Neonectria neomacrospora – Species variation in genus *Abies*

&
present studies of a new fungal pathogen

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Field/lab work:
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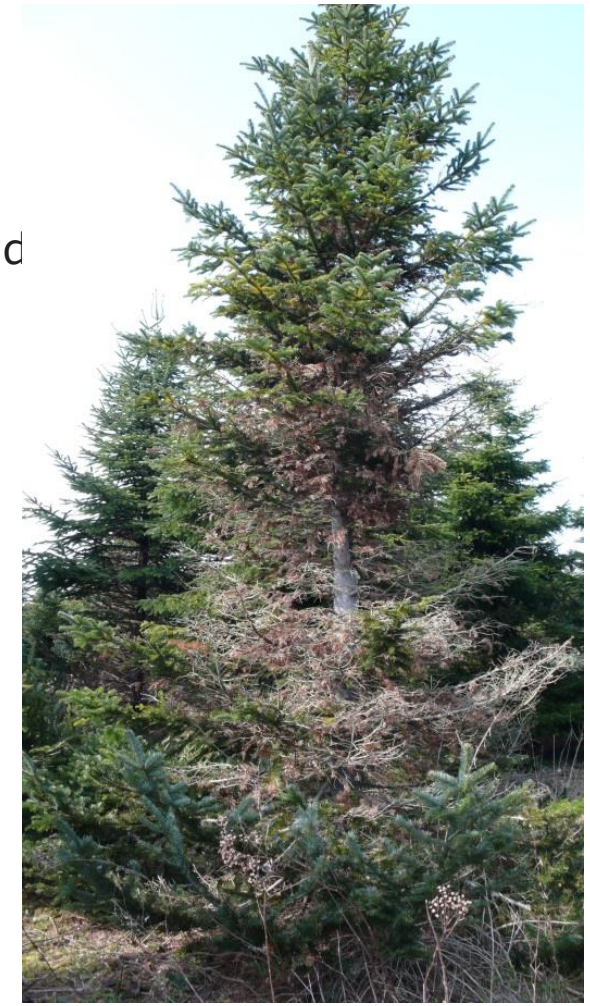
Content

1. The new problem of a fungus

2. Results from Arboretum Hørsholm in the field
   - *38 species/subspecies of genus Abies*

3. Inoculation tests 38 species

4. Present work on *Neonectria neomacrospora*
   - dispersal (timing and distance)
   - origin and population genetics



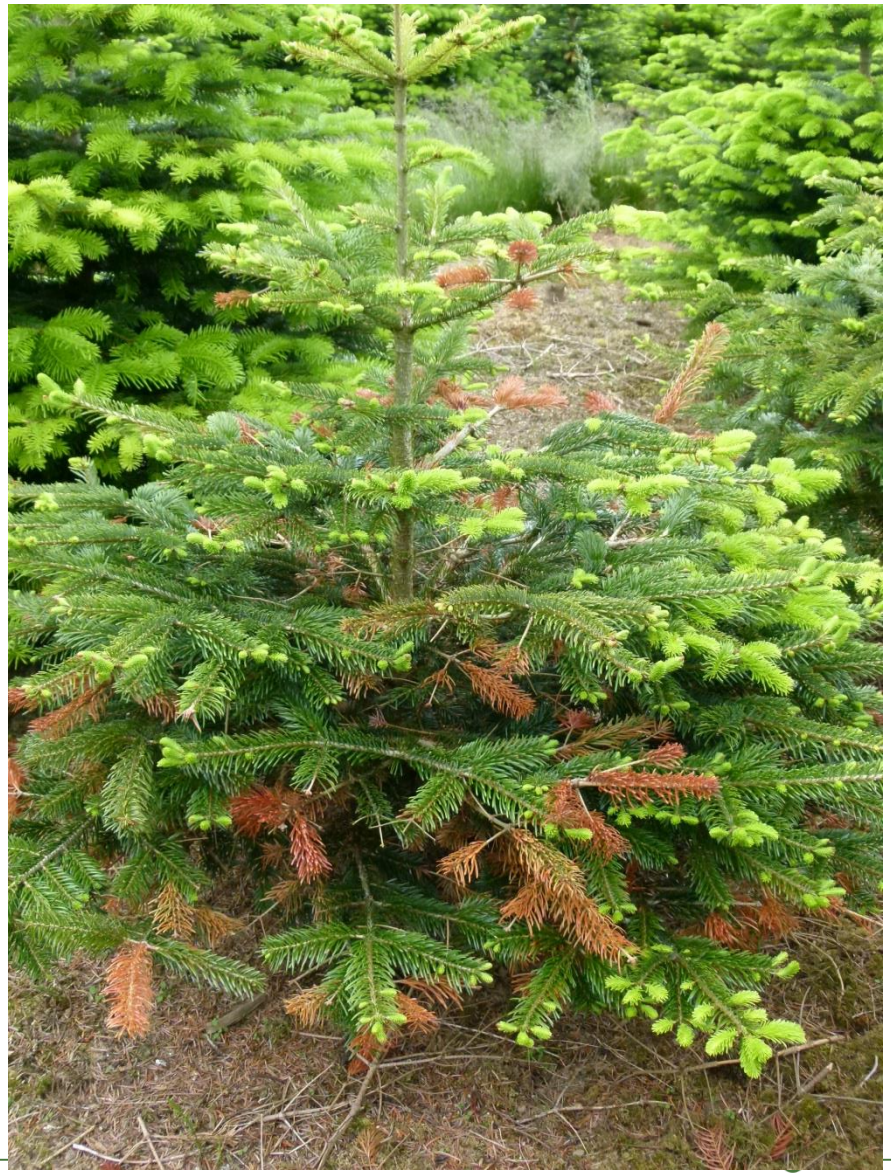
Background: A canker disease on *Abies sp*.

