



UNIVERSITÀ  
DEGLI STUDI  
FIRENZE

## FLORE

# Repository istituzionale dell'Università degli Studi di Firenze

### **Portable LAMP (loop mediated isothermal amplification): a new molecular assay to detect *Phytophthora ramorum***

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

*Original Citation:*

Portable LAMP (loop mediated isothermal amplification): a new molecular assay to detect *Phytophthora ramorum* / Aglietti, Chiara; Ghelardini, Luisa; Santini, Alberto; Luchi, Nicola. - ELETTRONICO. - (2017), pp. 188-188. (Intervento presentato al convegno IUFRO 125th Anniversary Congress Interconnecting Forests, Science and People tenutosi a Freiburg (Germany) nel 18-22 September 2017).

*Availability:*

This version is available at: 2158/1096454 since: 2017-10-05T23:40:14Z

*Publisher:*

IUFRO

*Terms of use:*

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

*Publisher copyright claim:*

(Article begins on next page)

All Division 7 (Forest Health) Meeting

122 - Invasive species surveillance: New methods and tools for survey and early detection

---

K9 (Konzerthaus Freiburg)

IUFRO17-1853 **Biosurveillance of Alien Forest Enemies (bioSAFE) - creating new genomic tools to meet the challenges posed by forest alien invasives**

Roe, A. D.\* (1); Bernier, L. (2); Bilodeau, G. J. (3); Blanchette, M. (4); Cusson, M. (5); Doucet, D. (1); Duff, C. (6); Griess, V. C. (7); Hintz, W. (8); Landry, C. (9); Levesque, R. C. (9); Porth, I. (10); Sinclair, B. J. (11); Tanguay, P. (5); Uzunovic, A. (12); Yemshanov, D. (1); Hamelin, R. (13)

(1) Natural Resources Canada, Canadian Forest Service, Sault Ste. Marie, ON, Canada; (2) Université Laval, Centre d'Etude de la Forêt (CEF), Québec, QC, Canada; (3) Government of Canada, Canadian Food Inspection Agency, Ottawa, ON, Canada; (4) McGill University, School of Computer Science, Montreal, QC, Canada; (5) Natural Resources Canada, Canadian Forest Service, Québec, QC, Canada; (6) Plant Health Science Directorate, Canadian Food Inspection Agency, Ottawa, ON, Canada; (7) University of British Columbia, Faculty of Forestry, Vancouver, BC, Canada; (8) University of Victoria, Dept. of Biology, Victoria, BC, Canada; (9) Université Laval, Institut de Biologie Intégrative et des Systèmes, Québec, QC, Canada; (10) Université Laval, Dept des sciences du bois et de la forêt, Québec, QC, Canada; (11) Western University, Dept. of Biology, London, ON, Canada; (12) FPInnovations, Pointe-Claire, Canada; (13) University of British Columbia, Dept of Forest and Conservation Sciences, Vancouver, BC, Canada

**Abstract:** The world's forests face unprecedented threats from invasive insects and pathogens. This threatens the ecological and economic stability of our natural and urban forests. New introductions and interceptions of Forest Invasive Alien Species (FIAS) are escalating at an alarming rate and managing this risk requires vigilant biosurveillance. Prevention and early detection are keys to successful biosurveillance programs, but are challenging to achieve. We will address these challenges by developing a biosurveillance pipeline to rapidly generate genomics tools that will provide: 1) accurate identification; 2) assignments to source populations and invasion pathways; 3) identification of Fitness and Outbreak-related Epidemiological (FORE) traits that can impact invasion outcomes; 4) reduced uncertainty of invasion outcomes and can inform decision support systems; 5) transferable biosurveillance tools to end users. In Canada four FIAS have been identified as current and urgent threats: Asian longhorned beetle, Dutch Elm disease fungi, Sudden oak death pathogen, and Asian gypsy moth. We will develop a biosurveillance pipeline using genomic tools developed for these four species to speed up and inform novel decision-support systems for FIAS mitigation and management.

diagnostics epidemiology WGS GBS target enrichment

---

K9 (Konzerthaus Freiburg)

IUFRO17-1441 **Portable LAMP (loop mediated isothermal amplification): a new molecular assay to detect *Phytophthora ramorum***

Aglietti, C.\* (1); Ghelardini, L. (1); Santini, A. (2); Luchi, N. (2)

(1) DISPAA-Università degli studi di Firenze, Firenze, Italy; (2) IPSP-CNR, Sesto fiorentino (Firenze), Italy

