Fungi in Wood Pellets

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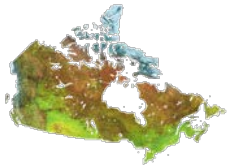
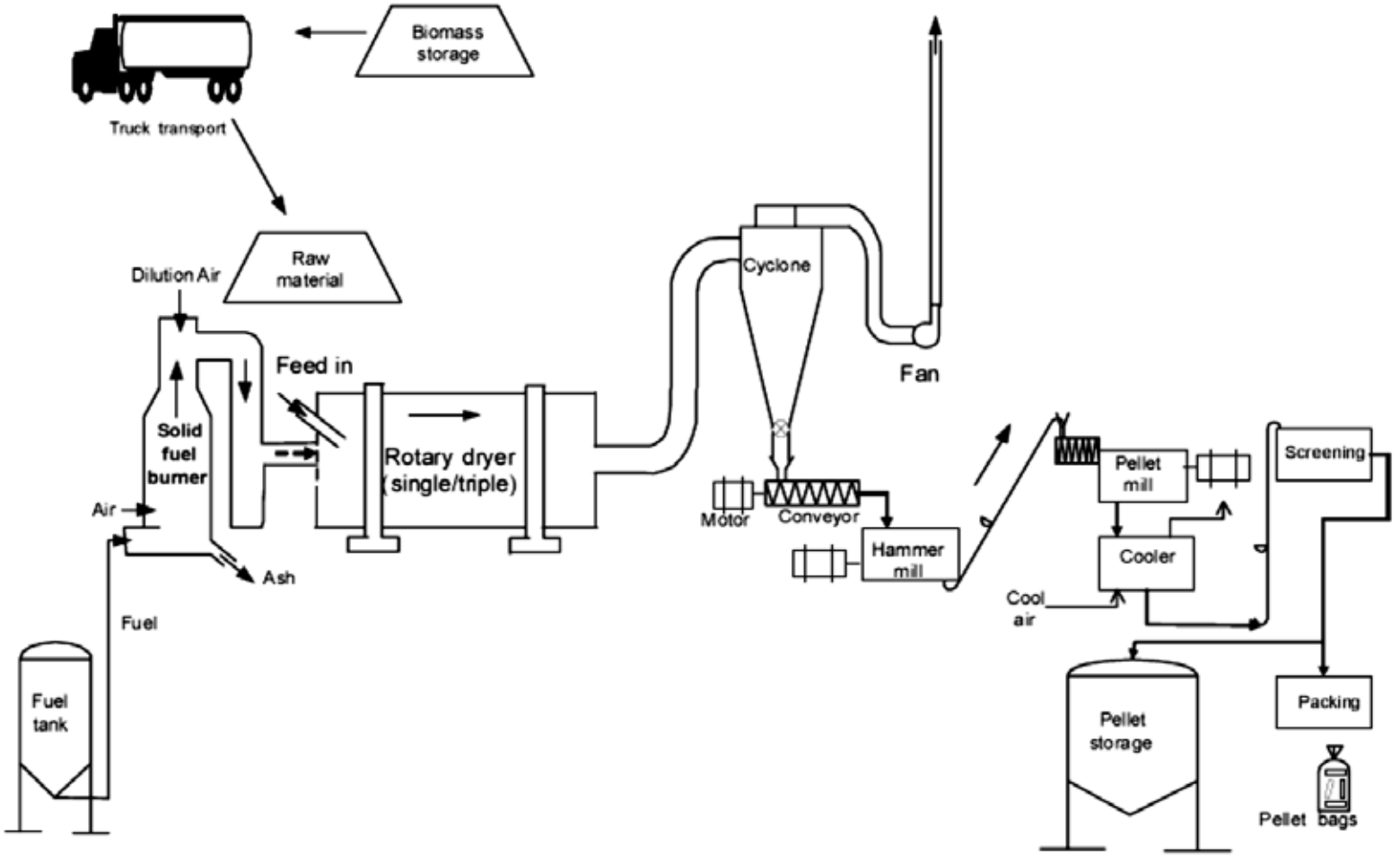


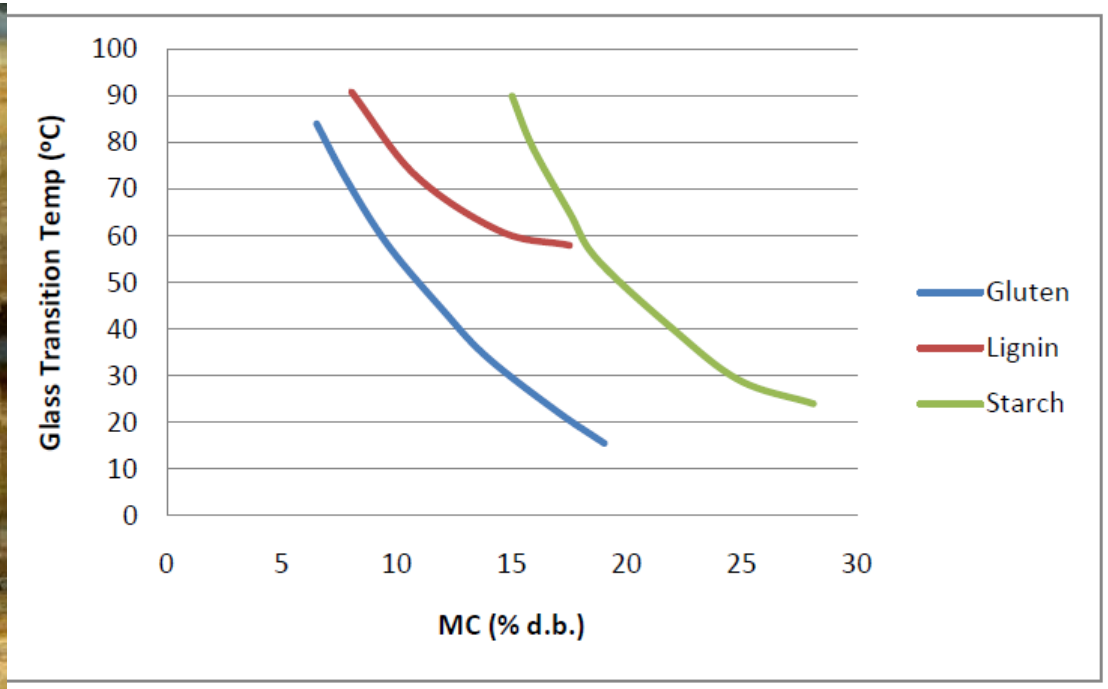
Does the wood pellet manufacturing process remove or reduce fungi that might be of phytosanitary concern?

The Manufacturing Process

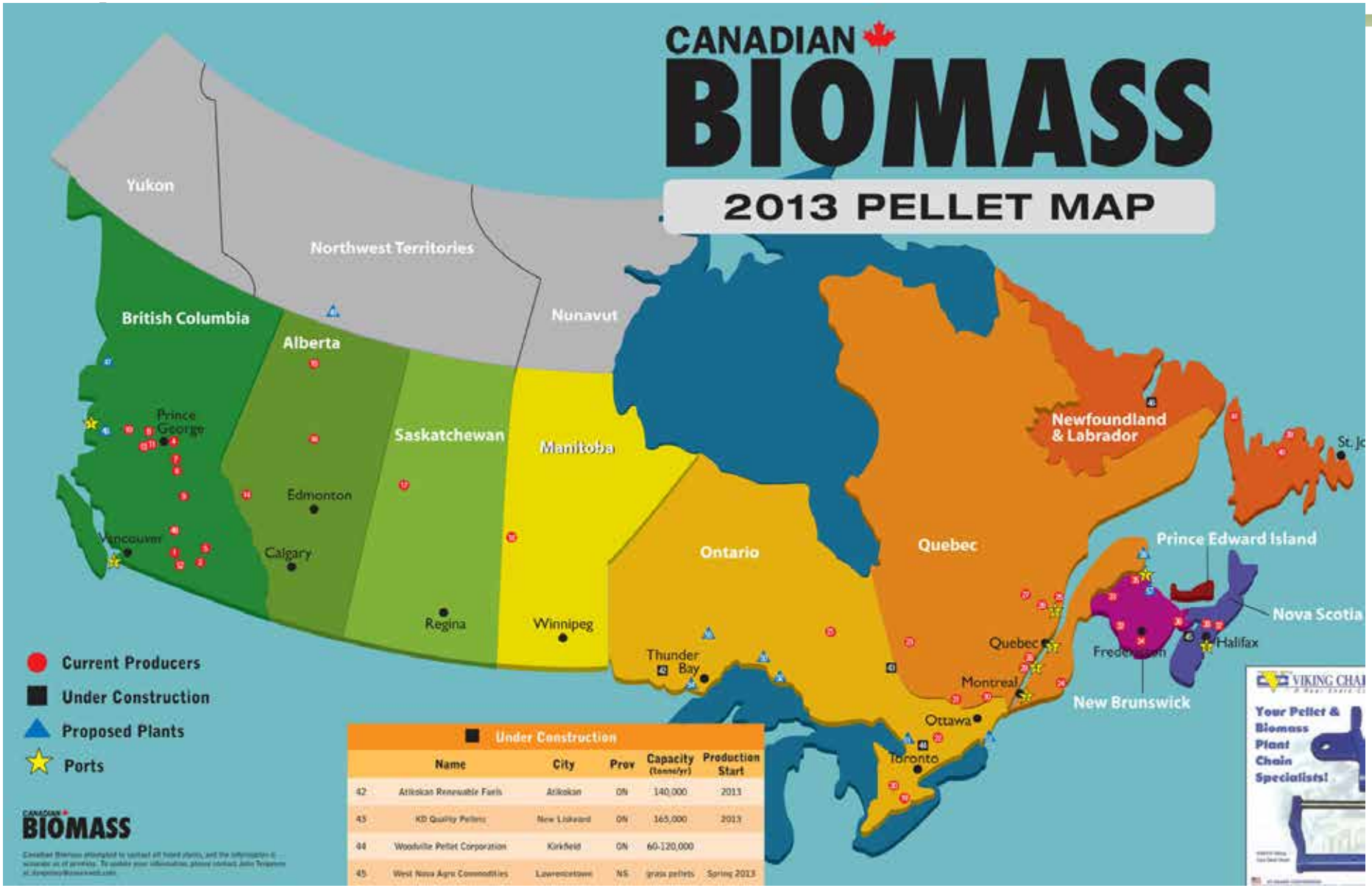


Source: Mani, Sokhansanj, Bi, & Thurhollow, Biomass & Bioenergy Research Group, University of British Columbia

High pressure during extrusion and glassification of the lignin on the surface wood holds the pellet together



– Effect of moisture on glass transition temperature (recreated from Chirfe and Del Pilar Buera, 1994)



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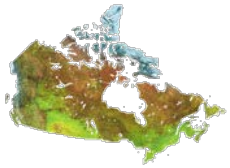
invasive species



Samples examined

Source	Type	Number	Amounts sampled per source
Storage silo in Vancouver (conifer), multiple plants	Pellets and fines	2	6 g pellets 4 g fines
Individual BC pellet plants (conifer)	Pellets and fines	4	6 g pellets 4 g fines (if present)
Individual QC pellet plant (mixed conifer and hardwood)	Pellets	1	6 g pellets 4 g fines
BC pellet plant, raw material, conifer	Chips	1	10 g
BC pellet plant, material after dryer, conifer	Ground wood chips	1	6 g

Total number of Petri plates examined was > 700

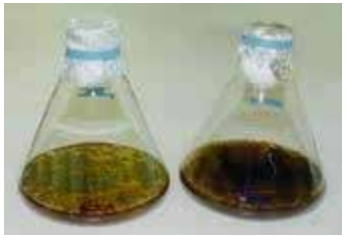
Examples of fungi identified from wood pellets identified using morphological and molecular techniques

Hundreds of isolates of common mold genera, ubiquitous on plant material and wood: *Penicillium, Aspergillus, Trichoderma, Paecilomyces, Rhizopus*

Many isolates of *Hormoconis resinae*, a mold common on wood, creosoted wood and petroleum products

Yeasts, and black yeasts such as *Aureobasidium, Cephaloascus*

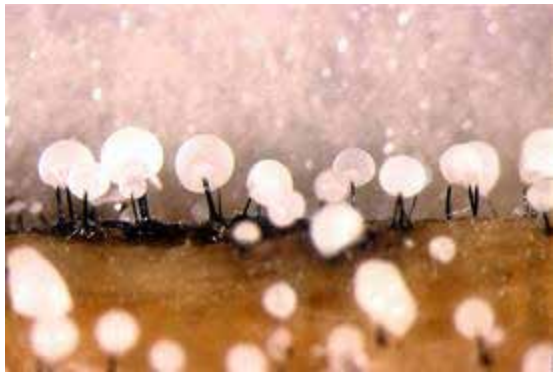
Many of these moulds are oligotrophic, adapted to growing in areas with low levels of nutrients. Many of these yeasts and moulds are also xerophilic, able to survive dry environments



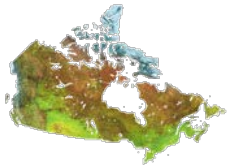
***Hormoconis resinae* growing on aviation fuel**

Fungi on Raw Material

- Some wood decay fungi (*Fomitopsis cajanderi*) – bracket fungus associated with brown rot of conifers
- Some wood stain fungi (*Graphium/Pesotum* complex, many associated with beetles, pending molecular identification)



None found in finished pellets



Results

- Some wood decay and stain fungi of potential phytosanitary concern were detected in the samples of raw and dried pre-pellet material cultured
- To this date, no fungi of phytosanitary concern (plant pathogens or decay fungi) have been detected in the final pellet products from any of the sources
- Fungi were isolated from the pellets but were molds that are adapted for dry conditions such as storage containers
- Fines contained more fungi than the pellets

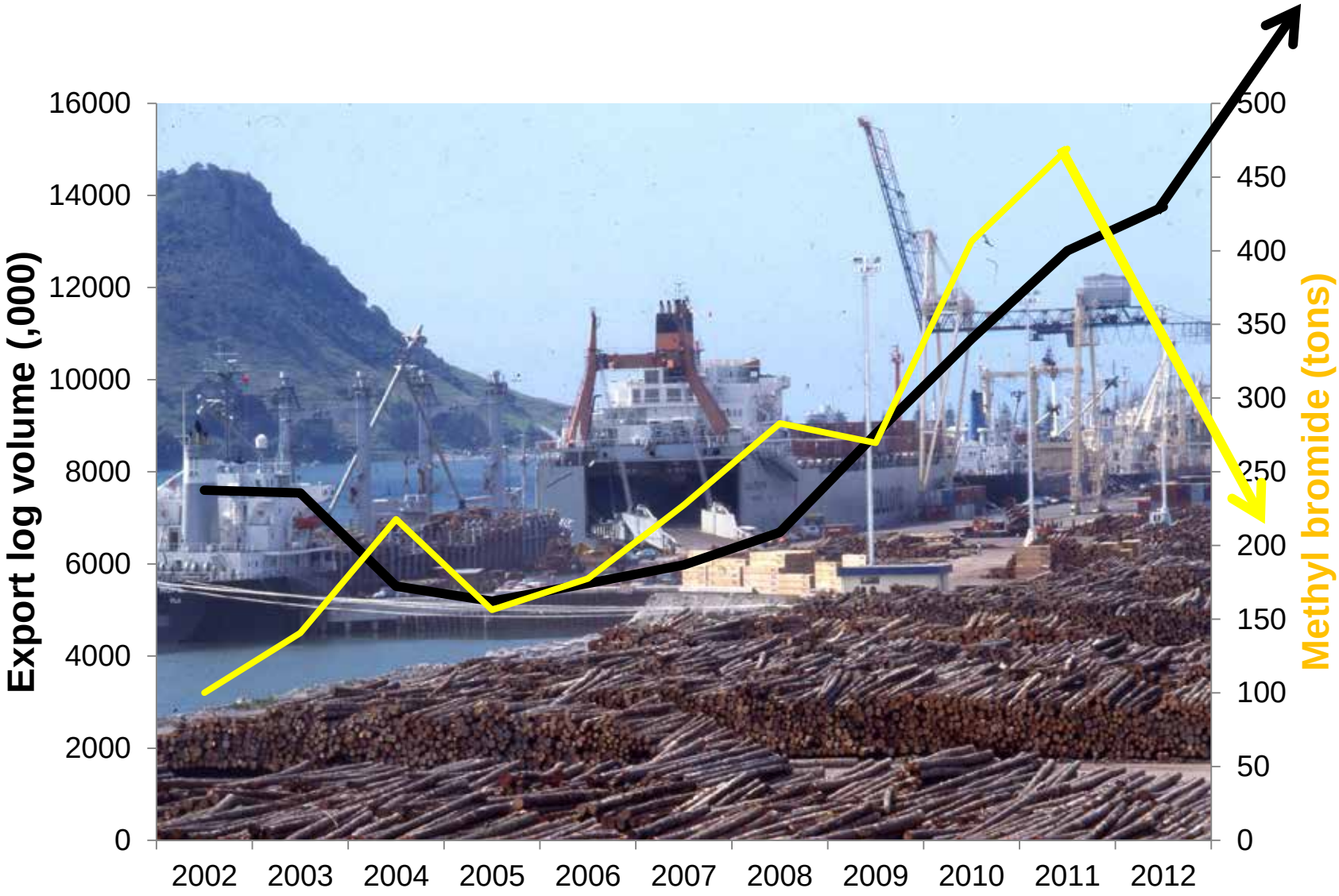
Thermal tolerance of phytosanitary pests

Stephen Pawson

Reducing rates of MeBr use

- IPPC recommendation to reduce the use of MeBr for quarantine and pre-shipment purposes.
- New Zealand is a significant user of MeBr to support the phytosanitary requirements of countries that import our logs.

Reducing rates of MeBr use



Alternatives to MeBr

- Alternative chemical fumigants is one potential solution.
- Alternative non-chemical treatments is another option:
  - Debarking
  - Radiation
  - **Heat**

Treatments

- Two potential pest species
  - *Arhopalus ferus* (Cerambycidae)
    - § Introduced species, native to Europe, Nth Africa, and Nth Asia
    - § Listed on ICPR for wood exported to Australia

  - *Hylurgus ligniperda* (Scolytinae)
    - § Introduced species, native to Europe
    - § Listed on ICPR for wood exported to China

Treatments

- Life stages
  - *Arhopalus* (eggs, larvae, adults)
  - *Hylurgus* (eggs, larvae, pupae, adults)

- Temperature ranges
  - 42 to 72 degrees
  - Temperatures dependent on species and life stage
  - 5, 15, and 30 minute treatments

Methodology

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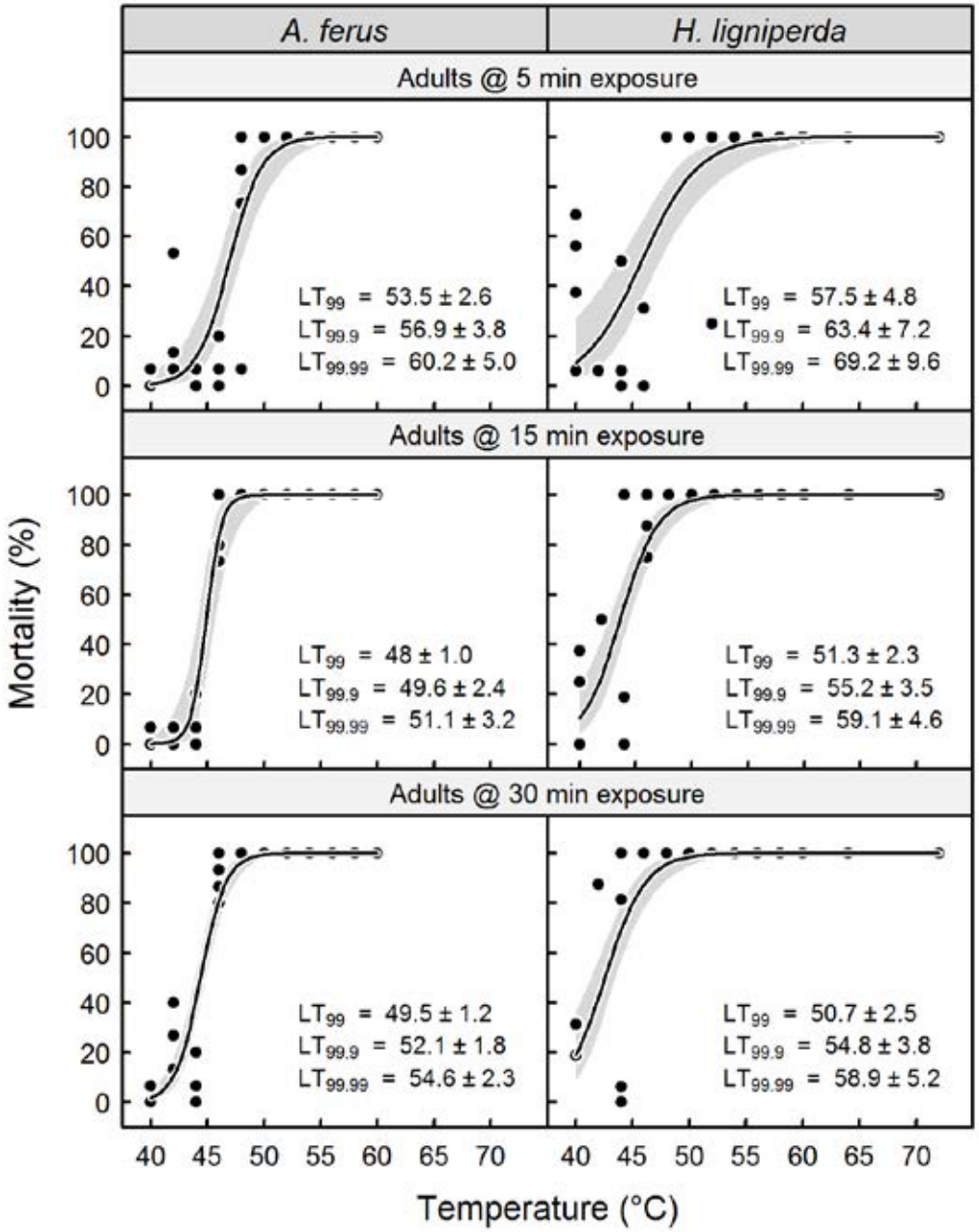
Adults



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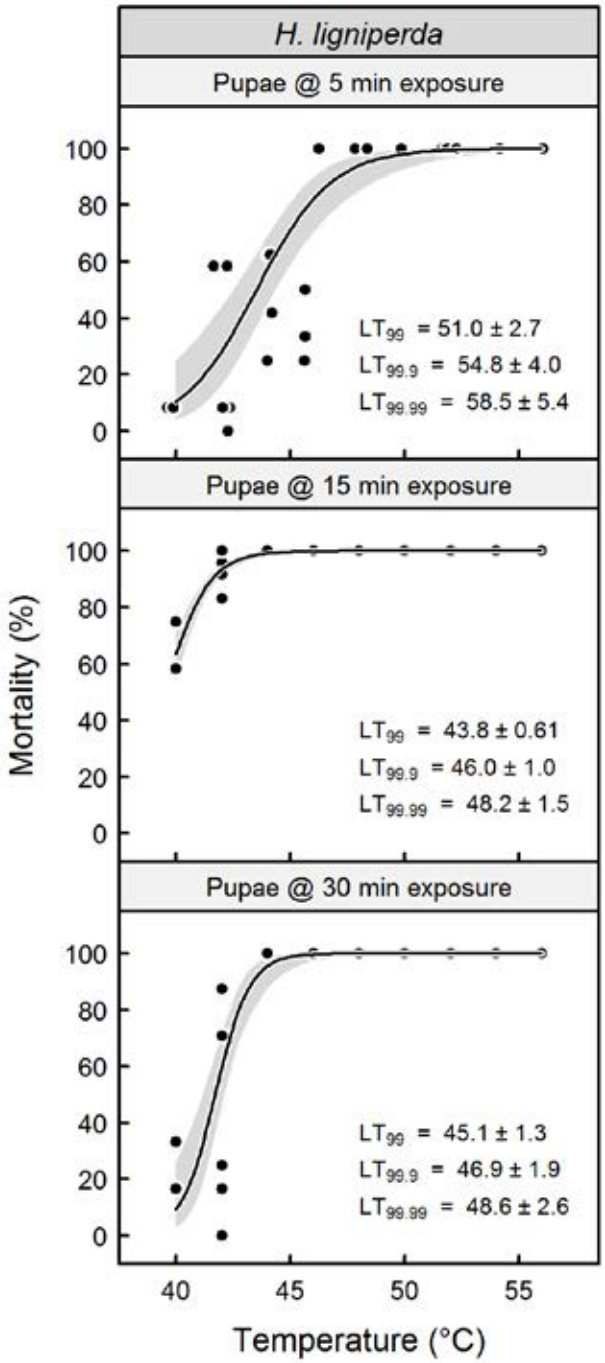
Pupae