- 1913: The anamorph stage was first described from grafted *A. concolor* in a German nursery
- 1930-1960s: Associated with dieback of *A. balsamea* in east Canada
- 1960s: Found in Norway

Recent events

- Germany: 1998
- Norway: 2008. On *A. concolor*, *A. sibirica and A. lasiocarpa*
- Denmark: 2011
- Great Britain: 2013
- Estimated losses to the Christmas tree industry due to this specific patogen: 50 million DKR in 2013 alone

Damages Christmas trees



Damages older trees

Damages on branches




Arboretum Hørsholm - 39 species/subspecies
SPECIES VARIATION ?

Material

40 taxons

390 individuals

Species/subspecies/variety	Botanical section (group)	Code geo	Distribution	Trees no.
alba	Abies	1	centrale_og_S_Europa	10
borisii-regis	Abies	1	Bulgarien_og_Grækenland	3
cephalonica	Abies	1	Grækenland	12
cilicica_subsp_saurica	Abies	1	Tyrkiet	1
nebrodensis	Abies	1	Sicilien	2
nordmanniana	Abies	1	Kaukasus_og_N_Tyrkiet	12
nordmanniana_subsp_equi-tro	Abies	1	V_Tyrkiet	10
amabilis	Amabilis	2	V_Nordamerika	8
mariesii	Amabilis	6	Japan_Honshu	5
lasiocarpa	Balsamea	2	V_Nordamerika	50
balsamea	Balsamea	3	Ø_Nordamerika	10
balsamea_var_phanerolepis	Balsamea	3	Ø_USA	4
fraseri	Balsamea	3	Ø_USA	4
veitchii	Balsamea	6	Japan_Honshu_og_Shikoku	9
veitchii_var_sikokiana	Balsamea	6	Japan_Shikoku	8
koreana	Balsamea	7	Sydkorea	106
nephrolepis	Balsamea	7	Kina_Korea_og_Russisk_TjernØsten	14
sachalinensis	Balsamea	9	Russisk_TjernØsten_og_N_Japan	7
sibirica	Balsamea	9	N_Russland	1
concolor	Grandis	2	Mexico_og_V_USA	8
concolor_subsp_lowiana	Grandis	2	V_USA	5
grandis	Grandis	2	V_Nordamerika	8
chensiensis_subsp_salouenen	Momi	4	Tibet_Indien_V_Kina	2
pindrow	Momi	4	Himalaya_Afghanistan_Indien_Nepal	3
recurvata	Momi	5	SichuaN_Kina	3
firma	Momi	6	Japan	2
homolepis	Momi	6	Japan	26
holophylla	Momi	7	Korea_Kina_og_SØ_Sibirien	10
kawakamii	Momi	7	Taiwan	2
magnifica_var_shastensis	Nobilis	2	V_USA	1
procera	Nobilis	2	V_USA	9
pinsapo	Piceaster	1	S_Spanien	5
pinsapo_var_marocana	Piceaster	8	N_Marokko	4
spectabilis	Pseudopicea	4	Himalaya_Afghanistan_til_Kina	8
fabri	Pseudopicea	5	SichuaN_Kina	2
fargesii	Pseudopicea	5	centrale_Kina	1
fargesii_var_faxoniana	Pseudopicea	5	SichuaN_Kina	4
fargesii_var_sutchuensis	Pseudopicea	5	sydcentrale_Kina	1
forrestii_var_georgei	Pseudopicea	5	Tibet_og_S_Centralkina	2
squamata	Pseudopicea	5	centrale_Kina_og_Tibet	8
				390

Objectives

- quantify the natural infestation among 33 species and some of their subspecies
- compare those data with lab results from artificial inoculation test.
- Evaluate differences among botanical sections and geographic areas,
- discuss the results in relation to Christmas tree production

Methods

Field *Neonectria* damage - score

Score:

0	Healthy
1-3	Little damage
4-6	Moderate damage
7-9	Severe damage
10	Dead

Evaluated:

May 2014

Nov 2015

RESULTS

Taylor & Francis
Taylor & Francis Group

Scandinavian Journal of Forest Research

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Species variation in susceptibility to the fungus Neonectria neomacrospora in the genus Abies

