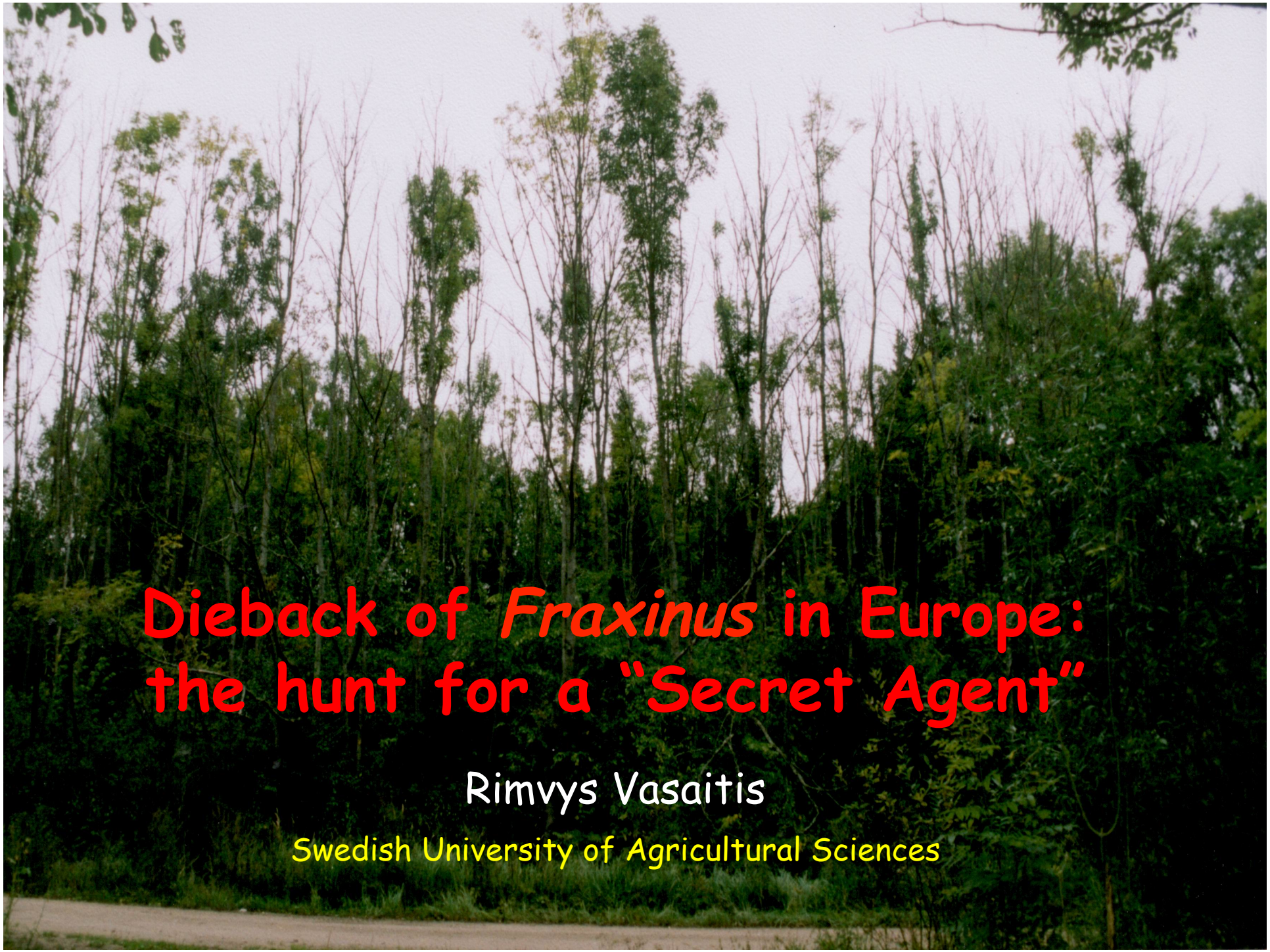


Dieback of *Fraxinus* in Europe: the hunt for a "Secret Agent"

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Contents

1. The Phenomenon

2. Role of *Armillaria*

3. Identification of the Pathogen

4. Mitigation


Completely new phenomenon, initially
nothing at all was known

History & current situation

- Lethal disease of *Fraxinus*
- Start in Poland and Lithuania, problem since early 1990s
- Large proportion of stands damaged (60-80%)
- In some areas <2% trees remaining symptomless
- Massive tree dieback in Europe
- Threat for the existence of host tree species

Symptoms & Environment

- Root and butt rot
- Crown dieback
- No correlation with tree age, soil (moisture & fertility), environment (forest, urban, nursery, roadside …)
- First wild guess: *Armillaria,* as it is everywhere (but why?)






Root and butt rot





Yes, Armillaria rot

Enderle R, Sander F, Metzler B (2017) *i-Forest* (in press)