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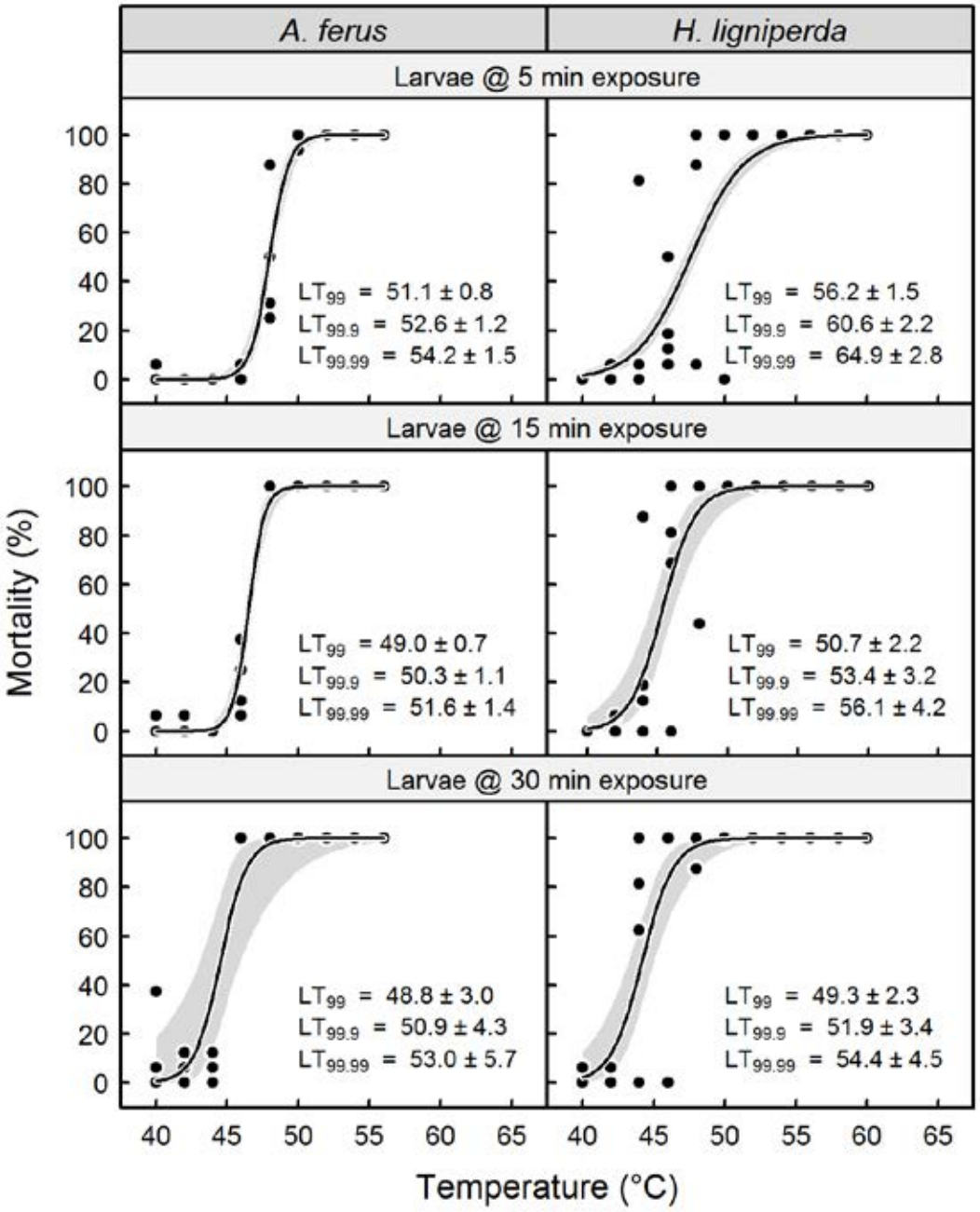
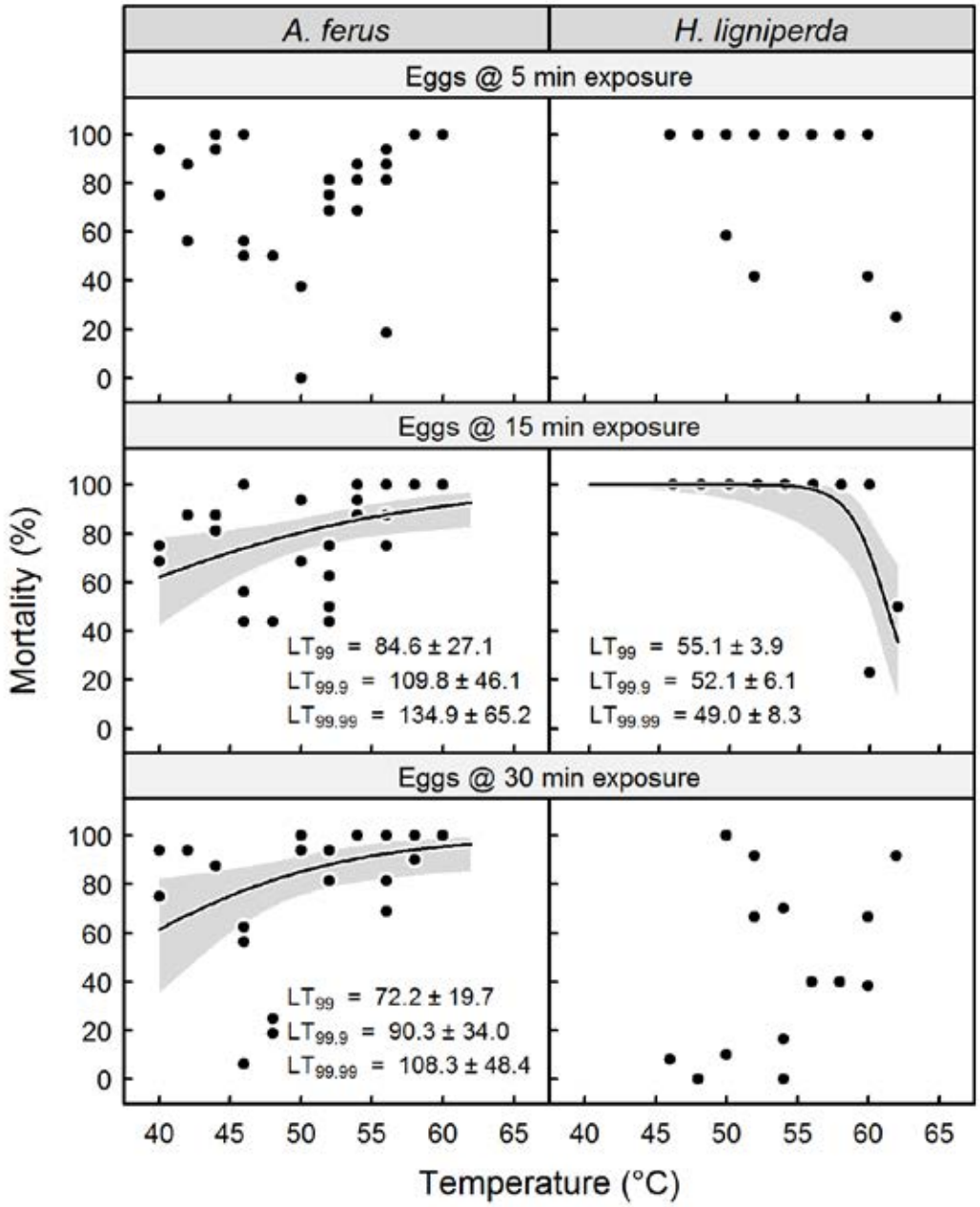
Larvae


A. ferus
H. ligniperda
Larvae @ 5 min exposure
LT99 = 51.1 ± 0.8
LT99.9 = 52.6 ± 1.2
LT99.99 = 54.2 ± 1.5
LT99 = 56.2 ± 1.5
LT99.9 = 60.6 ± 2.2
LT99.99 = 64.9 ± 2.8
Larvae @ 15 min exposure
LT99 = 49.0 ± 0.7
LT99.9 = 50.3 ± 1.1
LT99.99 = 51.6 ± 1.4
LT99 = 50.7 ± 2.2
LT99.9 = 53.4 ± 3.2
LT99.99 = 56.1 ± 4.2
Larvae @ 30 min exposure
LT99 = 48.8 ± 3.0
LT99.9 = 50.9 ± 4.3
LT99.99 = 53.0 ± 5.7
LT99 = 49.3 ± 2.3
LT99.9 = 51.9 ± 3.4
LT99.99 = 54.4 ± 4.5
Mortality (%)
0
20
40
60
80
100
40
45
50
55
60
65
Temperature (°C)

Eggs



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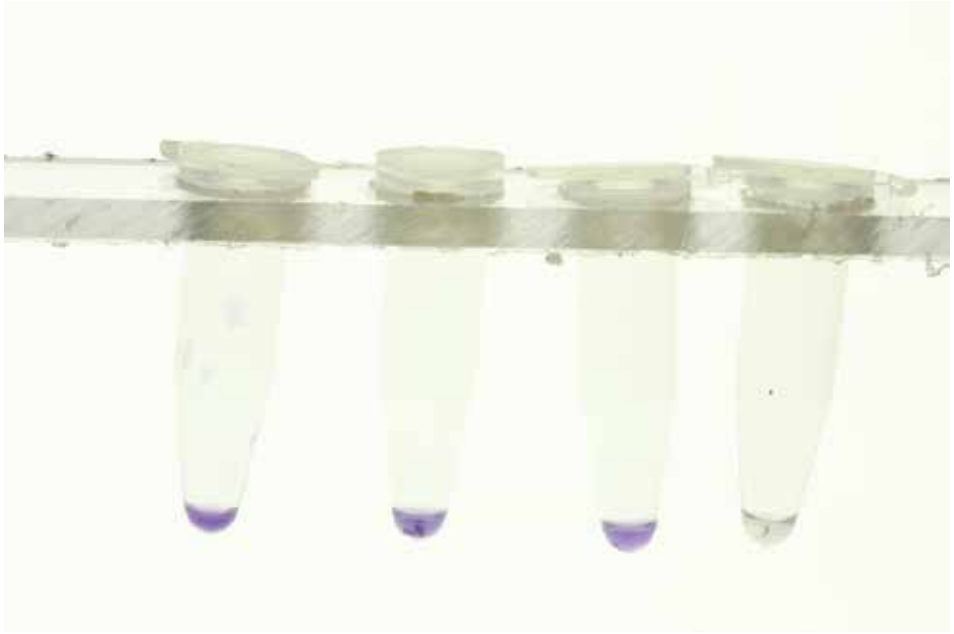
Eggs

- Small and difficult to locate in large numbers
- Were using a biochemical viability test to determine effectiveness of treatments
- Going to move towards an emergence test



What temperature were they exposed to?

- Calibrated temperature baths are an effective means of delivering a constant temperature to a metal block.
- Difficult to determine the actual temperature insects have been exposed to.
- Using micro-temperature probes to calibrate the relationship between temperature bath and temperature insect was exposed to.

Results summary

- A total of 3,786 individuals were assessed.
- No larvae, pupae, or adults of either species survived a treatment of 50 °C (or more) applied for 30 minutes
- LD_{99} of all life stages except eggs for a 30 minute treatment were below the ISPM 15 requirement.
- More work is needed to confirm thermal tolerance of eggs, difficult.
- Assessing the internal temperature that insects were exposed to my reduce LD_{99} by 2 to 3 °C

Acknowledgments

- Stakeholders in Methyl Bromide Reduction and Better Border Biosecurity for funding.
- Jessica Kerr, Alwin Sky, and Brooke O’Connor for testing of insects.
- Dr Martin Bader for assistance with the analysis of data.

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Vacuum/Steam Phytosanitation

Vacuum/steam treatment of pallet, firewood and logs

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Supports also were provided by Pallet One INC, Turman Lumber Co. PSP Marketing, Cosmed Group INC. and IMA Univ.

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Topics to be covered

- Overall Goal
- Background
- Special features of Vacuum/Steam Treatment
- VST of Wood Pallet
- VST of Veneer Grade Logs
- VST of Asian longhorned beetle infested wood
- VST of Firewood and low grade logs

Goal

The goal is to develop a treatment technology that will be used to effectively sanitize the forest products, including logs, firewood, chip and pallets as an ISPM alternative.

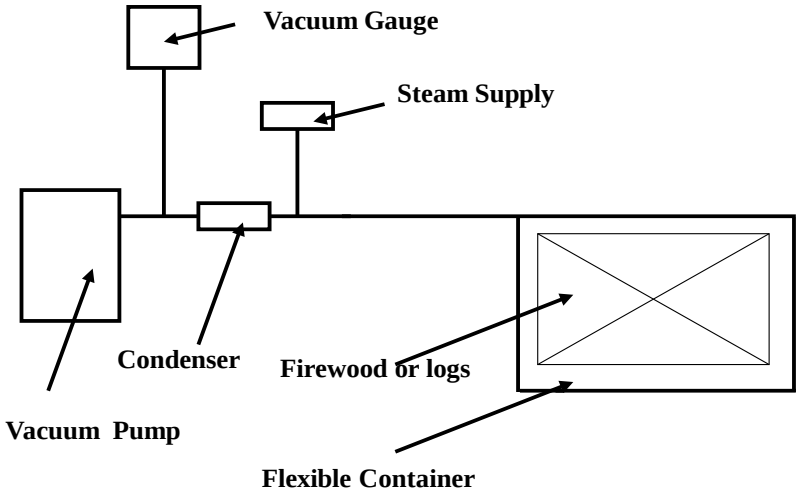
Principle of vacuum/steam treatment

- The ambient pressure is lowered which creates low pressure inside the wood.
- When steam is added, the steam pressure is higher than the pressure inside the wood.
- Vapor is transferred due to a total pressure difference and the amount of moisture transferred is directly proportional to the permeability.
- Water vapor condenses and changes into liquid water during vacuum/steam treatment.
- Large amount of heat is released to the wood to increase the temperature of wood.

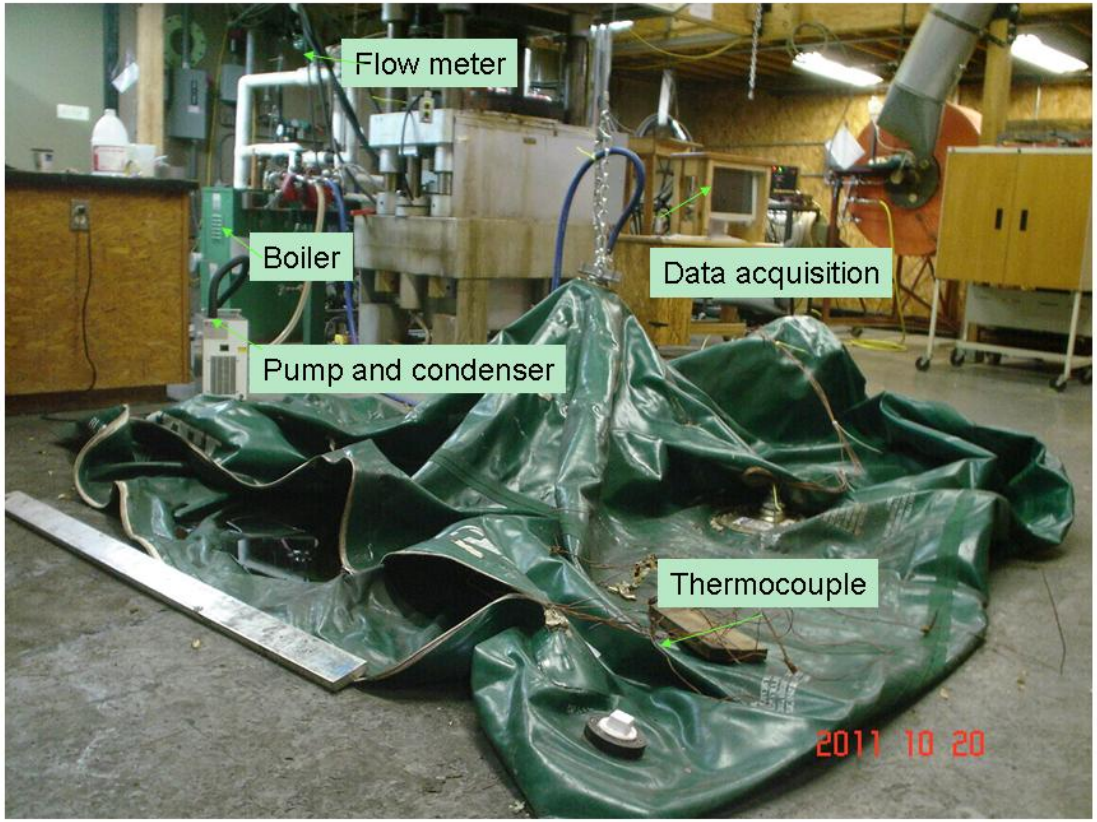
Vacuum steam treatment consists of four steps,

- 1) Vacuum,
- 2) Steam heating,
- 3) Holding,
- 4) Cooling.

Schematic diagram of vacuum steam treatment



Laboratory vacuum steam system



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Flexible container used during the test, size of 10’ by 11’

Condensation heat transfer

- Occurs when a vapor is cooled
- Changes its phase to a liquid.

- A fast process.
- Large amount of heat involved that includes both latent and sensible heats.

Two ways of condensation

- **Filmwise condensation**
- **Dropwise condensation**

Both forms of condensation occur during vacuum/steam treatment of wood, initially dropwise followed by filmwise condensations.

Heat transfer coefficient for steam condensation from 200 to 1900 W/m2·°C

Heat transfer coefficient (W/m2·°C)

Free air convection	Free water convection	Forced air convection	Forced water convection	Condensation
5	440	17	1911	200-1900

Two common latent heats

The latent heat refers to the amount of energy released or absorbed by a substance during a change of state without changing its temperature.

Phase transition such as the melting of ice (latent heat of fusion) or the boiling of water (latent heat of vaporization).

Comparison of sensible heat and latent heat

- 419 kJ of energy is required to heat 1 kg of water from 0ºC to the saturation temperature 100ºC.
- Another 2,257 kJ of energy is required to evaporate the 1 kg of water at 100ºC to steam at 100º C.

2257/419=5.38

Another feature of the vaporization

Comparing that to the volume of the liquid water, the volume expands by a factor of 1700 when vaporized into steam.

A mole of water is 18 grams, 18 cm3. A mole of gas occupies 22.4 liters at 273K. Temperature changes, 373K, and volume changes to 30.6 liters. 30600/18 = 1700.

Vacuum/Steam Heat Treatment of Pallets

The mixed hardwood 48 by 40 inch pallets

Stringer size 1.375×3.375×48, deck board thickness 0.625 inches

Collapsed flexible container after vacuum was applied to stack of 11 pallets.

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Thermocouple probe in stringer

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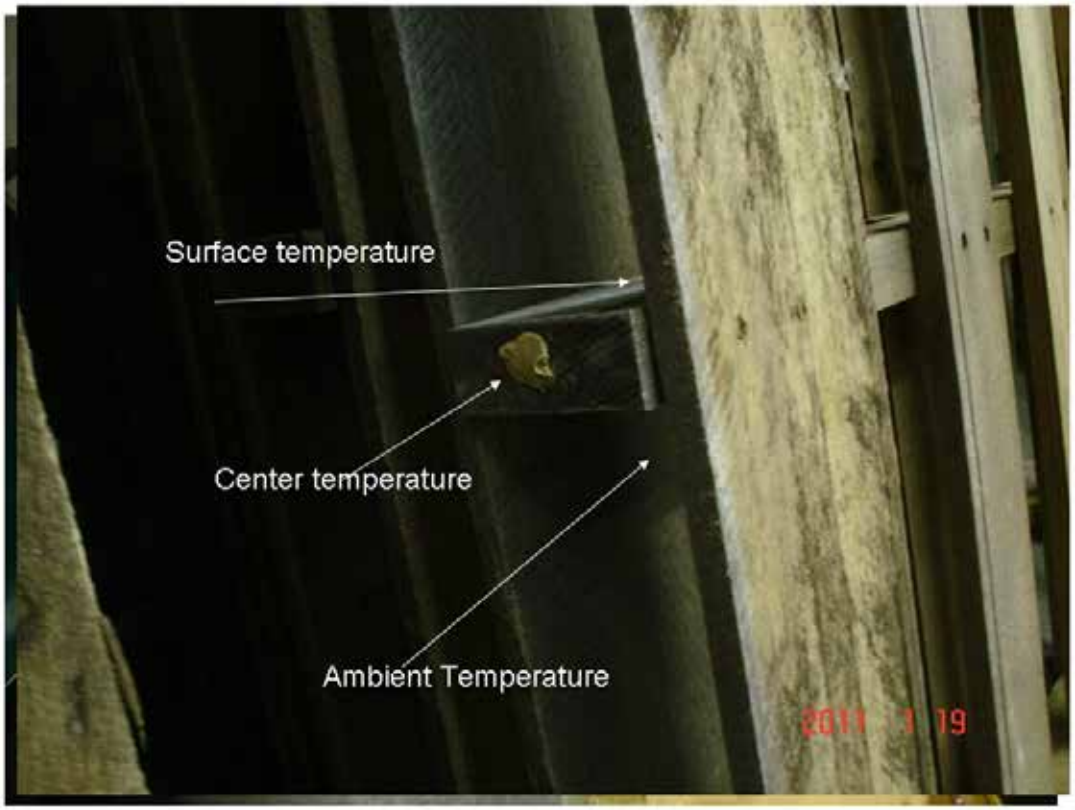
Locations where the temperatures taken



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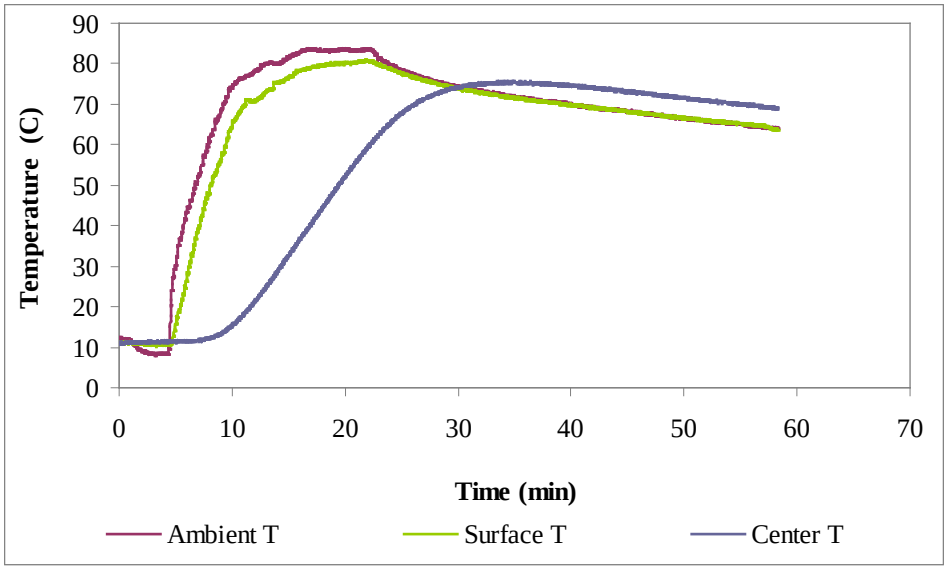
Moisture sections were cut for measuring the MCs



Temperature profile during the vacuum steam treatment of dry yellow-poplar pallets at the pressure of 300 mmHg.