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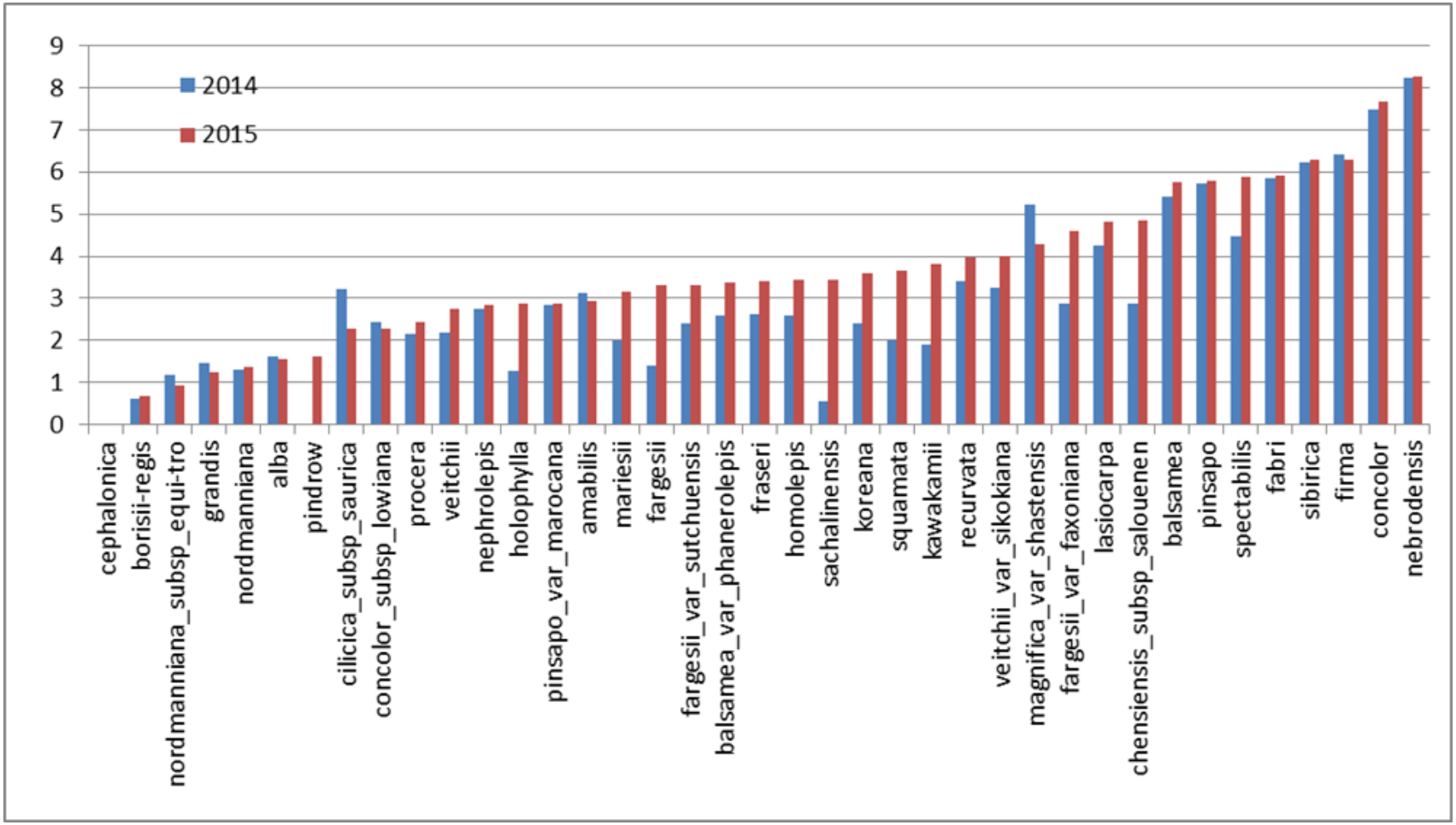
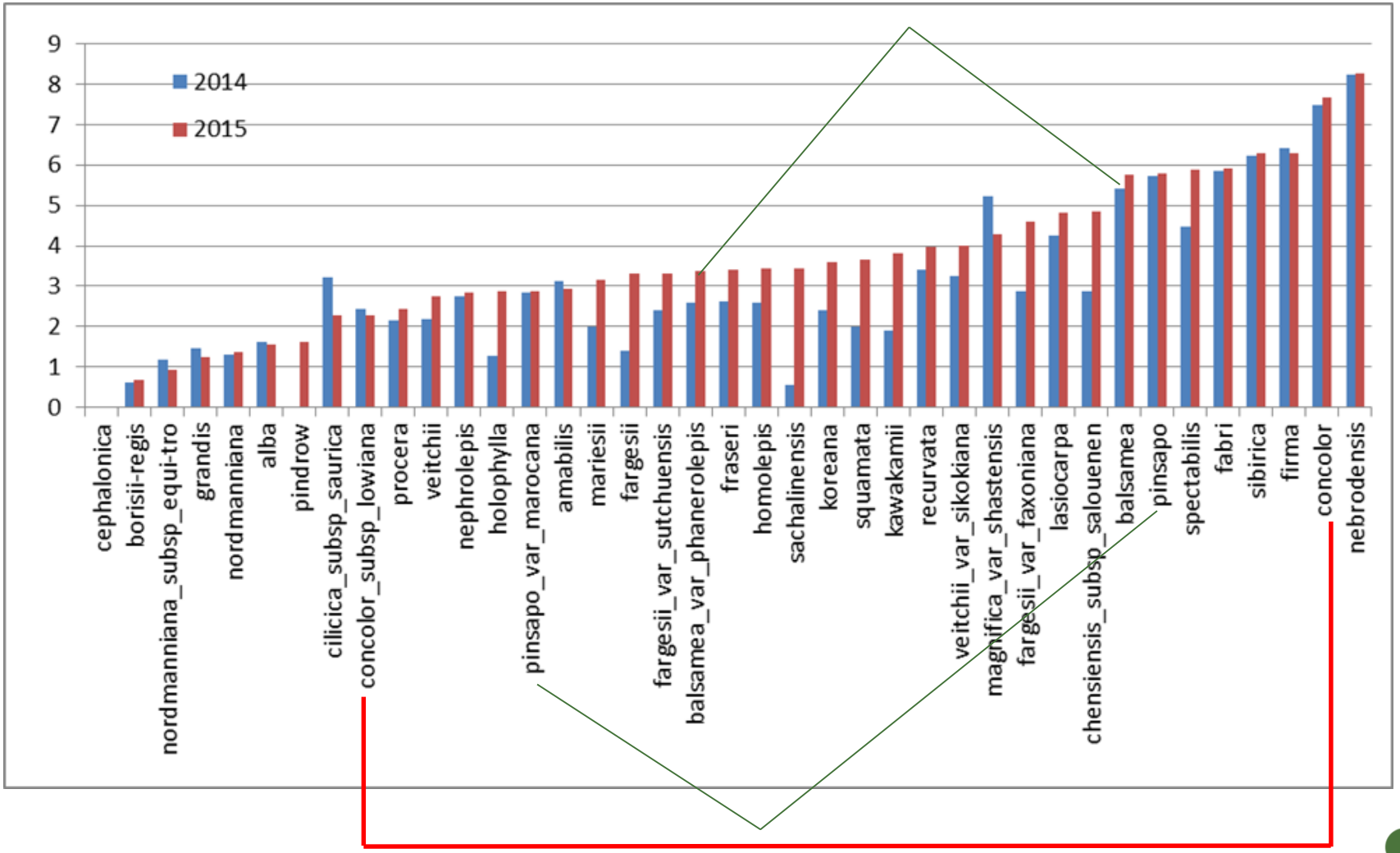
Field data: Lsmean score – species/subspecies