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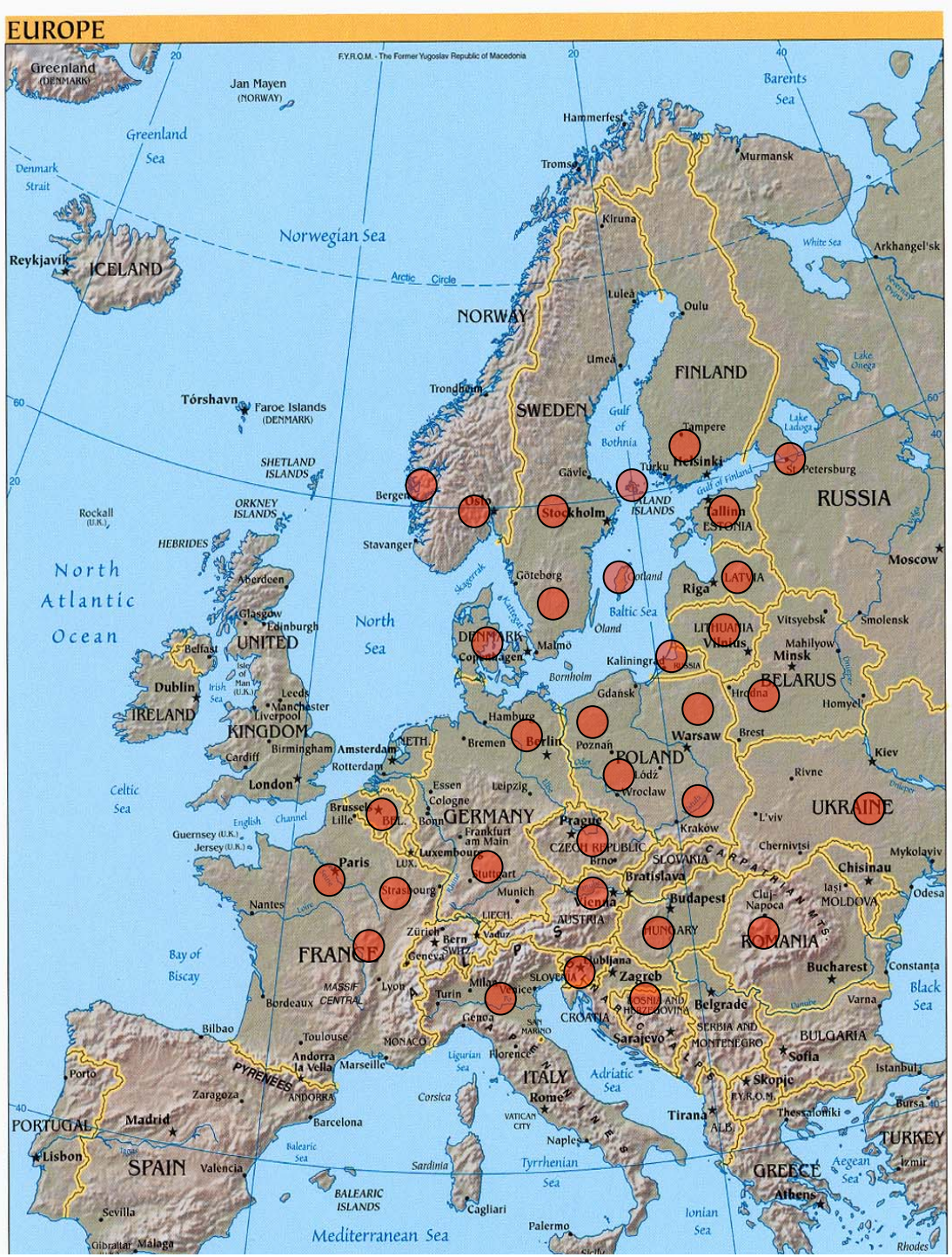
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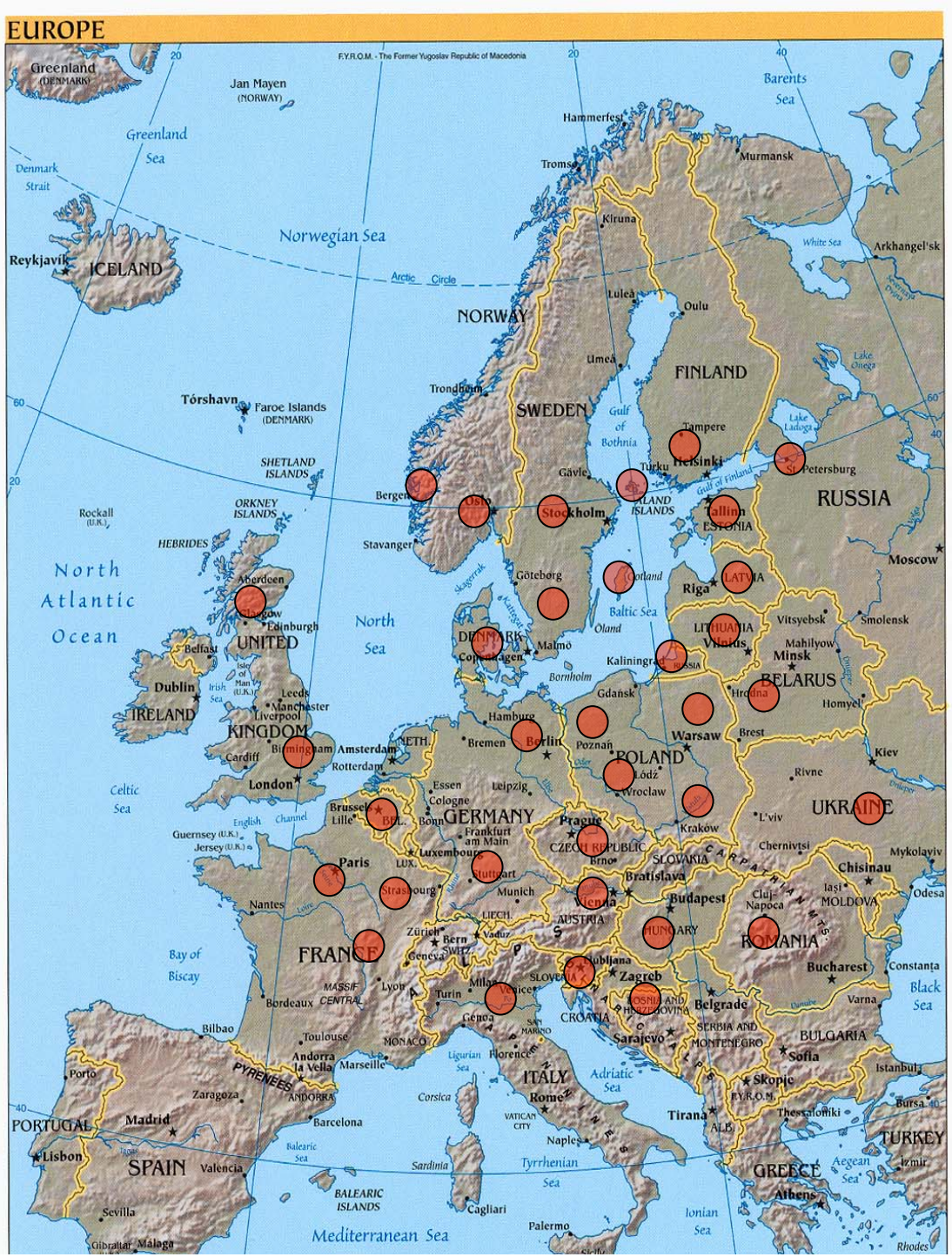
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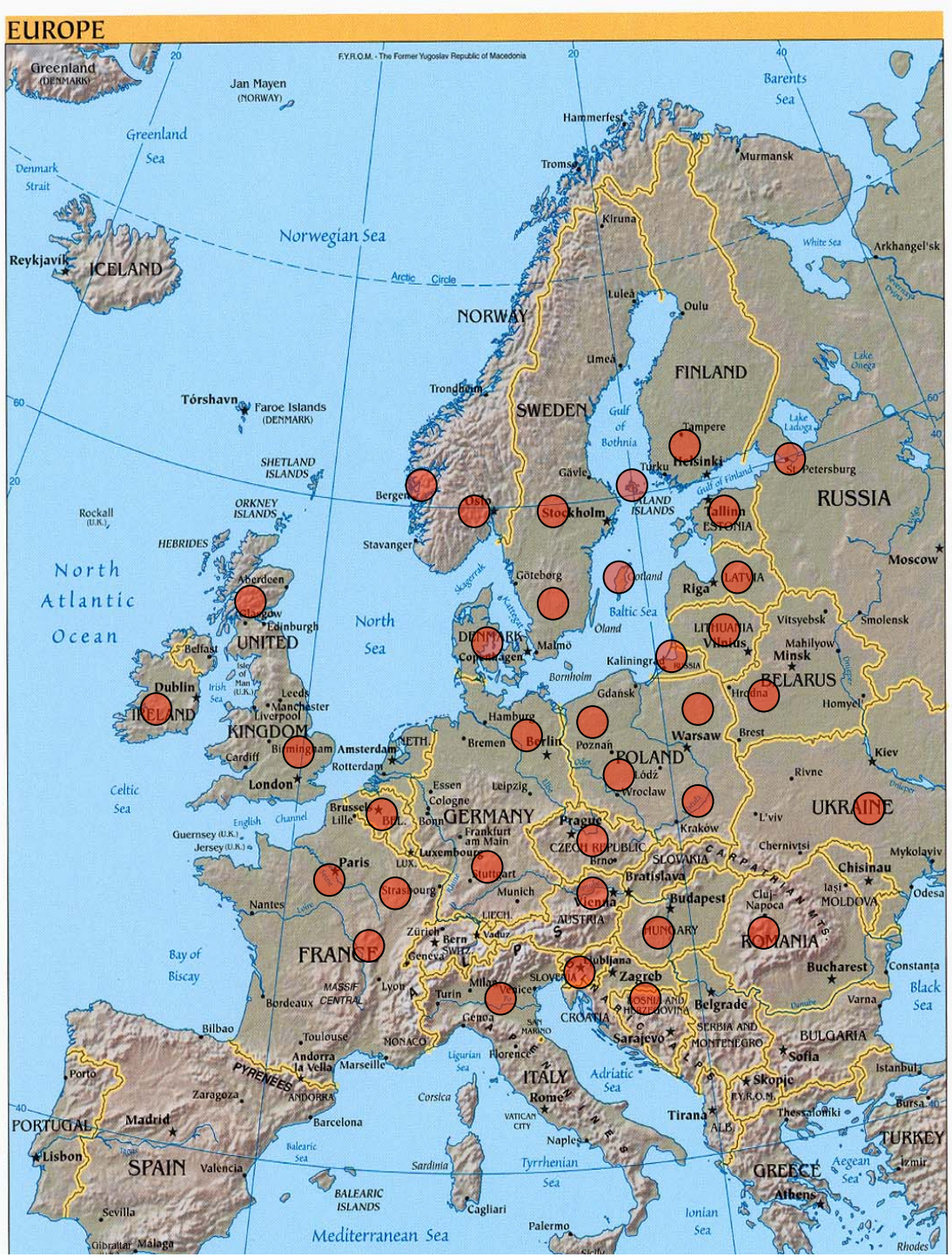
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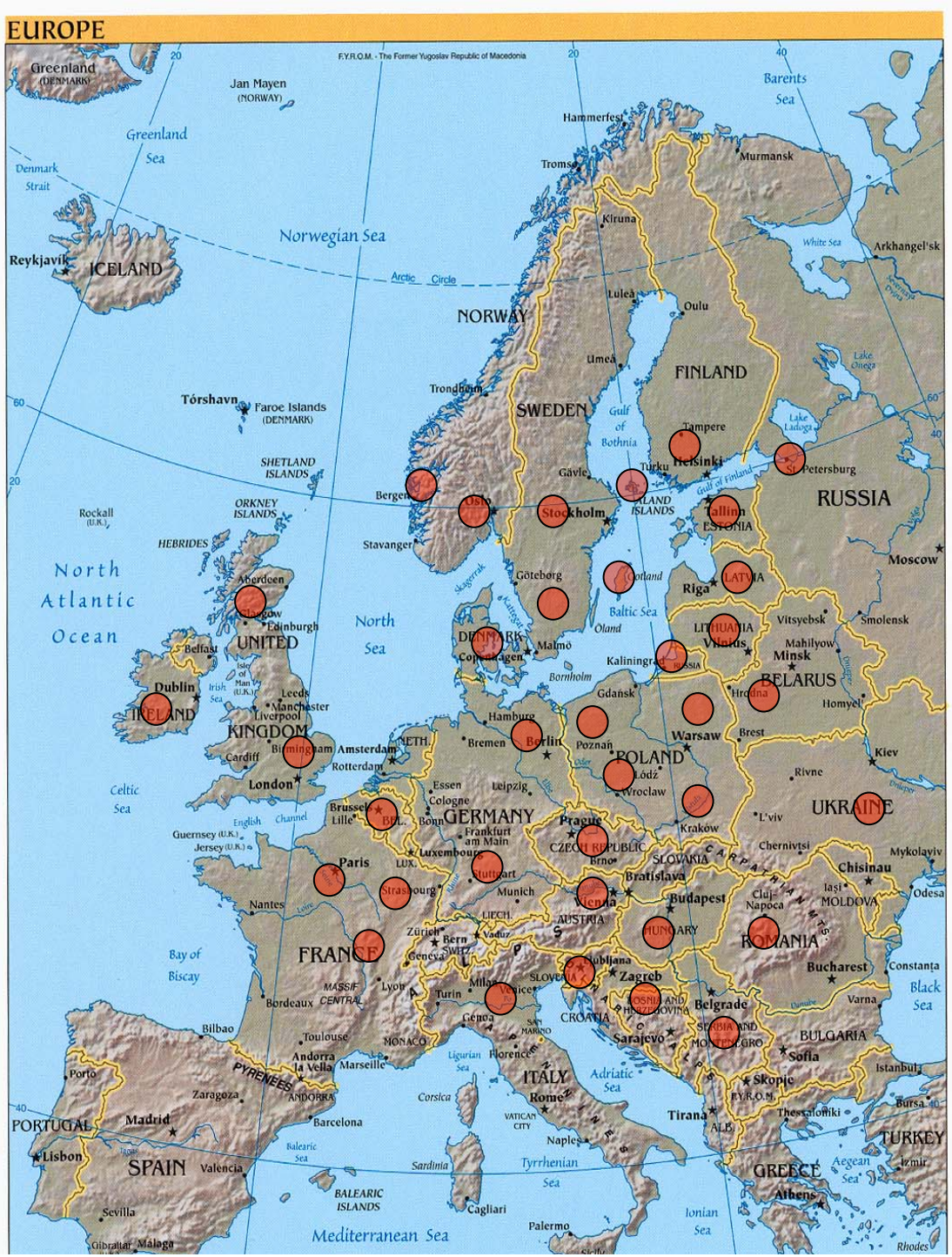


2012
UK (national disaster)



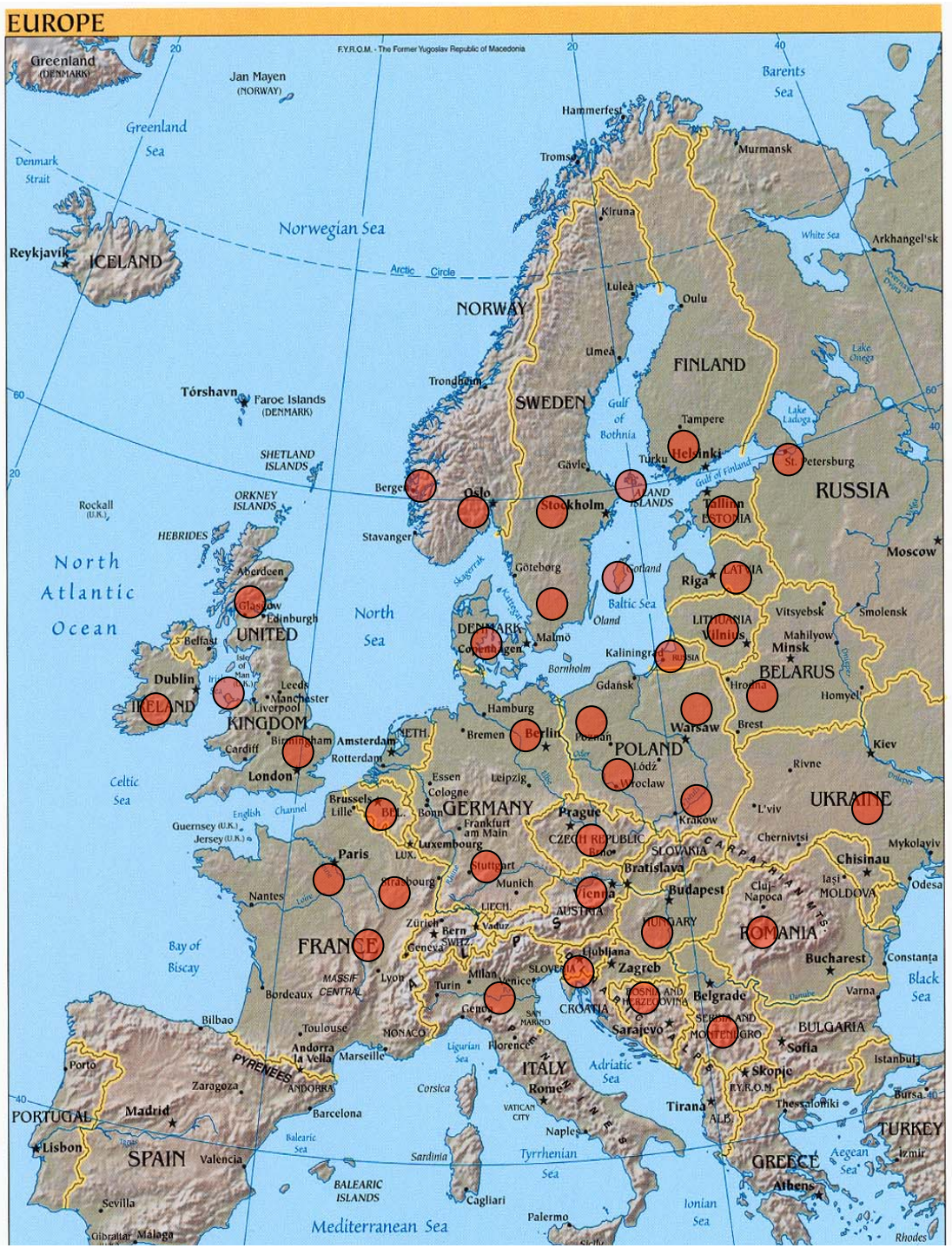
2013

Ireland (national disaster)