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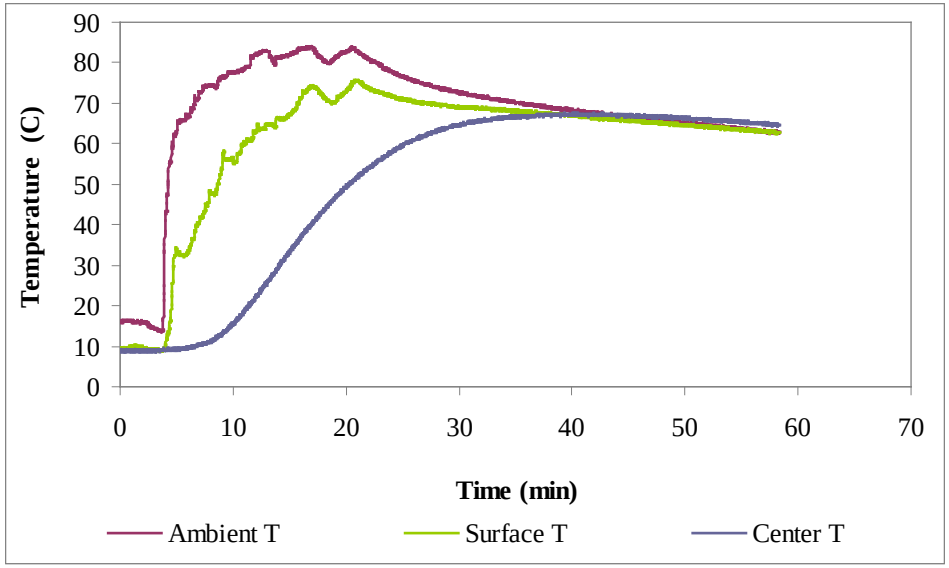

Temperature profile during the vacuum steam treatment of dry red oak pallets at the pressure of 500 mmHg.

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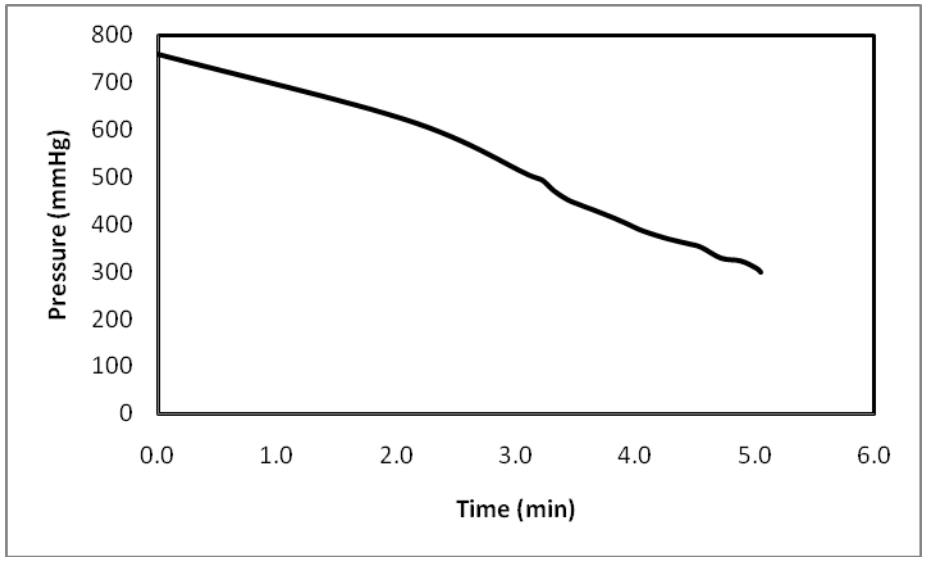

5.1 min. vacuum time from 760 to 300 mm Hg (about 9 psi applied stress to the contents in the flexible container)

Pressure curve when vacuum was drawn to 300 mmHg during test.

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Treatment of yellow-poplar dry pallets at 300 mmHg and temperature of 80 ºC with 30 minute hold time.

Test	Vacuum Time (min)	Steam Time (min)	Wood Initial Temp. (ºC)	Total Treatment Time (min)	Temp. Increase Rate (ºC/min)
1	6.8	14.4	20.1	51.1	2.50
2	5.6	15.6	12.8	51.1	2.78
3	5.1	12.9	16.0	48.0	3.11
4	4.4	16.7	11.0	51.1	2.69
5	6.4	16.4	13.7	52.8	2.58
6	5.5	12.0	24.4	47.5	2.63
Avg	5.6	14.7	16.3	50.3	2.7

Treatment of mixed hardwood species green pallets at treating pressure of 300 mmHg and treating temperature 80 ºC with hold time 30 minutes.

Test	Vacuum Time (min)	Steam Time (min)	Wood Initial Temp. (ºC)	Total Time (min)	Temp. Increase Rate (C/min)
1	5.2	22.9	7.2	58.1	2.13
2	5.6	22.2	11.3	57.8	2.01
3	5.2	21.3	5.2	56.5	2.38
4	4.6	25.5	0.0	60.1	2.20
5	7.2	23.1	9.6	60.3	2.01
6	5.7	27.4	0.0	63.1	2.05
7	5.6	22.9	10.0	58.4	2.01
8	6.1	20.6	16.6	56.7	1.91
9	6.5	23.0	17.6	59.5	1.67
10	7.0	20.5	9.9	57.5	2.25
Avg.	5.9	22.9	8.7	58.8	2.06

Summary of the treating result of pallets at 80(°C) with 30 minutes hold time

	MC Of wood	Pressure (mmHg)	Avg. Vacuum Time (min)	Avg. Steam Time (min)	Avg.Wood Initial Temp. (°C)	Avg. Total Treatment Time (min)	Avg. Temp. Increase Rate (°C/min)
Red Oak Pallets	Dry	300	7.0	22.8	14.7	59.8	1.8
Y-Poplar Pallets	Dry	300	5.6	14.7	16.3	50.3	2.7
Red Oak Pallets	Dry	500	3.4	16.9	14.4	50.3	2.5
Y-Popar Pallets	Dry	500	3.7	13.6	21.8	47.4	2.5
Mixed Hardwood Pallets	Green	300	5.9	22.9	8.7	58.8	2.1
Mixed Hardwood Pallets	Green	500	3.8	21.1	7.0	54.9	2.3

The dry pallets had brighter and cleaner wood surfaces after treatment

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Moisture content change during the vacuum treatment for green hardwood pallets at pressure of 300 mmHg.

Pallet	MC before treatment (%)	MC after treatment (%)	MC change (%)
1	108.4	107.8	-0.6
2	98.9	102.9	4.0
3	102.1	99.1	-3.0
4	70.4	75.9	5.5
5	37.9	39.2	1.3
6	37.6	43.3	5.7
7	38.6	43.0	4.4
8	40.0	42.5	2.5
9	42.6	45.8	3.2
10	43.3	44.0	0.7
Mean	62.0	64.3	+2.4

Conclusion on VST of pallets

Treatment Time

- Vacuum/steam treatment is fast.

Less than 65 minutes for pallet (both dry and green) including the vacuum time of 5 minutes and the holding time of 30 minutes.

Moisture Content

- The dry pallets were treated faster than green pallets.

The average treatment times of dry pallets were 7.1% and 10.4% faster than green pallets at 300 mmHg and 500 mmHg respectively.

Wood Species

- Wood species affects the treatment time.

The average treatment time of dry yellow-poplar pallets was 15.9% faster than that of dry red oak pallets at 300 mmHg.

Treating pressure

Treating pressure had some effect the on the treatment duration between 300 and 500 mmHg.

Moisture Change

- The moisture content of pallets increased after the treatment.

The average moisture content change was measured to be less than 3% for both green and dry pallets.

Wood Species

- Wood species affects the treatment time.

The average treatment time of dry yellow-poplar pallets was 15.9% faster than that of dry red oak pallets at 300 mmHg.

Vacuum/Steam Heat Treatment of Hardwood Veneer logs

Phase I

Lab scale, logs were treated in the flexible container to study the heating dynamics related temperature increasing rates.

Test specimens: Red Oak, Hickory, Black Cherry, Black Walnut, and Yellow-Poplar

Log size: 17 to 21 inch by 8 feet long

Three treated and one control of each species

Vacuum/steam treatment of veneer grade logs

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Log in the flex chamber

Veneer log were treated

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Veneer logs of hardwood species

Cherry, walnut, hickory, red oak and yellow-poplar

Diameters range from 17 to 21 inches in the small end.

Treating time varies from 18 to 29 hours.

Treatment temperature was 90 deg C

Treatment pressure 200 mm Hg

Target temperature was 56 deg C for 30 minutes

Initial log temperatures varied from +14 deg C to +25 deg C

10 % of logs treated, showed visible signs of end split progression during treatment. This was most common in black walnut and hickory

Based on a comparison veneer from the treated and control logs, the vacuum/steam heat treatment process has no significant effect on the quality and yield of veneer .

Logs were flitched at Danzer Veneer Edinburgh, Indiana

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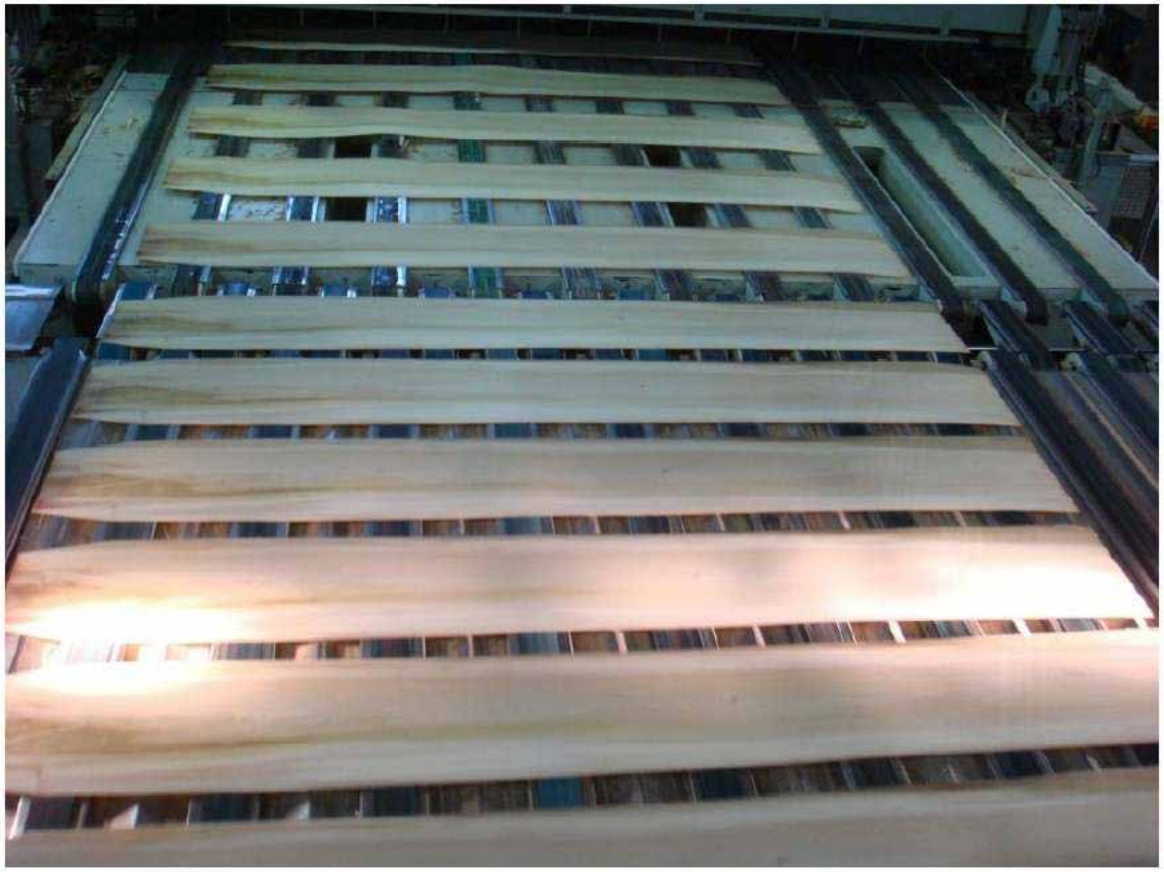
Flitches are “cooked”prior to slicing



Red oak veneer To dryer

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Veneer was sampled and graded

Comparison of the colors of veneers from both control and treated logs.

Species	Log Id.Number	Test Number	Test Date (m&d)	Surrounding Temp. °C	Wood Initial Temp. °C	Treatment time (h)
Pignut Hickory	649322	3	9.13	23	24.3	22
	649331					Control Sample
	649333	6	9.19	15	15.8	29.33
	649334	5	9.16	15	17.1	24.5
Black Cherry	629246	14	10.12	19	14.5	26.53
	649137	13	10.11	22	15.6	21.58
	649325					Control Sample
	649329	10	9.27	26	20.1	17.28
American Walnut	619480	4	9.14	25	22	24.5
	619481	1	9.9	20	23.5	18.25
	649323					Control Sample
	649326	7	9.21	25	17.8	28.17
Yellow Poplar	646653	8	9.23	25	18.7	21.86
	646676					Control Sample
	646690	2	9.12	20	19.2	20.25
	646707	9	9.26	25	20.5	24.5
Red Oak	649171	15	10.15	21	16.7	23.62
	649328	11	9.28	26	20.3	24.58
	649330					Control Sample
	704191	12	10.6	22	15.5	

Treatment times varied from 17 to 29 hours

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Phase II

Logs were treated at the commercial setting. The effect of vacuum/steam treatment on the potential damage to log quality and veneer grade was investigated.

- Twenty hardwood tree trunks from five species have been acquired in the eastern US. Two logs were cut from each trunk; one served as treated sample and anther one as control sample.
- Log diameters ranged from 15 to 32 inches in the small end and their lengths were about 8.5 to 10.7 feet.

- Four different treating schedules were used. The treating duration and energy consumption were also measured.
- During treating, the geometric centers of logs was heated to 60°C and held for 60 minutes.

Commercial scale of treating chamber was used

Data loggers were installed to measure temperatures

End grain photo images of logs before and after treatment

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W·S


W·S

The iron stain can be seen after the treatment especially for red oak.

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There is no difference in the veneer colors between the controlled samples and treated samples for both red oak and yellow poplar species. For hickory, there is no difference in the colors of heartwood veneers for treated and controlled samples. For the sapwood, the veneers from treated logs were darker that from controlled logs. For walnut species, there is no difference in the veneer color between treated and controlled logs.

Vacuum/Steam Treatment of Asian Long-horned Beetle infested wood

Inner Mongolia Agriculture University

Hohhot, China

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Treatment temperature: 90 deg C

Treatment pressures: 350 mm Hg and 500 mm Hg

Target temperature: 60 deg C for 60 minutes

Logs: willow, 4 to 10 inches in diameter,
temperature -3 deg C to 22 deg C

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Frass around the base of an infested willow tree

Infested logs for treatment

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Flex chamber showing vacuum and steam lines

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dissecting
willow logs
to measure
mortality of
ALB
after treatment

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Dead ALB larvae and adults

Treatment times: 176 to 510 minutes depending on log size and temperature

Mortality of ALB larvae, adult, and pupae stage: 100% in treated logs and 33% in control untreated logs

Logs gained from 4 to 14% moisture

60 deg C for 60 minutes is an effective ALB treatment

Vacuum/steam treatment of ash logs and firewood

Project objectives

To investigate the heat treatment of firewood grade ash logs and firewood using vacuum and steam technology.

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Testing Materials

The white ash (*Fraxinus americana*) logs were acquired from a local lumber company located in Christiansburg, Virginia.

They were harvested from the Southern Virginia.

Firewood grade ash logs were used in the test.

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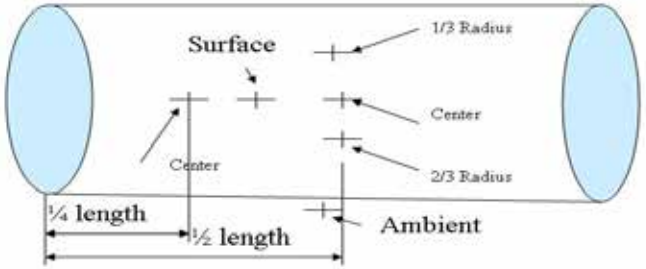
The ash logs together with the pallet were loaded into the flexible container



Schematic diagram showing the locations of the thermocouples in the firewood log.

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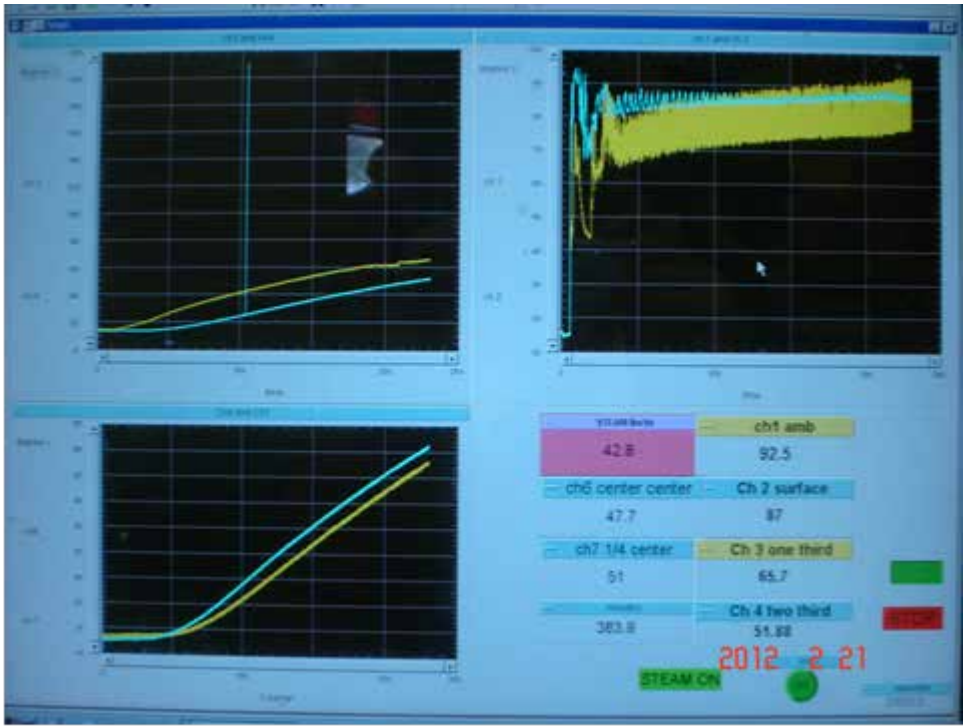

The monitor screen showed that the experimental progress.

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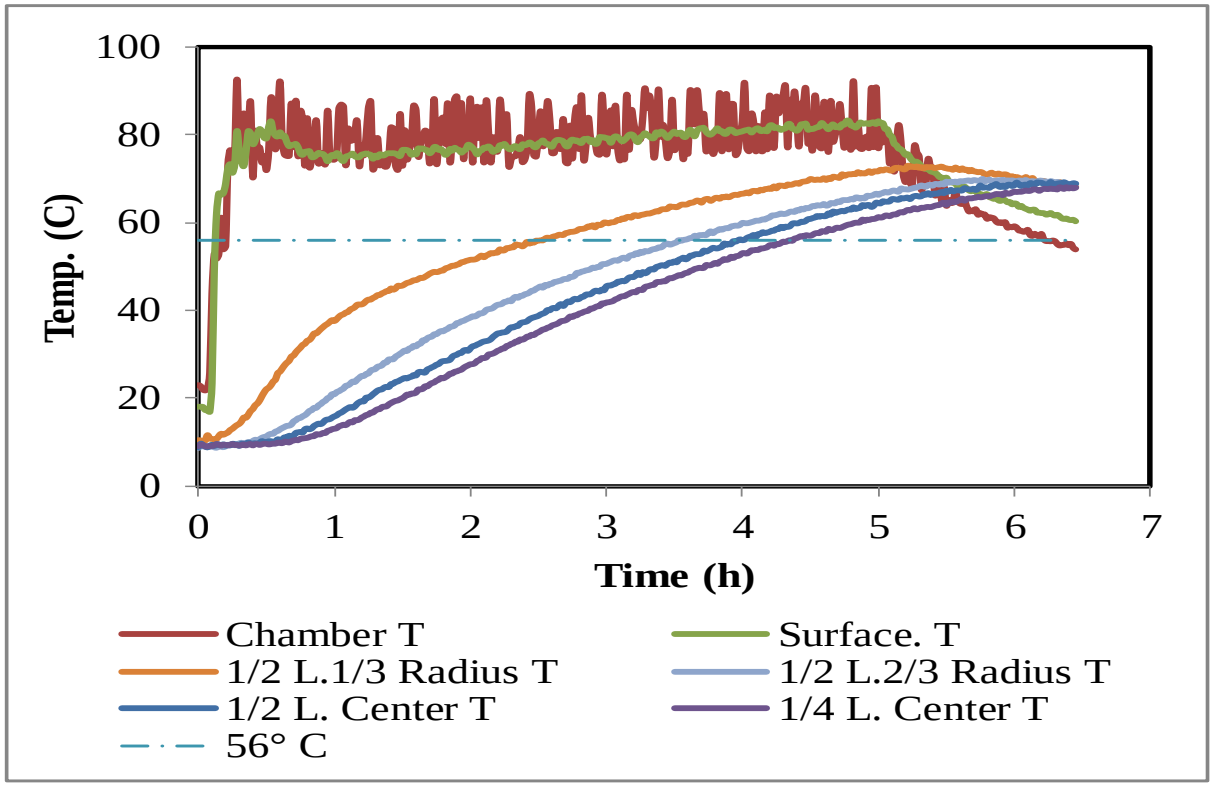
The treating time of ash logs at the temperature of 90ºC.