Field data: LSmean score – species/subspecies



Effects of inoculum on *Neonectria* infection in *Abies* cut branches

Treatment 1: Edge part inoculum of *Neonectria* cultures inoculation

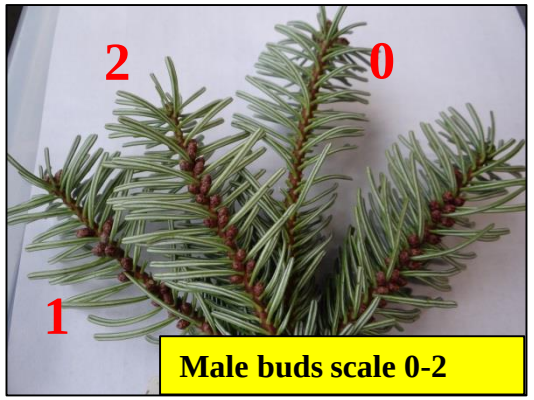
Treatment 2: Center part inoculum of *Neonectria* cultures inoculation

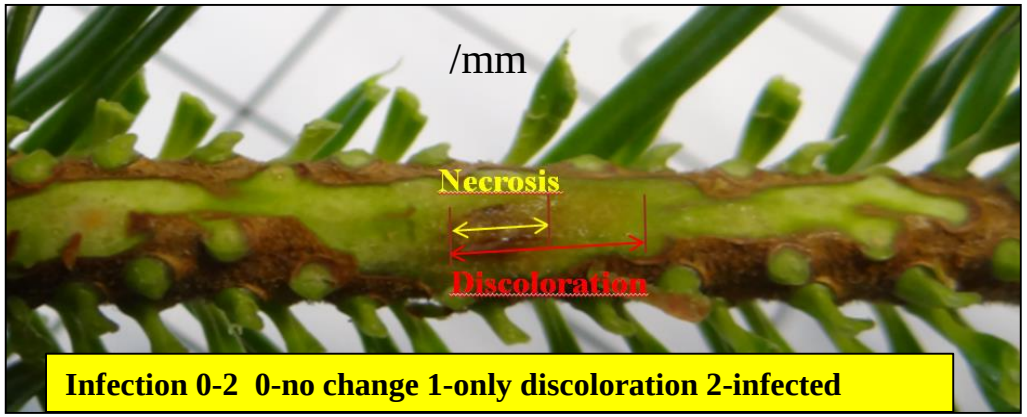
(Slide: Jing Xu)

Artificial inoculation methods

Evaluation twigs in inoculation trial - after 3 weeks


Shoot length/mm





(Slide: Jing Xu)

Statistical models

Field:

$Y = \mu + b + h + s + e$ [1a] by year analyses

$Y = \mu + b + h + r + s + sr + e$ [1b] across year analyses

Where
μ was overall mean,
b: random effect of block,
h: random effect of shade, s: fixed effect of species/subspecies,
r: fixed effect of year, sr: interaction subspecies and year, and
e: error variance assumed NIID?

The following models include furthermore botanical section or geographic area

$Y = \mu + a + s(a) + e$ [2a]

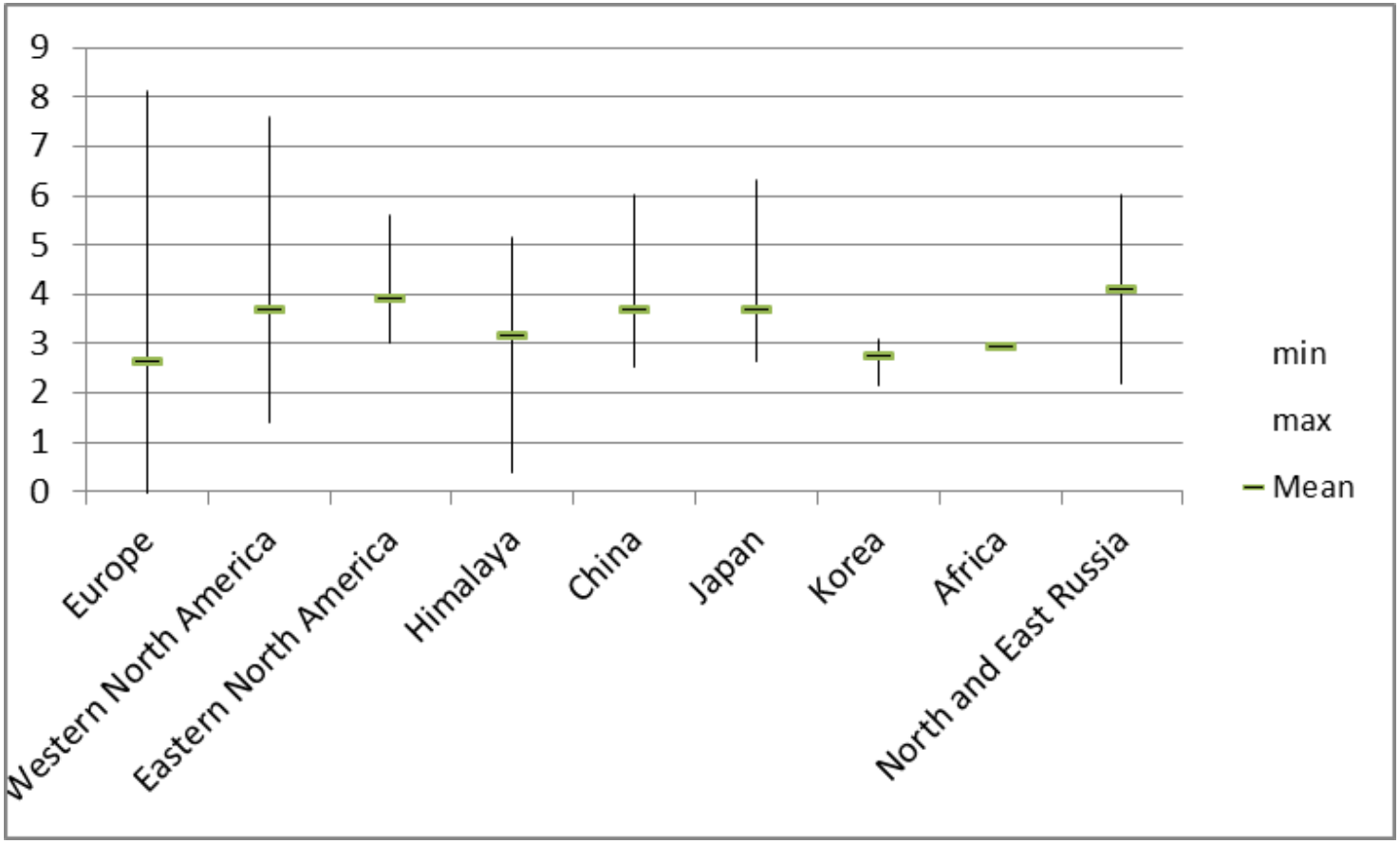
$Y = \mu + b + h + r + a + s(a) + ar + s(a)r + e$ [2b]

