GLOBAL WARNING

MULTITRAP PORTRAP

Delivering Alien Invasive Species Inventories for Europe

PRATIQUE

ISEFOR

Novel strategies for early warning and detection of “unknown” invaders

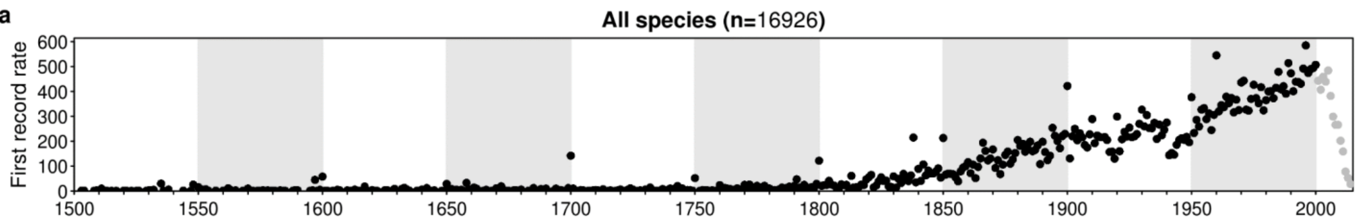
Alain ROQUES

INRA Zoologie Forestière Orléans, France

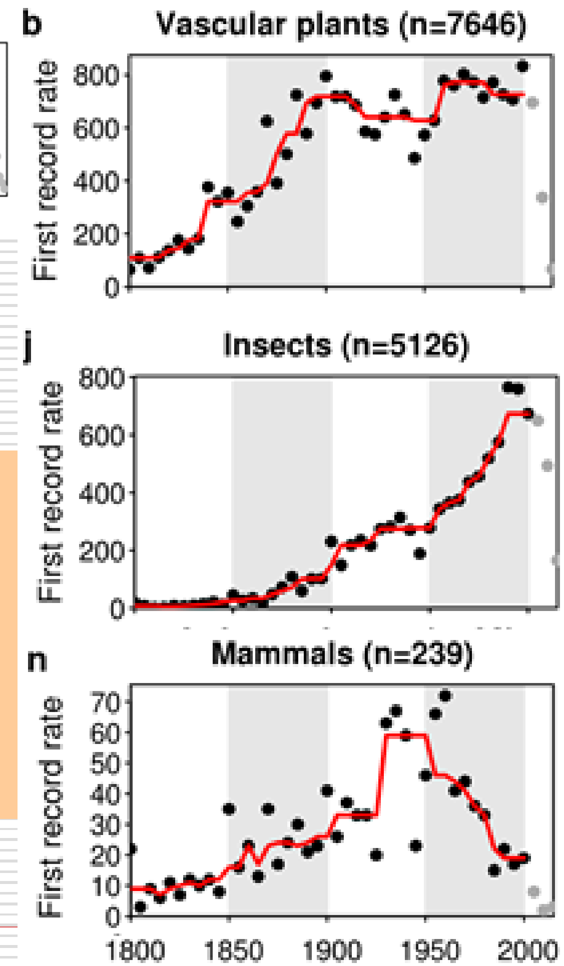
PLURIFOR, Oeiras, 25 Jan 2018

INRA SCIENCE & IMPACT

No saturation at world level in the establishment rate of alien species



Global temporal trends in first record rates for all animal and vegetal species at world level *(Seebens et al., NatureCom. 2017)*



But
Differences between taxa as well as regions

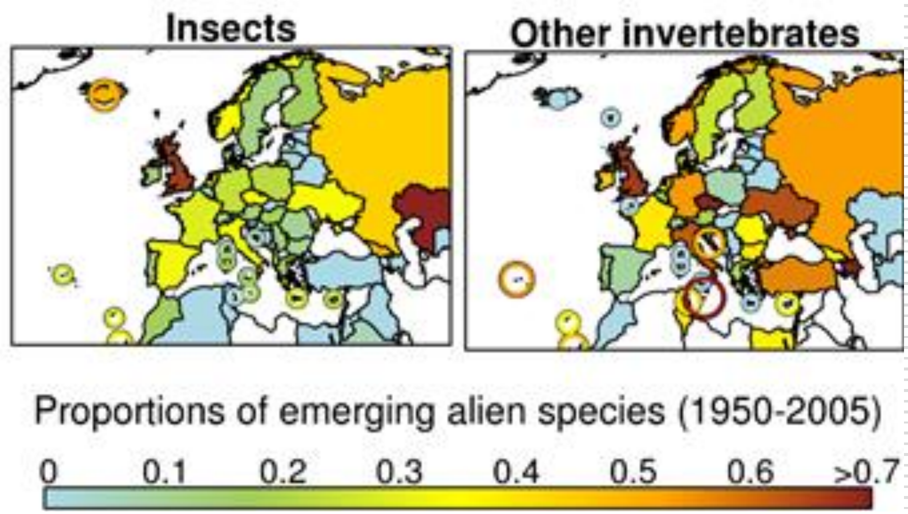
Major changes in the modes of introduction :
Accidental introductions predominate at present compared to intentional ones

Another key pattern:

The increasing proportion of « emerging » species

By "emerging species", we consider species that have never been observed as introduced in a continent other than the native

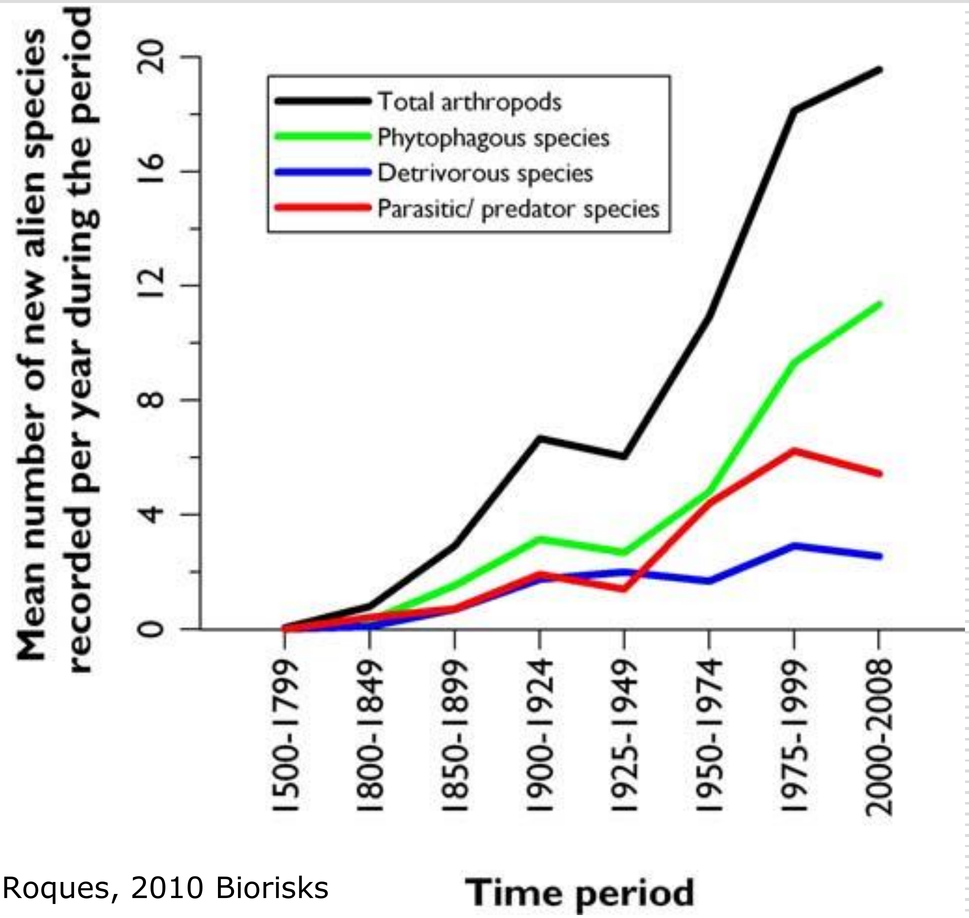
Typically EAB- *Agrilus planipennis* and ALB- *Anoplophora glabripennis* were emerging species when introduced to the USA in the late 1990s; Asian hornet similarly when introduced to France, and so on