EUROPE
F.Y.R.O.M. - The Former Yugoslav Republic of Macedonia
Greenland (DENMARK)
Jan Mayen (NORWAY)
Greenland Sea
Denmark Strait
Norwegian Sea
Barents Sea
White Sea
Arctic Circle
Reykjavik
ICELAND
Hammerfest
Tromsø
Murmansk
Arkhangel'sk
Kiruna
Luleå
Oulu
NORWAY
Umeå
FINLAND
Lake Onega
Lake Ladoga
Trondheim
Tórshavn
Faroe Islands (DENMARK)
SWEDEN
Gulf of Bothnia
Tampere
Helsinki
St. Petersburg
Gulf of Finland
Gävle
Turku
ÅLAND ISLANDS
RUSSIA
SHETLAND ISLANDS
Bergen
Oslo
Stockholm
Tallinn
ESTONIA
ORKNEY ISLANDS
Rockall (U.K.)
HEBRIDES
Stavanger
Moscow
North Atlantic Ocean
Aberdeen
Göteborg
Gotland
Riga
LATVIA
Glasgow
Edinburgh
North Sea
Baltic Sea
Oland
Vitsyebsk
Smolensk
UNITED
Belfast
DENMARK
Malmö
Copenhagen
LITHUANIA
Vilnius
Mahilyow
Minsk
Kaliningrad
Bornholm
BELARUS
Dublin
Irish Sea
Isle of Man (U.K.)
Leeds
Manchester
Liverpool
Gdańsk
Hrodna
Homyel'
IRELAND
KINGDOM
Hamburg
Bremen
Berlin
Poznań
Warsaw
Brest
Birmingham
Cardiff
NETH.
Amsterdam
Rotterdam
POLAND
Łódź
Wrocław
Kiev
Rivne
London
Celtic Sea
Essen
Leipzig
Cologne
Brussels
Lille
Bonn
GERMANY
Prague
Kraków
UKRAINE
L'viv
English Channel
Guernsey (U.K.)
Jersey (U.K.)
Frankfurt am Main
CZECH REPUBLIC
Brno
SLOVAKIA
Chernivtsi
Mykolayiv
Paris
Luxembourg
LUX.
Stuttgart
Bratislava
Chisinau
Strasbourg
Munich
Budapest
Cluj-Napoca
Iaşi
MOLDOVA
Odesa
Nantes
Loire
Vienna
AUSTRIA
HUNGARY
Zürich
Bern
LIECH.
Vaduz
SWITZ.
ROMANIA
Bay of Biscay
FRANCE
Geneva
Ljubljana
Zagreb
Bucharest
Constanța
Black Sea
MASSIF CENTRAL
Lyon
Milan
Turin
Venice
SLOVENIA
Bordeaux
Belgrade
Varna
Danube
CROATIA
Genoa
SAN MARINO
Bilbao
Toulouse
MONACO
Ligurian Sea
Florence
Sarajevo
BULGARIA
Sofia
Andorra la Vella
Marseille
ITALY
Adriatic Sea
Skopje
Istanbul
Porto
PYRENEES
ANDORRA
Zaragoza
Corsica
Rome
F.Y.R.O.M.
Bursa
Thessaloniki
PORTUGAL
Madrid
Barcelona
VATICAN CITY
Tirana
ALB.
TURKEY
Lisbon
Balearic Sea
Naples
Sardinia
Tyrrhenian Sea
Aegean Sea
Izmir
SPAIN
Valencia
GREECE
Athens
BALEARIC ISLANDS
Sevilla
Cagliari
Palermo
Ionian Sea
Mediterranean Sea
Gibraltar
Málaga
Rhodes

2016

Serbia



2017

Isle of Man

(areal spread)

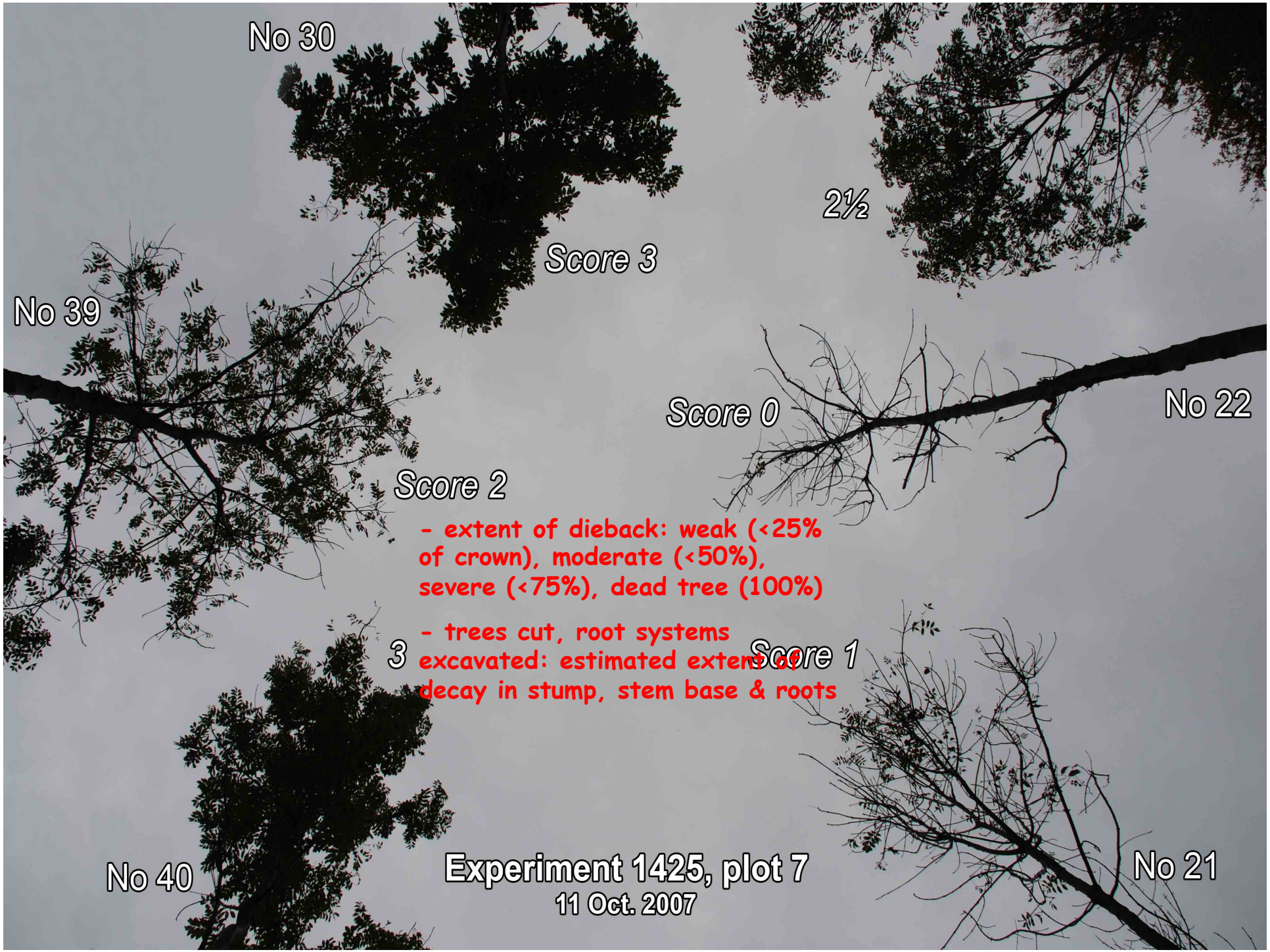
… but *Armillaria* is present everywhere across Europe (and globally) for thousands of years, thus observed gradual stepwise disease expansion in a tidy frontline over large geographic range indicated that there must be something new coming …

… also very diverse symptoms that cannot be attributed to *Armillaria*

Nevertheless, checking impact of *Armillaria*: impact of root & butt rot on ash dieback intensity

The questions

- What fungi decay root systems?
- Are they the primary cause of tree death?
- Root rot and eventual future self-regeneration


No 30
Score 3
2½
No 39
Score 0
No 22
Score 2
- extent of dieback: weak (<25% of crown), moderate (<50%), severe (<75%), dead tree (100%)
- trees cut, root systems excavated: estimated extent of decay in stump, stem base & roots
3
Score 1
No 40
Experiment 1425, plot 7
11 Oct. 2007
No 21

- **Severity of the dieback positively correlated with:**
  - amount of decayed roots (r = 0.86);
  - extent of decay over stump cross-section (r = 0.87);
  - length of decay at stem base (r = 0.57)

The work

- Mapping of trees to be sampled: healthy, declining and dead
- Sampling of wood and fungi
- Isolation to pure cultures and identification
- Vegetative compatibility tests: determining pathways of fungal individuals





Results of isolation & identification

pure culture



352 isolates from a total of 210 trees

75 distinct species, 60 (80%) of which identified

20 spp. of basidiomycetes

51 spp. of asco- and deuteromycetes

4 spp. of zygomycetes

Basidiomycetes – active decomposers of wood in roots and stem

Species	Tree condition			
	Sound	Declining	Dead	All 210
Armillaria cepistipes	46.7	37.9	46.0	41.0
Bjerkandera adusta	6.7	0.0	0.0	0.5
Ceratobasidium sp.908	0.0	0.8	0.0	0.5
Coprinus disseminatus	0.0	0.8	0.0	0.5
Fomitopsis pinicola	0.0	0.0	3.2	1.0
Ganoderma applanatum	0.0	0.8	0.0	0.5
Gloeoporus taxicola	0.0	0.0	1.6	0.5
Mycena sp.915	0.0	0.0	1.6	0.5
Mycoacia uda	0.0	1.5	0.0	1.0
Peniophora cinerea	0.0	0.8	0.0	0.5
Peniophora limitata	0.0	1.5	0.0	1.0
Phlebia rufa	0.0	0.8	0.0	0.5
Phlebia sp.964	0.0	0.0	1.6	0.5
Psathyrella candolleana	0.0	0.8	0.0	0.5
Resinicium bicolor	0.0	0.0	1.6	0.5
Steccherinum sp.912	0.0	0.8	1.6	1.0
Trametes hirsuta	6.7	0.0	0.0	0.5

Anamorphic fungi – a seldom cause of wood decay (endophytes, necrotrophs, saprotrophs)