Inoculation trial:

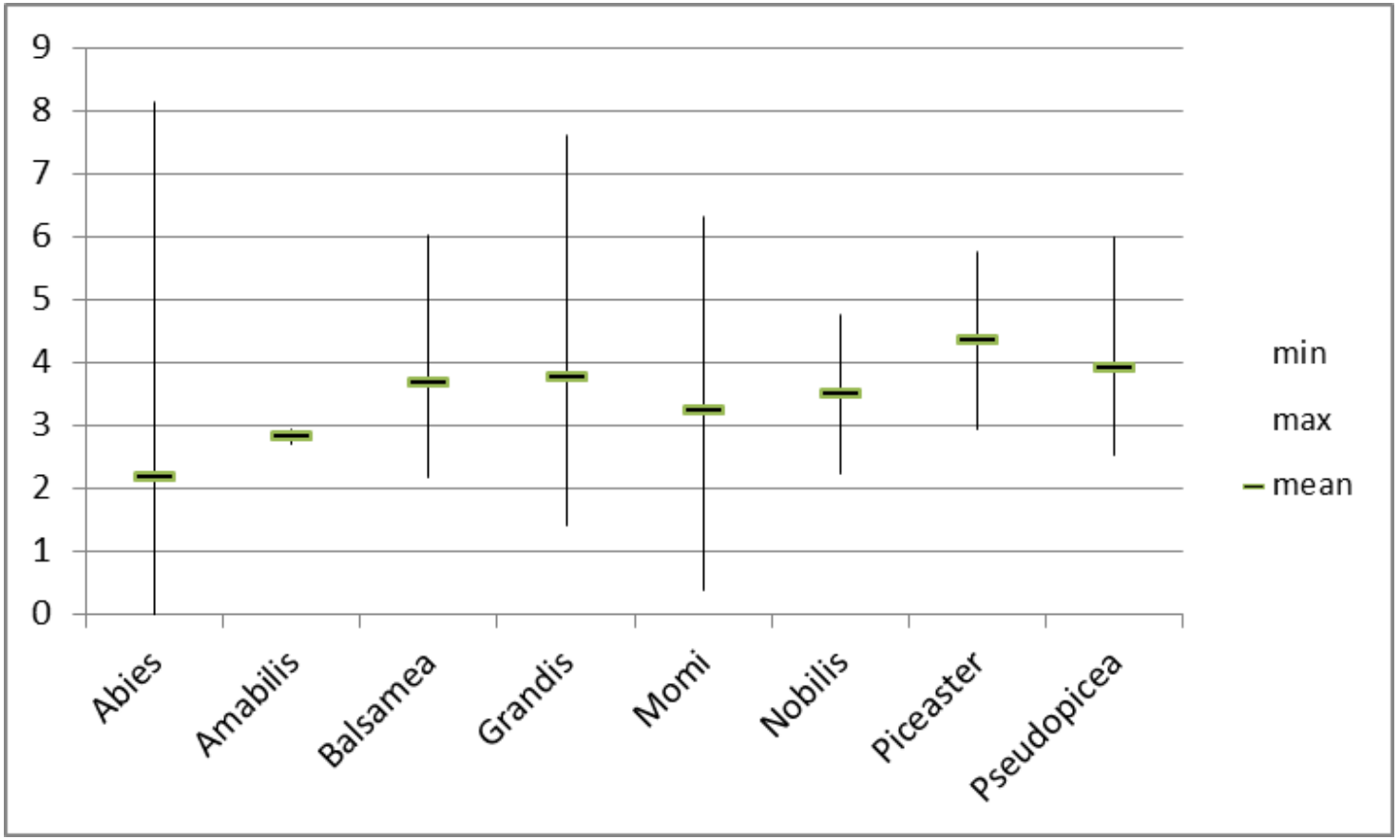
$Y = \mu + i + B(i) + s + e$ [3a]

$Y = \mu + i + B(i) + area + subspecies(area) + e$ [3b]