(Seebens et al., PNAS 2018)

Focus on terrestrial arthropods in Europe

Globalization is exponentially accelerating establishment of exotic species



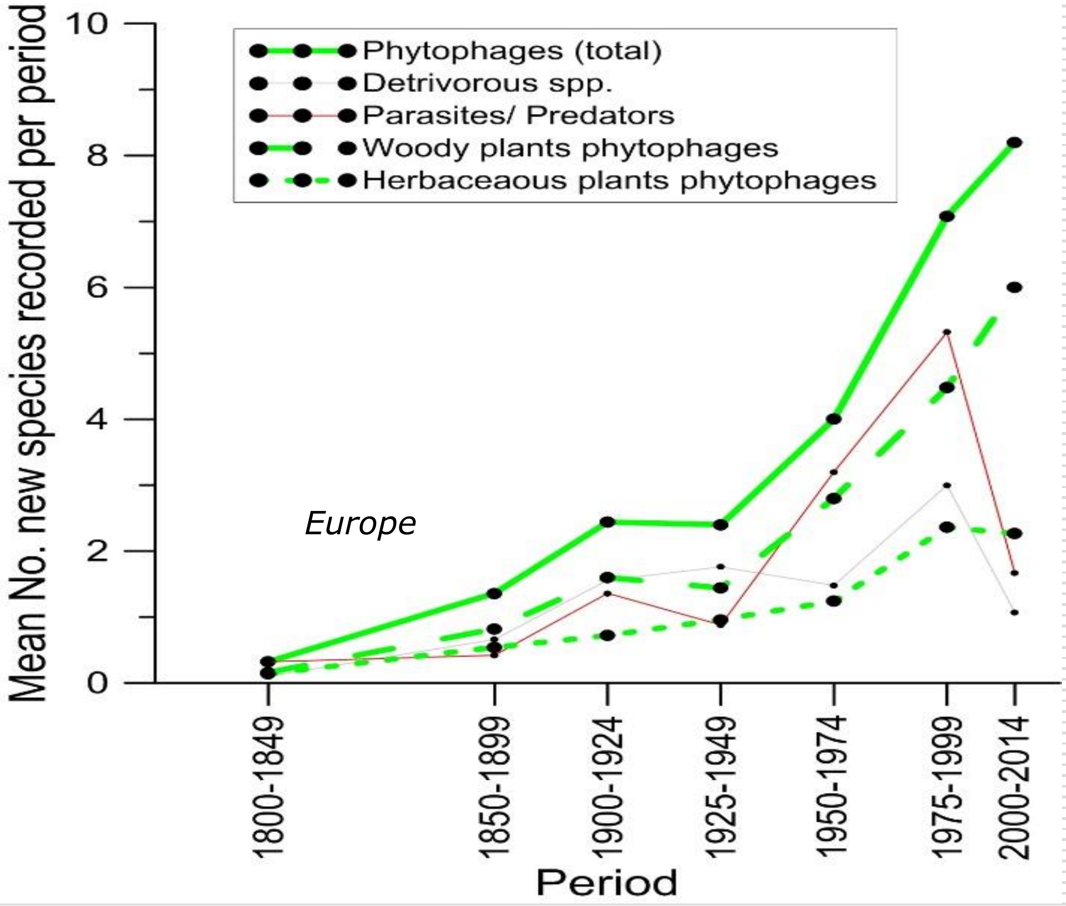
Roques, 2010 Biorisks

Increasing rate due to phytophages whilst the other groups decreased during the last period

ca. 11.5 new phytophagous species per year since the 2000s (4.5 during 1950-75)

Basic data:
EU DAISIE 2005- 2008
Update:
EU JCR- EASIN 2014
www.easin.org

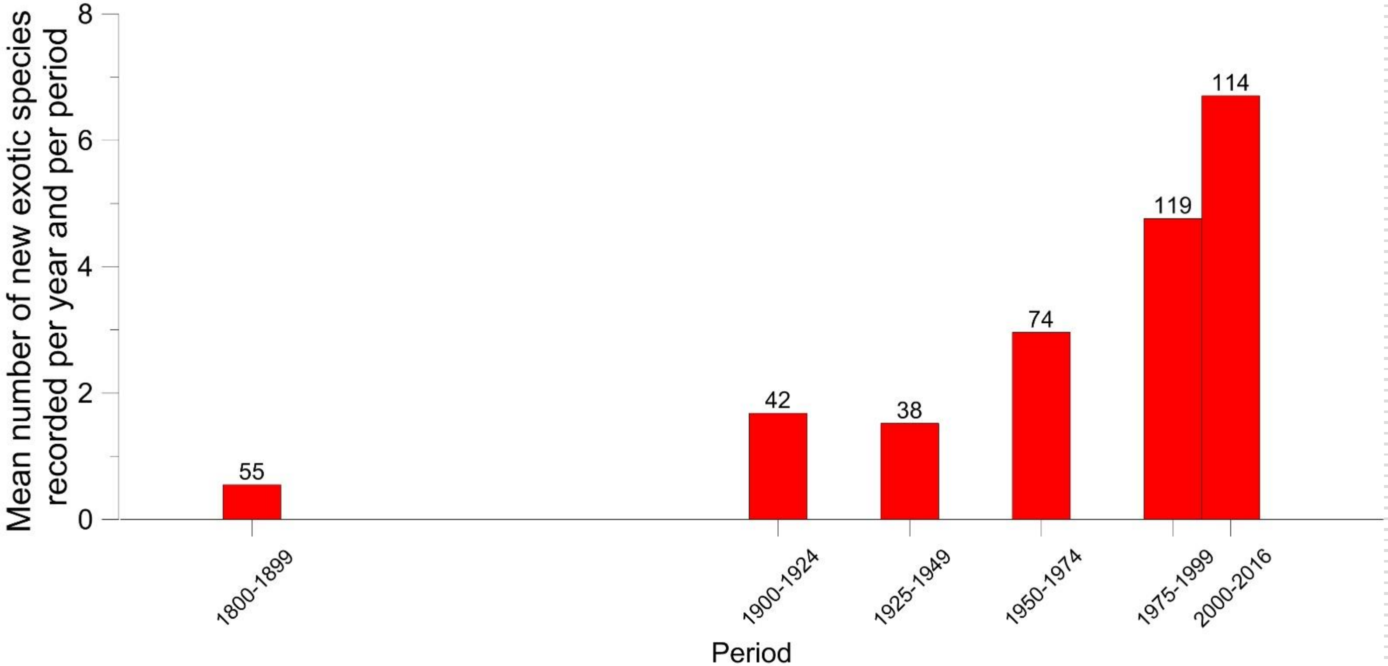
The increase in alien phytophages due to species associated to woody plants more than to those related to herbaceous and crops



Data for insects only
EU DAISIE 2005- 2008
EU JCR- EASIN 2017
www.easin.org

(Roques et al., Biol. Inv. 2016)

2-fold more establishments of exotic arthropods associated to woody plants during 2000-2016 than during 1950- 1975
6.7 vs. 2.9 species per year