Species	Tree condition			
	Sound	Declining	Dead	All 210
Ascocoryne sarcoides	0	4.5	11.1	6.2
Botryosphaeria stevensii	0	2.3	4.8	2.9
Cladosporium magnusianum	0	0.8	1.6	1.0
Hymenoscyphus sp.970*	6.7	3.8	0	2.9
Leptodontidium elatius	0	10.6	11.1	10.0
Nectria haematococca	0	2.3	0	1.4
Nectria radicicola	0	3.0	6.3	3.8
Nectria viridescens	13.3	4.5	3.2	4.8
Phialophora fastigiata	6.7	0.8	0	1.0
Phialophora malorum aggreg.	13.3	3.8	9.5	6.2
Phoma exigua	0	0.8	0	0.5
Xylaria sp.920	0	0.8	1.6	1.0

* slow grower in culture

Dominant fungus, -
Armillaria cepistipes:

- rhizomorphs on 100% trees
- mycelial fans on 98% trees
- isolated from 55% trees*



A.cepistipes, facts:

- oportunistic fungus
- weak pathogen
- in historical sources, noted as very seldom fruiting on *Fraxinus**

*in monograph by Sokolov (1964), *Fraxinus* is not listed as the host for *Armillaria*, although the records are provided for several hundred other plant species

Rhizomorphs:

robust mycelial structures enabling the fungus for independent growth throughout soil

Implications:

- single *Armillaria* genet can invade several trees

- occupying discrete teritory

- fungal territorial clones

- informative in regard to disease history and pathways of infection in a given forest stand



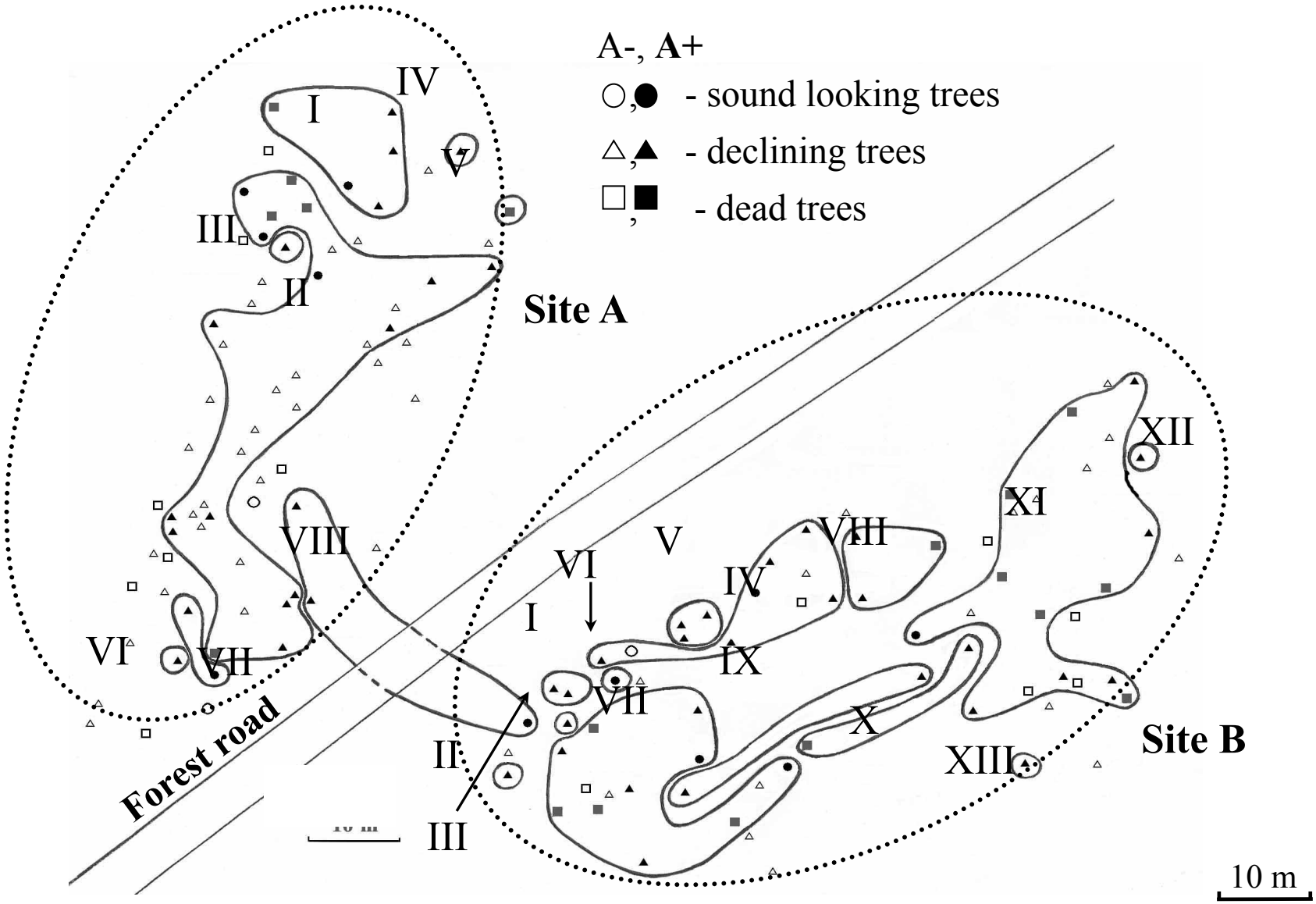
- Spread of *Armillaria* in declining *Fraxinus excelsior* stands

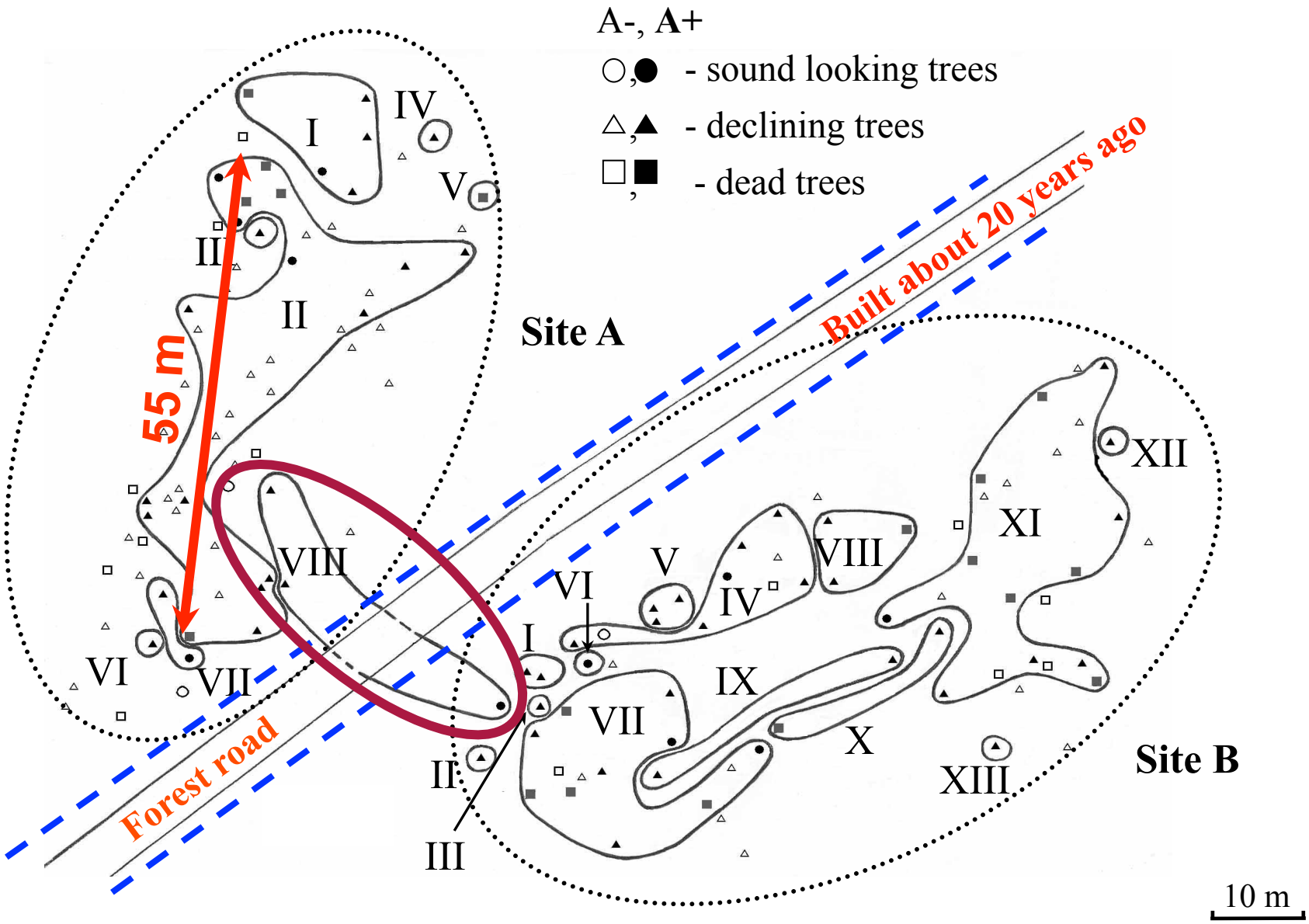
- Investigating local population structures

- Vegetative compatibility tests: self / non-self recognition of fungal individuals


30 m
3 m

Distribution of vegetative individuals of *A. cepistipes*




A-, A+
○,● - sound looking trees
△,▲ - declining trees
□,■ - dead trees
Site A
Site B
Built about 20 years ago
Forest road
55 m
10 m
I
II
II
III
IV
V
VI
VII
VIII
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII

Conclusions & implications

1. High diversity of fungi

2. *Armillaria* NOT a primary agent, - its individuals in the sites much older than the decline

3. Common cause of butt decay in live (declining) trees – contributes to the decline

…again, a closer look to the symptoms …



Enderle R, Sander F, Metzler B (2017), *i-Forest* (in press)