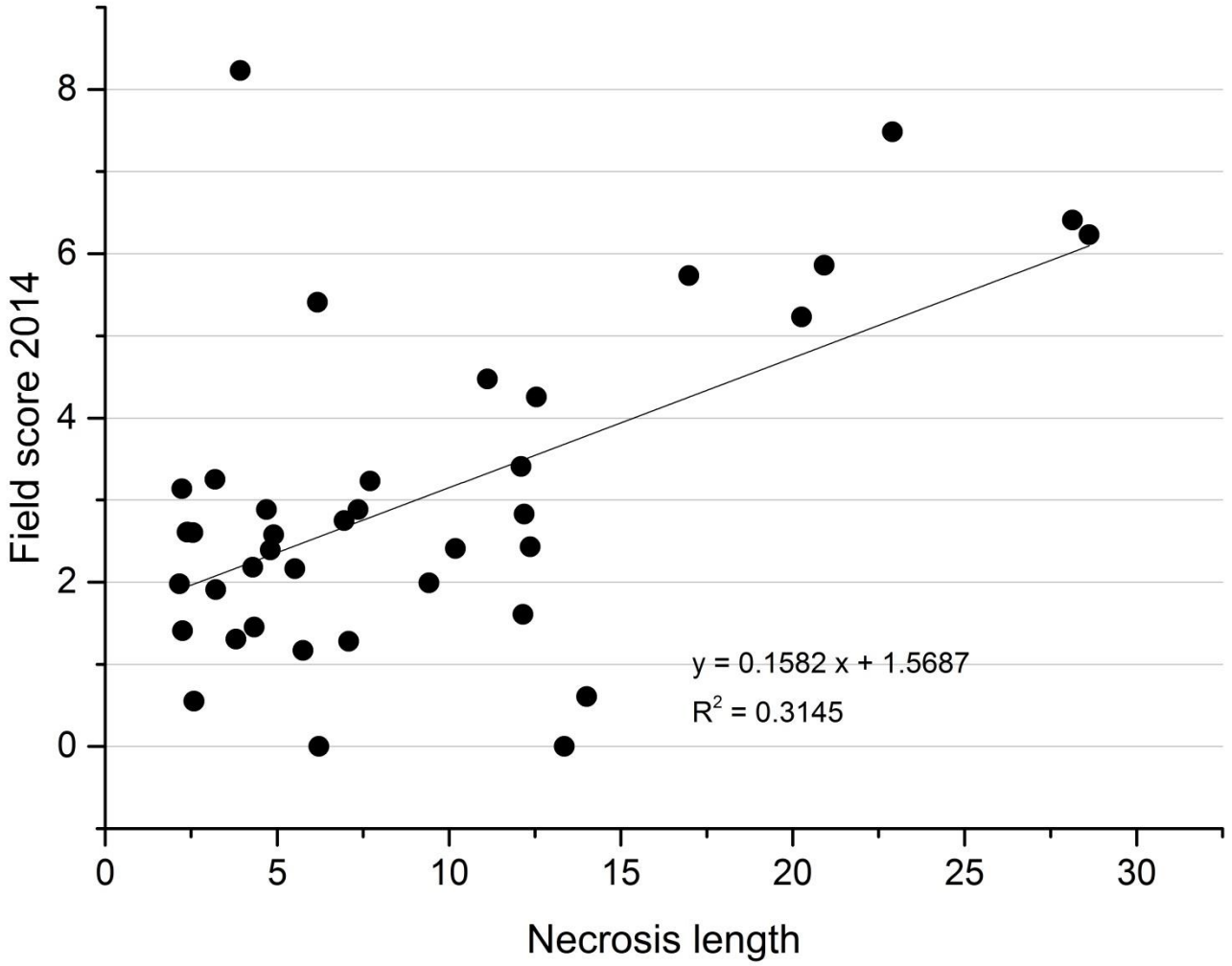
Geographic area: average min/max species



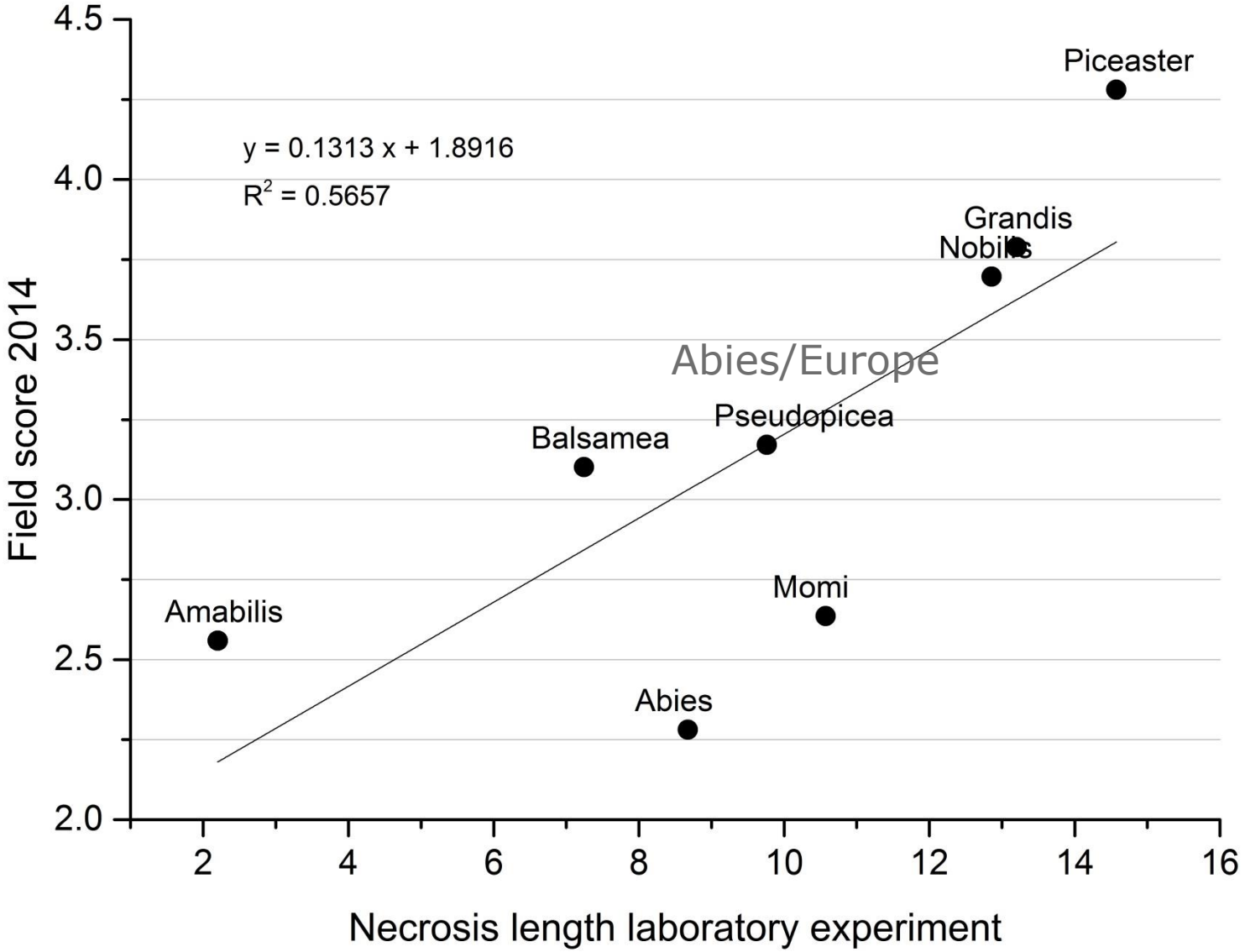
Botanical section: average min/max species