512 exotic arthropods related to woody plants established in Europe by 2016

- *448 insects*
- *64 mites*

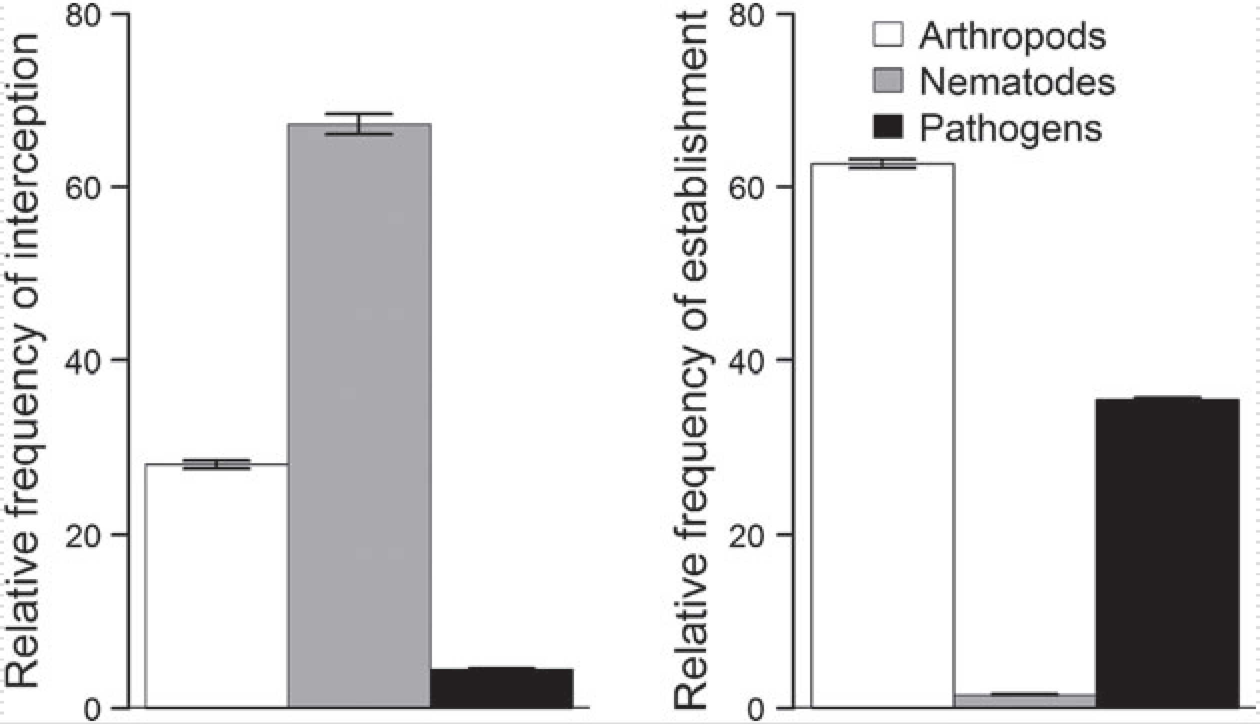
(Roques, 2010 NZJF updated EASIN 2017)

Relying on interceptions to predict the arrivals ?
Large discrepancies with establishments !

The example of invertebrates related to woody plants

Intercepted vs established 1995- 2012: *only 7 species over 117 established !*

(Eschen, Roques & Santini, 2014, Div & Dist.)



The major problem: only A1 and A2 pests targeted
Most new species: unknown as pests in countries of origin (cf EAB)

How to forecast and detect precociously the next arrivals ?

A number of EU projects aimed at developing novel strategies

- **Preventive warning:**
  - *Lists based on the pests known in the native range… but most newly-arrived species were not considered as pests in the native range … or unknowns!*
  - Sentinel plantings of European plants in exotic countries
  - Sentinel nurseries of exotic plants in exotic countries
  - Survey of arboreta and botanical gardens with European plants

    Former EU projects PRATIQUE, ISEFOR, PERMIT, COST 'GLOBAL WARNING' and IPSN (International Plant Sentinel Network); HOMED in future

- **Early detection at arrivals:**
  - Test of multiplex traps and lures with generic attractivity in ports-of entry

    French project PORTRAP and EUPHRESCO project MULTITRAP "*Multi-lure and multi-trap surveillance for invasive tree pests*"; now HOMED *in future*

First tests of a sentinel planting strategy

- Since China turned to be the main supplier of insect invaders

- First experiment: Sentinel plantings of 7 species of European trees in China (2008-2011): *Abies alba, Cupressus sempervirens, Carpinus betulus, Fagus sylvatica, Quercus ilex, Q. petreae; Q. suber*
  - Survey for 3 years of colonization by Chinese insects and pathogens

- Second experiment taking into account the role of ornamental trade (2012-2014)
  - Selection of the 6 most imported woody plants from China to Europe during 2008-2011 (*Buxus microphylla; Acer palmatum; Fraxinus chinensis; Ilex cornuta; Zelkova schneideriana)*
  - Settlement of 'scientific sentinel nurseries in China without treatments to survey their « freely » colonization by insects and pathogens

The same sentinel plots in China for both experiments

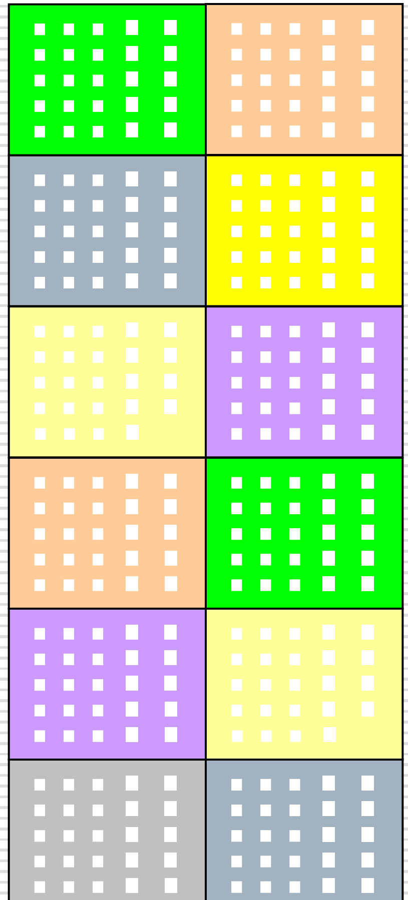
Beijing suburban area
Continental conditions
INRA- CABI

Fuyang, nr. Hangzhou
Warm and humid climate
INRA- IOZ- ZAF

1st experiment:
Planting European seedlings in China

- Abies alba
- Quercus suber
- Cupressus sempervirens
- Quercus petraea
- Fagus sylvatica
- Quercus ilex
- Carpinus betulus

All seedlings individually tagged



Designed for statistic analysis

100 seedlings per site and species

Random planting of blocks of 25 seedlings

Colonization by Chinese insects

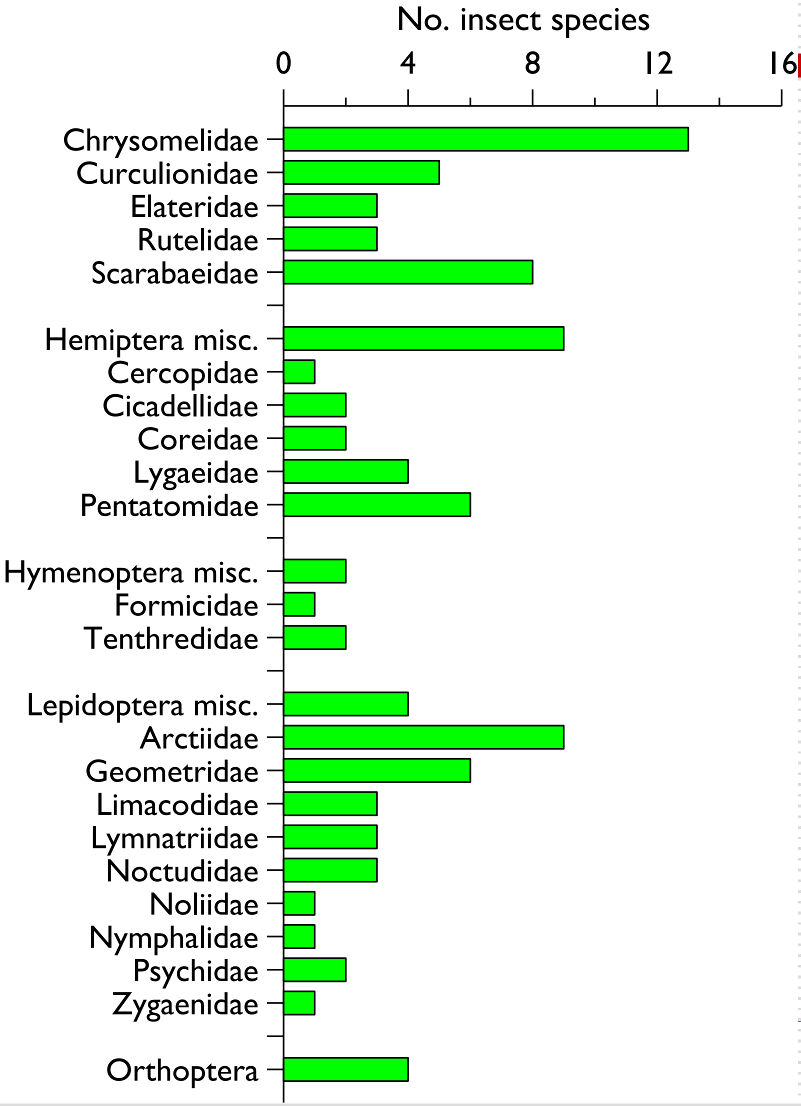
Relating damage morphotypes with insect species

Not so easy ! Only by rearings on the same trees

105 insect species colonized the seedlings in 3 yrs

Large diversity (25 families) but defoliators dominate (101 spp.)