Test Number	Test Date (M&D)	Pressure (mmHg)	Surrounding Room Temp. (°C)	Log Length (inch)	Log Large end dia. (inch)	Log Small end dia. (inch)	Total Treatment Time (h)
1	10-Feb	250	12	75	10.5	10	14.5
2	15-Feb	250	20	74	13	11	12.0
3	18-Feb	250	16	73	8	6.5	9.5
4	21-Feb	250	19	72	10.5	9	8.3
5	23-Feb	250	20	76	10	8.5	7.6
6	27-Feb	250	20	76	10	8	9.5
7	29-Feb	250	20	72	10	9	7.4
8	1-Mar	250	20	74	9	7	5.5
9	4-Mar	250	15	73	8	7.5	6.8
10	6-Mar	250	16	73	8	7	5.8
11	7-Mar	500	17	78	11	9	7.7
12	8-Mar	500	17	70	10	6.5	5.5
13	9-Mar	500	21	73	8	8	6.5
14	11-Mar	500	22	72	10	9	7.5
15	12-Mar	500	20	72	10	10	9.8

Test number	Wood Initial Temp. (°C)	Small end dia. (inch)	Steam consumed (lb)	Log weight (lb)	Steam use (lb/ lbwood)	Energy Use		
						KWh used	KWh/lb	KWh/lb/ C
1	12	10	141.3	406	0.348	105.65	0.260	0.0059
2	20	11	168.1	440	0.382	125.68	0.286	0.0079
3	16	6.5	130.8	316	0.414	97.81	0.309	0.0077
4	19	9	138.2	360	0.384	103.35	0.287	0.0078
5	20	8.5	115.0	363	0.317	86.03	0.237	0.0066
6	20	8	112.9	350	0.322	84.41	0.241	0.0067
7	20	9	95.2	350	0.272	71.18	0.203	0.0056
8	20	7	108.2	327	0.331	80.91	0.247	0.0069
9	15	7.5	69.7	338	0.206	52.08	0.154	0.0038
10	16	7	110.3	327	0.337	82.47	0.252	0.0063
11	17	9	96.9	378	0.256	72.49	0.192	0.0049
12	17	6.5	89.5	316	0.283	66.96	0.212	0.0054
13	21	8	119.5	340	0.351	89.33	0.263	0.0075
14	22	9	124.9	367	0.340	93.38	0.254	0.0075
15	20	10	160.3	406	0.395	119.89	0.295	0.0082
Avg.			118.7	358.99	0.33	88.77	0.256	0.0066



Temperature profile during vacuum/steam treatment of ash log at the pressure of 250 mmHg.

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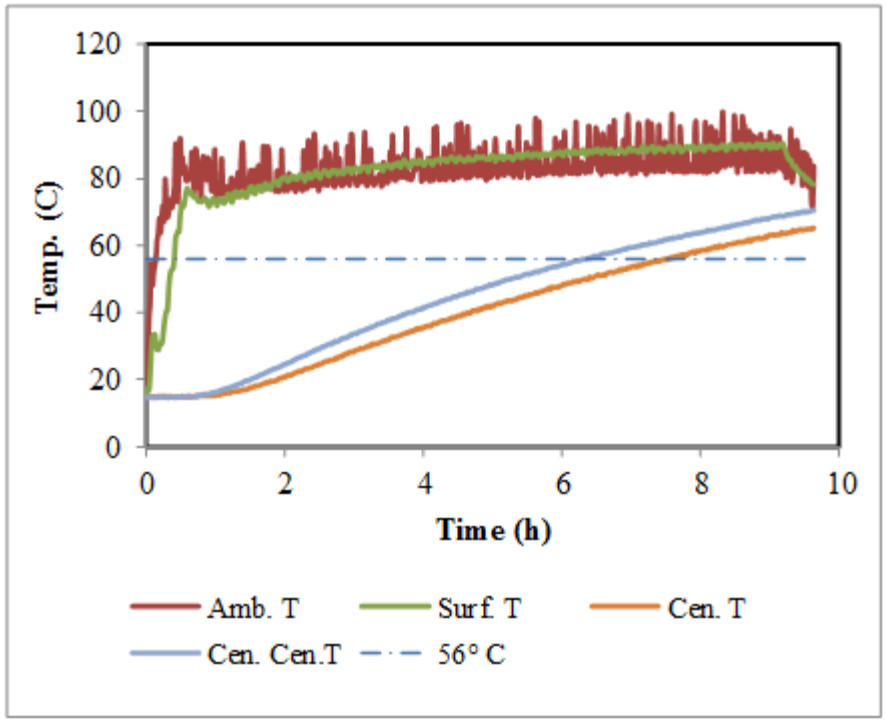

Temperature profile during vacuum/steam treatment of ash log at the pressure of 500 mmHg.

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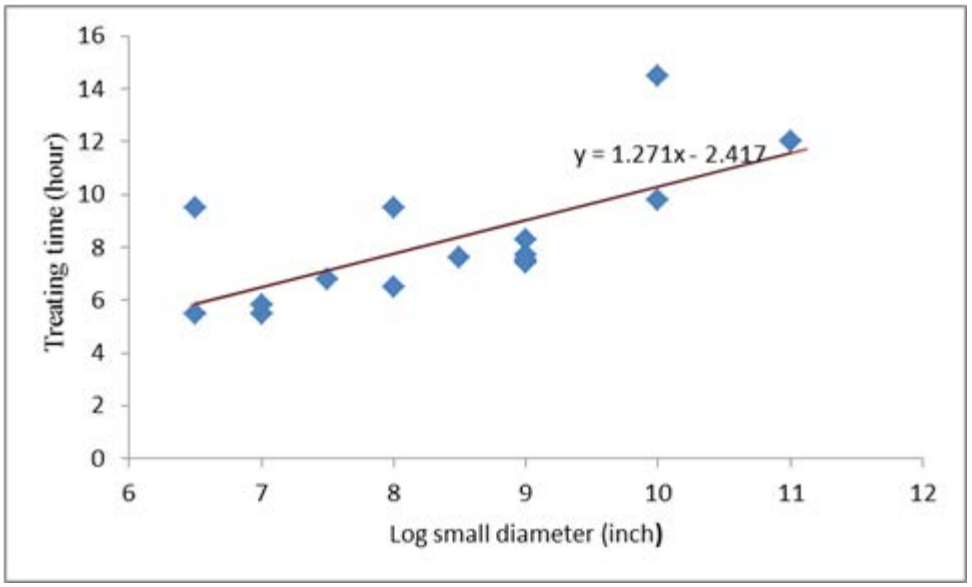

The relationship between the firewood log diameter and the treatment duration.

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Huskee log splitter

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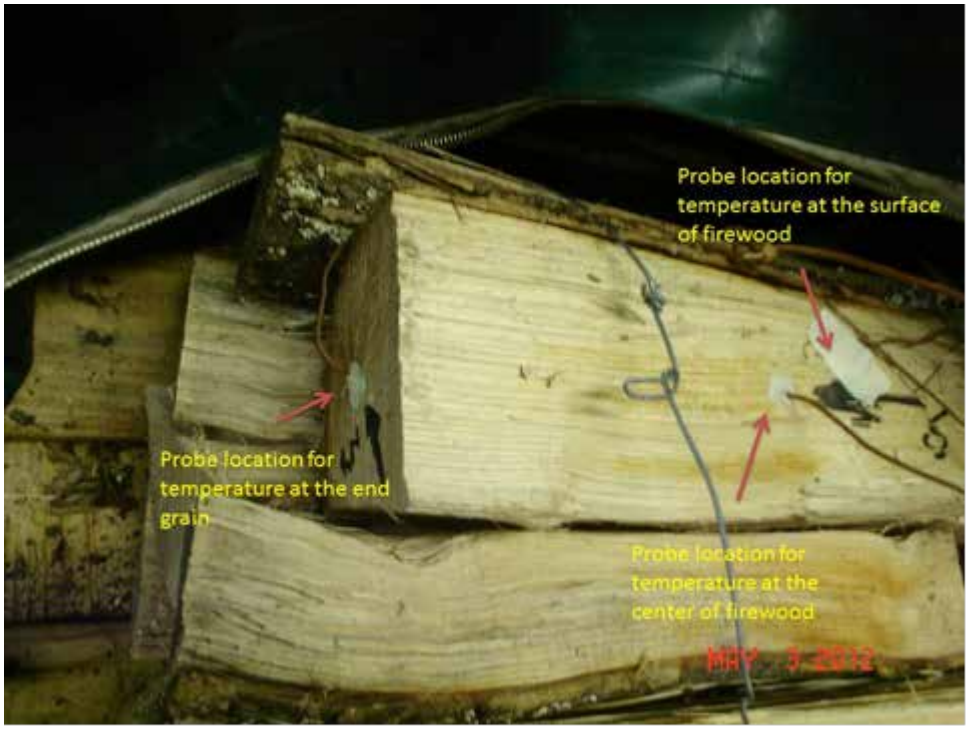
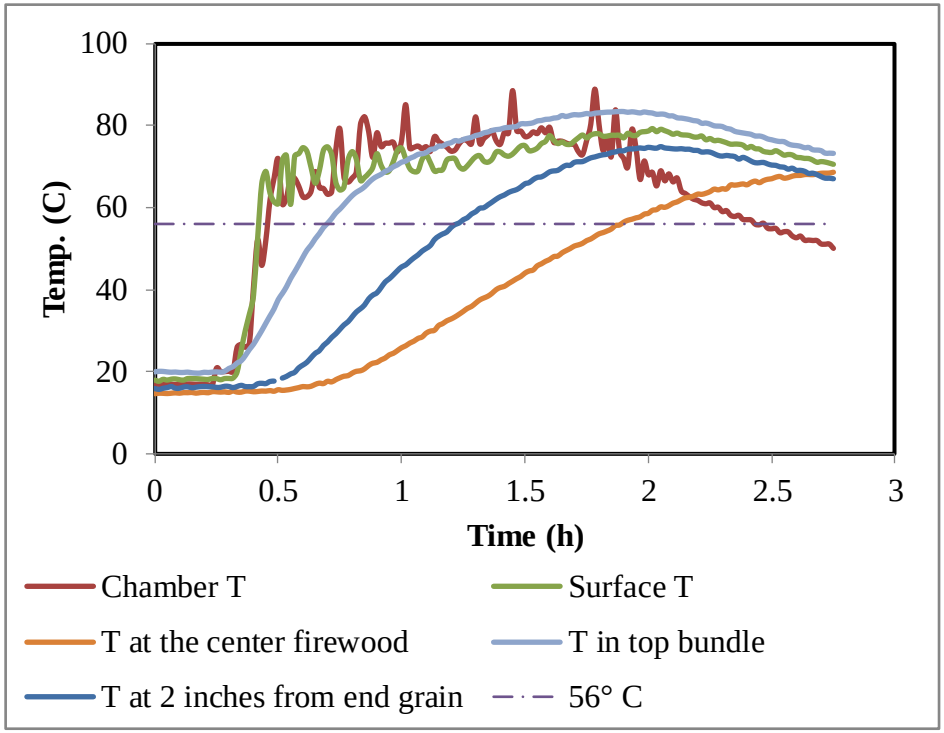
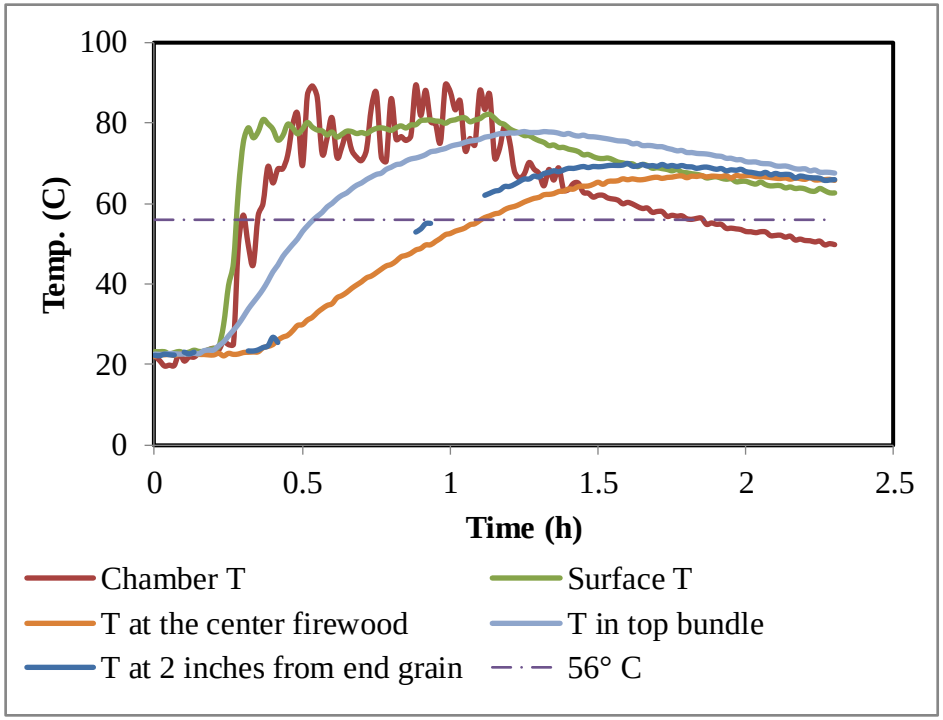
Firewood bundles were loaded into the container



Temperature probes embedded in the firewood for temperature measurements



Temperature profile during vacuum/steam treatment of ash firewood at the pressure of 300 mmhg.



Temperature profile during vacuum steam treatment of ash firewood at the treating pressure of 500 mmhg.

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The treatment time and energy consumption during the ash firewood vacuum/steam treatment at 300 mmHg.

Testing number	Treatment Time (min)	Wood Initial Temp. (° C)	Steam consumed (lb)	Firewood weight (lb)	Steam use per lb of wood	Energy Consumption		
						KWh used	KWh/lb wood	KWh/lb/C
1	70	22	19.4	130.08	0.149	14.48	0.111	0.0033
2	92	24	29.5	132.08	0.223	22.04	0.167	0.0052
3	112	21	32.2	134.75	0.239	24.04	0.178	0.0051
4	113	22	37.4	135.11	0.277	28.00	0.207	0.0061
5	70	22	21.3	131.57	0.162	15.89	0.121	0.0036
6	93	18	33.9	151.14	0.225	25.38	0.168	0.0044
7	92	17	22.1	131.14	0.168	16.50	0.126	0.0032
8	140	17	33.7	148.94	0.227	25.23	0.169	0.0043
9	110	18	39.9	149.09	0.267	29.80	0.200	0.0053
10	137	16	44.8	152.79	0.293	33.53	0.219	0.0055

The treatment time and energy consumption during the ash firewood vacuum/steam treatment at 500mmHg.

Testing number	Treatment Time (min)	Wood Initial Temp. (° C)	Steam consumed (lb)	Firewood weight (lb)	Steam use per lb of wood	Energy Consumption		
						KWh used	KWh/lb wood	KWh/lb/C
11	103	15	39.4	150.86	0.261	29.49	0.195	0.0048
12	87	19	34.6	149.38	0.232	25.89	0.173	0.0047
13	107	20	31.1	146.43	0.213	23.28	0.159	0.0044
14	107	20	34.3	150.5	0.228	25.64	0.170	0.0047
15	112	20	34.0	148.85	0.228	25.39	0.171	0.0047
16	86	20	37.6	149.2	0.252	28.13	0.189	0.0052
17	102	21	44.9	153.41	0.293	33.59	0.219	0.0063
18	98	19	34.6	150.36	0.230	25.86	0.172	0.0046
19	104	18	35.5	149	0.238	26.56	0.178	0.0047
20	90	20	27.8	146.6	0.190	20.80	0.142	0.0039
21	100	20	33.8	148.42	0.228	25.29	0.170	0.0047
22	80	18	32.4	150.9	0.215	24.22	0.160	0.0042

Energy Consumption

The treating energy were measured and varied 0.15 to 0.31 kwh/lb or an average of 0.256 kwh/lb. log

The energy required to treat firewood is 0.12 to 0.22 kwh/lb firewood with average of 0.169 kwh/lb. This is significantly less than that consumed when treating the logs. This is because of the smaller dimension of the firewood.

Moisture Gain

As expected, during the steam treatment, firewood absorbed some moisture and weights of bundles increased.

The firewood weight gains were measured and MC changes calculated.

The average increase in MC was calculated to be 2.24%.

Internal Factors that may affect the vacuum steam treatment time

- Wood density
- Wood specific heat
- Wood permeability
- Wood conductivity
- Wood MC

External Factors that may affect the vacuum steam treatment time

- Initial temperature of lumber,
- Ambient temperature,
- Vacuum level,
- Even steam distribution

Conclusion

1. Steam and vacuum can effectively heat treat ash firewood and firewood logs to comply with ISPM 15, 56°C for 30 minutes at the core.
2. Treatment times were 5.5 to 14.5 hours which includes the holding time of 30 minutes for log diameters ranged from 6.5 to 11 inches in the small end and the logs were cut into 6 feet long.

3. Treatment times were 70 to 137 minutes which include vacuum and the holding time of 30 minutes for the split firewood.

4. The treatment of split firewood consumes 34% less energy per pound of wood than treating the logs before splitting. Treating the split firewood is more efficient.

5. During treatment of split firewood, moisture content of firewood increase an average of 2.2%. This should not affect firewood drying process as costs.
6. An increase in vacuum from 500 mmHg to 300 mmHg has no significant effect on treatment time and energy consumption. Using less vacuum can be more practical at lower cost equipment designs.

7. The results indicate a steam/vacuum system with a 400 kw boiler can treat about one ton of split firewood per hour.

8. By comparing the energy consumption with treating pallet as 0.02 kwh/lb, it took more to treat firewood.

Demonstration of vacuum/steam treatment on the live EAB infested ash firewood

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Thank You

Questions and Comments Are Welcome.

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European wood packaging industry

Filipa Pico
Embar - National Association of Recovery and Recycling of Packaging and Wood Wastes – Portugal

FEFPEB - Fédération Européenne des Fabricants de Palettes et Emballages en Bois
European Federation of Wooden Pallet and Packaging Manufacturers

11th IFQRG
Qingdao, China
October 2013

Embar

- National Association of Recovery and Recycling of Packaging and Wood Wastes
- 38 members: packaging producers, recyclers, waste management
- Work with the green dot system - recycling

FEFPEB

- Established in 1948
- Representative stakeholder for the EU wooden pallet and packaging industry
  - 16 countries - Netherlands, Belgium, Austria, Switzerland, Germany, France, United Kingdom, Italy, Portugal, Sweden, Spain, Turkey, Lithuania, Denmark
  - 5 pallet pools - PRS, IPP Logipal, LPR, EPAL, CHEP
  - 10 associated members

European Timber Pallet & Packaging Market

- Annual New Pallet Production – 505 million
- Timber Consumption - 24 million m^3
- Timber Pallets in Circulation in EU* - 2,8 billion
- Total number of Employees*
  - Directly - 80,000
  - Indirectly - 300,000

*estimated

Major challenges

- ISPM 15 phytosanitary standard
- Competition from Renewable Energy
- Chain of Custody / Certification
- Competition from other materials

Our work

- continue to engage in ongoing dialogue with NPPOs and with DG SANCO* at the European Commission
- working with DG SANCO about potential future extension of ISPM 15 in Europe for intra-Community movements of wooden pallets and packaging

* Directorate-General for Health and Consumers

ISPM 15

- passport for our products
- allows them to travel around the world

ISPM 15 – Problems?

- Different country interpretations and lack of harmonization

  e.g.

  – different marking systems in different countries

  – repairs – multiple marking, use of reclaimed timber

ISPM 15 – Global forum

- convened at Interpal VIII in Bordeaux with industry representatives: Europe, USA, Canada, China, Australia and South Africa
- agreed, in principle, to form a global industrial alliance to speak with one voice - in particular, it is intended to co-ordinate industry news to share with IPPC

ISPM 15 – Future developments

- concerned about lack of harmonization of the individual country regulations
- interested in developing future approved measures to meet ISPM 15
- application of dielectric treatment to industrial scale – both HT and KD
- need for a field verification test for HT – research project - ESR (electronic spin resonance)

Our message to IFQRG

- Remain highly committed to work with plant health scientists – forest protection
- defend the use of wood as a safe, sustainable and environmentally friendly material for pallets and packgaging

European wood packaging industry

Filipa Pico
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European Federation of Wooden Pallet and Packaging Manufacturers

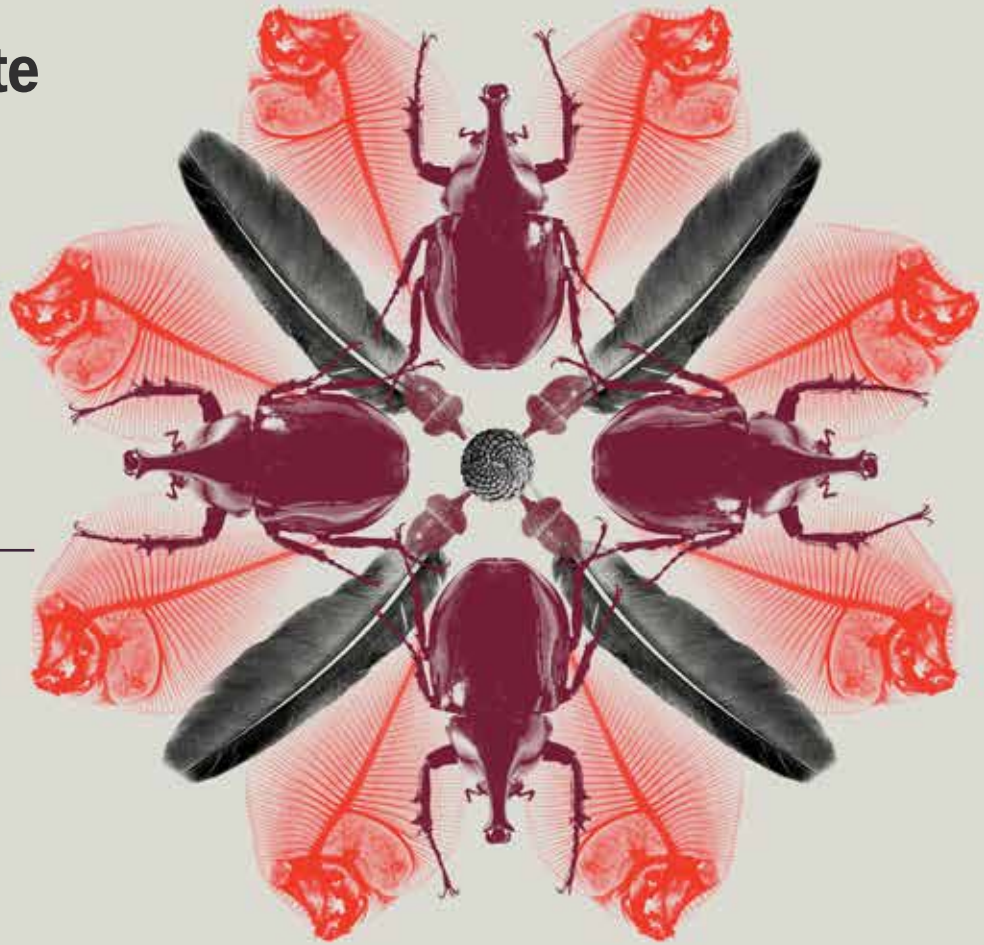
11th IFQRG
Qingdao, China
October 2013

Australian Government
Department of Agriculture

Australia's use of remote sensing to predict risk maritime pathways for gypsy moths

Our approach & operational results for 2011-2013

Plant Biosecurity



John E. Nielsen & David P. Rees

October 2013

Asian Gypsy Moths (AGM) are among the most serious quarantine and forestry pests for Australia

AGM are forest defoliators as larvae

Of the 1800+ recorded host plants for one gypsy moth species[1], many are important species for forestry in Australia

Most intercepted egg masses arrive via maritime vessels that have visited risk seaports

Australia considers AGM to be the five *Lymantria* taxa associated with maritime vessel pathways:

Lymantria dispar asiatica[2]

Lymantria dispar japonica & Lymantria umbrosa

Lymantria mathura

Lymantria monacha

[1] *Lymantria dispar* <u>*sensu lato*</u>

2 Specimens ex Australian National Insect Collection & JEN private collection

Australia has shifted to a risk-based approach to gypsy moth management

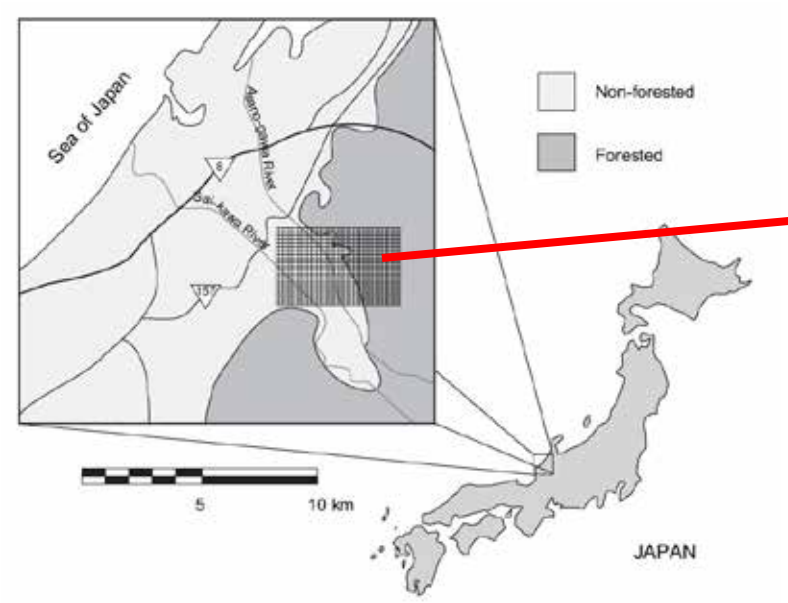
Remote sensing provides an objective method of managing the gypsy moth risk posed by maritime vessels

- Seaports are only a risk for AGM if they are within flight range of AGM host plants
- We can predict AGM habitat based on forest composition
- Forest composition can be determined by reflectance data collected by satellite
- Use satellite data to predict which seaports are close enough to risk habitat to pose a gypsy moth risk

Base study: Liebhold *et al.* 2008

Gypsy moth are strongly attracted to lights in areas of human habitation

- Measured flight penetration of gypsy moth into cities is ~1500 metres
- With increasing distance, more moths land and lay eggs at light sources
- Gypsy moth *can* fly further, but we are concerned with penetration vs. endurance
- Any port within 2000 metres of risk habitat patches is potentially high risk



Remote sensing risk assessment method

- Assessed 121 seaports in temperate Asia (China, Japan, Korean Pen., Russia)
- Seaports identified from listings at www.portsource.com and Google Earth
- Proprietary forest cover satellite data (Globcover) used to provide maps
- 54 ports found to be within 2000 metres of risk vegetation were assessed further
- Maps overlaid on Google Earth to verify forest cover, identify vessel berthing sites and exclude military and non-commercial sites

Results – inspection manual with risk ports and maps

Seaport reference location
2 km radius from seaport
4 km radius from seaport
8 km radius from seaport
10 km radius from seaport

Water bodies
Forest/cropland mosaic
Closed broadleaved deciduous forest
Open broadleaved deciduous forest
Open needleleaved deciduous forest

Closed needleleaved deciduous forest
Shrubland-grassland mosaic
Grassland-forest/shrubland mosaic
Broadleaf deciduous shrubland
Low-risk AGM habitat type

4.7.7 Petropavlovsk-Kamchatsky, Russia

Risk estimate

Risk port located in an area with one endemic *Lymantria* taxon and potential host vegetation within 2 km of known berthing sites for vessels.