Field score versus inoculum trial – species/subspecies



Field score versus inoculum trial – botanical sections



Conclusions/discussion

- Weakness: one site, not fully randomized, for most trees few trees per taxon (1 to 106)

Field:

- Increasing field damage from May 2014 to November 2015
- Large species variation
- Indication of differences between subspecies/varietes

Inoculum trial

- Species differences also significant
- Different 'damage' measures correlates

Field vs inoculation trial

- For all taxon's correlation, R^2=23%, for botanical sections R^2=43%

Christmas tree production

- Commercial important *A. balsamea*, *A. lasiocarpa* are very susceptible, and also (*A. concolor*)
- Breeding may diminish risk, interesting examples of variation within species

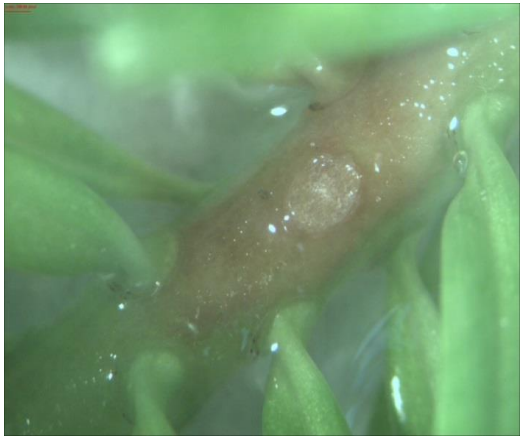
Other organisms complicating the picture?

Balsam wolly adelgid in A. lasiocarpa

Silver fir woolly adelgid in Nordmann fir

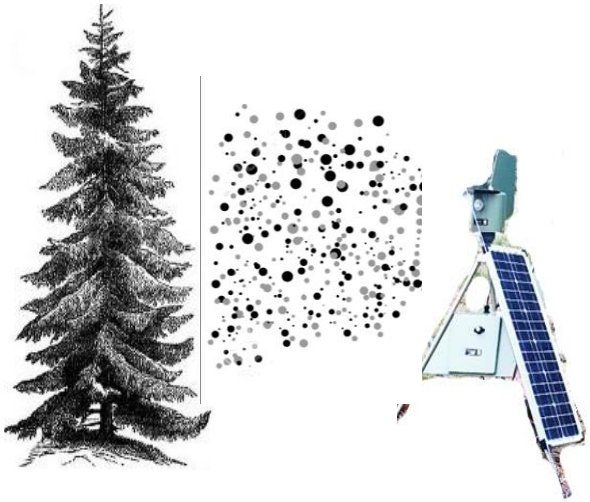
The bark beetle *Cryphalus piceae*

Inoculation by spraying spores – Jing Xu

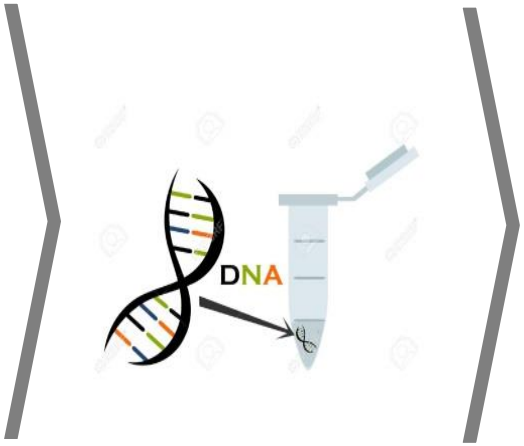


Dispersal of *Neonectria*

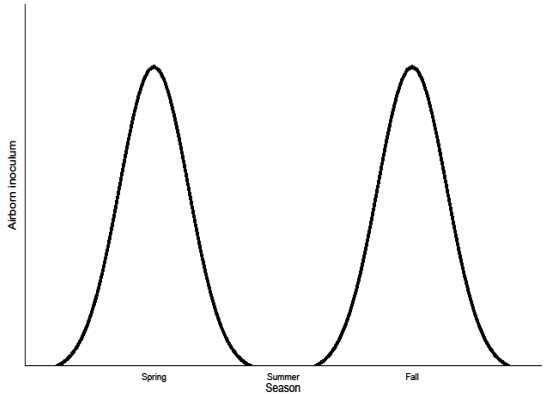
– Dispersal timing, i.e. season, and climatic conditions



Field collection of fungal spores



Molecular quantification



Data

Dispersal range

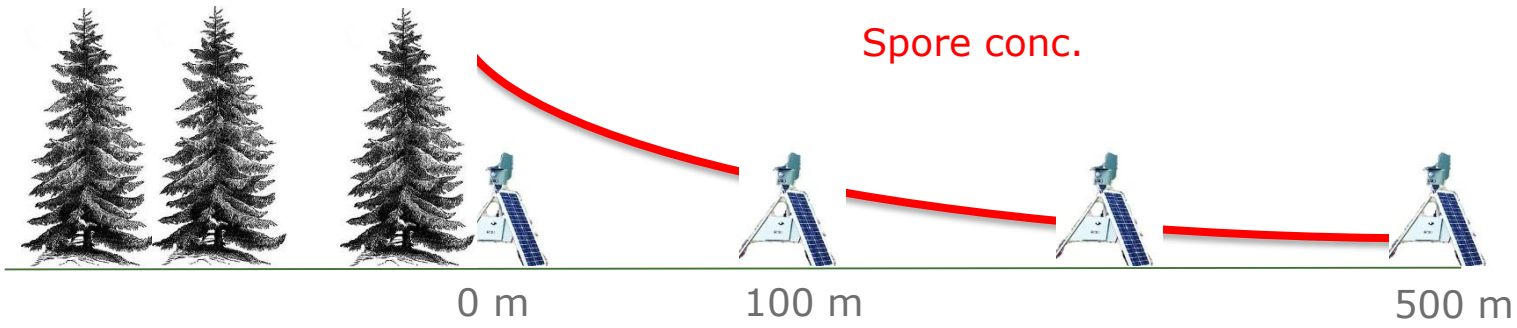
Dispersal phenology

Each sampler produces one sample per day (0400 to 2200: 18 h)