**Abstract:** Plant health emergencies due to invasive pathogens have increased in Europe as in others countries. These pathogens can cause huge ecosystem changes and biodiversity losses if introduced to areas with suitable conditions and potentially susceptible hosts. To contain the environmental and economic damage that they may cause in forests and urban environments, specific and sensitive diagnostic tools are necessary. An effective plan for early warning and rapid response is a crucial element of any policy aimed at reducing impacts of biological invasions or preventing the establishment of pathogens as *P. ramorum*, a recently described quarantine species that causes mortality on conifer and native broadleaved species in California (mainly *Lithocarpus densiflorus* and *Quercus spp.*), and on ornamental plants (mainly *Rhododendron* and *Viburnum spp.*) in Europe. PCR-based methods are often favored for their sensitivity and specificity but require a well-equipped laboratory. Advantages might be gained from moving testing closer to the site of sampling, reducing delays. A diagnostic assay based on LAMP (Loop mediated isothermal amplification) was developed to detect *P. ramorum*. The assay, optimized on the portable instrument Genie II (Optigene, UK) and based on ITS2 target region, can recognize the pathogen with a high level of specificity (only *P. ramorum* was identified with a melting temperature of 88,7 °C) and sensitivity (DNA was detected as low as 0,128 pg/μl), results that equal those obtained with the *P. ramorum* qPCR diagnostic assay. The use of LAMP method for detecting quarantine pathogens like *P. ramorum* on symptomatic and asymptomatic samples, could assist to check imported and exported live plants for planting, limiting the uncontrolled spread of these pathogens. Great simplicity, sensitivity and specificity, high speed (only 30 min) and minimum required equipment make the assay ideal for application in the field and for routine plant testing both in cities and forests.

Early detection, in field diagnostic

All Division 7 (Forest Health) Meeting

83 - Are we doing the right things to deal with invasive forest pest and pathogens? Lessons from history

---

KG I - 1199 (Uni Freiburg)

IUFRO17-156 Tree Resistance as a Primary Tool for Managing Forest Pathogen and Insect Invasions in Defense-Free Space

Showalter, D. (1); Smith, J. (2); Raffa, K. (3); Snieszko, R. (4); Herms, D. (1); Liebhold, A. (5); Bonello, P.\* (1)

(1) Dept. of Plant Pathology, The Ohio State University, Columbus, Ohio, United States; (2) School of Forest Resources and Conservation, 212 Newins-Ziegler Hall, Gainesville, Florida, United States; (3) Dept. of Entomology, University of Wisconsin, Madison, Wisconsin, United States; (4) U.S. Forest Service, Dorena Genetics Resource Center, Cottage Grove, Oregon, United States; (5) U.S. Forest Service, Northern Research Station, Morgantown, West Virginia, United States

**Abstract:** Massive global trade of goods is facilitating movement of alien phytophagous insects and phytopathogens (PIPs) of trees. A fraction of these organisms defeat measures intended to exclude them from naïve forest ecosystems, e.g. those designed to prevent transport or to eradicate early infestations. Eventually, some cause severe economic and environmental impacts by killing vast numbers of trees, occasionally on continental scales.

It is becoming increasingly clear that many of the most devastating alien PIPs become invasive largely due to a lack of bottom-up control that is otherwise exerted by host defenses in their native region. These PIPs, acting in the "defense-free space" of naïve environments, are often intimately and cryptically associated with their hosts, which makes early detection very difficult, and damage tissues with high fitness value and low compensatory mechanisms, thereby quickly killing their hosts and limiting the effectiveness of density-dependent biological control.

We posit that once such PIPs become established, responses should immediately and sustainably integrate strategies that transition valuable tree populations toward an environment in which these particular PIPs exist in "defense-constrained space", i.e. through the development of host resistance. A combination of traditional selection and breeding, rapidly advancing genomic and phenotypic marker techniques, and targeted genetic engineering, offers great potential to accelerate development and deployment of diverse populations of locally-adapted, PIP-resistant trees. Integrated host resistance development programs have considerable potential to conserve and/or restore threatened tree species and their ecosystem services, as productive components of urban, plantation and naturally regenerating forests.

invasions, pests, pathogens, tree resistance

---

KG I - 1199 (Uni Freiburg)

IUFRO17-4044 Role of sea containers in unintentional movement of invasive "hitchhiker" pests - a closer look at a barely managed pathway

Brockerhoff, E.\* (1); Liebhold, A. (2); Bulman, L. (3)