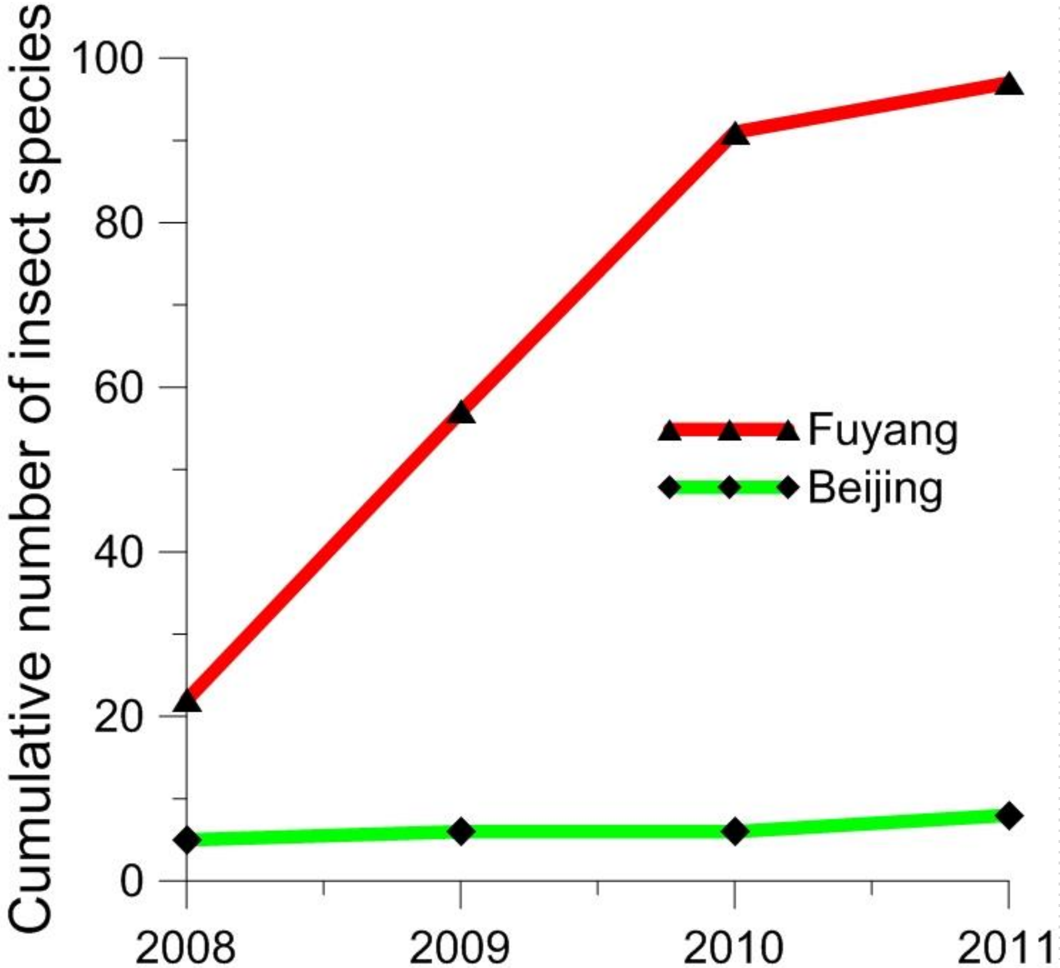
No xylophagous species



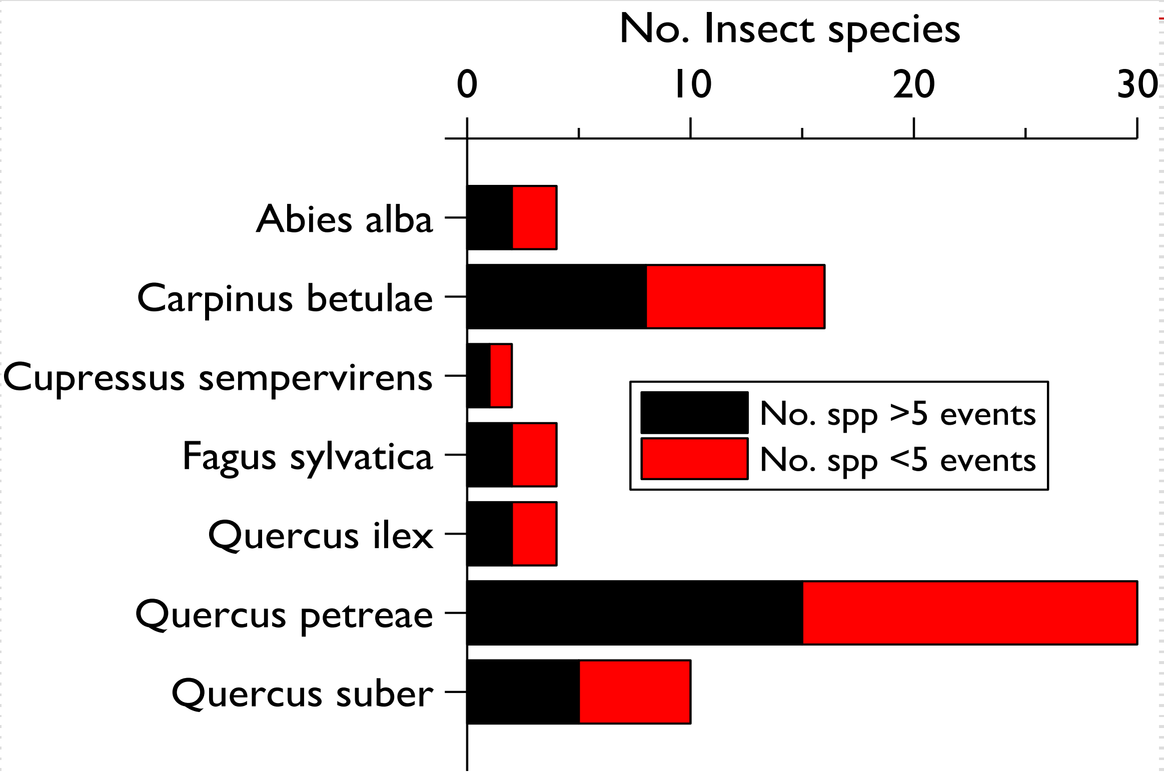
Root feeders (Scarabaeidae): 4 spp.

3 years enough to check the potential for species recruitment of folivores and root feeders

- More insect species in Fuyang: 97 spp.
- Quite no insects in Beijing: 8 spp. but pathogens
- No insect species in common between the 2 sites
- Rate of accumulation of new species important in year 2 and 3; fewer later



Which species are incidental ?
Which ones are really capable of switching on European trees ?



