted on these organisms, several were considered threats to native plants present in Europe. Based on these first results, sentinel nurseries are the central topic of the recently accepted COST Action FP1401 "Global Warning" and of the EUPHRESCO-funded project, *International Plant Sentinel Network*, that includes several ISEFOR partners.

Rapid (accelerated) PRAs were carried out on selected organisms found on the sentinel nursery plants, and further supported the use of this technique in conjunction with the sentinel plantings for determining risk to European plants.

Further development and more widespread use of the sentinel nursery method will be of great benefit to NPPOs and RPPOs, as it will delineate regions where there is a danger of particular pests and pathogens affecting plants and being accidentally imported into Europe. It will also allow the identification of many more pests and pathogens, some of which will probably be unknown to science, or attacking hosts not previously considered susceptible. Hence, this approach, has numerous economic and social benefits, reducing the time taken in diagnostics, and the likelihood of establishment and spread of many potentially damaging alien organisms.

The database of commercial flows developed in WP4 is available for use in any pest risk assessment for the EU involving the movement of plants for planting. The database will allow more accurate assessments of the risks of entry of any given organism. As the data demonstrated that substantial changes occurred in commercial flows over time (plant species imported, volumes, countries of origin), it is important that NPPOs take these points into consideration; it would also be desirable that the database be maintained and updated as further information become available. We hope that our results encourage NPPOs or the EU to establish a standardized, real-time system of data collection at ports of entry, to allow real-time risk assessments. Further analysis of the database is is underway in the framework of COST Action FP 1002 PERMIT and in EFSA procurement “Probabilistic pathway models for risk assessment of plant pest invasions” (Lot 2 Non-edible plant products).

Workpackage 5: Both Cellular automaton modelling methods and GIS-based mapping with regional climatic models were very useful in generating relevant pest and pathogen spread models applicable at wide geographical scales. The modelling protocols developed in ISEFOR WP5 are of great value in decision support for many European institutions with responsibility for phytosanitary issues, in addition to ISEFOR partner institutions themselves. Moreover, it is likely that these protocols will be tested, possibly adopted, by other scientists and end-users around the world who work on parallel problems.

The restricted number of invasive species used in the modelling does not preclude the use of the same protocols for other organisms: it is mainly a matter of changing the climatic tolerance inputs for a given organisms and running the models with those changes. Modelling and mapping of climatic suitability and forest susceptibility to invasive forest pests is a valuable contribution to pest risk analysis. Attendees at ISEFOR workshop II in Joensuu stated that the models would be of value to their work in the plant phytosanitary units of several EU and near-neighbour countries.

Modelling tools are widely used for various applications in forestry, and by phytosanitary organisations wishing to predict the consequences of pest or pathogen invasions. Hence, the application of these techniques is essential for the assessment of European forest vulnerability to potentially invasive pests and pathogens. As these tools can indicate which geographic areas are more likely to be invaded by particular alien pests and pathogens under different climate change conditions, it is then possible for appropriate authorities to target their efforts more logically and efficiently. Moreover, modelling assists stakeholders in understanding how changing environmental conditions may impact forest health.

Other impacts of ISEFOR Research

The reduction in research fragmentation was a significant point in the objectives for work Framework 7. ISEFOR was extremely successful in addressing this point, as it combined the efforts of several different disciplines, including entomologists, pathologists, molecular biologists and modellers, along with the largest forest certification company in the world (PEFC), and the lead organisations representing forest owners in Europe, CEPF and EUSTAFOR. The impacts of these collaborations made the sum of the work greater than each individual part.

For example, research on forest pests has, traditionally, been divorced from work on forest pathogens, despite the clearly artificial nature of this divide. Both groups work on forest health. Interactions in ISEFOR greatly reduced the artificial barriers between entomologists and pathologists and a great deal of collaborative work was undertaken.