(1) Scion (New Zealand Forest Research Institute), Christchurch, New Zealand; (2) USDA Forest Service, Morgantown, WV, United States; (3) Scion (New Zealand Forest Research Institute), Rotorua, New Zealand

**Abstract:** The volume of international trade is at unprecedented levels, and much of this is moved with so-called "sea containers". An unwanted by-product is the transport of "hitchhiker pests" on the external or internal surfaces of sea containers, which may become invasive species. Hitchhiker pests found on sea containers, such as gypsy moth, giant African snail, Argentine ant and brown marmorated stink bug, threaten forests and other environments worldwide. Soil contamination of sea containers may contain seeds of invasive plants, nematodes and plant pathogens. A summary will be presented of records from sea container inspections by quarantine officers along with a review of previous work on risks associated with this pathway and an evaluation of the likely benefits of mitigation measures. Inspection records from the United States, Australia, China and New Zealand indicate that thousands of organisms from a wide range of taxa are being moved unintentionally with sea containers. Records of more than 100,000 consignments of empty sea containers recently arriving in New Zealand indicated a contamination rate of nearly 15%. A sea container hygiene system implemented in New Zealand showed that a range of measures can be highly effective in reducing infestation rates. There is a strong case to implement such measures internationally, to address pathway risks that are currently largely unmanaged.

invasive species, pathway risk management

---

KG I - 1199 (Uni Freiburg)

IUFRO17-1331 Different invasive forest pathogens need different management strategies, a view from a Mediterranean perspective

Santini, A.\* (1); Luchi, N. (1); Pepori, A. L. (1); Ghelardini, L. (2)

(1) Institute for Sustainable Plant Protection, National Research Council (CNR), Sesto fiorentino, Italy; (2) DISPAA, Firenze, Italy

**Abstract:** Historically, Mediterranean countries were invaded by many alien forest pests. Among causal factors, large human population and population density, and high imports were identified. A great number of invasive pathogens is thus the result of a long history of continue commercial exchange and human activity.

Moreover, Mediterranean countries host high environmental variety and biodiversity that, with a generally mild climate, favor the establishment of various organisms. Such a high environmental diversity likely increased the arrival and spread rates of microbes helping the establishment of many forest pathogens with diverse niches.

Army supply during WWII was the most likely introduction pathway for three serious pathogens spreading today in the Mediterranean area: *Seiridium cardinale*, the agent of cypress canker, *Ceratocystis platani*, the agent of plane canker stain, and *Heterobasidion irregulare*, the cause of root and butt rot in pines.

*S. cardinale* reproduces asexually and parasexually, while *C. platani* reproduces asexually and sexually, being hermaphroditic and self-fertile. *H. irregulare* reproduces mainly gamically, but also by asexual spores. *S. cardinale* is mainly spread by occasional insect vectors, the dispersal of *C. platani* is mostly human-mediated, while *H. irregulare* is air-dispersed.

Distinctive traits and differentiated management strategies for each pathogen led to different outcomes. *S. cardinale* spread in the Mediterranean basin until an equilibrium between pathogen and host was reached. Despite huge control efforts, *C. platani* spread in Italy, southern France and Switzerland; jumped to Greece and Albania, and spreads now eastward to the center of *Platanus orientalis* native range. *H. irregulare*, despite no control actions, is present in *Pinus pinea* stands on 103 km of seacoast near Rome and spreading at 1.3 km/year.

Specific lifestyle and bio-ecological traits of these fungi that possibly influenced their invasiveness, were compared in this work.

alien pathogens, canker, rot, reproduction, spread

Poster Exhibition Friday

192 - Global decline of *Fraxinus* species caused by invasive pests and pathogens

KG II - HS 2121 (Uni Freiburg)

IUFRO17-1805 Natural infection of *Fraxinus angustifolia* by *Hymenoscyphus fraxineus* in Slovakia

Kadasi-Horakova, M. (1); Pastircakova, K. (1); Adamcikova, K.\* (1)

(1) Institute of Forest Ecology, SAS, Nitra, Slovakia

**Abstract:** The fungus *Hymenoscyphus fraxineus* is responsible for dieback of common ash (*Fraxinus excelsior*) and in some parts of Europe also of narrow-leaved ash (*F. angustifolia*). The first symptoms of ash dieback have been recorded on *F. excelsior* in Slovakia since 2004. This study reports about the first natural occurrence of *H. fraxineus* on *F. angustifolia* in Slovakia. The field investigation was carried out in 2014. The segments of diseased shoots and last year's petioles were collected in clonal seed orchard situated in southwest part of the country. The fungus was isolated from infected host tissue and identified using molecular techniques (DNA extraction from pure cultures and apothecia, conventional PCR).