Quercus petreae significantly more colonized

Species with > 5 colonization events
List of 39 potential insect threats to European trees

INRA

Ranking the top species
> 15 occurrences over several years

Holotrichia titanis
Holotrichia diomphalia

Pteroma nr. pendula
(positive tests under quarantine conditions)

Compsapoderus continentalis

- Prove they can completely develop from egg to a new generation on the European host
- Check if they can survive travel and can be introduced

Efficient also for pathogens

PLOS ONE

RESEARCH ARTICLE

Planting Sentinel European Trees in Eastern Asia as a Novel Method to Identify Potential Insect Pest Invaders

Alain Roques[1]*, Jian-ting Fan[2], Béatrice Courtial[1], Yan-zhuo Zhang[3], Annie Yart[1], Marie-Anne Auger-Rozenberg[1], Olivier Denux[1], Marc Kenis[4], Richard Baker[5], Jiang-hua Sun[3]

1 INRA UR 633 Zoologie Forestière, Orléans, France, **2** School of Forestry and Bio-technology, Zhejiang Agriculture and Forestry University, Lin'an, China, **3** State key laboratory of Integrated Management of pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China, **4** CABI, Delémont, Switzerland, **5** Department for Environment Food and Rural Affairs, Sand Hutton, York, United Kingdom

* alain.roques@orleans.inra.fr

CrossMark
click for updates

PLOS ONE

RESEARCH ARTICLE

Sentinel Trees as a Tool to Forecast Invasions of Alien Plant Pathogens

AnnaMaria Vettraino[1], Alain Roques[2], Annie Yart[2], Jian-ting Fan[3], Jiang-hua Sun[4], Andrea Vannini[1]*

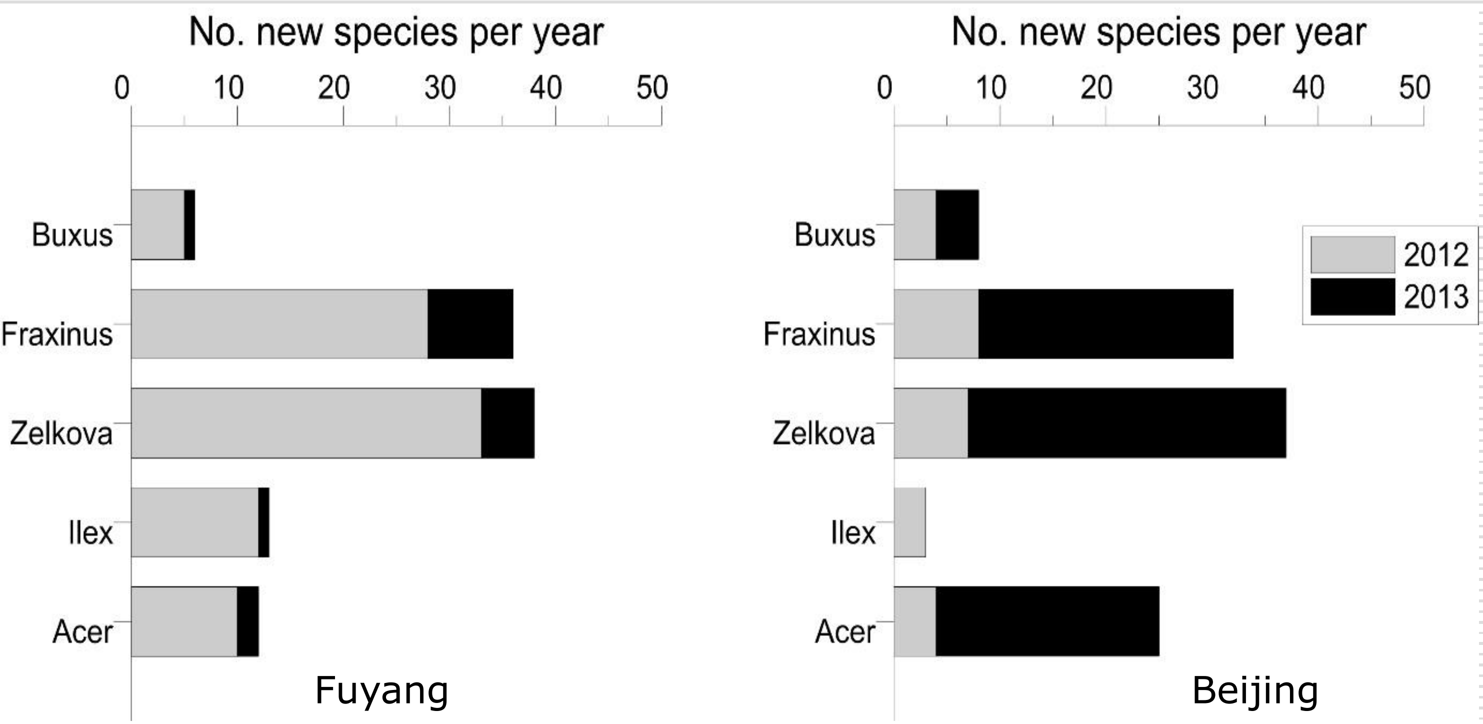
1 DIBAF, University of Tuscia, Viterbo, Italy, **2** INRA-UR633, Zoologie Forestière, Centre de recherche d'Orléans, Orléans, France, **3** School of Forestry and Bio-technology, Zhejiang A & F University, Lin'an, China, **4** State key laboratory of Integrated Management of pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

* vannini@unitus.it

But ensure that unexpressed endophytes were not present before seedling translocation ! Need of prior NGS!

2nd experiment: sentinel nurseries

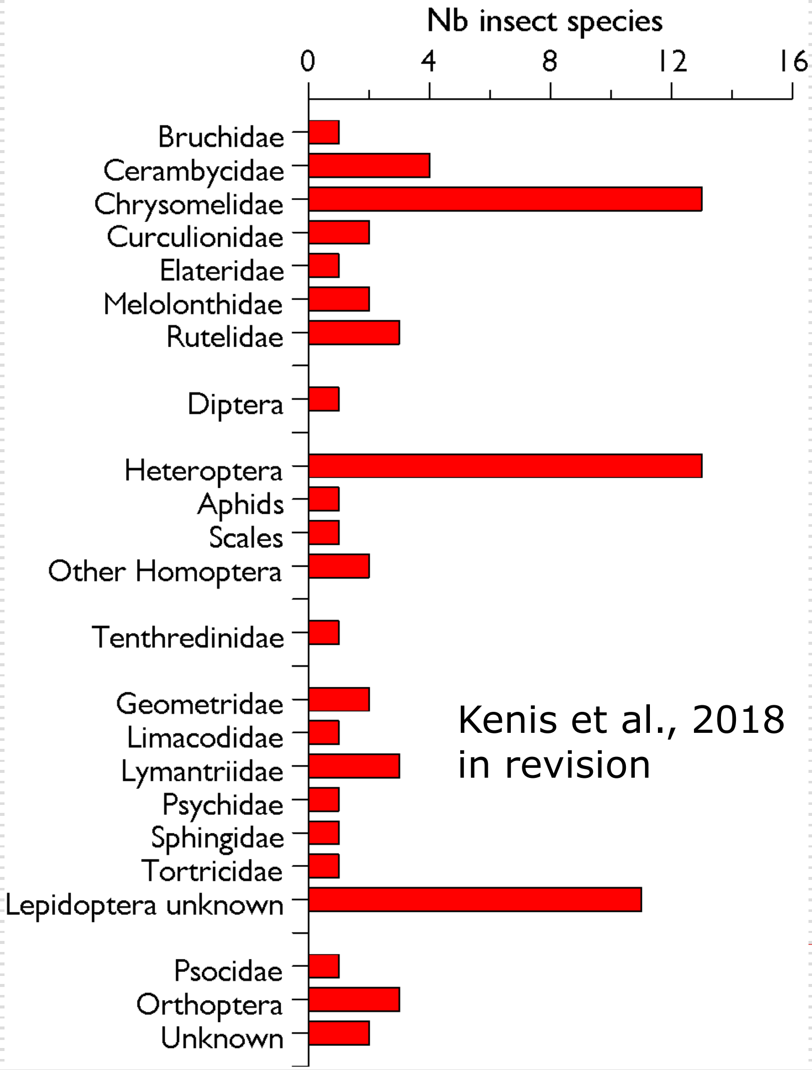
Tree and shrub spp. the most exported to Europe



Large differences among tree species after 3 yrs

Kenis et al., 2018 in revision

105 insect species x host associations



Kenis et al., 2018
in revision

90% not found in previous literature survey
Nearly 80% of these associations not found in *a posteriori* literature survey

The best example: *Cydalima perspectalis* (invasive *Buxus* moth) would have been detected prior to introduction in Europe !