Fragmentation in forest health and phytosanitary matters was also greatly reduced: all partners and participants in the ISEFOR consortium became more aware of the problems from alien invasive pests and pathogens overall, rather than the previous focus on a given group of organisms.

Teams from a wide range of EU states were involved in the work. International participation in ISEFOR also was high, with two partners from Switzerland, three organisations contributing to the Russian partnership, and the inclusion of the Chinese team, with access to sentinel tree experiments set up under other research programmes. PEFC is also an international organisation, with offices in many countries around the world.

The diagnostic tools and increased knowledge of the most highly threatening pest organisms to European plant biosecurity in the work of ISEFOR, along with the intensive work on the Plants for Planting Pathway and databases of potential threats, is of great relevance to a range of stakeholder groups, especially within horticulture and forestry, to NPPOs, national plant health laboratories, policy makers and to the wider public. RPPOs, such as EPPO, will be able to list far greater numbers of threatening organisms on the A1 list, providing details, such that phytosanitary inspectors can obtain the required data and maintain their level of expertise.

The greatly improved ability to detect and diagnose alien invasive organisms using the diagnostic platform developed in ISEFOR will also help secure outputs from rural-based industries, protecting jobs in areas of low population.

ISEFOR made significant contributions to the expected impacts of the Framework 7 Knowledge Based Bioeconomy programme, and indirectly to many others. In generic terms, the molecular biology programme in ISEFOR contributed to the this subject area overall, a major aim of Framework 7, Theme 2. The project was very successful in 'exploit[ing] the new and emerging research opportunities' arising from rapid developments in molecular biology, particularly in the application of next generation sequencing technologies.

Clearly, alien invasions of plant pests and pathogens with potentially very serious impacts on forest productivity and ecosystem function remain major challenges to Europea, at many levels, including European Society, yet with world-wide connotations. Such pathogens also pose major threats to sustainability of timber production, to renewable bio-resources and to ecosystem function. In the US, much attention is focused on the possible use of plant pathogens in bioterrorism, where highly virulent or otherwise genetically modified pathogens may be introduced in order to disrupt food production, and destroy the economies of affected regions: these possibilities apply equally to our forest and other wild land ecosystems. These concerns underline the need to greatly improve our abilities to detect and, therefore better manage, such potential threatening organisms, which the outputs from ISEFOR do.

Forest production contributes greatly to the economics of renewable biological resources through both the tangible and non-tangible benefits of natural and semi-natural ecosystems, all of are threatened by invasive pathogens. The significance of the methods and protocols and the outputs of ISEFOR are, therefore, clear. The ecological disasters in forest and tree-based ecosystems of the 20th Century have continued into the 21st Century, with the emergence of the huge threat to European ash posed by *Hymenoscyphus pseudoalbidus* and *Agrilus planipennis*, for example. The work in ISEFOR has and will continue in the future to contribute greatly to measures undertaken at an EU-wide scale, and beyond, aimed at reducing the likelihood of further invasions of damaging organisms in the future.

Protection to forest trees and autochthonous forest ecosystems arising from work in the ISEFOR project will help in supporting many aspects of EU policy, including the Common Agricultural Policy, Forestry Strategies at the EU and national levels, the Forestry Action Plan, and in the horticultural trade.

ISEFOR has contributed knowledge which will assist in fulfilling EU directives on ecology, biodiversity, forest ecosystems and forest productivity, for example The European Climate Change Programme, the Habitats Directive 92/43/EEC and NATURA 2000, Sustaining ecosystem services for human well-being.

Biological security is a global problem. Pests and pathogens do not recognise state borders, and it is essential, therefore, that the problems these organism present are tackled at an international scale. ISEFOR contributed to essential international cooperation in the field, with various international partners directly funded in the work, and in having an advisory team comprising experts from South

Africa, North America, Russia and Australia. Each of these countries/continents has been pro-active in developing links with European research on alien invasive pests and pathogens. For example, the South African institute FABI is extremely highly regarded throughout the world, being one of the leading institutes dealing with plant protection issues globally.