Risk taxa endemic to the region surrounding this seaport

Lymantria umbrosa is believed to occur on the Siberian mainland by Pogue and Schaefer (2007). As the Kamchatka Peninsula is the closest part of Siberia to the known range of *L. umbrosa*, it is regarded as a potential risk area for *L. umbrosa*.

Evidence for Risk estimate

Forest cover data for the region surrounding the Petropavlovsk-Kamchatsky seaport shows three types of potential risk habitat are within *Lymantria* flight range of the seaport. The main risk vegetation type for *L. umbrosa* is deciduous needle-leaved forest, which the satellite data shows as the dominant vegetation type for this region.

The forest type of concern occurs over hills rising to 400 meters within 2000 meters of the Petropavlovsk-Kamchatsky seaport. The nature of these hills may act to concentrate and direct *L. umbrosa* into the seaport itself. Human development at Petropavlovsk-Kamchatsky is mostly support infrastructure and housing for the seaport.

Quarantine measures required for this port

Petropavlovsk-Kamchatsky was not historically subject to existing AQIS vessel certification and the mandatory inspection requirements applying to AGM vessels from Russia. However, Plant BSG recommends that the AGM certification program for Russian vessels be extended to this seaport due to its proximity to risk vegetation types.

Interception history

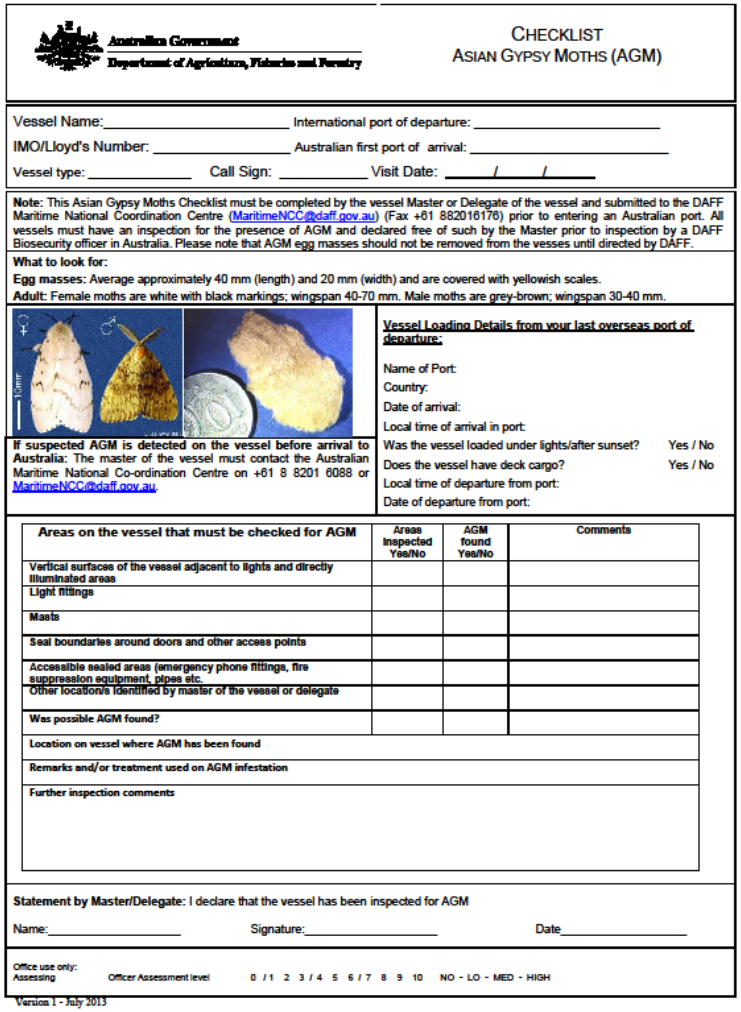
No *Lymantria* interceptions are recorded from this seaport to date. Interception records of *Lymantria* taxa from this port will assist DAFF maintain appropriate import policies and help protect Australia from an incursion.

Results – Egg mass interceptions 2011-2013

Country	Source seaport	Vessel breakdown	AGM egg masses intercepted
2011			
Japan	Fukuyama	4 x Bulk Carrier 1 Cargo	19
	Mizushima	Bulk carrier	10
	Tachibana	Bulk carrier	2
2012			
China	Nanjing	1 x Bulk Carrier	26
Japan	Fukuyama	4 x Bulk Carriers	186
	Matsushima	1 x Bulk Carrier	55
	Mizushima	7 x Bulk Carriers	110
	Tachibana	1 x Bulk Carrier	5
South Korea	Dangjin	2 x Bulk Carriers	16
	Gwangyang	1 x Cargo	16
2013			
Japan	Fukuyama, Japan	Bulk carrier	4
	Nagoya, Japan	2 x Bulk carriers	3
	Nanano, Japan	Bulk carrier	6
	Tsuruga, Japan	Bulk carrier	19
South Korea	Taean, South Korea	Bulk carrier	1
Total number of AGM egg masses intercepted (2011-2013)			**478**

Vessel Master's questionnaire

- Checklist for crew inspections of vessels underway at sea
- Document is faxed to Masters of Vessels from risk ports after notification of arrival
- Prompts inspection of the vessel for AGM egg masses and dead adults offshore
- If egg masses are found, the Master notifies Australia of the detection
- Prior notice of contamination allows preparation of human & materiel resources
- Reduces inspection effort by Australian authorities
- Reduced border detection of AGM substantially



Australian Government
Department of Agriculture, Fisheries and Forestry

CHECKLIST
ASIAN GYPSY MOTHS (AGM)

Vessel Name:_____ International port of departure: _____
IMO/Lloyd's Number: _____ Australian first port of arrival: _____
Vessel type: _____ Call Sign: _____ Visit Date: ____/____/____

Note: This Asian Gypsy Moths Checklist must be completed by the vessel Master or Delegate of the vessel and submitted to the DAFF Maritime National Coordination Centre (MaritimeNCC@daff.gov.au) (Fax +61 882016176) prior to entering an Australian port. All vessels must have an inspection for the presence of AGM and declared free of such by the Master prior to inspection by a DAFF Biosecurity officer in Australia. Please note that AGM egg masses should not be removed from the vesses until directed by DAFF.

What to look for:
Egg masses: Average approximately 40 mm (length) and 20 mm (width) and are covered with yellowish scales.
Adult: Female moths are white with black markings; wingspan 40-70 mm. Male moths are grey-brown; wingspan 30-40 mm.

If suspected AGM is detected on the vessel before arrival to Australia: The master of the vessel must contact the Australian Maritime National Co-ordination Centre on +61 8 8201 6088 or MaritimeNCC@daff.gov.au.

Vessel Loading Details from your last overseas port of departure:

Name of Port:
Country:
Date of arrival:
Local time of arrival in port:
Was the vessel loaded under lights/after sunset? Yes / No
Does the vessel have deck cargo? Yes / No
Local time of departure from port:
Date of departure from port:

Areas on the vessel that must be checked for AGM	Areas Inspected Yes/No	AGM found Yes/No	Comments
Vertical surfaces of the vessel adjacent to lights and directly illuminated areas			
Light fittings			
Masts			
Seal boundaries around doors and other access points			
Accessible sealed areas (emergency phone fittings, fire suppression equipment, pipes etc.			
Other location/s identified by master of the vessel or delegate			
Was possible AGM found?			
Location on vessel where AGM has been found			
Remarks and/or treatment used on AGM infestation			
Further inspection comments			

Statement by Master/Delegate: I declare that the vessel has been inspected for AGM

Name:_____ Signature:_____ Date_____

Office use only:
Assessing Officer Assessment level 0 / 1 2 3 / 4 5 6 / 7 8 9 10 NO - LO - MED - HIGH

Version 1 - July 2013

The future

Australia would like to reduce inspection effort for AGM

Can we use climate data to predict AGM flight times?

- Use degree-day models to predict risk at individual seaports
- Develop Seaport Risk Forecasts based on climate data
- *Lymantria* only affect specific locations for v. limited time
- Help industry avoid AGM risk – reduce costs & delays

Can we move away from blanket-style certification?

- Remote sensing & inspection data may eliminate many ports from inspection
- Reduce international quarantine effort & industry expense

Do we need to inspect at all?

- AGM have an obligate diapause requirement – min. 60 days of cold chilling
- Crossing the equator to Australia prevents eggs from receiving this cold
- Will eggs arriving on maritime vessels hatch on arrival in Australia?
- What time of year can allow eggs on vessels to hatch?

Development of a reverse transcription loop-mediated isothermal amplification (RT-LAMP) method to detect living *Bursaphelenchus xylophilus* in wood

IFQRG-11
Qingdao, China
October 2013

Isabel Leal, Eric Allen, Jennifer Anema, Brett Foord, Caralyn Reisle, Adnan Uzunovic, Aniko Varga, Delano James

Natural Resources Canada | Ressources naturelles Canada
Canadian Forest Service | Service canadien des forêts

- **Countries should only regulate against live pests**

Ø **How to determine if pests are alive**

Methods for detection of PWN

- **Microscopy**
  - reliant on morphological diagnostic expertise
  - challenges: morphological similarity of adult PWN to *B. mucronatus*; inability to distinguish the juvenile stages
- **DNA-based molecular techniques**
  - dead vs. alive?

Possibility for false positives

DNA from dead nematodes passes through Baermann funnel

Objective:

To develop a molecular diagnostic tool that can differentiate living from dead PWN

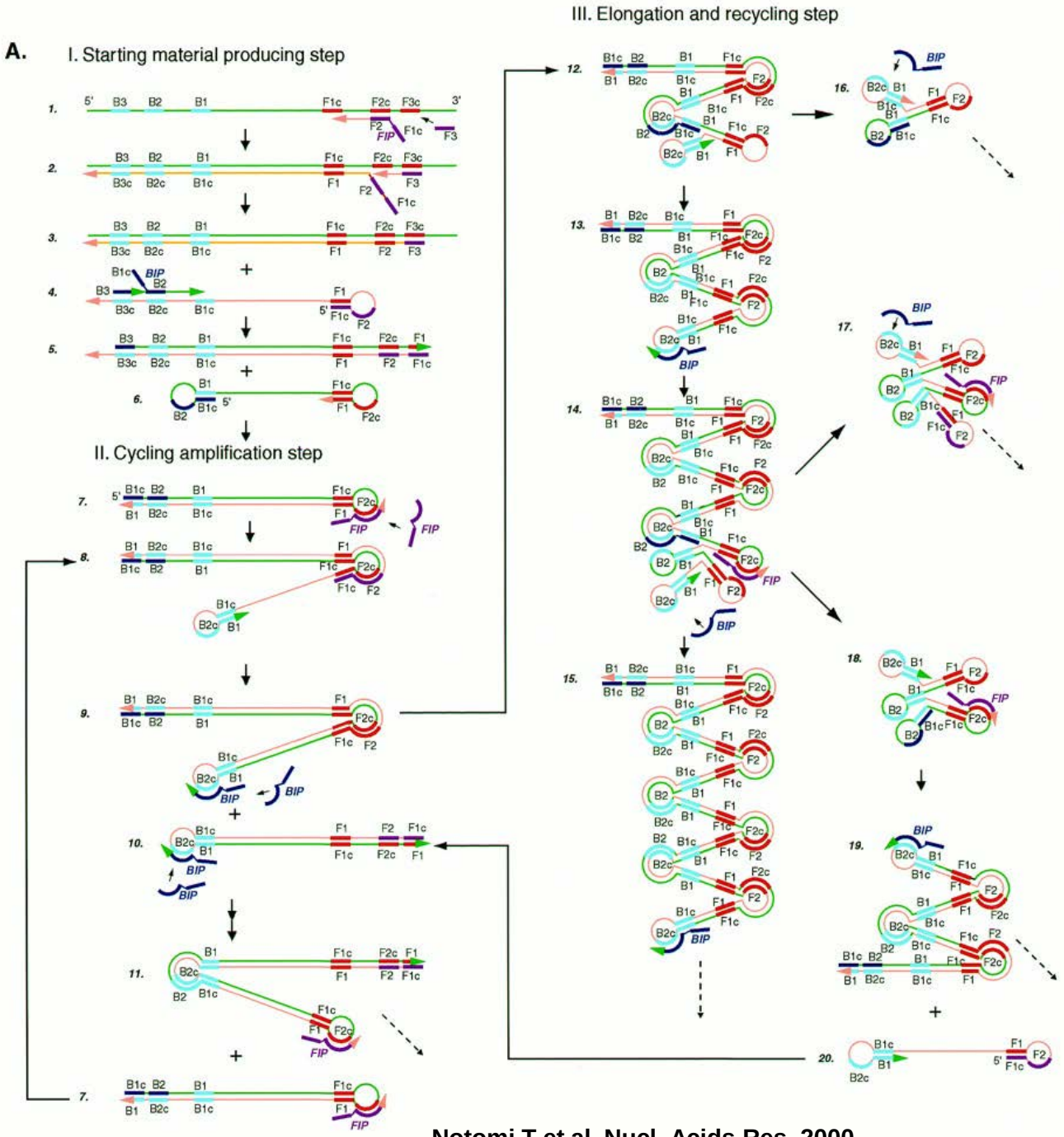
Methodology and Rationale

- RT-LAMP assay based on the use of expansin mRNA as a viability marker
- Rapid degradation of mRNA compared to DNA in dead nematode cells

Loop mediated Isothermal Amplification (LAMP)

- DNA/RNA amplification method that is based on the principle of auto cyclic strand displacement activity by a DNA polymerase (Bst)
- The basic LAMP reaction involves:
  - Two inner primers that form the basic cycling product, and continue amplification after this structure is formed
  - Two outer primers that function at the initiation of amplification in displacing the basic cycling structure
- The accelerated LAMP reaction involves the addition of loop primers and/or stem primers

Schematic representation of LAMP



Notomi T et al. Nucl. Acids Res. 2000

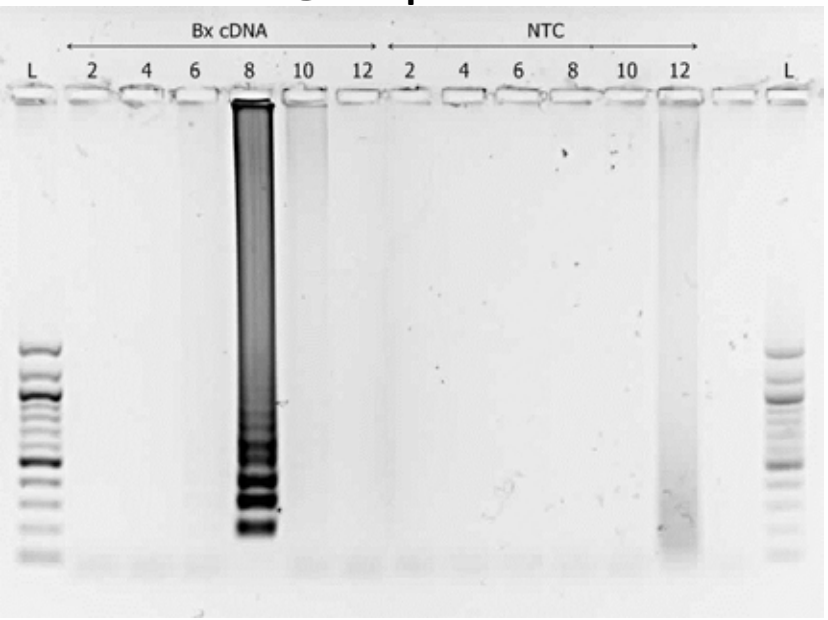
Key differences between LAMP and PCR

- LAMP uses Bst Polymerase whereas PCR uses Taq Polymerase.
- LAMP has six primer binding sites (or 8-10 when using loop and stem primers) whereas PCR has only 2.
- LAMP can be amplified isothermally whereas PCR requires thermocycling
- LAMP has been shown to have both a higher sensitivity, specificity and is more robust.

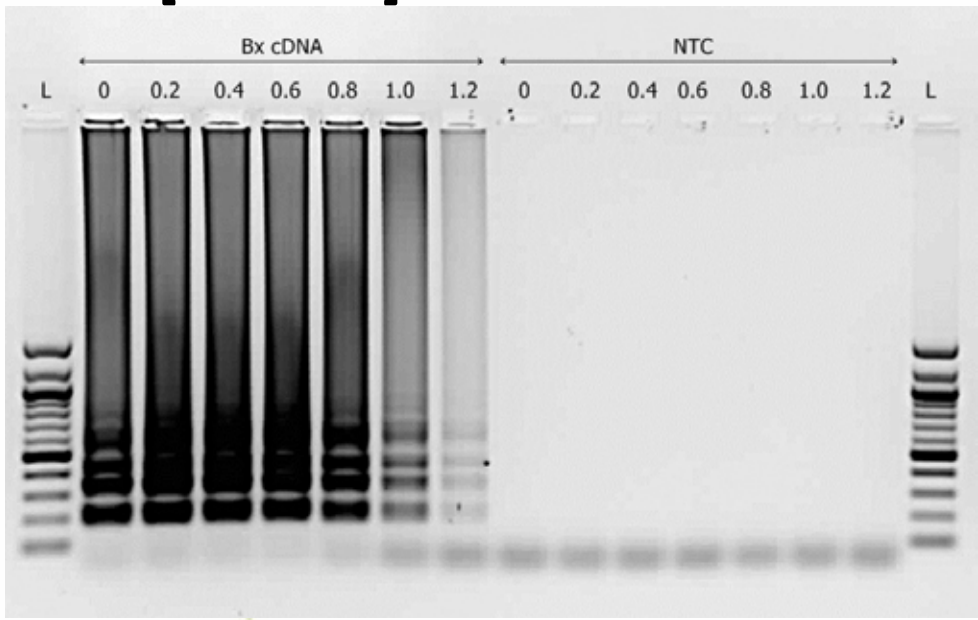
Bursaphelenchus spp. isolates

Species	Isolate	Origin
B.xylophilus	USDE-2	USA
	Ne12 Shandong (SD)	China
	Pt3	Portugal
	Q52A Q1426 Ne15 AB BC FIDS St.John (StJ) St.William(StW)	Canada
B.mucronatus	Chiba	Japan
	DE30	USA
	Fin287	Finland
B. fraudulentus	DE-10w	Germany
B. hoffmani	DE-6w	
B. singaporensis	Ne 7/04	Singapore
B. conicaudatus	Ne 5b/05	China
B. thailandae	KR-2w	Korea
B. doui	Ne 2/046	Taiwan