Yes, Armillaria rot

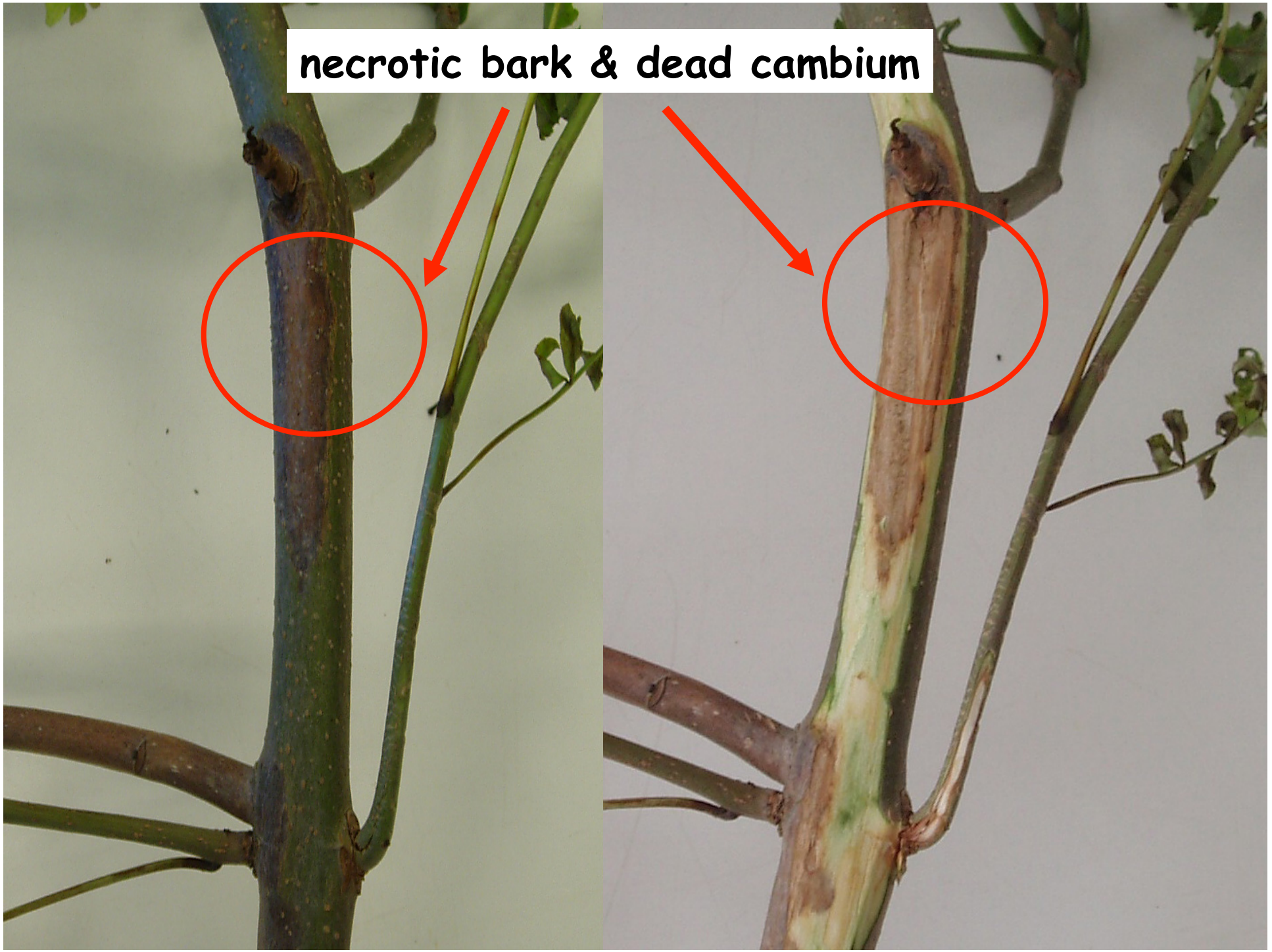
Enderle R, Sander F, Metzler B (2017) *i-Forest* (in press)

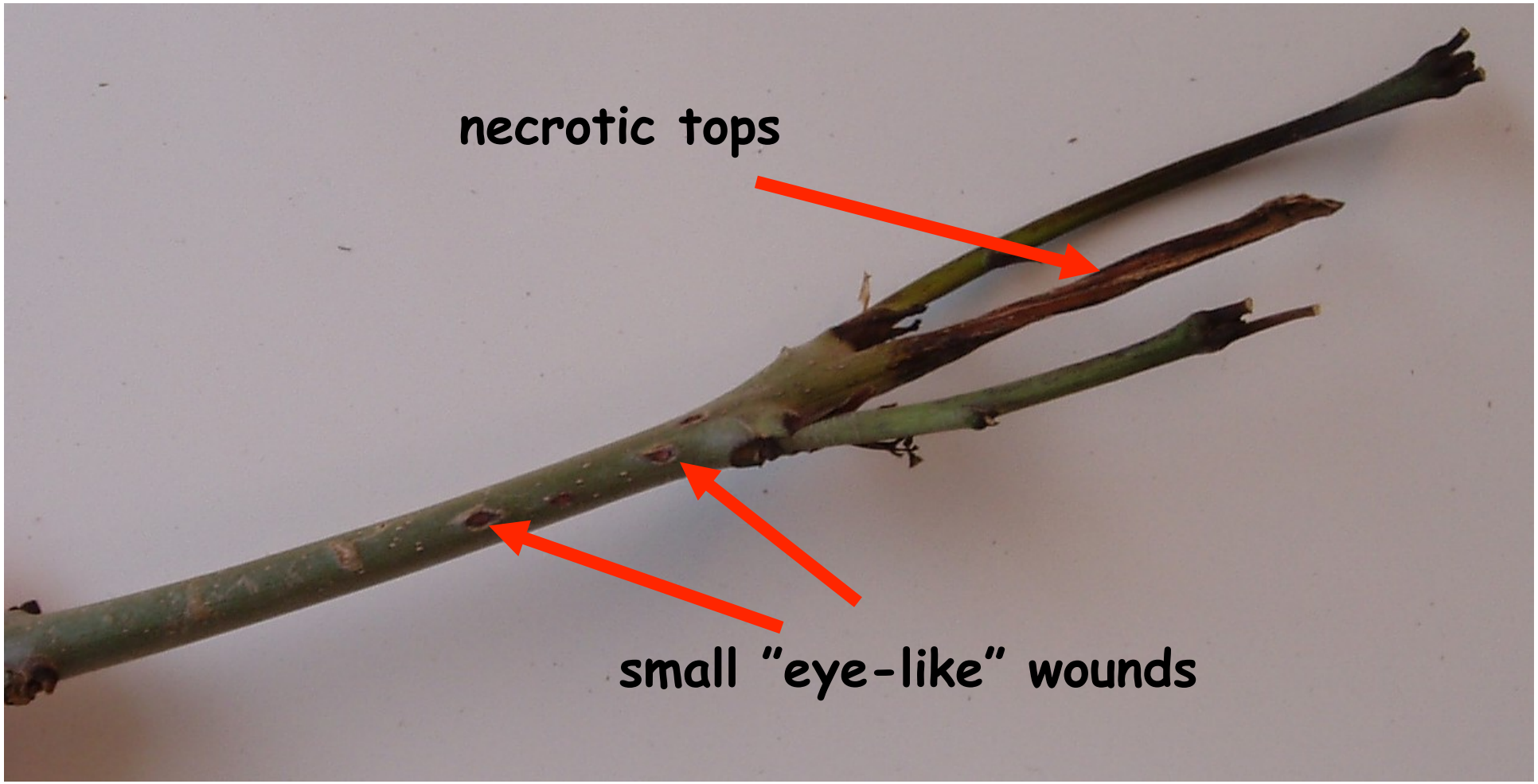
… yet apart root & butt rot also very diverse other symptoms that cannot be attributed to *Armillaria*

yet many more & different external symptoms:

on shoots …




necrotic bark & dead cambium


necrotic tops
small "eye-like" wounds


bigger bark wounds


canker wounds

symptoms on leaves

wilt



necrotic leaves & petioles





- Sprouting is weaker from decayed root systems & stumps
- Eventual sprouts repeatedly and heavily attacked by "crown fungi"

… but then what is the identity of the "Secret Agent", and where does it come from?

Attention to declining crowns: crown dieback



The questions:

1. What fungi do attack declining crowns?

2. Which of them is/are the dieback agent(s)?

The work: three different methods

1. Fungal isolations into pure culture

2. Identification of the cultures by ITS rDNA sequencing and by morphology

3. Direct analyses of fungal DNA (T-RFLP) from bark and leaves

   (certain fungi are difficult to isolate on artificial media, in particular biotrophs)

Fungal isolations

1st study: flame sterilization

- symptomless bark & wood (58 samples)
- bark & wood with initial necrosis (58)
- bark & wood with advanced necrosis (58)

2nd study: sodium hypochlorite sterilization

- small eye-like bark wounds (60)
- bigger bark wounds (45)
- stem cankers (30)
- symptomless petioles (40)
- necrotic petioles (45)

Flame sterilization; 200 isolates, 36 spp.; most common known fungi:

- *Gibberella avenacea*
- *Alternaria alternata*
- *Epiccocum nigrum*
- *Lewia* sp.924
- *Botryosphaeria stevensii*
- *Phomopsis* sp.R57
- *Phoma glomerata*
- *Cladosporium tenuissinum*

Przybył (2002)
Zaspel & Nirenberg (2002)
Kowalski & Łukomska (2005)
Lygis et al. (2005)

+ Hymenoscyphus sp.970 (seldom)

slow grower that was also occasionally isolated from stems during *Armillaria* work

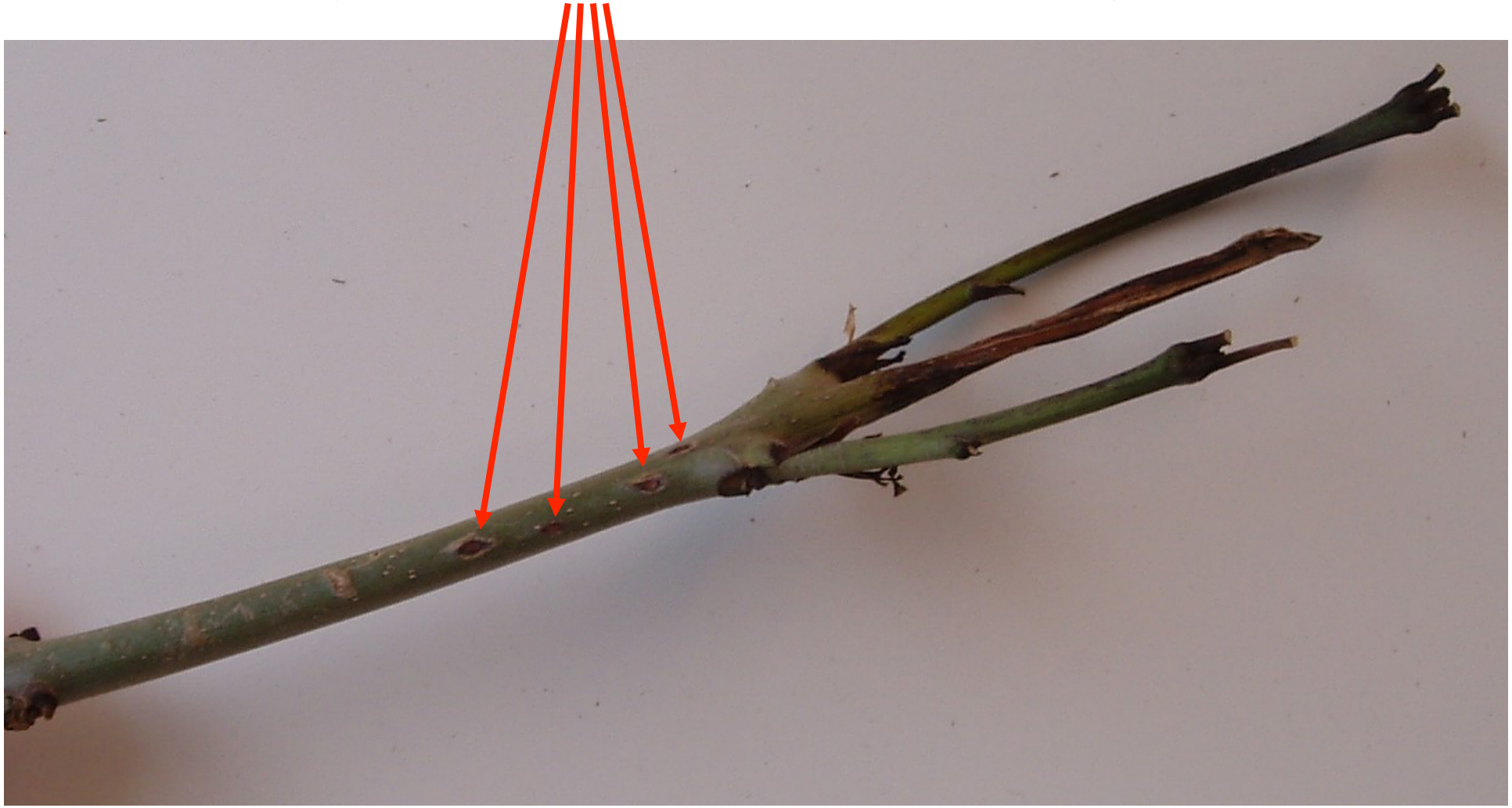


2nd study: sodium hypochlorite sterilization

Hymenoscyphus sp.970

dominant in:

- small "eye-like" wounds: 16 out of 60 samples, or 27%



Hymenoscyphus sp.970

dominant in:

- bigger bark wounds:

11 out of 45 samples, or 28%



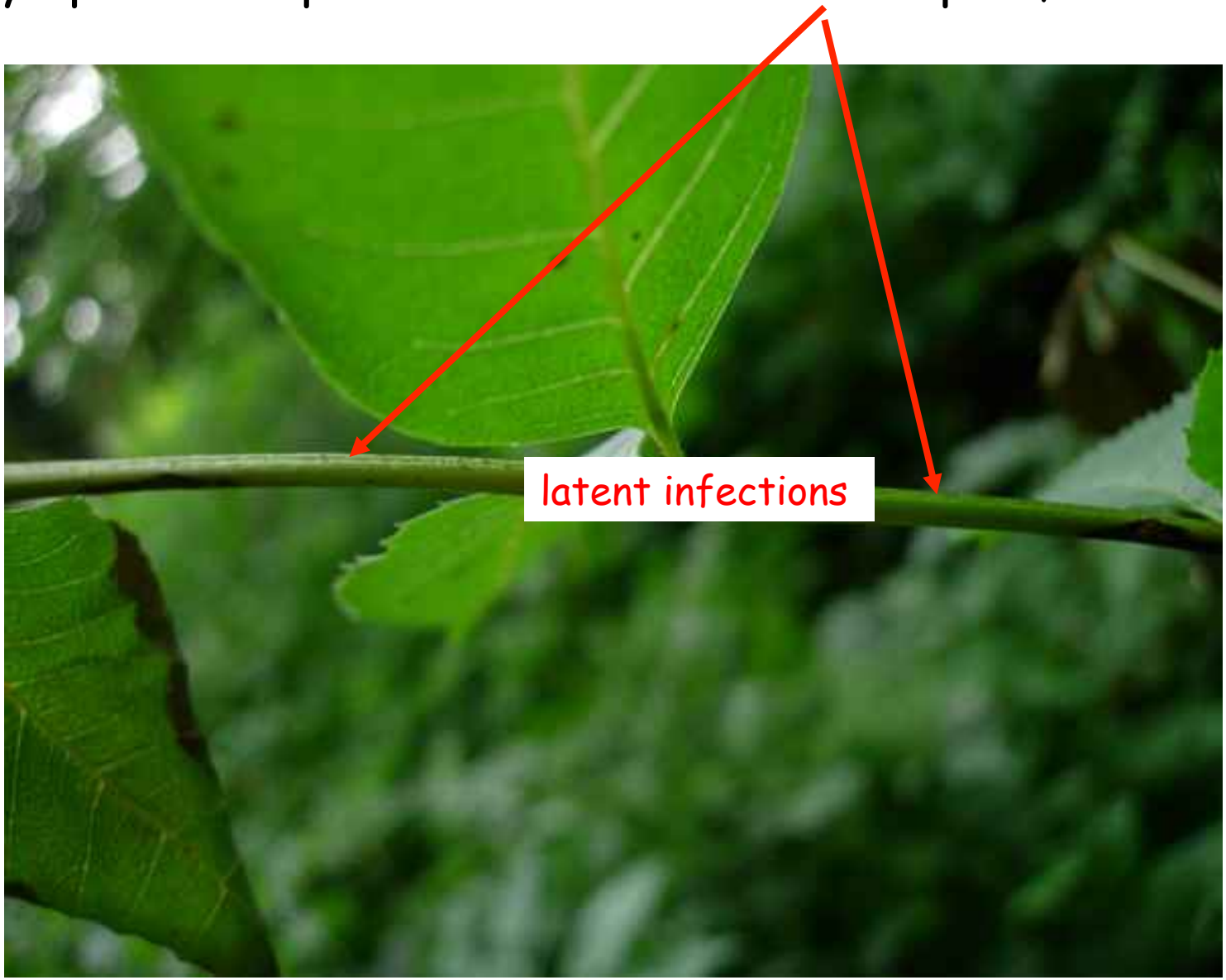
Hymenoscyphus sp.970 – dominant in:

- stem cankers: 28 out of 30 samples, or 93%



Hymenoscyphus sp.970 - dominant in:

- non-symptomatic petioles: 12 out of 40 samples, or 30%



Hymenoscyphus sp.970 - dominant in:

- necrotic petioles: 41 out of 45 samples, or 91%



Infections

- universal colonizer
- occurs in physiologically, biochemically and physically different plant tissues
- e.g. leaves, petioles, bark, wood

3. Direct DNA analyses from diseased tissue (T-RFLP)

- necrotic bark (32 samples)
- necrotic petioles (32)
- necrotic leaves (32)

- revealed presence of 27 fungal species
- up to 12 spp. per sample, e.g.:
  - 5 × 5 mm piece of bark
  - 7 - 10 mm long petiole
  - 1 cm^2 of leaf

Direct analyses (T-RFLP), % of samples

Fungi (in 34-70% of all 96 samples)	bark	petioles	leaves
Cryptococcus foliicola ?	48	63	100
Cryptococcus sp. ?	38	72	93
Hymenoscyphus sp.970	41	97	39
Phoma glomerata	76	13	71
Phoma exigua	66	13	68
Cladosporium sp.	38	28	79
Dioszegia sp.	31	28	54
Bullera coprosmaensis	17	34	54
Chalara sp. ?	28	56	14

Pathogenicity tests

- 45 fungal species from crowns, stems and roots of declining trees

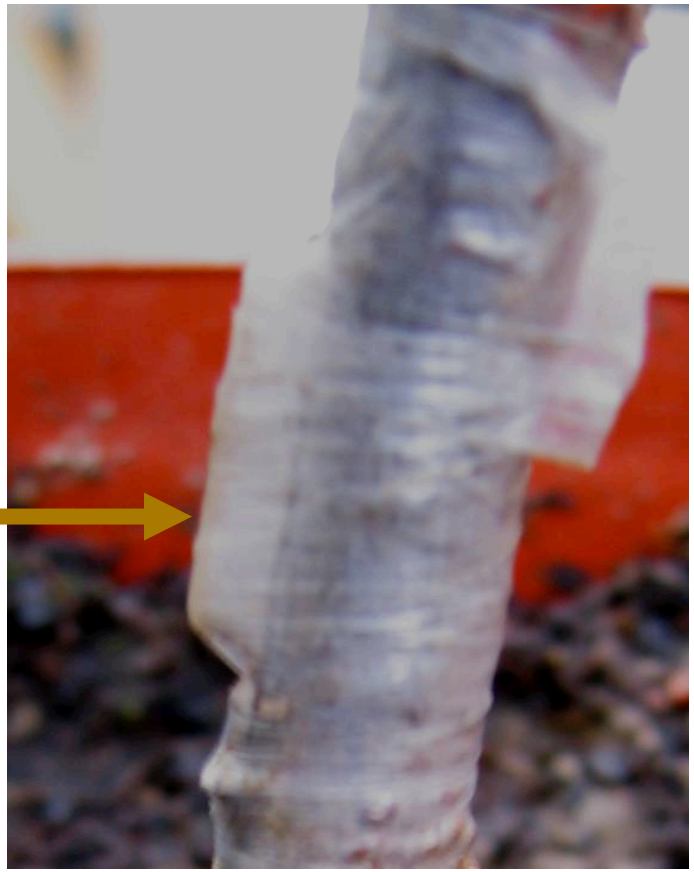
- 700 one-year-old *Fraxinus excelsior* seedlings

- bare root nursery and greenhouse



Pieces of pre-colonized wood (1x1x5 mm) …

… taped to a 1x5 mm wound.



The only one of those causing symptoms:

Hymenoscyphus sp. 970 !!!


The test with
Hymenoscyphus sp.970



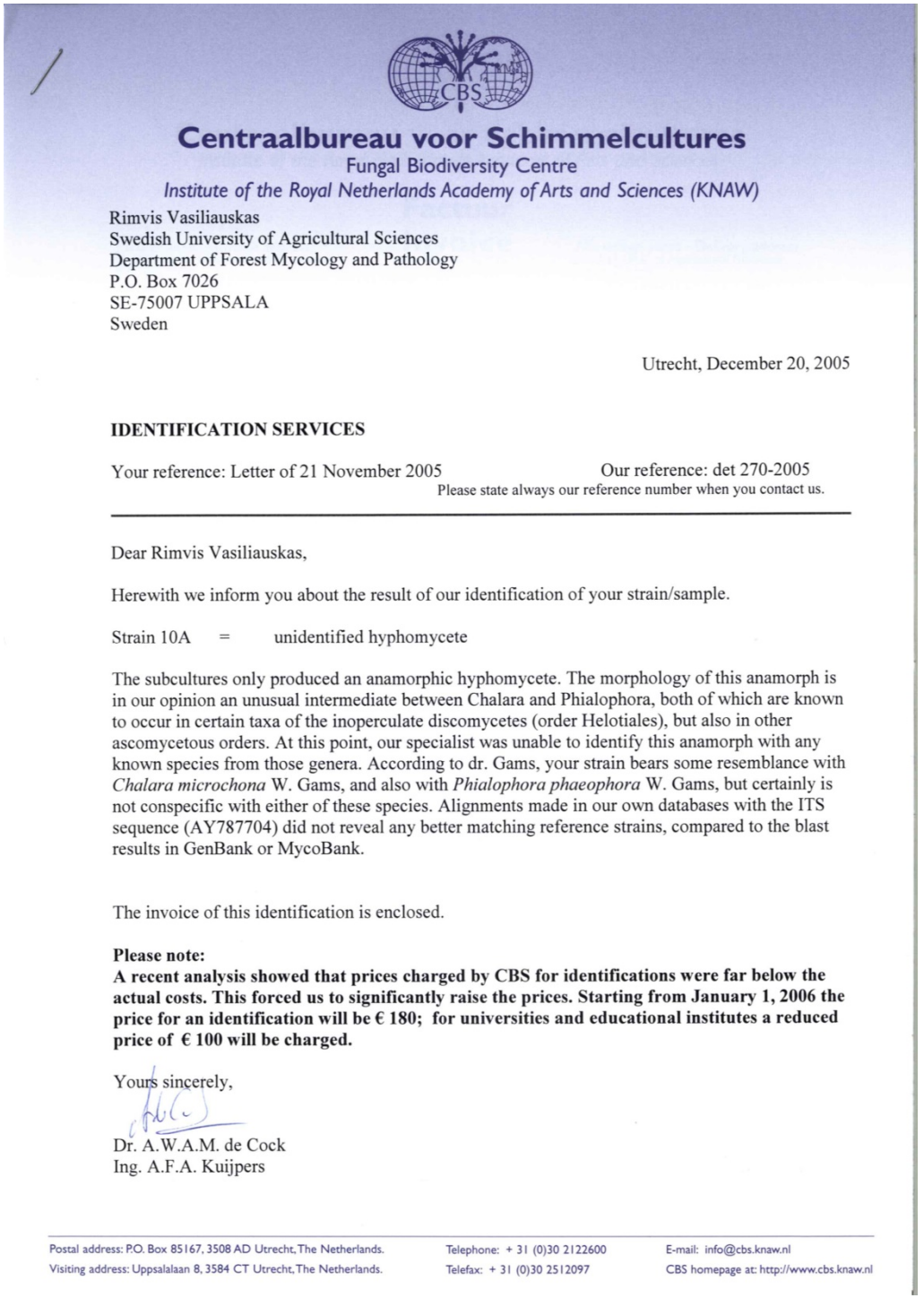


Koch's postulates

- Inoculation with a test species
- Yielding similar symptoms
- Reisolation of the test species from obtained symptoms
- Proof that reisolated strain is identical to the inoculated one (e.g. vegetative compatibility test)

The identity of Hymenoscyphus sp. 970?