The results will greatly assist policy makers in their decision-making capacities concerning the phytosanitary status of different plant pathogens threatening European biosecurity. The target industries are based in rural areas, and greater protection from invasive pathogens will assist in protecting the rural economy, maintaining agricultural and forest production and thereby maintaining jobs in these sectors. The results will also reduce the need to apply agrochemicals at all stages in plant production processes, as threats will be detected earlier. In these ways, ISEFOR will contribute directly to more sustainable and improved rural livelihoods.

ISEFOR established an interdisciplinary network within Europe, creating a critical mass of scientists with relevant expertise, and through the Advisory Board, extension workers and other stakeholders, and successfully utilised a hitherto unparalleled opportunity for improving the detection and diagnosis of alien organisms, and also reducing the likelihood of transmission within Europe.

Continued impacts beyond the end of ISEFOR

As alien invasive pests and pathogens will continue to threaten to food and native ecosystem security globally, the results of ISEFOR will be of significance for many years beyond the end of the project. The results will provide benefits in terms of:

- ✓ Increased security for more sustainable yields of better quality timber from European forests
- ✓ Increased protection for autochthonous forest ecosystems
- ✓ Reductions in losses of amenity values of forests, at the local and landscape scales
- ✓ Increased security to the woody plant ornamentals industry
- ✓ Decreases in damage to non-timber benefits from forest ecosystems, such as potential impacts on water catchments and flood control
- ✓ Improved employment prospects for rural areas and protection of rural livelihoods;
- ✓ Protection of forest production systems, including timber supplies and non-tangible forest benefits such as water catchment protection,
- ✓ Protection of biodiversity
- ✓ Protection to landscapes at local, regional and national scales
- ✓ Protection of rural livelihoods

In addition, of course, the technological advances in ISEFOR have already enhanced scientific development in the immediate and medium-term, adding greatly to knowledge on the biology, ecology and management of the target organisms, the mechanisms of spread of invasive species and have opened possibilities for further avenues of research on alien invasive pests and pathogens.

Main dissemination activities

Dissemination, was the focus of **Workpackage 6**, has proven remarkably successful in ISEFOR. The aim was to get the project results to end users in all European states and other participating countries as rapidly and effectively as was feasible. The project web site was advertised widely by all partners, and the project leaflet was distributed to all known interested parties, and taken to many conferences and other meetings. For example, the leaflet was distributed in the delegate pack at a World Trade Organisation Conference on alien invasive pests and pathogens held in Geneva in July 2012, at which the ISEFOR coordinator spoke.

A powerful tool for dissemination to end-users is through directed workshops. Interfacing directly with plant health authority and quarantine organisation personnel at the first ISEFOR Workshop held in Brussels in February 2011 illustrated how significant the problem is thought to be by the authorities and policy makers. The second workshop (Joensuu, February 2014) was focused on modelling outputs, but again was attended by representatives of the plant health authorities from several countries, both within and outside the EU. At this second workshop, delegates gained hands-on experience of using the models, and were able to adjust climatic parameters to determine the potential effects of increased numbers of entries of the organisms, or of time on the development of epidemics

Apart from the outputs related directly to the plant health industries, we also communicated project results to the general public in a non-technical manner, partly through distribution of the publicity leaflet early in the project and through the project web site. After ISEFOR began, there was a big upsurge in public awareness of the problems of alien invasive pests and pathogens, largely through realisation in the press of the significance of ash dieback in Europe. The increased public awareness is a positive thing both for increasing research focus on these issues and for facilitating the passage of new legislation through parliaments in different EU member states. This point was particularly evident from the numerous request ISEFOR partners had to provide interviews to various media outlets, including newspapers, radio and television, and is also reflected in the number of popular articles published.

The scientific dissemination activities within ISEFOR included presentations of project results at numerous local, national and international conferences/meetings of relevance, and the publication of almost 100 papers in peer-reviewed journals.

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