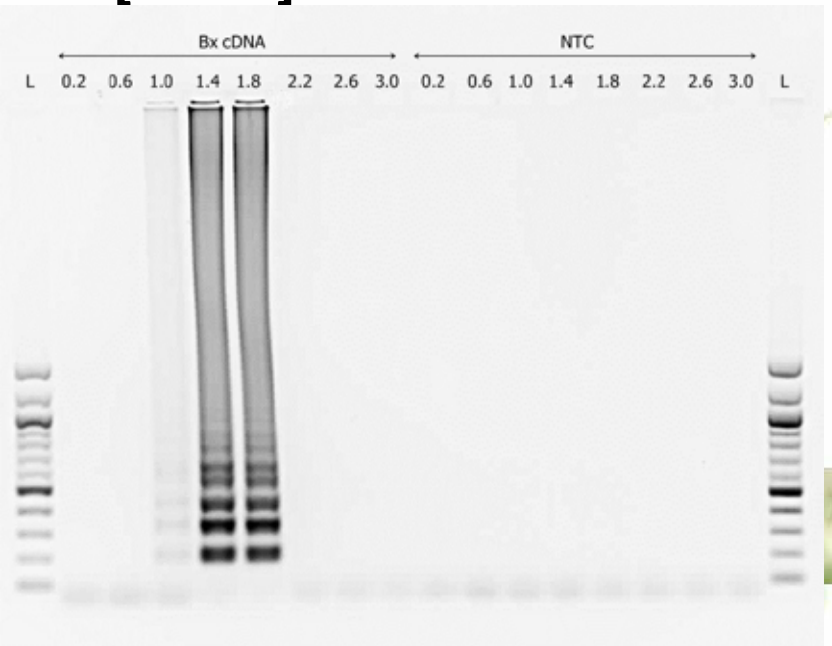
[$MgSO_4$] 8mM



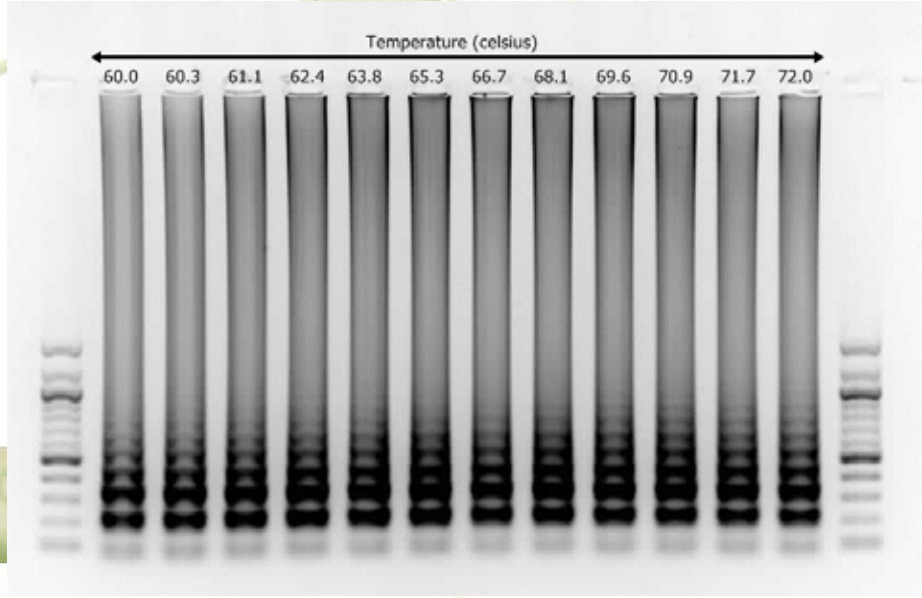
[Betaine] 0.6mM



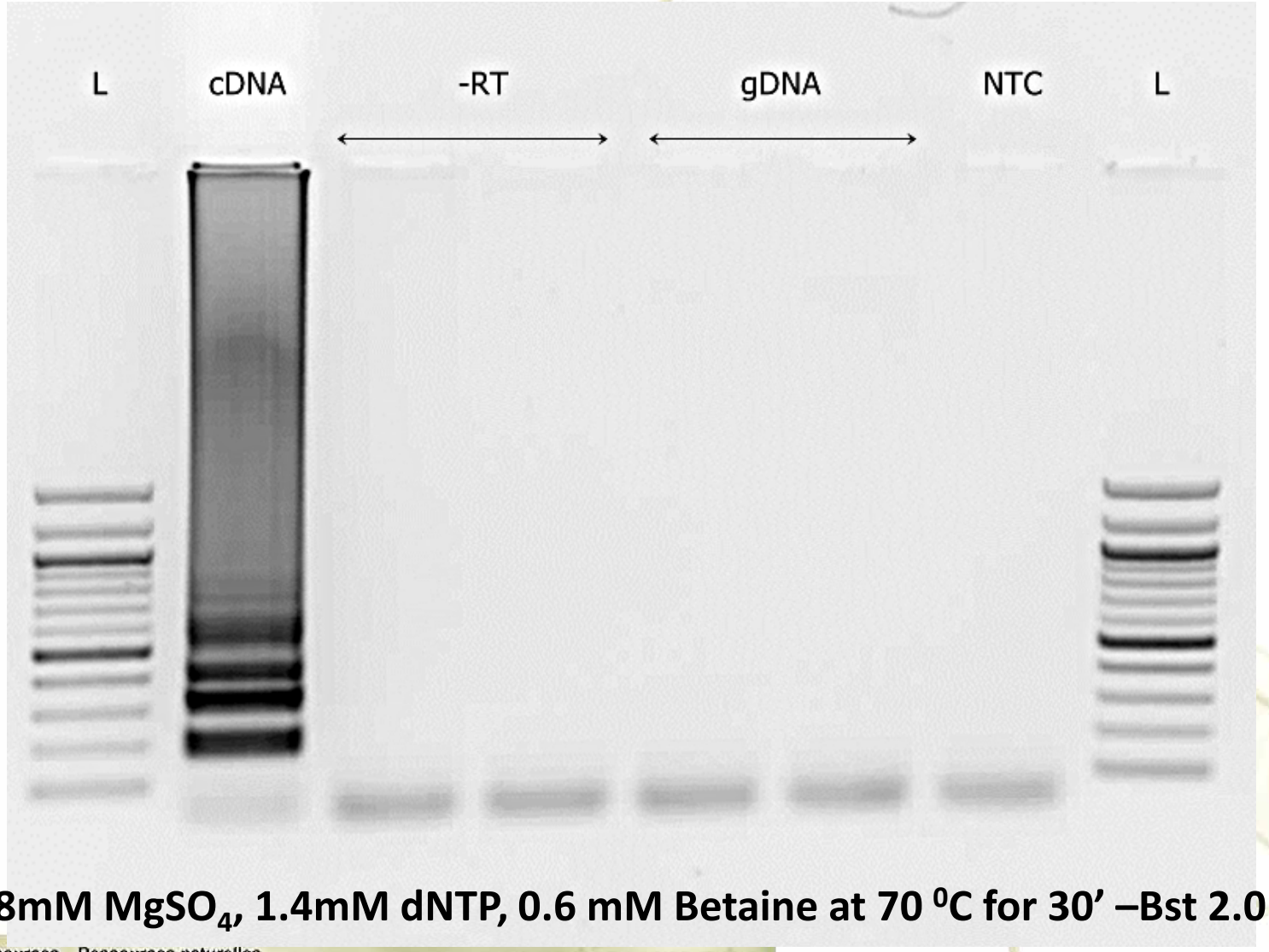
[dNTP] 1.4mM



Temperature 70 ^{0}C

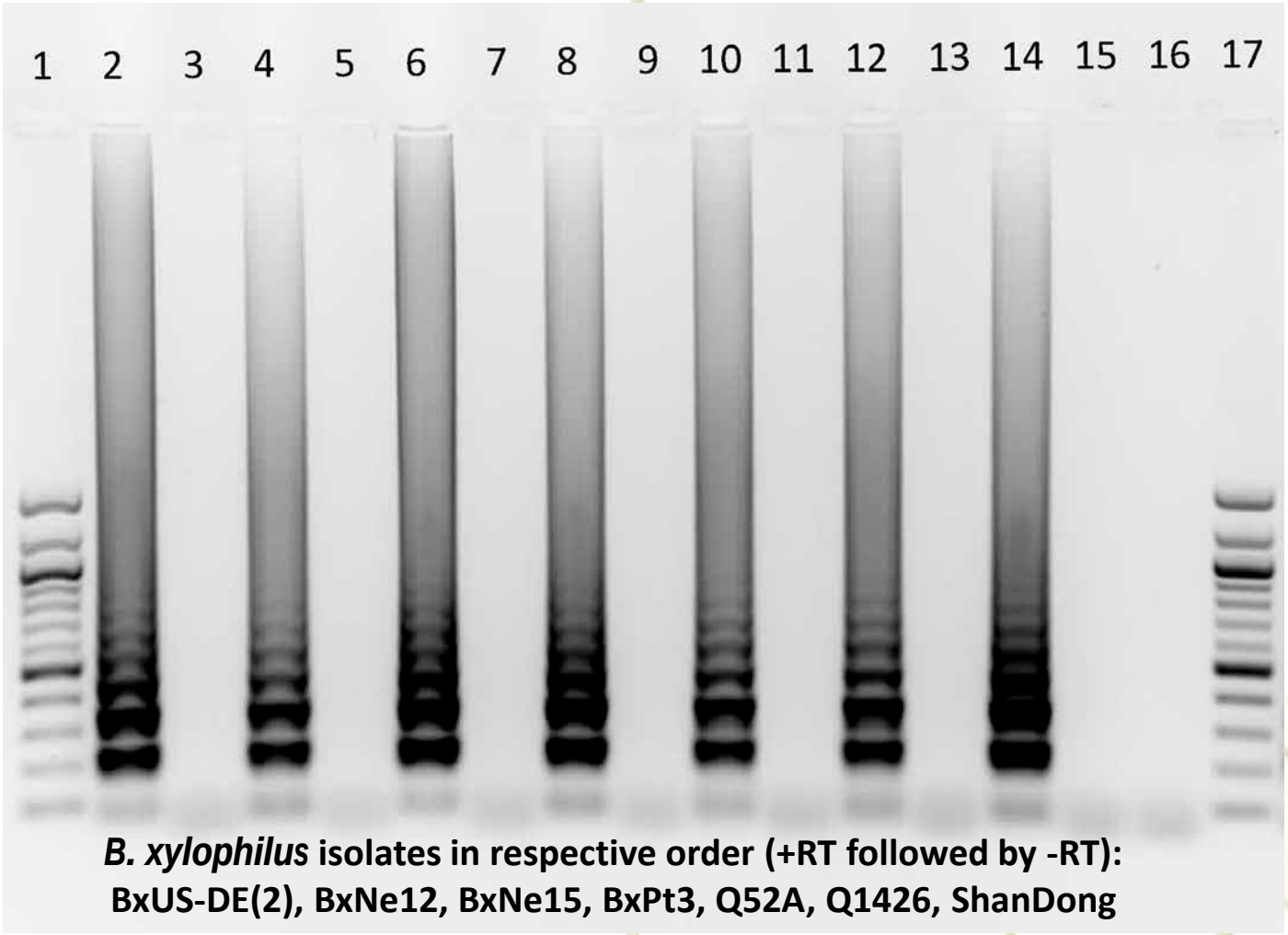


RT-LAMP specificity for cDNA:



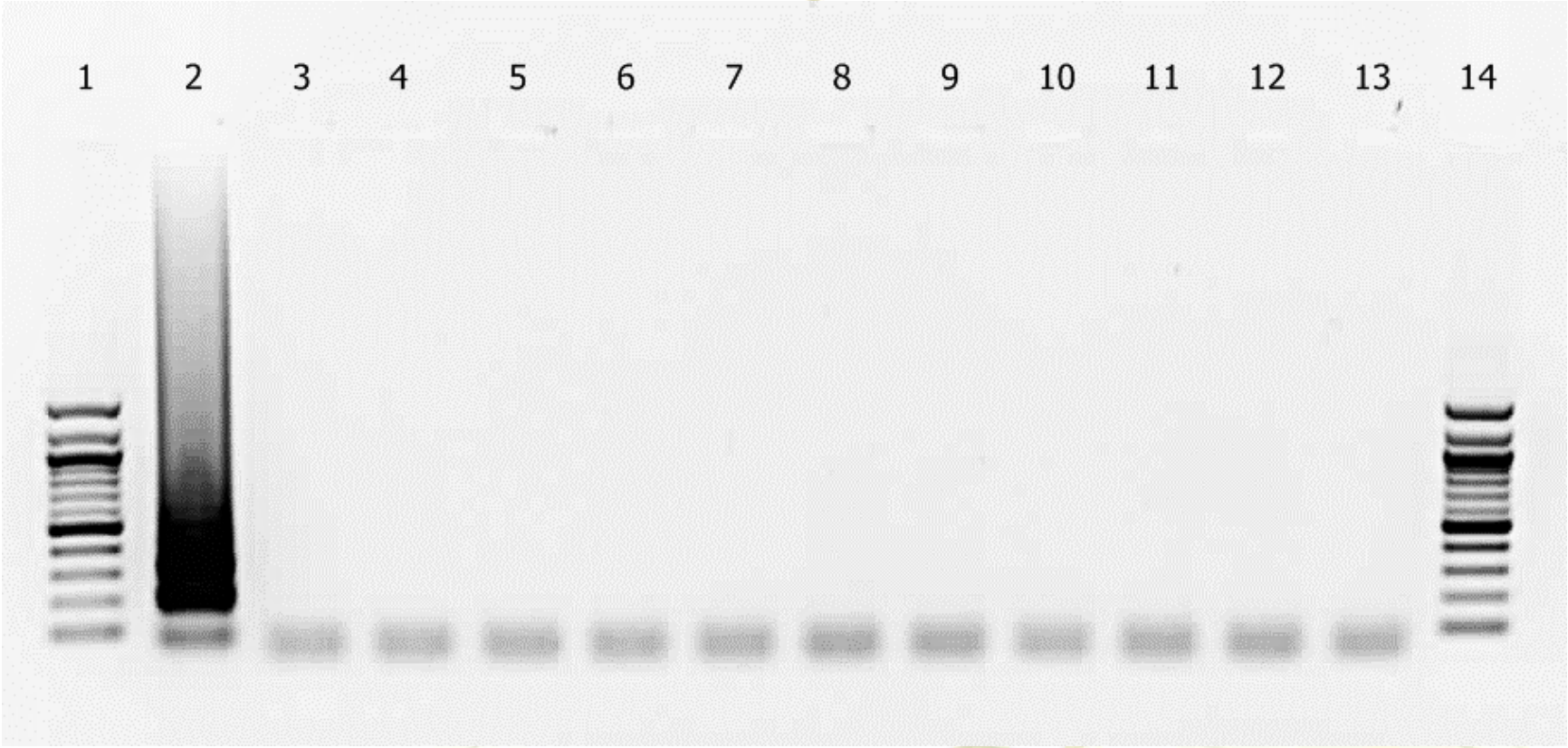
8mM $MgSO_4$, 1.4mM dNTP, 0.6 mM Betaine at 70 ^{0}C for 30' –Bst 2.0

Assay efficacy with several *B. xylophilus* isolates:



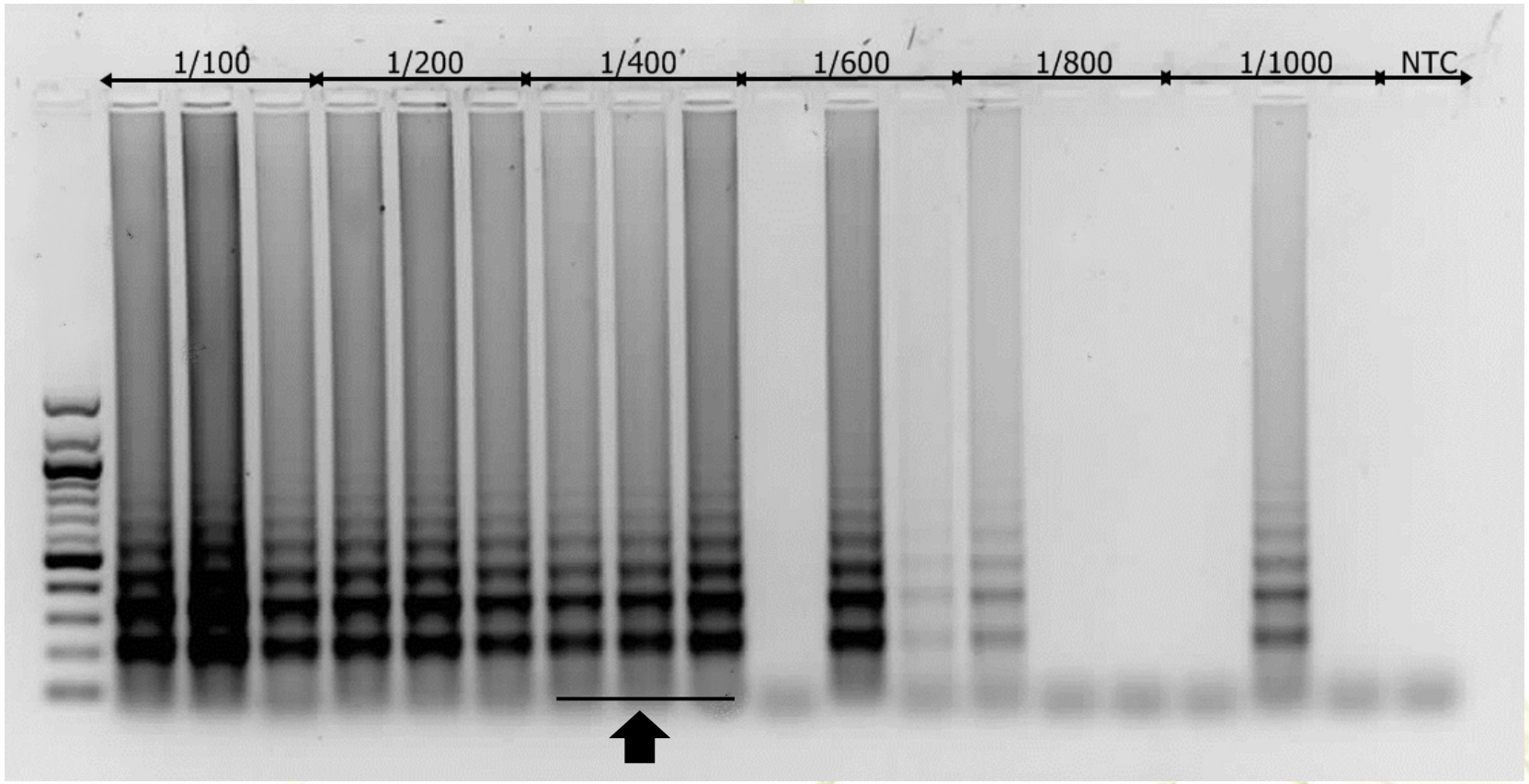
B. xylophilus **isolates in respective order (+RT followed by -RT):**
BxUS-DE(2), BxNe12, BxNe15, BxPt3, Q52A, Q1426, ShanDong

Species Specificity:



B. xylophilus **(2),** ***B.fraudulentus*** **(3),** ***B.doui*** **(4),** ***B.hoffmani*** **(5),** ***B.conicaudatus*** **(6),** ***B.thailandae*** **(7),** ***B.singaporensis*** **(8),** ***BmDE30*** **(9),** ***BmDE18*** **(10),** ***BmFin287*** **(11),** ***BmChiba*** **(12).**

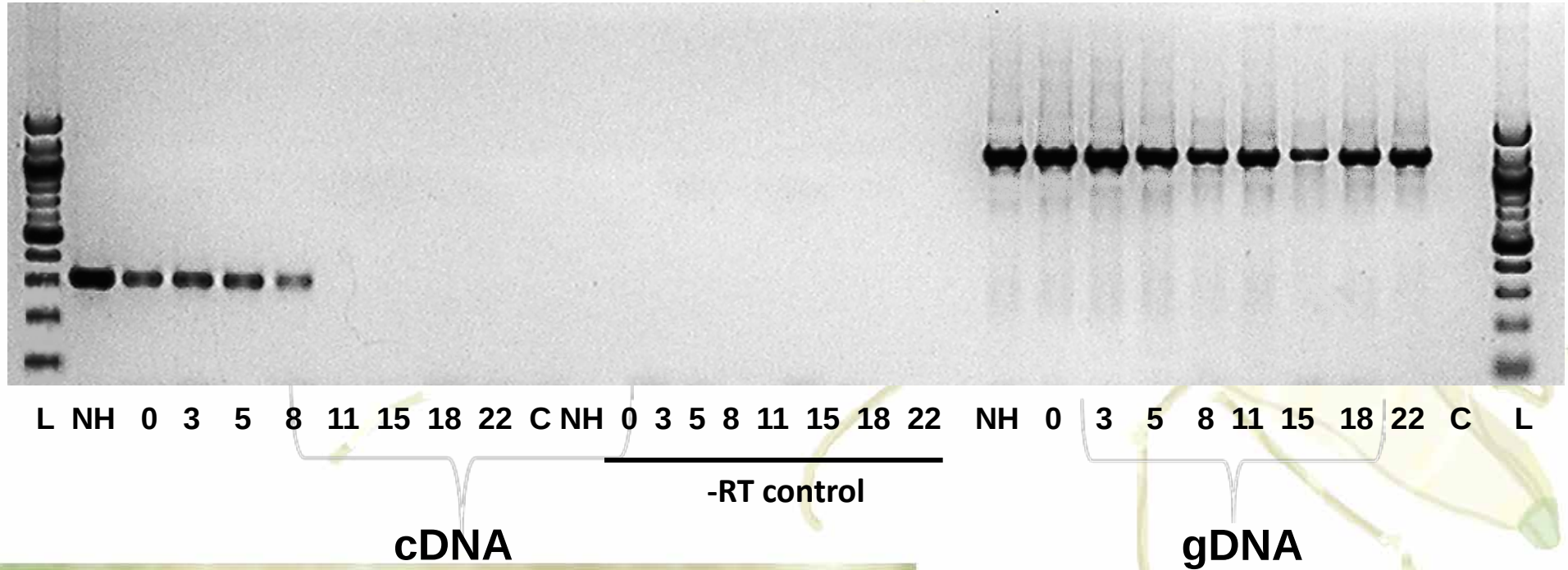
Sensitivity of the assay from PWN pure cultures (serial dilution):



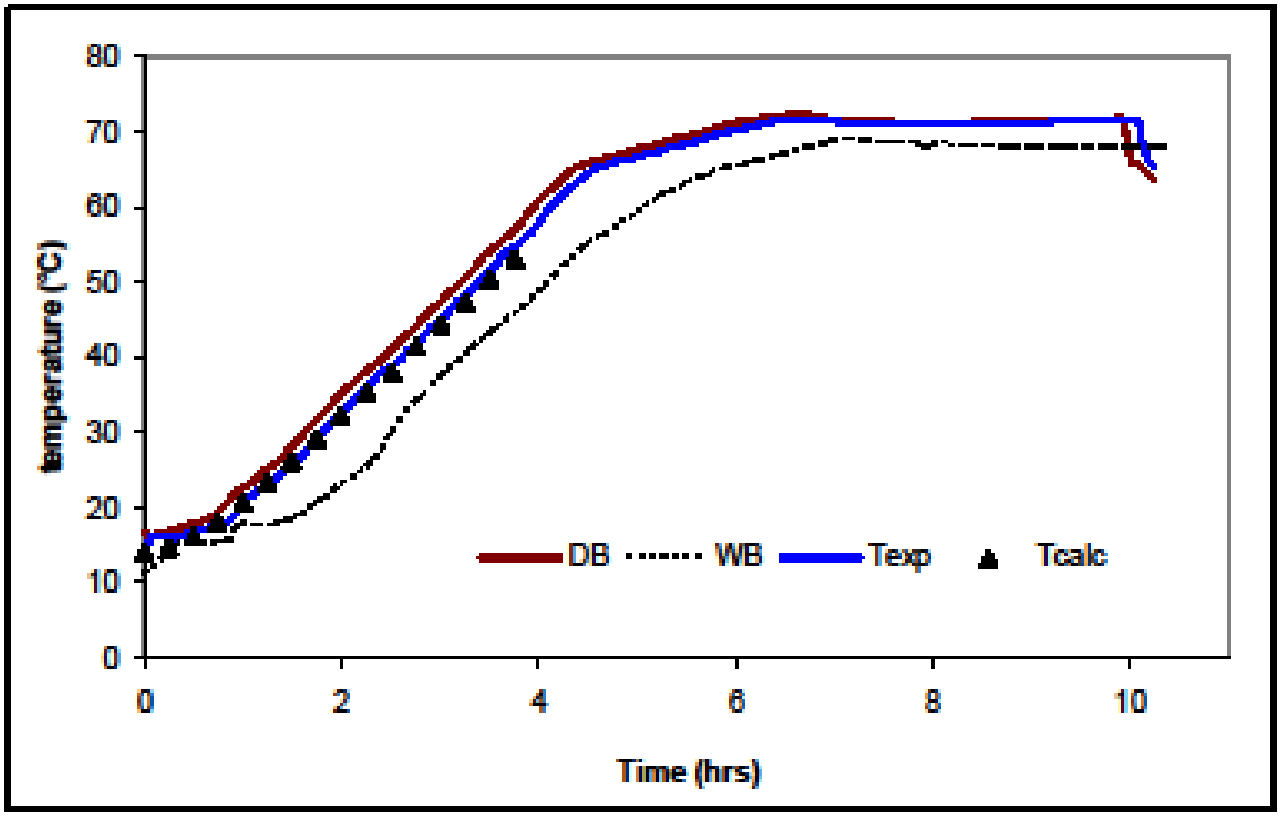
Amplification observed at the 1/400 times dilution detection: 0.25 nematodes/rxn

Time Course:

- Heat treatment of wood: 56°C for 30 minutes – kills nematodes
  - Placed at room temperature
  - Collected at time points
    - No heat (NH),0,3,5,8,11,15,18 and 22 days after heat treatment treatment