isolation, apothecia, DNA extraction, ash dieback

KG II - HS 2121 (Uni Freiburg)

IUFRO17-3135 Assessing the potential invasiveness of *Hymenoscyphus fraxineus* in the Mediterranean mountains

Aglietti, C.\* (1); Luchi, N. (2); Cantini, F. (1); Capretti, P. (1); Papini, S. (1); Santini, A. (2); Ghelardini, L. (1)

(1) DISPAA-Università degli studi di Firenze, Firenze, Italy; (2) IPSP-CNR, Sesto fiorentino (Firenze), Italy

**Abstract:** *Hymenoscyphus fraxineus* is an aggressive ascomycete that in just a few years invaded large parts of Central and Northern Europe causing dieback in *Fraxinus excelsior* and *F. angustifolia*. The fungus, present in the Alps for some time, was recently discovered in a small area of the Northern Apennines, which is the southernmost disease focus to date. The pathogen's potential to adapt to the local environment, spread and become established in the Mediterranean mountains is unknown. Tests were carried out to characterize both ecologically and genetically the population found in the Italian Apennines. In vitro growth tests were performed to understand the population's reaction to the relatively high summer temperatures that might limit fungal growth or survival in the Mediterranean mountains. In vitro growth tests on leaf enriched media were performed to understand how leaf age influences mycelial growth, and to provide circumstantial evidence about the relation between host leaf phenology and fungal infection, which might undergo major changes with climate either impairing or improving disease development. Finally vegetative compatibility between local and non-local isolates of the fungus was assessed as to obtain a measure of the population's genetic variability. A number of isolates had optimum growth temperature higher than 20 °C, the most common value also in populations from Central Europe. All isolates resumed growth with no apparent damage after thermal stress at 28 °C for 6 weeks. A great genetic variability was found in the population that, together with the tolerance to relatively high temperature, may increase the population's potential to survive and adapt to the Mediterranean environment. The competence of host leaves to support fungal growth was maximum in late spring but declined during summer. On synchronization between optimal sporulation pressure and leaf receptivity in this climate will depend the capacity of the pathogen to become invasive in the area.

Italian Apennines, growth tests, population studies

KG II - HS 2121 (Uni Freiburg)

IUFRO17-1987 *Hymenoscyphus fraxineus* at eastern border of its secondary range in Europe

Zviagintsev, V. (1); Seraya, L. (2); Panteleev, S. (3); Yarus, A. (1); Baranchikov, Y.\* (4)

(1) Belarusian State Technological University, Minsk, Belarus; (2) All-Russian Research Institute of Phytopathology, Bol'shiye Vyazemy, Russian Federation; (3) Institute of Forest of the NAS of Belarus, Gomel', Belarus; (4) Sukachev Institute of Forest SB RAS, Krasnoyarsk, Russian Federation

**Abstract:** In spite of quick distribution of ash dieback disease in Europe, till recently only limited information on invasive micopathogen *Hymenoscyphus fraxineus* Baral et al. has been available for the eastern part of natural range of European ash *Fraxinus excelsior* L. In 2014-2016, we covered about 14 000 km exploring most eastern corner of *F. excelsior* range in European Russia. The infected samples were collected in 162 plots situated in forest belts along highways, in cities and sometimes in natural forest stands of 23 administrative regions of Russia. Samples were taken from *F. excelsior*, as well as from *F. pennsylvanica* Marshall, *F. americana* L. and *F. ornus* L. which were present in the plots. Genetic analysis proved existence of *H. fraxineus* in 86% of the samples. We found no vivid regularities in distribution of fungus activity from Poland and Byelorussia across the region to the river of Volga. The mature trees of *F. excelsior* were the most resistant to the disease comparing to other ash species. Young trees and sprouts were infested more intensively. Their chronic injury took place at least during last 7-8 years.

Different hypothesis on the time of *H. fraxineus* invasion and its distribution in European Russia will be discussed. The work was supported by the Russian Foundation for Fundamental Research (grant 17-04-01486).