Taxonomic identification a big problem !

Although sytematic barcoding of all morphospecies

In 1st exp, 15 out the 39 spp. identified by morphological keys
Most larvae not identifiable- Some may be unknown to Science

- All larvae genetically analyzed (COI mtDNA barcode + nuclear ITSs)
- Tentative match with genetic databases (GeneBank and others)
- Allow to identify 10 more species with > 99% match
- Good for moths and sawflies, much less for other groups less sequenced

But these tests did no allow to warn for future xylophagous invaders ?

New survey in June 2016 and 2017
(ie 5 and 6 years after plantations) with tree/shrub heigth from 0.5m (*Buxus*) to 5m (*Zelkova, Fraxinus, Acer*), and diameter from 3 cm to 20 cm.

Significant increase in the number of associated species belonging to new guilds

- Gall insects on *Zelkova, Acer and Fraxinus:* 7 more species at least

- Xylophagous insects: 10 more species at least
  - 5 *Batocera horsfieldii* laying eggs on *Zelkova*
  - *Cossus* moths in trunks of *Fraxinus,* jewel and bark beetles

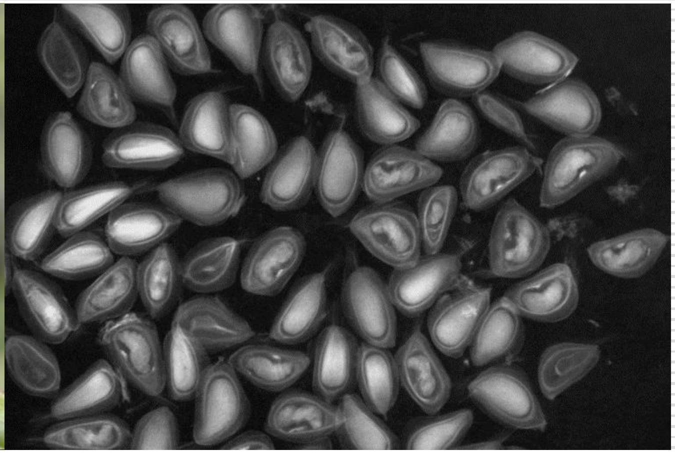
Another method for identifying potential invaders and for early detection of invaders: Arboreta surveys

- Inspection of European trees and shrubs in arboreta and other plantations in other continents

- Inspection of arboreta/ gardens in Europe

An example: infestation of rose hips by *Megastigmus* seed chalcids in botanical gardens

- Seed chalcids strictly associated with seeds of a given family/ genus
- 1 'native' species associated with Rosa seeds: *M. aculeatus*
- Are there alien species ?
- Easy to survey using X-raying of seeds



- MNHN Paris: 40 *Rosa* species sampled
- Kew: 77 *Rosa* species sampled

Both 'native' and exotics

Detection of non-native *Megastimus* species: 4 in MNHN, 5 in Kew

Morphological identification confirmed by genetics

M. alba
(Asia ?)

M. nigrovariegatus
(NA)

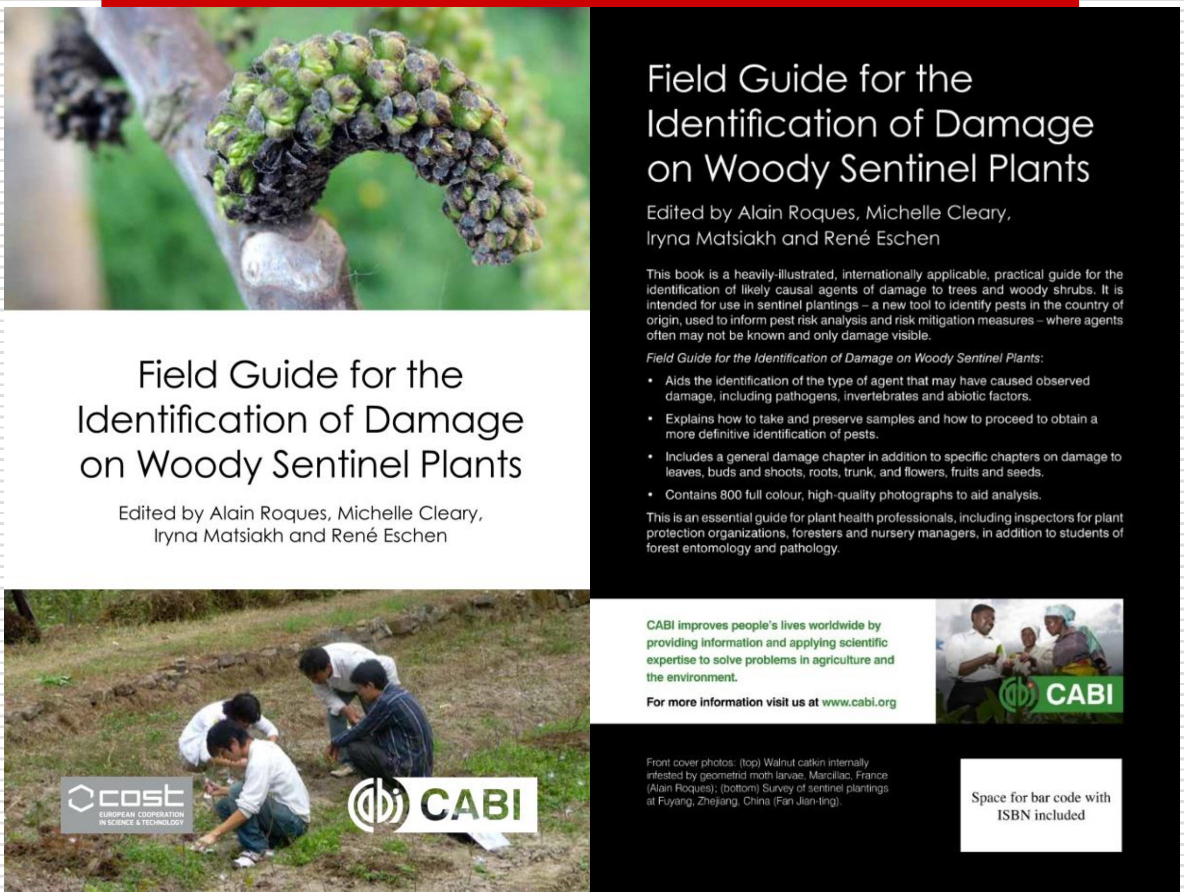
M. sp A
(?)

M. rosae
(Alps)

M. aculeatus
nigroflavus
(NA)

together with the 'native' M. aculeatus

Standardized methods allowing a quick identification of the damaging agents were still missing



Under the COST project "Global Warning", a team of scientists combining entomologists and pathologists realized a field guide proposing standard guidelines for assessing and identifying insect and pathogen damage in sentinel designs and arboreta

Available for free !

http://www.cabi.org/cabebooks/ebook/20173265430

General aims of the field guide

- Surveys in sentinel designs will often face the presence of organisms and symptoms that have never been observed before by the people in charge.
- The guide is aimed at aiding staff of sentinel/ arboreta and phytosanitary inspectors to characterize the observed damage in situations where most damaging agents are unknown
- The guide combines the description of symptoms of animal (insects, mites, nematodes, mammals, birds) damage, pathogen (fungi, Oomycetes, bacteria, viruses, phytoplasma) damage, and abiotic damage
- The guide only allows a tentative identification of a broad group of potential agents but NOT a definitive indentification of the causal agent.
- The guide explains how to collect, how to preserve the samples, and how to proceed to get the most probable identification of the causal agent.