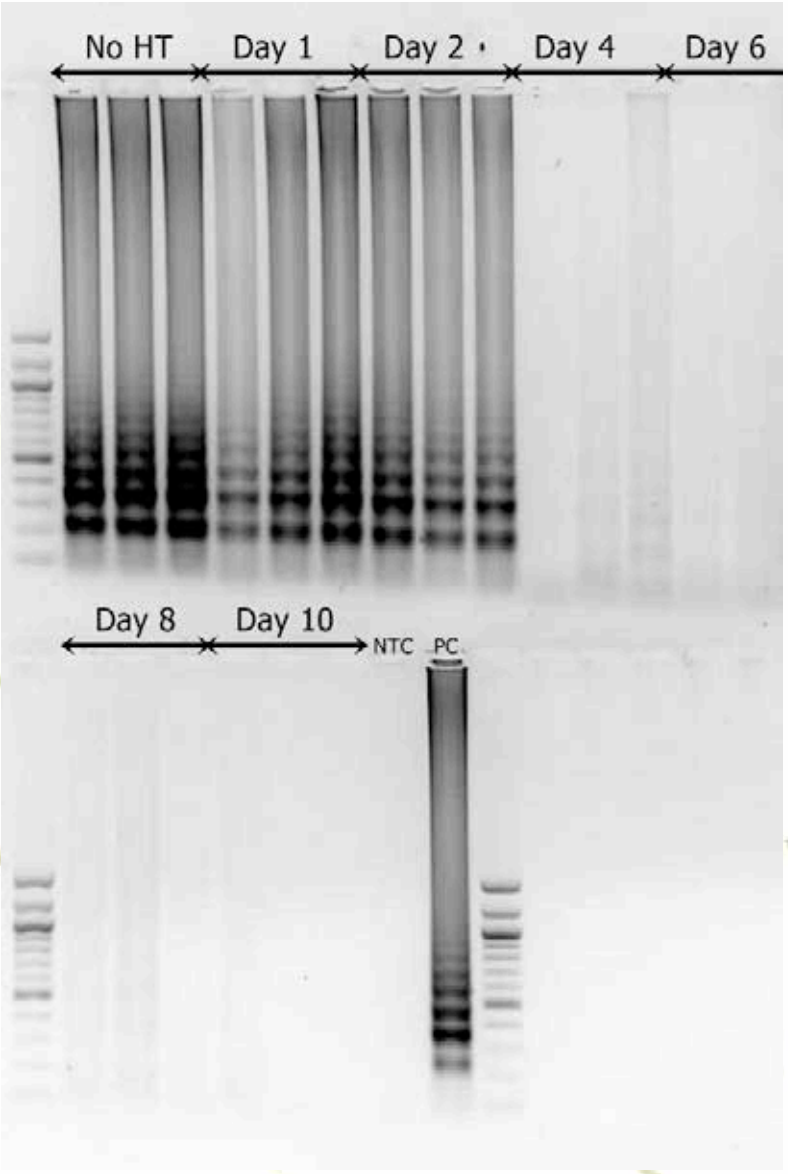


Industrial kiln-drying

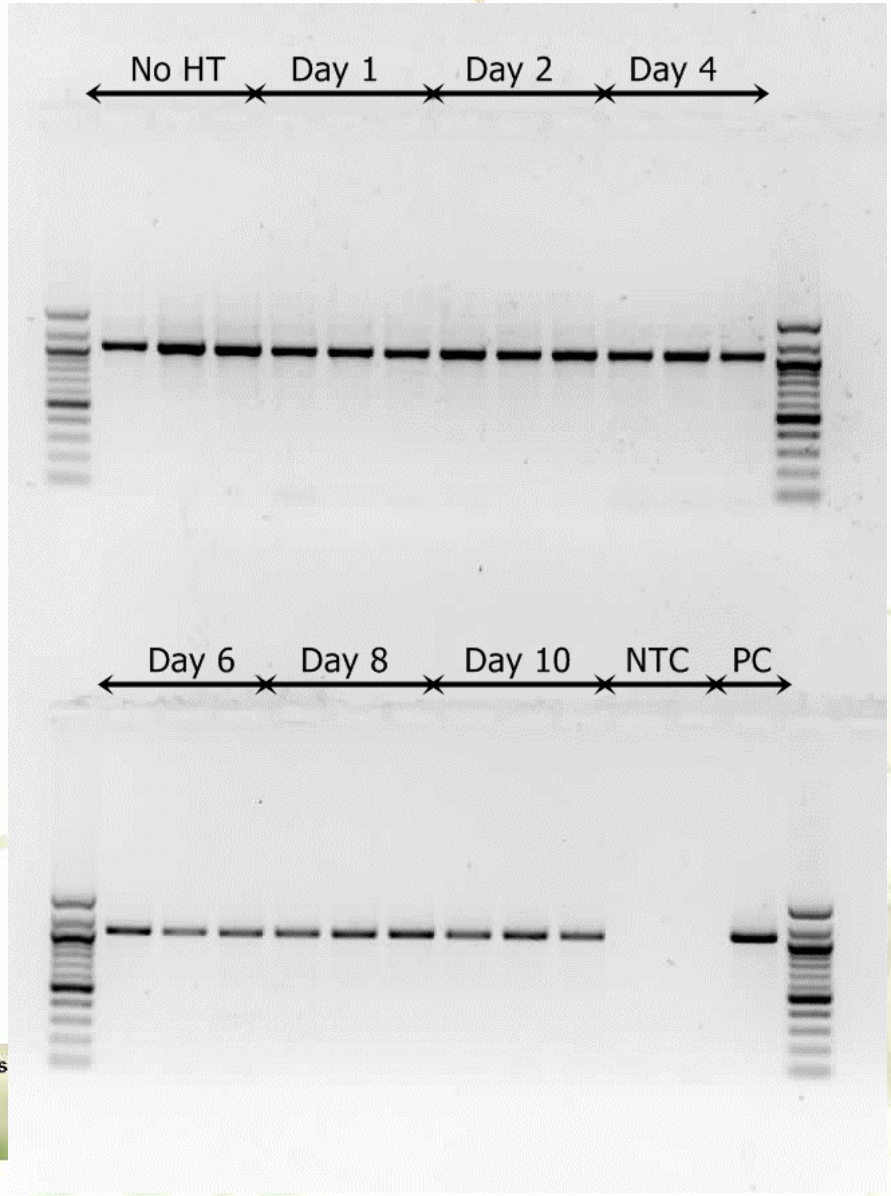


Time Course:

- Heat treatment (HT) of wood (kiln-drying schedule)
  - Included 56°C for 30 minutes – kills nematodes
  - Placed at room temperature
  - Collected at time points: No heat (NH), 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days after HT

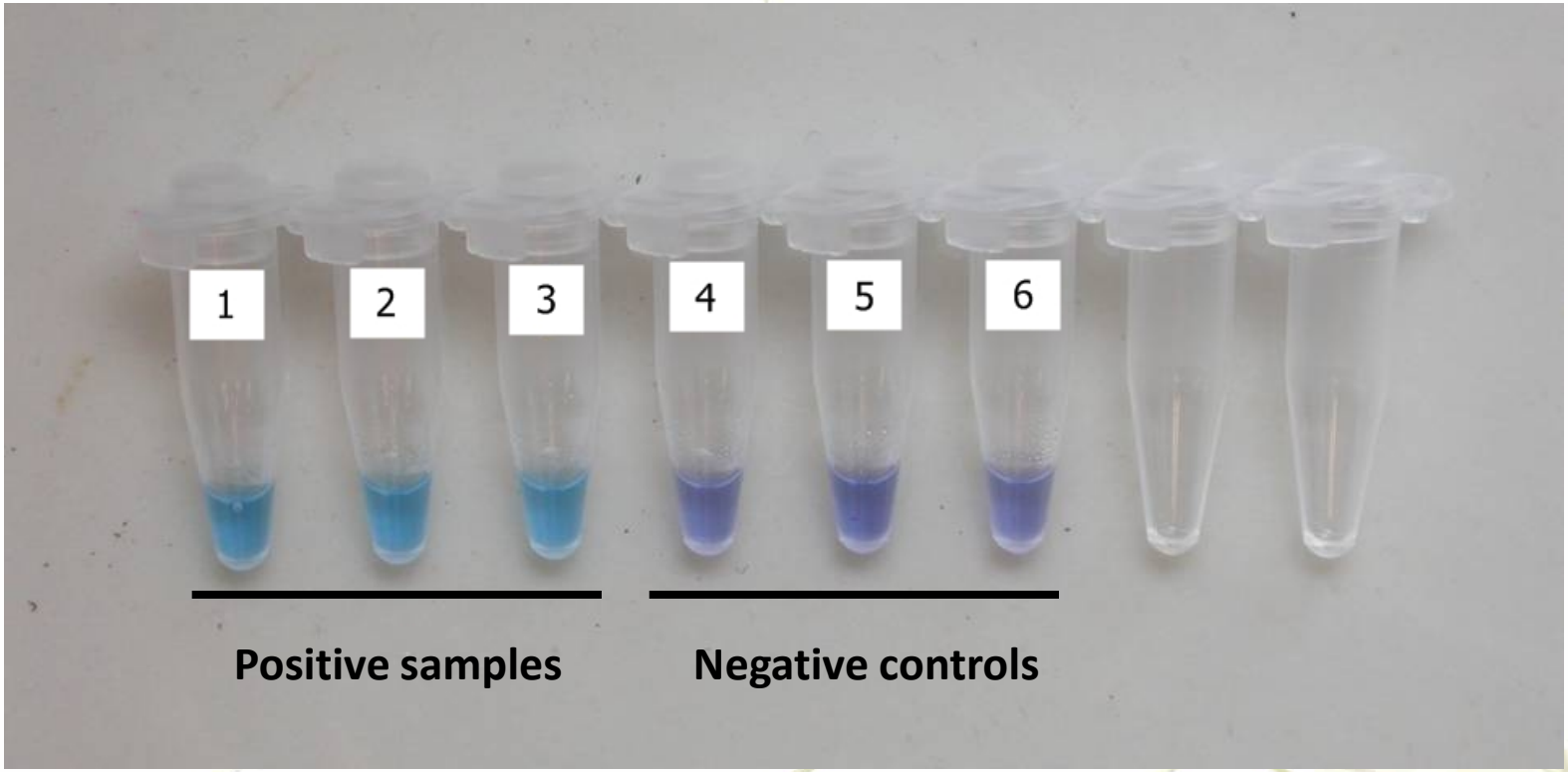


DNA is still present…….



Colorimetric Reaction:

This assay has the advantage of monitoring amplification of target cDNA by a change in colour



120 mM Hydroxyl Napthol Blue

Change from violet to blue is caused by a build-up of the magnesium pyrophosphate.

As the free $Mg2^{+}$ ions become sequestered and their concentration drops --- color change

PWN-RNA extracted directly from PWN-infected wood

RNA extraction method (modified from Chomczynski & Sacchi, 1987)

4 g of wood (mixed population of Canadian isolates of PWN):

Extraction buffer:

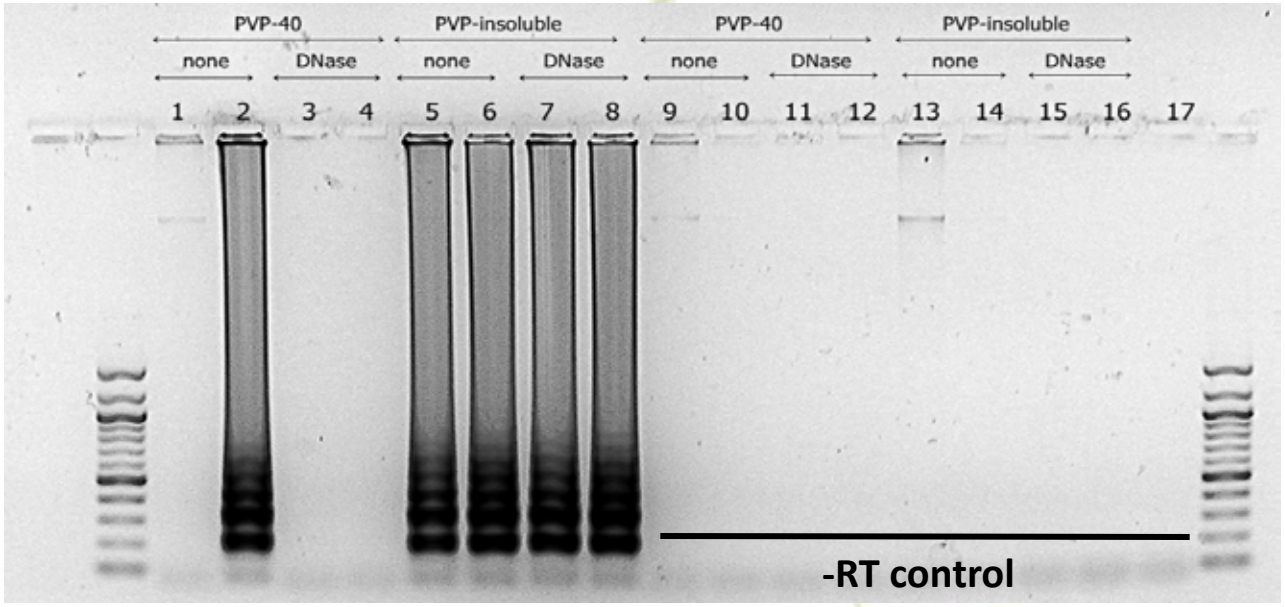
- **1.5 % PVPP (insoluble)**
- 4M guanidinium isothiocyanate
- **0.1M Na acetate**
- **0.2% sarcosyl**
- **β-ME (10uL/mL)**
- Adjusted to pH 7.0

- phenol:$CHCl_3$:isoamyl alcohol
- **Spin 10,000g for 10'**
- **isopropanol precipitation (once)**
- **Spin 10,000g for 10'**
- **EtOH wash, air dry**
- **re-suspend in RNAse-free H_2O**

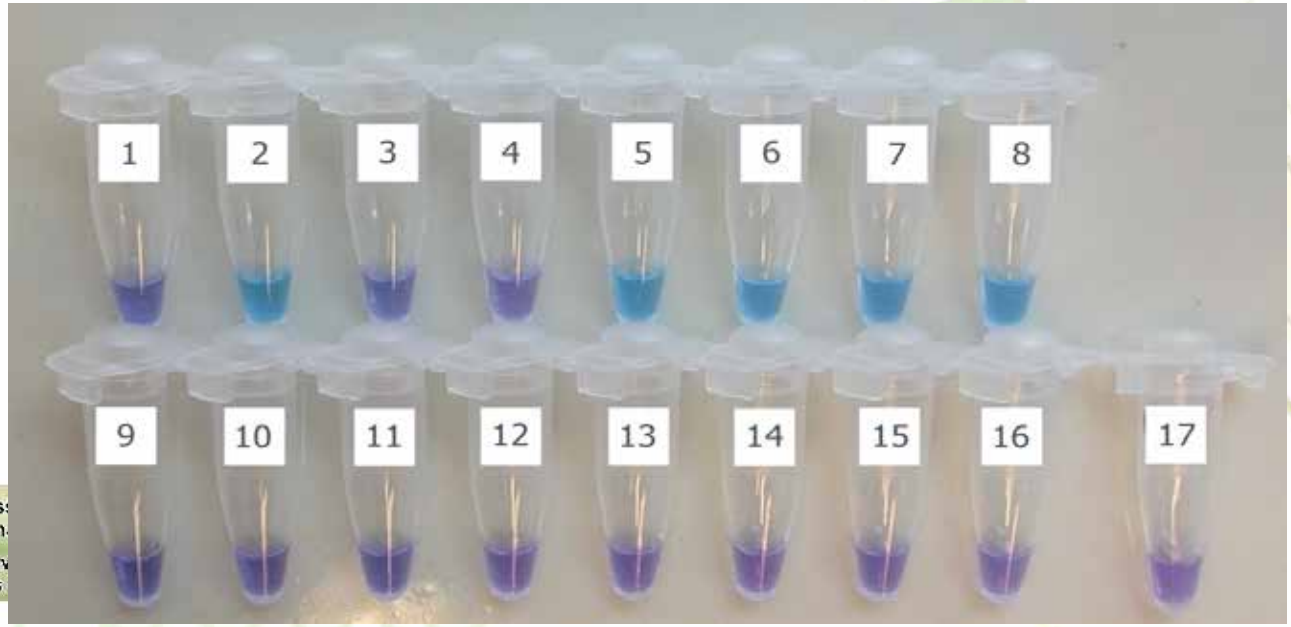
- **Relatively fast**
- **Cost-effective**

RT-LAMP assay- Validation of protocol on wood samples:

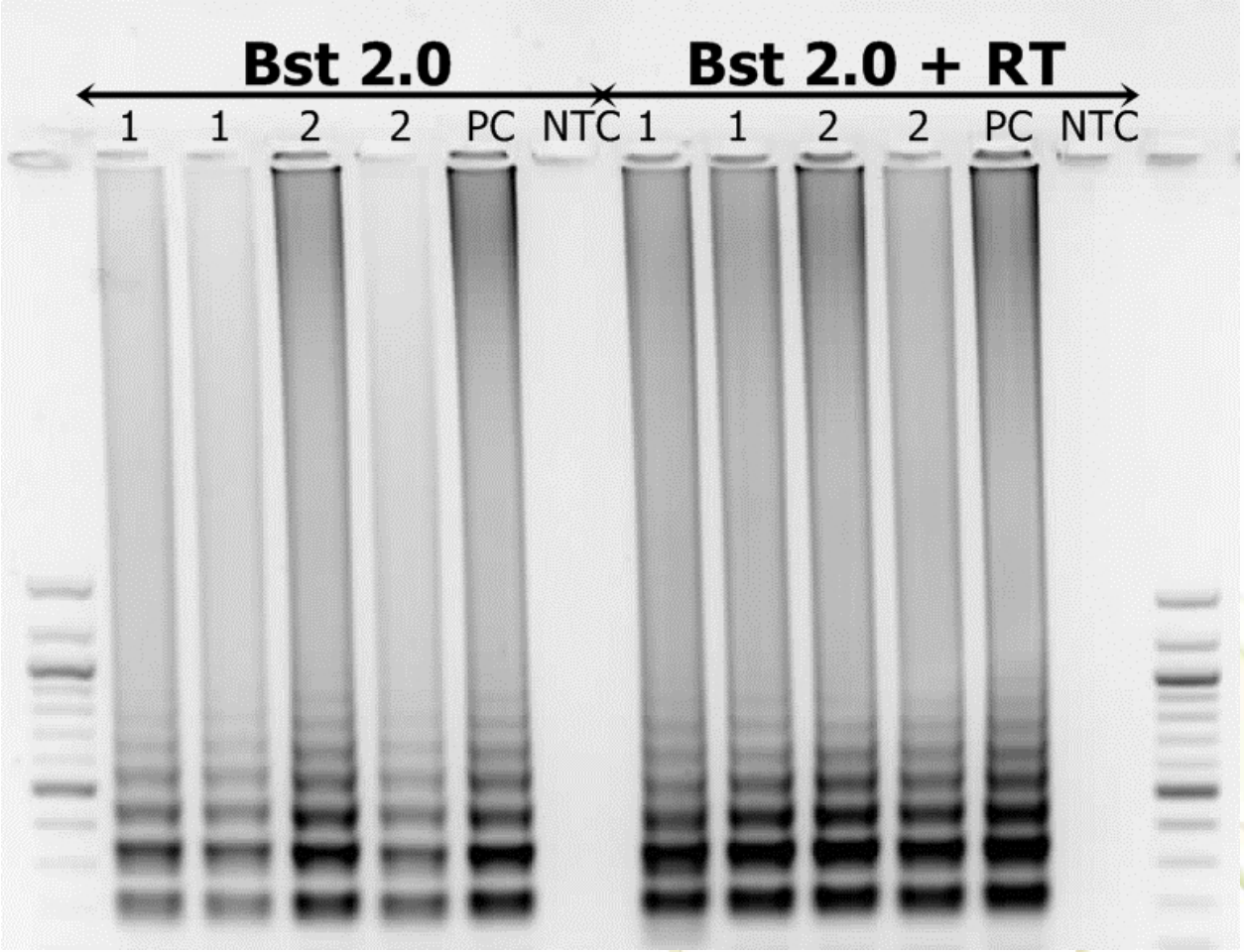
A



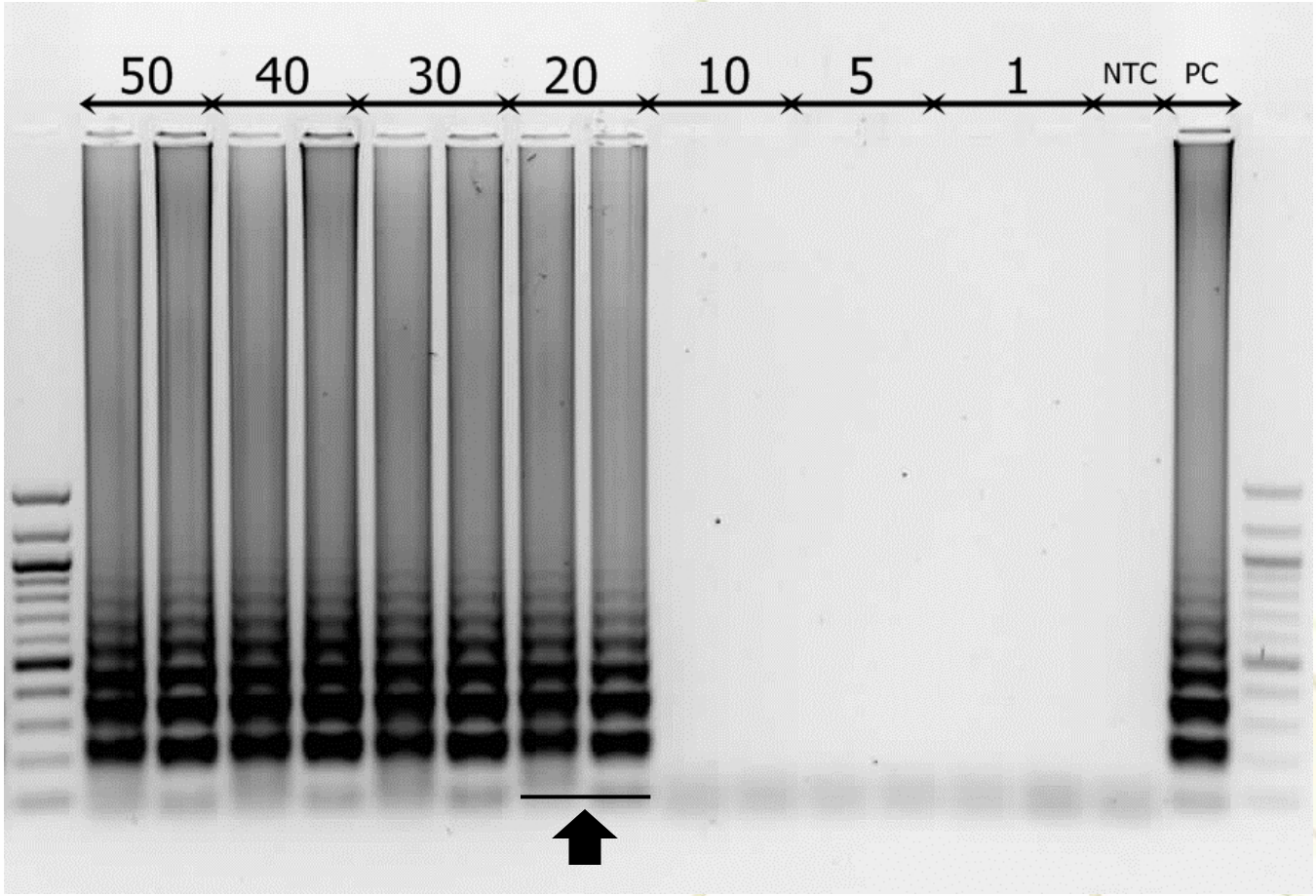
B



One-step RT-LAMP assay:



Sensitivity of the assay (LOD) from PWN-infected wood :



Amplification observed at a LOD of 20 nematodes: 0. 4 nematodes/rxn

Summary:

- **This mRNA method identifies whether wood contains live or dead PWN**

Ø **Prevent unnecessary trade disruption in internationally traded wood products**

Ø **Valuable in treatment evaluation (FPInnovations: Sulfuryl Fluoride and phosphine fumigation as alternative treatments to take the place of MeBr)**

Genome-based detection tools as a part of standardized diagnostic protocols

A. Uzunovic

IFQRG , Qingdao, China, Oct 28-Nov1, 2013

Increased interest in fungi?