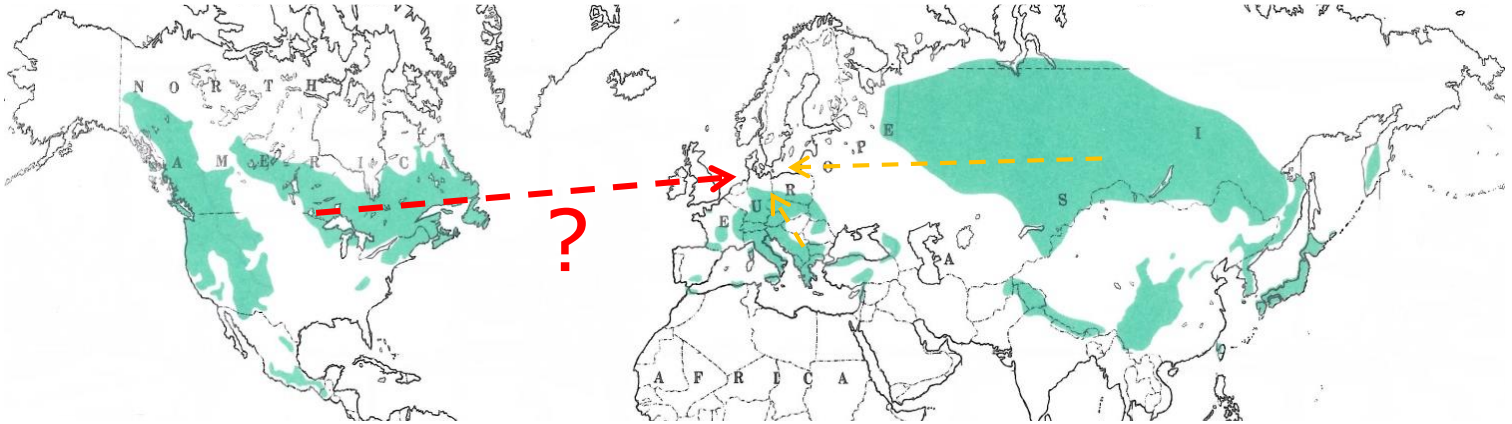
Burkard Multi-vial Cyclone Samplers + Weather station

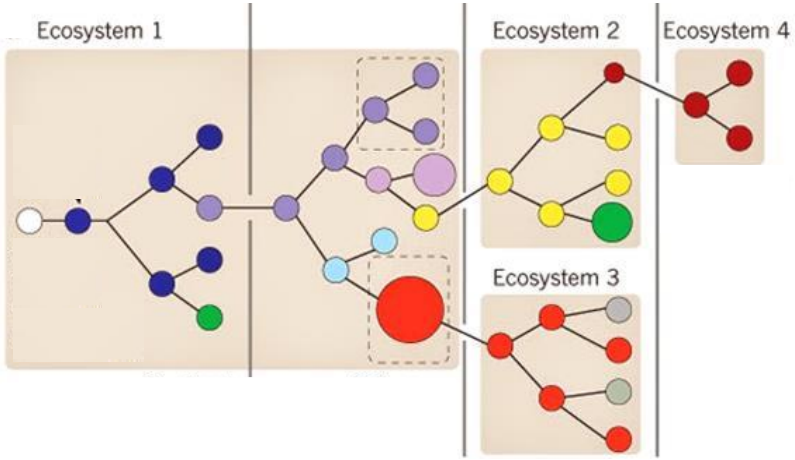
Dispersal distance

Four samplers at increasing distance from a source



The origin of the *Neonectria neomacrospora* epidemic





Possible origins of novel virulence

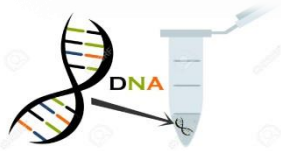
- Migration
- Mutation
- Hybridization
- Climate change

Populations genetics of the fungi

Isolating the fungus from infected trees

↓

Extract DNA from cultures



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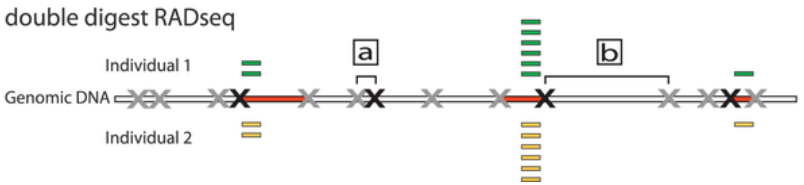
ddRAD-sequencing



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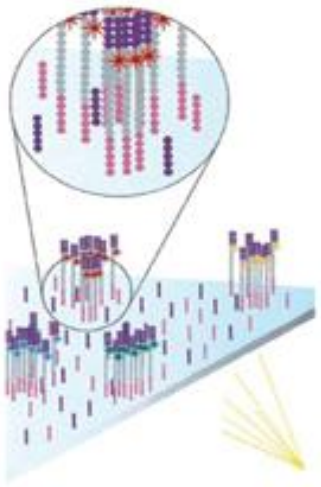
1000+ SNPs



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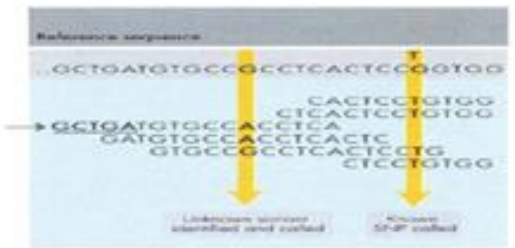
Analysis



Thank you for your attention !!

Questions or comments?

Photo: Kirstine Bräuner Nielsen