It goes the survey of the whole tree…

2
Field diagnosis of damaging agents of woody plants

A.C. Moreira, H. Bragança, C. Boavida and V. Talgø

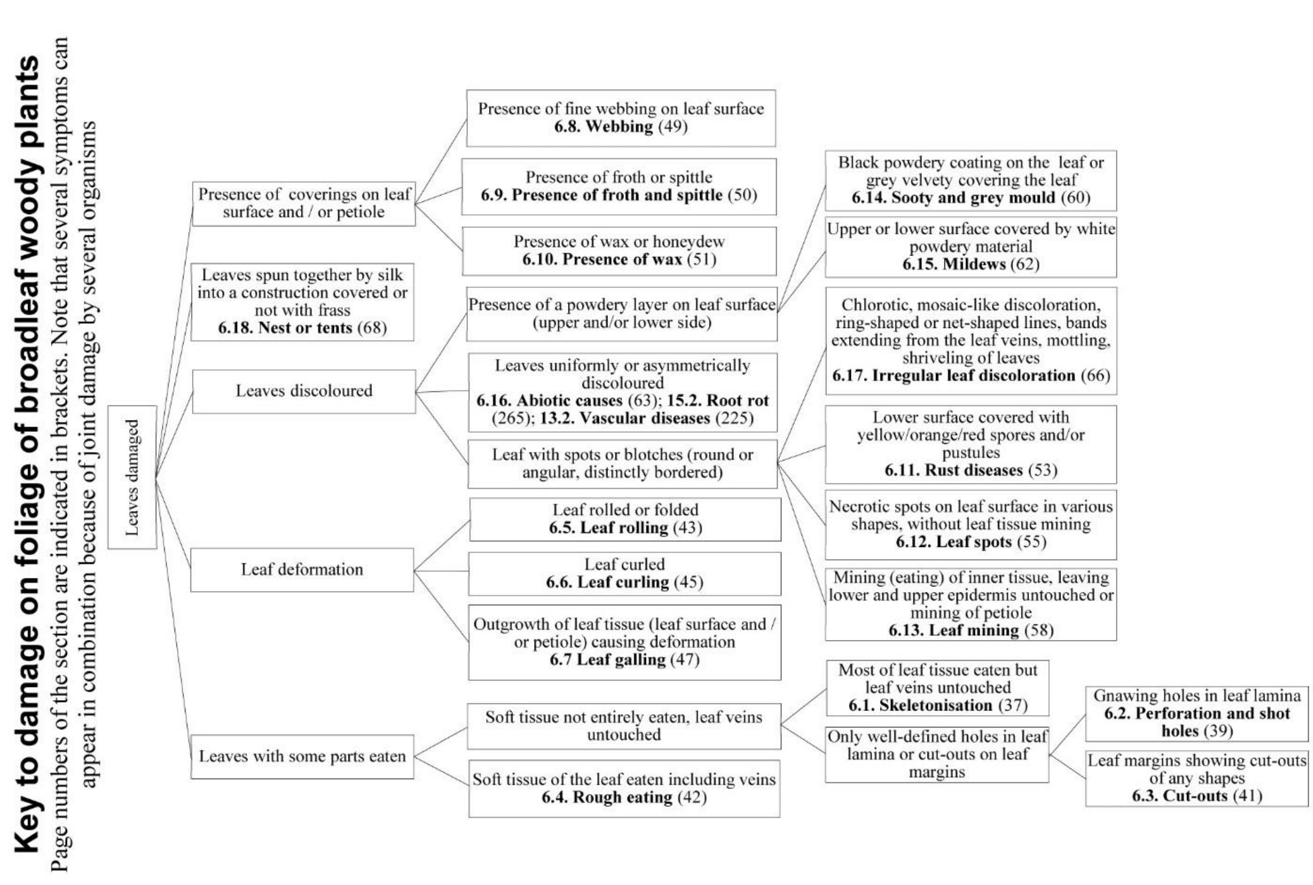
Introduction

The observation and evaluation of trees or shrubs with symptoms is the first step towards a diagnosis. Several damaging agents can give similar symptoms and because tree damage is often due to a combination of causes, field diagnosis is often complex. An overview of the system will be needed to assess the extent of the problem. For example, the symptom distribution pattern of affected trees is important to determine whether an observed pattern of damage is localised or widespread, which may be indicative of the problem being abiotic (e.g., soil flooding/drought) or biotic, caused by an arthropod pest (insect, mite) or by a pathogen) (Fig. 2.1.).

Fig. 2.1. Cork oak (*Quercus suber*) killed by Phytophthora root rot (*Phytophthora cinnamomi*) in the south of Portugal (left, ACM) and noble fir (*Abies procera*) killed by *P. cambivora* in western Norway (right, VT).

… to symptoms on specific organs of broadleaved and conifer trees …

A (dichotomic or multi-entries) key to aid at characterizing symptoms on all organs
or with regard to organ development stages



Each organ chapter has the same structure

6.4. Rough eating

Description: Eating away soft tissue of the leaf lamina including veins. The main leaf vein and some hard parts of other veins may be left untouched.
Possible damaging agents: Insects: Larvae of many Lepidoptera (Fig. 6.4.1), Coleoptera (Figs. 6.4.2, 6.4.3), larvae and adults of some Hymenoptera (Fig. 6.4.4).

Fig. 6.4.1. Leaf of *Acer tatatricum* roughly eaten by the moth larvae (Lepidoptera, Ypsolophidae: *Ypsolopha chazariella*). Novosibirsk, Russia, NK.

Fig. 6.4.2. Leaf of guelder-rose (*Viburnum opulus*) severely defoliated by mature beetle larvae (Coleoptera, Chrysomelidae: *Pyrrhalta viburni*). Novosibirsk, Russia, NK.

Fig. 6.4.3. Leaf of Chinese ash (*Fraxinus chinensis*) roughly eaten by an unknown insect. Sentinel plantation, Fuyang, China, AR.

Fig. 6.4.4. Leaf of willow (*Salix* sp.) roughly eaten by sawfly larvae (Hymenoptera, Tenthredinidae: *Nematus* sp.). Kerlavic, France, SA.

Additional information: Before checking leaves, especially with square damage (often due to weevils: Curculionidae), place a Japanese umbrella or an ordinary umbrella under the branches to catch insects. For insect collection and preservation see Chapter 3.

Description of the damage symptom

Examples of possible damaging agents
Only examples

Illustrations of possible damaging agents

Additional information about collection timing, techniques, …

Early detection at arrival, a major challenge !

Phytosanitary inspection, yes… but facing trade increase, a need for more automated tools !

- Settle multiple traps baited with a single, specific lure for a given targeted species … *high manpower and costs and most arriving species remain unknown*

vs.

- Settle a few traps with a multicomponent lure expected for a generic effect on families, subfamilies, tribes or genera … *limited costs and unknown arriving species may be trapped*

Implies to check possible repellency effects between components of the multilures through tests in forests, statistical treatments being not possible in ports-of entry

Which lures expected for generic activity ?

At first, only cerambycids were considered. Progresses in pheromone identification revealed well-conserved sex- and aggregation-sex compounds among subfamilies and tribes at world level (Hanks and Millar, 2016), and thus **possibilities of using generic attractivity for trapping non-natives at arrival**

Compound	Sex	Subfamily	Tribe	
Fuscumol + Fuscumol Acetate	M	Aseminae Lamiinae	Asemini Acanthocini Acanthoderini Obriini	Blend A (in isopropanol)
Geranyl acetone	M	Aseminae Lamiinae	Asemini Acanthocini	
Monochamol	M	Lamiinae	Laminii Monochamiini	
3-hydroxyhexan-2-one (C6-ketol)	M	Cerambycinae	Callidiini Clytini Hesperophanini Hylotrupini Molorchini	Blend B (in isopropanol)
Prionic acid	F	Prioninae	Prionini	
2-methylbutanol	M	Cerambycinae	Callidiini	
2R*,3S*-hexanediol	M	Cerambycinae	Clytini	

2014- 2017 PORTRAP trapping design: Yearly steps towards a unique blend

YEAR	Forests	Ports of entry
2011-2012	6 forests Single lures	-
2014	20 A B T	-
2015	9 C B T	11 C B T
2016	20 C B B+C	11 C B
2017	21 B+C BC	15 B+C