*Hymenoscyphus fraxineus*, distribution, W. Russia

Theme 4: Biodiversity, Ecosystem Services and Biological Invasions

9 - IUFRO Task Force on Biological Invasions in Forests

---

KG I - 1010 (Uni Freiburg)

IUFRO17-884 **Are non-native forest insects recently established in Europe spreading faster than before?**

Roques, A.\* (1)

(1) INRA, Zoologie Forestiere, Orleans, France

**Abstract:** Globalization is triggering an increase in the establishment of alien insects in Europe, with several species having substantial ecological and economic impacts. We investigate long-term changes in rates of species spread following establishment. We used the total area of countries invaded by 1171 insect species for which the date of first record in Europe is known, to estimate their current range radius. We estimated initial rates of radial spread and compared them among different groups of insects for all years (1800-2014) and for a subset of more recent decades (1950-2014). Decreasing spread rates over residence time were observed in herbivorous species associated with herbaceous plants and crops but much less in those related to woody plants. Initial spread rate was significantly greater for species detected after 1990, roughly 3-4 times higher than for species that arrived earlier. It is hypothesized that the political changes in Europe following the collapse of the Iron Curtain in 1989, and the further dismantling of Customs checkpoints within an enlarged European Union (EU) have facilitated the faster spread of alien insect species. Also, the number of species first recorded in the Eastern Bloc of the politically-divided Europe before 1989 was lower than for the rest of Europe. A detailed analysis of several recent invaders associated to woody plants indicated a dominant role of long-distance translocations related to human activities, especially with the plant trade, in determining rates of spread.

insect, invasion, spread, Europe, woody plants

---

KG I - 1010 (Uni Freiburg)

IUFRO17-3296 **Country regulations of non-native tree species in Europe**

Pötzelberger, E.\* (1); Lapin, K. (1); Brundu, G. (2)

(1) Institute of Silviculture, University of Natural Resources and Life Sciences, Vienna, Wien, Austria; (2) Dipartimento di Agraria, Università degli Studi di Sassari, Sassari, Italy

**Abstract:** Non-native tree species (NNT) play an important role in forest production in many European countries and management of NNT has a long tradition in Europe. The legal situation varies however considerably among the European countries, ranging from countries with almost no restrictions on growing NNT to countries where only very few NNT can be grown, and permission needs to be sought for every plantation. Similarly, countries have differing strategies and methods for the classification of NNT as invasive species. A standardised questionnaire on NNT regulations was sent to representatives of the 36 member countries of COST Action FP1403 'Non-native tree species for European forests: experiences, risks and opportunities' (NNEXT). We inquired if and how NNT are treated in the countries' hard laws (Forest Acts, Nature Conservation Acts) and soft laws (Guidelines, Forest certification standards, Lists of NNT of concern) and what measures have been taken so far in response to the regulation (EU) No 1143/2014 on invasive alien species. Countries could be categorised regarding their regulatory intensity. Differences in regulatory intensity across jurisdictions showed to be neither a clear predictor for NNT coverage, nor for the multitude of research activities in European countries.

Non-native trees, regulations, invasive species

---

KG I - 1010 (Uni Freiburg)

IUFRO17-1475 **From climate change to new diseases**

Ghelardini, L.\* (1); Santini, A. (2)

(1) DISPAA - University of Florence, Florence, Italy; (2) IPSP-CNR, Sesto fiorentino, Italy

**Abstract:** Sudden environmental changes may heavily impact the Earth biotas. The way climate change affects ecosystem components reducing the ability of the whole system to recover from disturbance are complex and difficult to forecast. However, the stresses associated with global change-induced disturbances will likely benefit plant pathogens both directly and indirectly, i.e. predisposing plants to infection. Abrupt alterations in weather patterns decrease ecosystem resistance to disease, and reduce ecosystem resilience facilitating the establishment of alien disease agents. It is well documented that warmer temperatures increase the risk of disease development in plants. At northern latitudes, plant growth, but also plant susceptibility to disease, are expected to increase following temperature rise. In fact, under optimal resource availability plants tend to allocate energy to growth rather than to differentiation processes, which include secondary metabolism components that have a general role in parasite defense. Non-pathogenic microbial associates, which commonly inhabit plant tissues, may shift to pathogens because environmental changes or stress factors have altered host physiology breaking the equilibrium of plant-endophyte interactions. Moreover, non-cryophilic, drought-intolerant or water-borne pathogen species, which include many fungal pathogens, may be directly favored by milder and wetter climate. Current climate change is indeed altering the distribution of organisms on Earth. In response to ongoing climate change the geographic range of many plant pests and pathogens has shifted polewards, while seasonal activities, migration patterns, species abundance of fauna and flora species, and the interactions among them, were modified. Introduction and establishment of exotic forest pests and pathogens originating from warmer regions and possibly more adapted to the changed environmental conditions is also affected.

migration, alien pathogens, cryptic pathogens