… so what species is it, and where does it come from?



Centraalbureau voor Schimmelcultures
Fungal Biodiversity Centre
Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW)

Rimvis Vasiliauskas
Swedish University of Agricultural Sciences
Department of Forest Mycology and Pathology
P.O. Box 7026
SE-75007 UPPSALA
Sweden

Utrecht, December 20, 2005

IDENTIFICATION SERVICES

Your reference: Letter of 21 November 2005 Our reference: det 270-2005
Please state always our reference number when you contact us.

Dear Rimvis Vasiliauskas,

Herewith we inform you about the result of our identification of your strain/sample.

Strain 10A = unidentified hyphomycete

The subcultures only produced an anamorphic hyphomycete. The morphology of this anamorph is in our opinion an unusual intermediate between Chalara and Phialophora, both of which are known to occur in certain taxa of the inoperculate discomycetes (order Helotiales), but also in other ascomycetous orders. At this point, our specialist was unable to identify this anamorph with any known species from those genera. According to dr. Gams, your strain bears some resemblance with *Chalara microchona* W. Gams, and also with *Phialophora phaeophora* W. Gams, but certainly is not conspecific with either of these species. Alignments made in our own databases with the ITS sequence (AY787704) did not reveal any better matching reference strains, compared to the blast results in GenBank or MycoBank.

The invoice of this identification is enclosed.

Please note:
A recent analysis showed that prices charged by CBS for identifications were far below the actual costs. This forced us to significantly raise the prices. Starting from January 1, 2006 the price for an identification will be € 180; for universities and educational institutes a reduced price of € 100 will be charged.

Yours sincerely,

Dr. A.W.A.M. de Cock
Ing. A.F.A. Kuijpers

Postal address: P.O. Box 85167, 3508 AD Utrecht, The Netherlands.
Visiting address: Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.
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E-mail: info@cbs.knaw.nl
CBS homepage at: http://www.cbs.knaw.nl

Then, described as a new species

For. Path. 36 (2006) 264–270
© 2006 Blackwell Verlag, Berlin

Chalara fraxinea sp. nov. associated with dieback of ash (*Fraxinus excelsior*) in Poland

By T. Kowalski

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E-mail: rltkowal@cyf-kr.edu.pl

Summary

A new species of the hyphomycete genus *Chalara* is described. It has been frequently isolated from stems and branches and sometimes also from roots of wilting and dying *Fraxinus excelsior* in Poland. It differs from previously described species of *Chalara* by its small, short cylindrical phialoconidia extruded in chains or in slimy droplets, morphological features of the phialophores and by colony characteristics. It is non-tolerant to cycloheximide.

1 Introduction

In Poland, intensive dieback of ash (*Fraxinus excelsior* L.) has been observed during the last 10 years. Initially, the dieback was observed only in the north-western part of Poland but currently it occurs all over the country. Trees are dying in all age classes, irrespective of site conditions and regeneration methods. Initially, small necrotic spots, without slime flux, occur on stems and branches. Later, they increase in size, resulting in wilting of leaves, top-dieback of branches and dying of whole trees (Przybył 2002; Kowalski and Łukomska 2005) (Fig. 1). A fungus from the genus *Chalara*, has been isolated from up to 70% of shoots at the beginning of the pathological process. It has also been found in dead roots of alive ash trees (Kowalski 2001; Kowalski and Łukomska 2005). Current studies confirmed that it is importantly involved in the dying of *F. excelsior* in Poland (T. Kowalski, unpublished results).

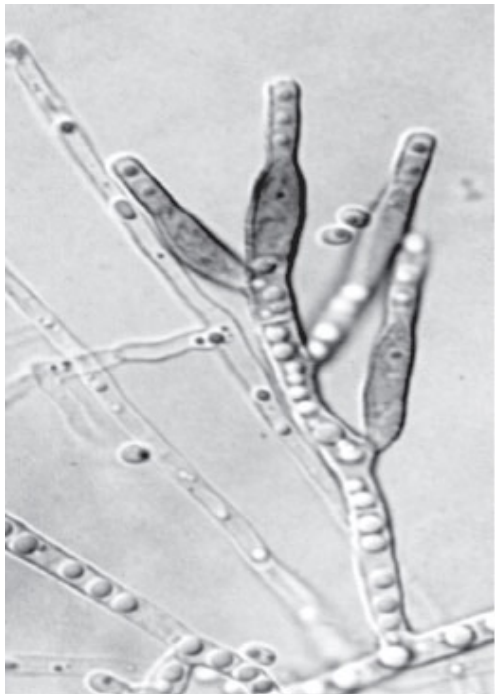
This fungus could not be assigned to any previously described species of *Chalara* (Nag Raj and Kendrick 1975; Gams and Holubová-Jechová 1976; Holubová-Jechová 1984; Kowalski and Halmschlager 1996; McKenzie et al. 2002). It is, therefore, proposed in this paper as a new species.

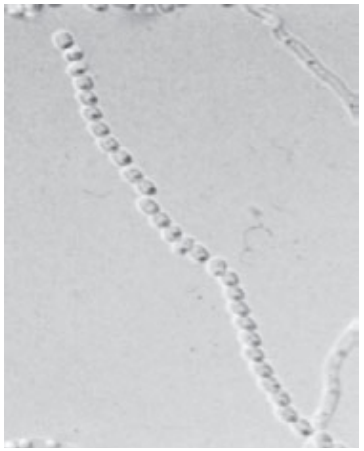
2 Materials and methods

Investigations were carried out on six strains isolated from ash main shoots and twigs. After surface sterilization (1 min ethanol 96%, 5 min NaOCl 4%, 30 s ethanol 96%) and removing surface bark, pieces of shoots 5 × 2 × 2 mm were removed and placed in Petri dishes on the surface of 2% malt extract agar (MEA; 20 g l^{-1} malt extract Difco, Sparks, MD, USA, 15 g l^{-1}agar Difco supplemented with 100 mg l^{-1} streptomycin sulphate). The cultures were then incubated at room temperature in the dark. Growing mycelium was transferred to new MEA plates and incubated at 20°C in the dark for at least 6 weeks. Dimensions of phialophores (n = 30) and phialoconidia (n = 30) were measured for each strain in distilled water. Photomicrographs were taken using a Zeiss Axiophot microscope (Carl Zeiss, Jena, Germany) in interference contrast.

Received: 01.12.2005; accepted: 02.02.2006; editor: O. Holdenrieder

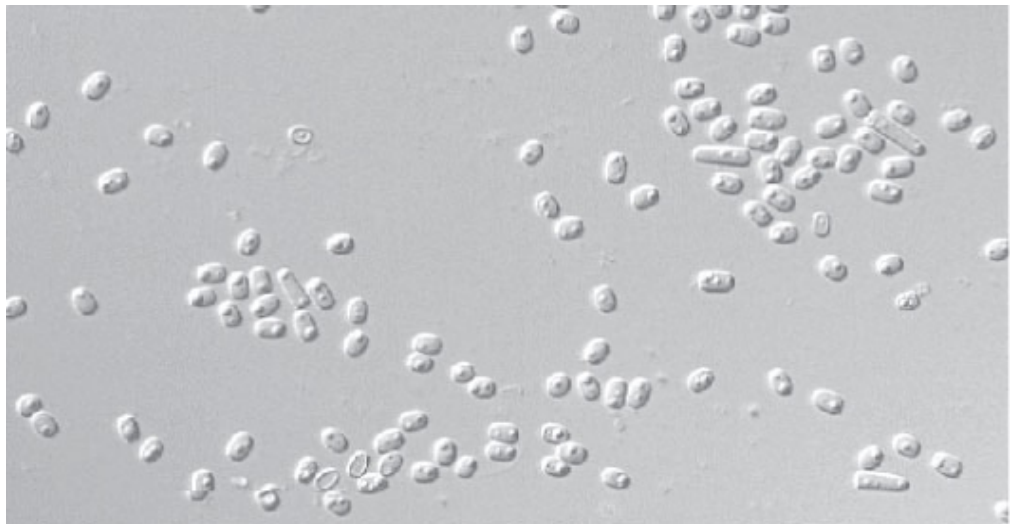








Kowalski (2006) *Forest Pathology*



Phialophores and conidia:

(asexual spores implying clonality)

Afterwards matched to teleomorph:

Hymenoscyphus albidus

For. Path. doi: 10.1111/j.1439-0329.2008.00589.x
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The teleomorph of *Chalara fraxinea*, the causal agent of ash dieback

By T. Kowalski[1] and O. Holdenrieder[2,3]

[1]Department of Forest Pathology, Faculty of Forestry, Agricultural University, Al. 29 Listopada 46, 31 – 425 Kraków, Poland; [2]Forest Pathology and Dendrology, Institute of Integrative Biology (IBZ), ETH Zurich, 8092 Zurich, Switzerland; [3]E-mail: ottmar.holdenrieder@env.ethz.ch (for correspondence)

Summary

Ash dieback, caused by the pathogen *Chalara fraxinea*, is an emerging lethal disease of *Fraxinus excelsior*, threatening the host species in large parts of Europe. The ascomycete *Hymenoscyphus albidus* (Helotiaceae, Helotiales) was identified as the teleomorph of *C. fraxinea* by culturing from ascospores, morphological comparison and nuclear ribosomal internal transcribed spacer (ITS) sequencing.

1 Introduction

Ash dieback, associated with the ascomycete anamorph *Chalara fraxinea* T. Kowalski (Kowalski 2006), is an emerging disease of major concern in Europe (Barklund 2005; Kowalski and Łukomska 2005; Schumacher et al. 2007; Thomsen et al. 2007; Halmschlager and Kirisits 2008; Kowalski and Holdenrieder 2008). The disease is characterized by a rapid dieback of the crown, associated with the presence of cankers and wilting. In active cankers, necrotic lesions frequently extend into the xylem beyond the margin of the superficial lesion. The pathogenicity of *C. fraxinea* was recently demonstrated under field conditions (Kowalski and Holdenrieder 2009); however, the biology of the fungus is largely unknown. The mucilaginous conidia of *C. fraxinea* have been observed very rarely in the field and no vector has been found. Therefore, we assumed that a teleomorph may play a crucial role in the epidemiology of the pathogen and searched for potential candidate ascomycetes.