Cross-vane traps coated with teflon

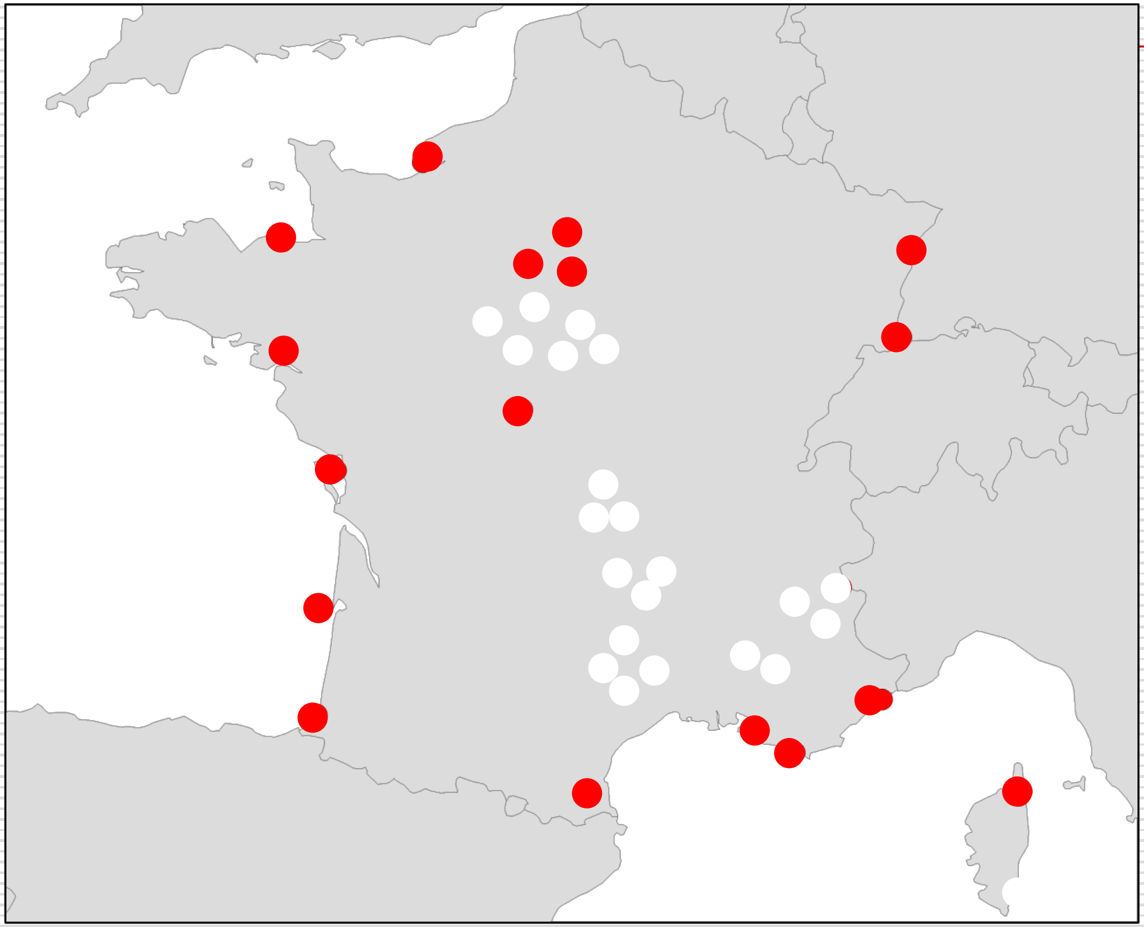
A grid for water at collector's bottom and insecticide bag (for DNA barcoding)

1ml of blend in polyethylene bags

T= control with 1ml isopropanol

To get bark beetles, a change needed in 2015: C= A+ α-pinene+EtOH
(test of C vs. A for cerambycids: no significant differences in the number of trapped species)

2014- 2017 PORTRAP Trapping sites

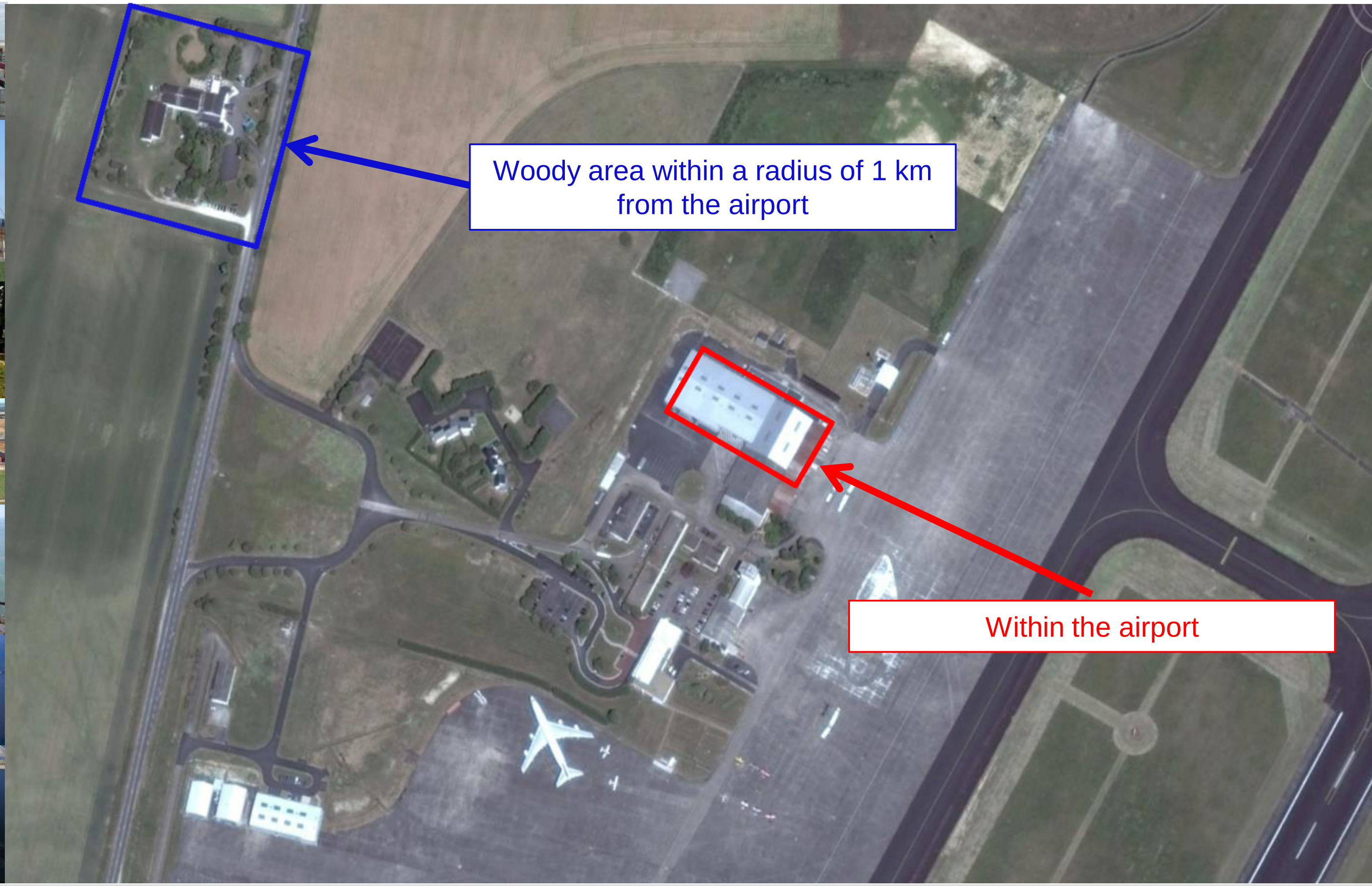


Forests
3 replicates of each multilure per site- necessary for statistics

Ports of entry (maritime, fluvial, aiport, national market)
4 replicates of each multilure per site: 2 within the port and 2 within a 1 km radius

Whenever possible near wood waste deposit areas (Rassati et al., 2014)

An example of trapping at ports-of-entry



Thank your for your attention !