2 Materials and methods

Ascomycete teleomorphs were investigated in affected ash stands in the Polish forest directorate Kraków (forest unit Stary Sącz, nursery: 49°33′51′N; 20°38′04.5′E; forest unit Miechów, forest office Goszcza: 50°1′06.6′N; 20°04′19.7′E) and in unaffected stands in Switzerland (close to the city of Zürich, Käferberg: 47°24′13′N; 8°31′18″E; Wehrenbachtobel: 47°21′15′N; 8°34′46′E; Küsnachter Tobel: 47°19′05′N; 8°35′22′E) during the 2008 vegetation period. The collections were studied by microscopy and isolations made from ascospores and from the substrate. Cultures were grown on malt extract agar (MEA: 20 g/l malt extract and 15 g/l agar) at room temperature in the dark. For comparison, two herbarium specimens of *Hymenoscyphus albidus* (Roberge ex Desm.) W. Phillips from the mycological herbarium, ETH Zürich (Z+ZT) were examined (ZT 87-243, Küsnachter Tobel, 460 m, 5.9.1987, J. Schneller and ZT 87–236, Sihlwald, 490 m, 26.8.1987, J. Schneller). Measurements were made in squash mounts in water (in herbarium material

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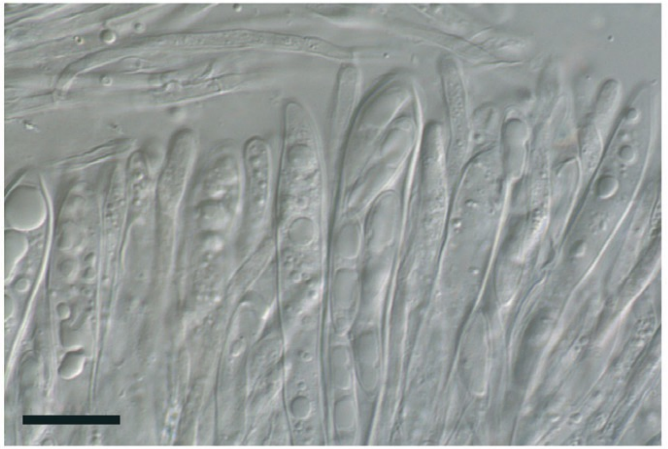
Kowalski & Holdenrieder (2009)

Forest Pathology



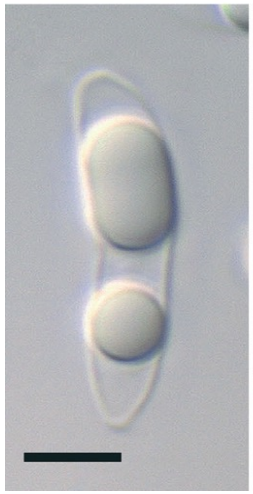




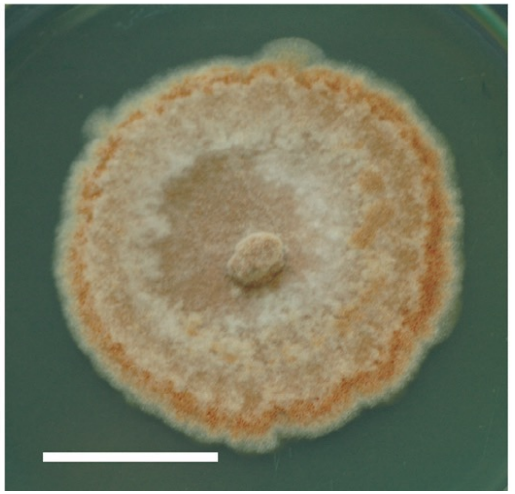




Ascospores: sexual spores implying high genetic diversity







So, was the mystery of *Fraxinus* dieback solved?

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NO!

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NO!

First, two interesting moments:

1) **ecology of the fungus**
2) **geographic pattern of the decline**

Ecology of *H. albidus* : a BIG surprise

- Indigenous, common and widespread fungus in Europe
- Known for over 100 years
- Saprotroph, decomposing petioles of shed *Fraxinus* leaves in tree litter

… therefore many Colleagues just refused to accept this identity, FLAT

… moreover, stepwise geographic spread of *Fraxinus* dieback throughout Europe: strongly suggested invasiveness

Puzzle of identity was then remaining

- invasive hybrid?
- invasive cryptic species? (morphologically indistinguishable, but genetically different)
- invasive mutant? (also)
- climate that chenged behaviour?

Or?

- indeed, indigenous oportunistic saprotroph (e.g., as *Armillaria*)

- Then, what factors trigger its pathogenicity, or host tree susceptibility, or both?
- Impact of climatic factors on disease development?
- Why the dieback spreads in all geographic directions, but not along climatic gradient?
- Why against the direction of prevailing winds?

… but in the meantime Japanese mycologists ITS-sequenced their native decomposer of leaves of *Fraxinus mandschurica*…

… and it was 100% match!!!
… with their described *Libertella fraxinea*

- Origin: Far East Asia
- Ecology: in native environment, (harmless) decomposer of shed ash leaves
- Identity: *Chalara fraxinea* / *Hymenoscyphus fraxineus* (to date called 4 names)
- Entrance to EU: imported plants for planting
- Spread: plant material & airborne spores (flying far)
- Genetic diversity in EU: not too high, indicating few entries

Airborne spread



H. albidus_ascospore release.wmv

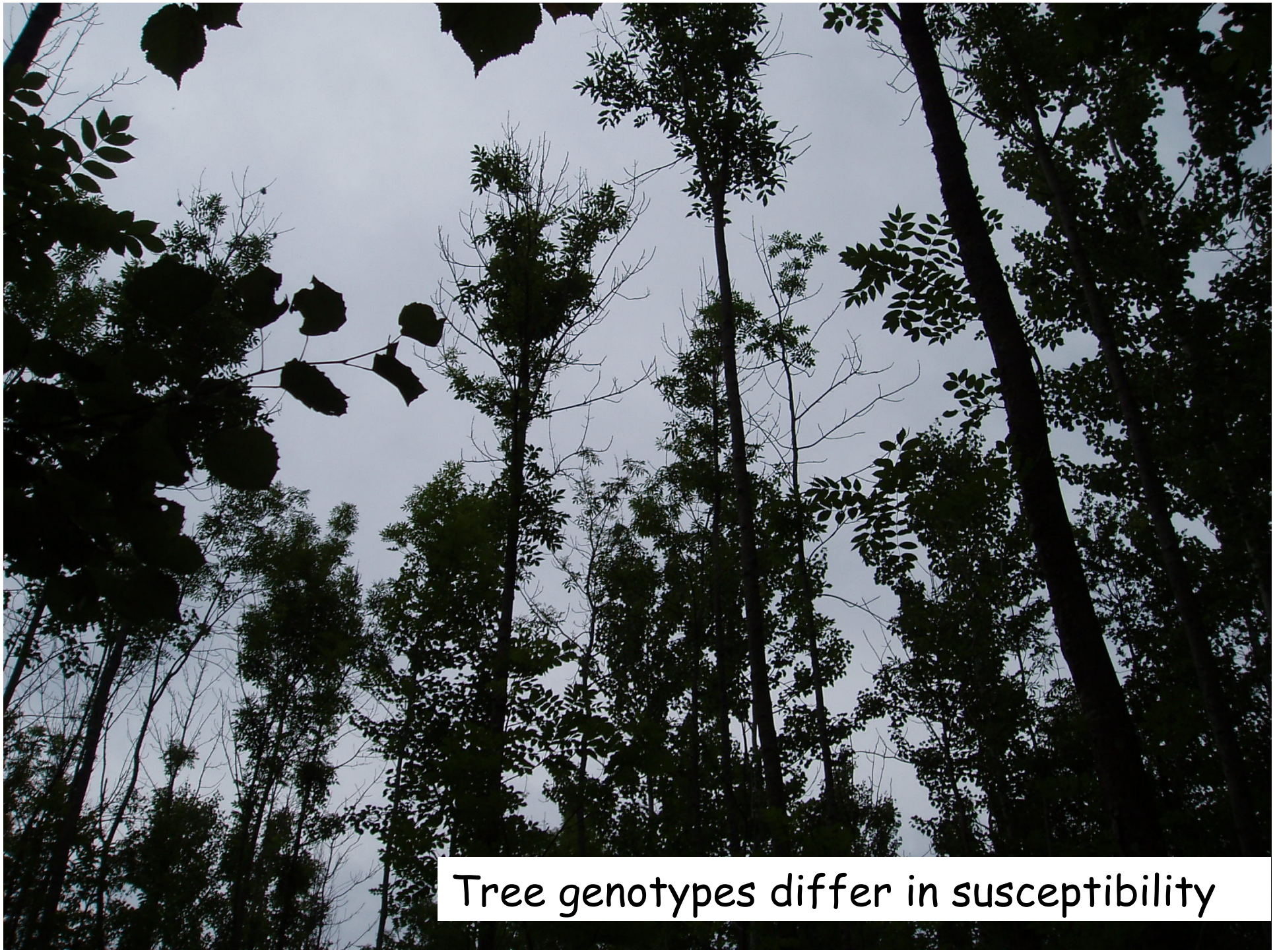
39 second long thriller by
Prof. Thomas Kirisits

Practical forestry

- what to do with diseased trees and stands?
- could the extent of the dieback be minimized by certain silvicultural practices (e.g. thinnings or sanitary felling)
- or even: does it make sense to grow ash at all?

Breeding for resistance: a posibility?

- Evaluations of clonal seed orchards of ash and progeny trials provide strong evidence for genetic variation in resistance
- The potential may be available for breeding for resistance
- Investigating how the initially observed variation between ash clones and progenies evolve over time


Tree genotypes differ in susceptibility

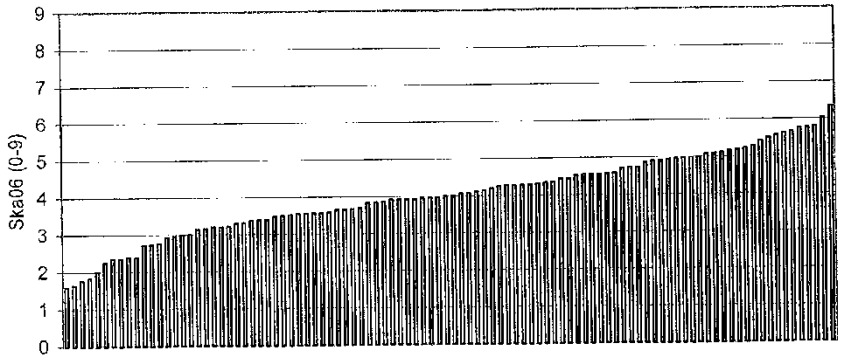
Different susceptibility of *F. excelsior* genotypes

Stener (2007):

- 15 yr-old clonal seed orchard

- 100 clones

- 6-29 (aver. 17) ramets per clone



Figur 1.
Genotypvärden för Ska06 sorterad i stigande ordning (ju högre värde desto större skada). Varje stapel representerar en plusträdsklon.

Perspectives: Breeding Ash

- Trials initiated approx. 10 yrs ago
- Inventory & mapping of healthy looking trees
- Following ”durability” of resistance over time
- Progeny trials & propagation
- Establishment of seed orchards / banks of dieback-resistant ash

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