- Historic focus on insects/nematodes in phytosanitary regulation
- Rapid evolution of international phytosanitary guidelines (ISPM 15 revision: appendix for evaluation of treatments , IPPC and NAPPO Standard for Wood Commodities, IPPC Plants for Planting Standard, NAPPO Standard for Christmas trees)
- More questions and concerns expressed around fungi especially in P4P

Diagnosing pathogens and challenges

- Accurate disease diagnosis and knowledge on biology and pathway are important step in effective pest management.
  - Diagnosis often based on symptomps, isolatio techniques, target known pathogens and presumed pathways
- Missed detection or misidentification can lead to infested commodity shipments being released or non-infested shipments being destroyed
- Accuracy is the key. There are many potential problems with quick identifications. There are grey zones
  - Symptoms for different diseases may be similar (e.g rotted roots)
  - Pathogens may look the same as saprotrophs.
- There are mote than 50,000 know plant diseases, variety of hosts

DNA based detection - a possible solution

Culture-based detection method

- Time consuming (culture purification)
- ~1% of microorganims are cultivable
- Need specialized taxonomist for identification
- Some species are very difficult/impossible to differentiate based on morphology
- Problematic with asymptomatic material

DNA-based detection method

- Rapid
- Sensitive
- Accessible
- Quantifiable
- Can detect pathogen on asymptomatic material
- Robust; more than one gene can be use for detection

The TAIGA Project – Started July 2011

Tree Aggressor Identification using Genomics Approaches

Richard Hamelin, Faculty of Forestry, University of British Columbia

Kermit Ritland, Faculty of Forestry, University of British Columbia

Steven Jones, Canada's Michael Smith Genome Sciences Centre

Jeremy Hall, Faculty of Business Administration, Simon Fraser University

Adnan Uzunovic, FPInnovations

André Marziali, Boreal Genomics and Physics Department, UBC

Phil Tanguay, Natural Resources Canada

Stéphan Brière, Canadian Food Inspection Agency

Canadian Food Inspection Agency

Agence canadienne d'inspection des aliments

Natural Resources Canada

Ressources naturelles Canada

BC Cancer Agency
CARE + RESEARCH
An agency of the Provincial Health Services Authority

Canada's Michael Smith Genome Sciences Centre

TAIGA - Targeted pathogen groups

- Most important pathogens of trees and crops with a history of invasiveness

Oomycetes: Sudden oak Death, Jarrah dieback, Chilean Pine Blight

Pucciniales: White pine blister rust, poplar and willow rusts

Dothideomycetes: Septoria canker of poplar, pine needle blight

Other pathogens to come of with top 50 most unwanted pathogens' list

CFIA and Sudden Oak Death

Development of a Highthroughput Molecular Diagnostic System

- CFIA started surveys and routine testing for *Phytophthora ramorum* in 2002
  - Almost 200,000 plant, soil and water samples tested
  - > 70,000 PCR, qPCR reactions performed
- Due to increasing sample load *P. ramorum* became a “test bed” for the development of a qPCR based highthroughput diagnostic system
- Current system is very flexible and is used routinely to run an increasing number of different qPCR assays

Objectives

- Assemble a culture collection and database of pathogens, including stakeholder identified 50 ‘most unwanted’ pathogens

- Develop a collection of qPCR detection assays, that will be arrayed, for the ‘most unwanted’ forest pathogens

List of target species

***Phytophthora* (15)**

Phytophthora quercina
*Phytophthora alni**
*Phytophthora cambivora**
Phytophthora cinnamomi
Phytophthora ramorum – all lineages
Phytophthora ramorum NA1*
Phytophthora ramorum NA2*
Phytophthora ramorum EU1*
Phytophthora ramorum EU2*
*Phytophthora lateralis**
Phytophthora kernoviae
*Phytophthora pinifolia**

Dothideomycetes (1)

*Phaeocryptopus gaeumannii**

Pucciniales (10)

Chrysomyxa abietis
Chrysomyxa himalensis
*Cronartium comandrae**
Cronartium comptoniae
Cronartium flaccidum
*Cronartium quercuum**
*Cronartium ribicola**
*Endocronartium harknessii**
*Melampsora allii-populina**
*Melampsora pinitorqua**

Others (24)

Amylostereum areolatum
Ceratocystis fagacearum
Ceratocystis fimbriata
Ceratocystis coerulescens complex
Ceratocystis fujiensis
Ceratocystis laricicola
Ceratocystis polonica
Chalara fraxinea
Fusarium circinatum
Geosmithia morbida
Gremmeniella abietina (EU race)
Heterobasidion annosum sensus lato
Heterobasidion irregulare
Heterobasidion occidentale
Lachnellula willkommii
Leptographium wageneri
Ophiostoma ulmi sensus lato
Ophiostoma ulmi sensus stricto
Ophiostoma himal-ulmi
Ophiostoma novo-ulmi
Rosellinia necatrix
Sclerotinia pseudotuberosa
Venturia nashicola
Xanthomonas populi

Culture collection and Assays

- Obtain and build a reference collection for the Top 50 'most unwanted' pathogens

- Choice of the taxa and cultures from recent published phylogenetic studies
  - **3-5 isolates** of the **target** species
  - **5-25 « sister »** species
  - **1-5 isolates** for each of the **« sister »** species

- Develop a collection of qPCR detection assays, that will be arrayed, for the 'most unwanted' forest pathogens

Culture collection

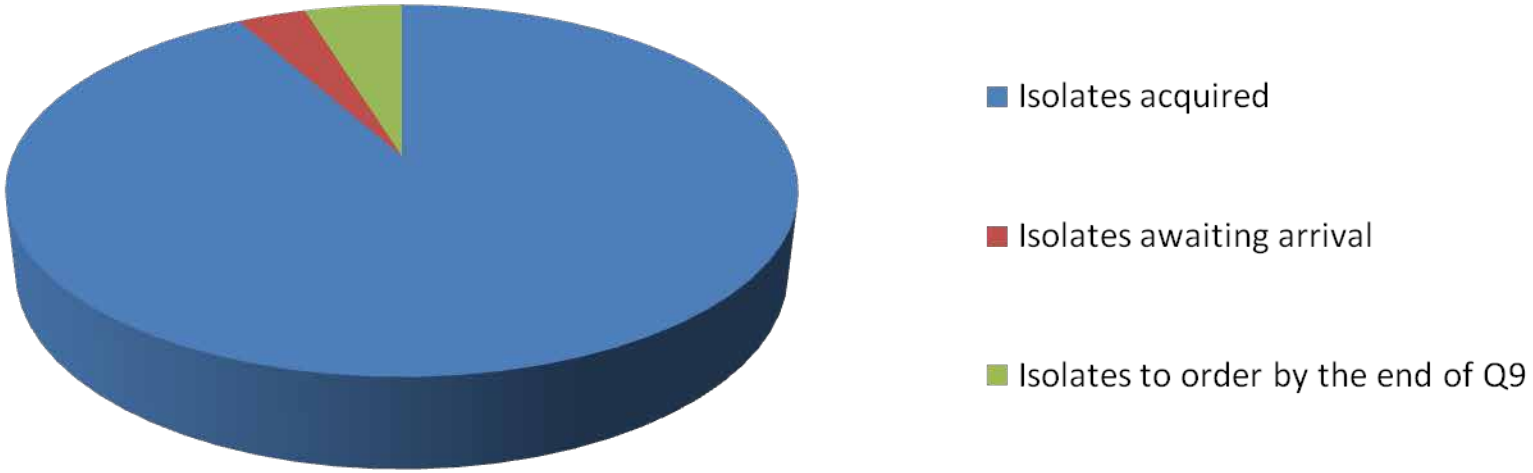
- Summary of the culture acquisition effort done so far:

Import permit applications	42
Total number of isolates	**590**
Culture collections[a]	186 (32%)
Collaborators[b]	267 (45%)
TAIGA team isolates	137 (23%)

[a] CBS, ATCC, UAMH, DAOM, World Oomycete Genetic Resource (WOC)

[b] From Canada, USA, Chile, France, Czech Republic, Switzerland, South Africa, Japan

- Isolate acquisition progress by Q8:



Update on assay development

Group of targets	No. of assays to be included	No. of assays in development	No. of culture-validated assays
Phytophthora	15	9	6
Dothideomycetes	1	1	0
Pucciniales	10	7	0
Others*	24	4	18
Total	**50**	**21 (42%)**	**24 (48%)**

* 5 more assays are at the *isolate acquisition* phase.

Conclusions

- So far, we have generated over 580 sequences (deposited in GenBank); over 600 more to be deposited by the end of Q10
- 45 / 50 of the assays to be included into the Top50 array are either culture-validated or in development
- The remaining 5 targets have been selected and lab work will be undertaken by the end of Q9

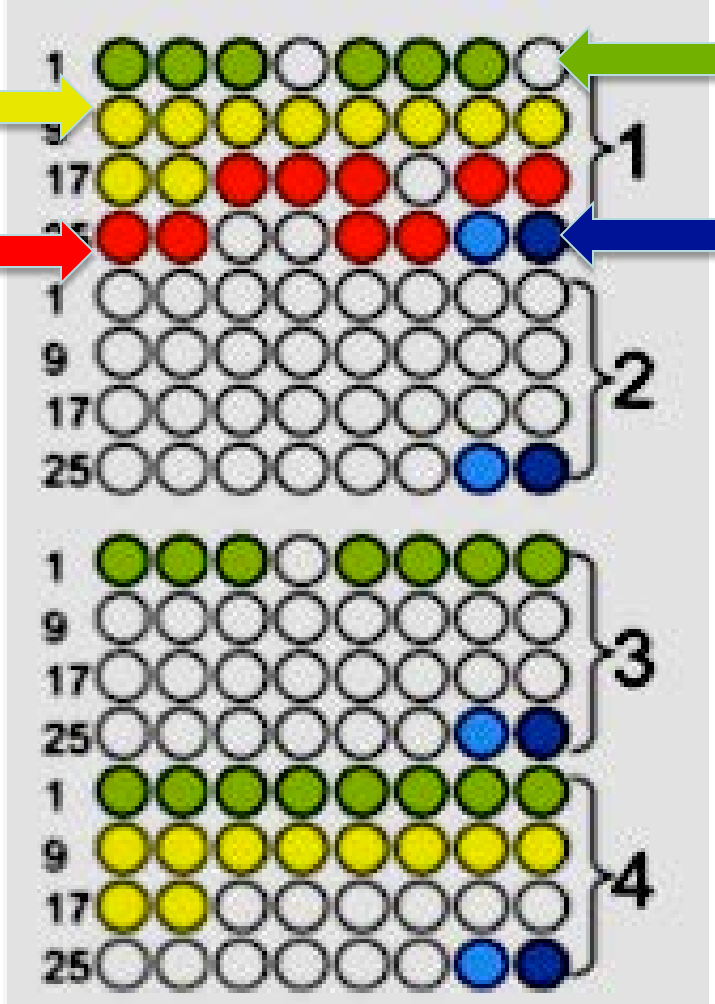
Hierarchical assays

Species probes

Pathogenicity probes

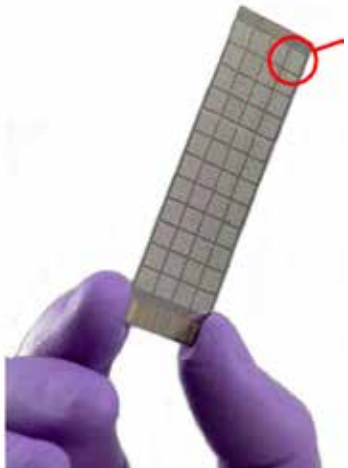
Genus probes

Control probes



Results interpretation

1) Positive for genus, species, and pathogenicity
2) Negative for genus, species and pathogenicity
3) Positive for genus
4) Positive for genus and species



Datapoints genotyping 3,072; real time PCR 2,688

Examples of DNA-based Diagnostics – Plant Protection Organizations Worldwide

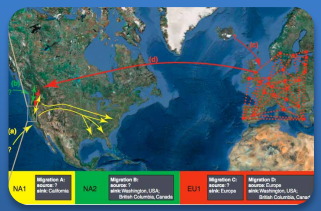
1. QBOL-EPPO Conference on DNA barcoding (2012)
2. Q-Detect – “multi-disciplinary research network focused on developing innovative tools that enhance the capacity of phytosanitary inspectors”
3. EUPHRESCO – e.g, “Development of validated procedures for whole genome amplification of DNA/RNA for quarantine plant pathogens and pests (Q-AMP)”
4. “DNA tech innovations could help solve potato scab” (FERA, UK)
5. “York scientists’ hi-tech approach to finding ash dieback” (FERA, UK)

Applications

Phytosanitary management

Phytosanitary certification

Surveys, monitoring



Pest risk assessment

TAIGA - GE^{3}Ls team review

G = Genomics *& its*
E^3 = Ethical, Economic, Environmental
L = Legal and
S = Social *Aspects*

- Determine commercialization and policy issues of pathogen detection technology using integrated framework of innovation uncertainties to explore **T**echnological feasibility, **C**ommercial viability, **O**rganizational appropriability and **S**ocial acceptability (**TCOS**) of new technology development

- **Output**: provision of early information and insights concerning costs and benefits, commercialization processes, public policy issues and social acceptance to inform technology development

http://www.genomecanada.ca/en/ge3ls/

DNA-based Sudden Oak Death Test - Acceptance in Canadian Policy

- "[We were] actually forced . . . to certify for the Sudden Oak Death, because they were growing our yellow cypress cuttings, which we produced at our site, and **they would not take them without us being certified because they, at the time, were shifting internationally and needed the certification for [the P. ramorum].**" (Nursery Representative)
- "In terms of the diagnostics, I believe Canada will be leading the way. We were the lead for the development of **sudden oak death testing [and] the DNA testing**. And **the USDA ended up taking on our test method because it was so good.** So Canada was already... we've already been leading the way with DNA-based methods for detecting forest pathogens, and I imagine we'll continue to be in the lead, especially with this project." (National Policy Actor)
- "We tended to focus on the negative, but in terms of the **diagnostic tool for Sudden Oak Death, I've been around long enough to have a couple of ministers go to standing committee for a variety of reasons, and for the last at least 3 or 4 years, as it relates to genomics, every minister has been more than willing to include that as a success story**." (National Policy Actor)
- "I'm certain [the TAIGA] project has a high chance of success . . . **we'll have to go through our regular diagnostic validation, but we can put it in place. We did it with the sudden oak death test, and I'm sure whatever comes out of this we'll be able to use as well**." (National Policy Actor)

Genomics-Based Pathogen Detection Kit: - Primary stakeholder concerns about uncertainty is a key hurdle

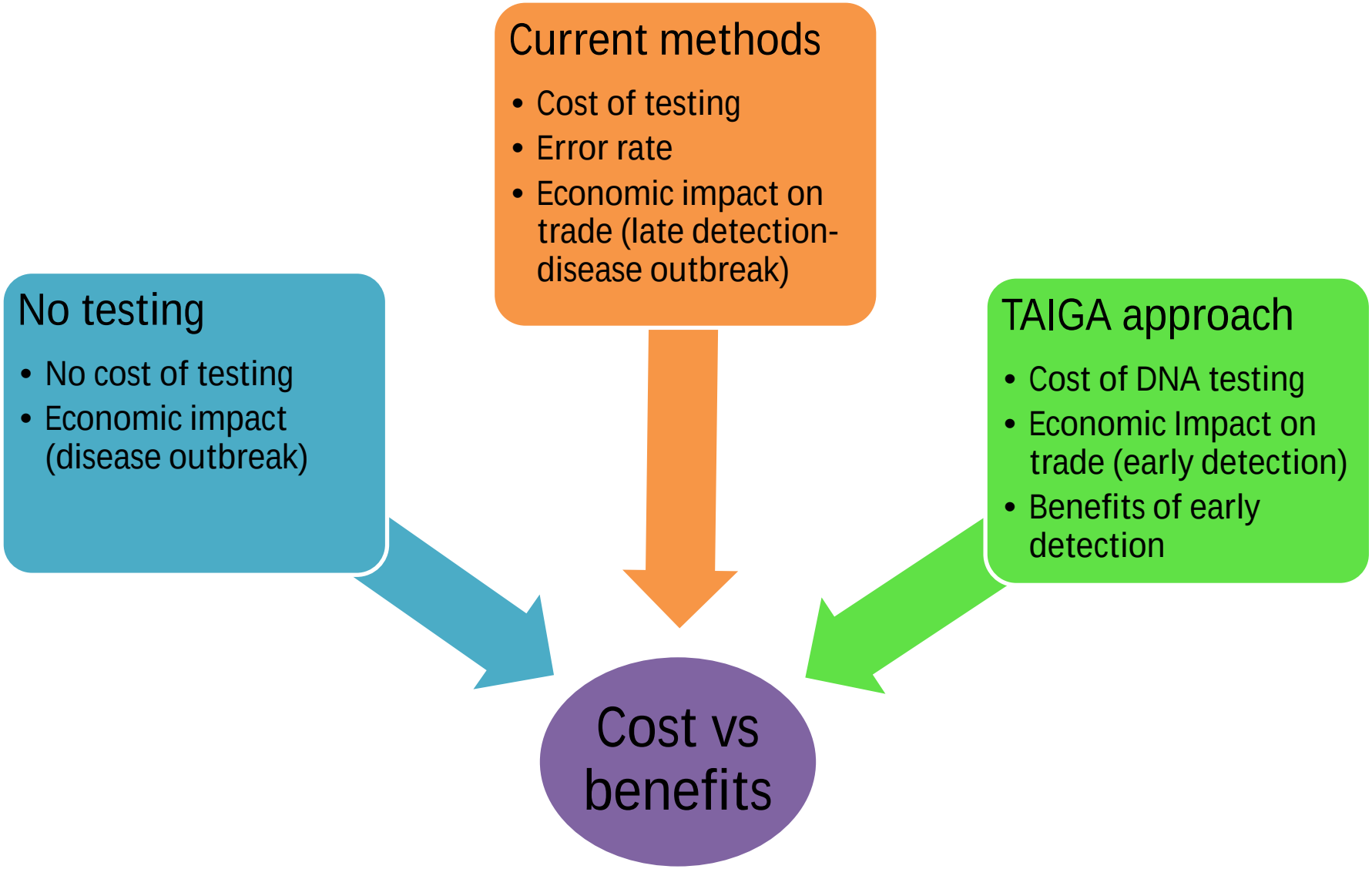
- So we usually use it as an indication that there might be a problem, and we would do further investigation, and it may need a different test. DNA might not give us the answer if we're looking to see if something's alive." (National Policy Actor)

- "[False Negatives] are an absolutely massive risk, and especially around the world of pathogens, because they're so varied, there are so many that we likely do not even know, and their ability to . . . cause problems is absolutely huge. It is a huge concern, that this would allow things in, because it wasn't one of the top [pathogens] that it was able to identify." (Provincial Policy Actor)

Genomics-Based Pathogen Detection Kit: Experts believe that simplifying risk assessments could be a key lever

- "You know, some of these pathways have upwards of a thousand species on them that we know about, an awful lot of information to go through and assessment to be done. If we can say, "Well here's a big bunch of fungi. **Let's not worry about all that. Let's just look for a particular group of pathogenic genes, and if we pick those up, we've got a problem, if we don't we're okay", it simplifies things enormously**" (International Policy Actor)

Cost benefit analysis



Nature of trade and commodities to be considered

- Plants for planting are serious threat and focus on this pathway
- Wood in trade often processed or treated (KD, HT), current inspection requirements for wood do not require pathogen ID but sooner or later someone will apply these tools to Wood
- Detection using DNA does not indicate if pathogen is alive opening a door for unnecessary regulatory actions
- Even if alive, pathways not necessarily established (e.g. pathogenic wood decay fungi need to produce conk to spread)

Bottlenecks in translating genomics from the lab into the real world

- How to manage the risk associated with the use of genomics based tools within a complex stakeholder environment, where some see that risk as a business opportunity, while others see it as a threat?

- There is a need to standardize the use of genomic based tools through Diagnostic protocols

Update on sentinel nurseries activities between Europe and Asia as tools for risk assessment

Two very different approaches with different objectives

1. Exotic plantations: To assess infestations by native pests and pathogens on exotic plants (in botanical gardens or specifically established sentinel plantations)
   - **Objective**: to detect species that are harmless on native plants but could be harmful if introduced elsewhere (e.g. EAB, HWA, Ash dieback, SOD, etc)

2. Native plantations: To plant and survey native plants commonly exported to obtain lists of potential pests that can be expected on these commodities
   - **Objectives**: To provide a tool for commodity risk assessment and test whether the presently used methodology based on literature surveys is adequate; to provide information for NPPOs of importing countries for inspection and surveillance

Use of arboreta surveys and sentinel tree plantings in Asia to identify potential forest pests in Europe

Marc Kenis
CABI Europe-Switzerland

Alain Roques
INRA Orléans, France

With

In Russia: Natalia Kirichenko, Yuri Baranchikov, Maria Tomoshevich, Svetlana Gorokhova, Pavel Ostrogradskiy
In China: Jian-tin Fan, Jiang-hua Sun
In France: Annie Yart
In Italy: Anna-Maria Vettraino and Andrea Vannini

Emerald ash borer

Hemlock and balsam woolly adelgids

Sirex woodwasp

Chestnut blight

Dutch elm disease

Sudden oak death

Photos: Forestry Images

Two methods to identify potential alien Asian forests pests and their threat to Europe before their introduction:

- Inspection of European trees and shrubs in Arboreta and other plantations (Asian Russia – CABI and RAS)

- Exposure of European sentinel trees (China – INRA and CAS)

Inspection of European trees and shrubs in Russian Arboreta

Pathogens:

Dr. Maria Tomoshevich, plant pathologist of the botanical garden of Novosibirsk has recorded hundreds of fungal foliar pathogens on trees/shrubs (incl. European spp.) in recent years, with levels of damage

Pathogens:

Tomoshevich et al (2013). Foliar fungal pathogens of European woody perennials in Siberian arboreta and cities: an early warning of new encounter diseases? Forest pathology.

- 106 symptomatic infections
- 75 fungal species
- 56 woody plants
- Issues:
  - Identification – geographic distribution
  - Only foliar pathogens

Insects – own surveys:

- In 2008-2010: Surveys in Siberia

- All European trees and shrubs are inspected 1-4 times /year
- Any particular conspicuous damage of the tree or shrub is noted
- If a pest is found:
  - It is collected and identified
  - We investigate among foresters and arboreta managers if the pest is common
  - We check in the literature and on neighbouring native congeneric if the species if common

Insects – own surveys:

- In 2008-2010: Surveys in Siberia
- Many insects found (mainly foliar)
- Some identified, some not

Ypsolopha chazariella

But:

Climate not suitable for many European plants

Inspection of European trees and shrubs in Russian Arboreta

Surveys in Russian Far East 2010-2011

Mountain-Taiga Station Ussuriysk

Surveys in Russian Far East 2010-2011

Ussuriysk – *Fraxinus* spp.

Surveys in Russian Far East 2010-2011

Ussuriysk – *Juglans* spp.

J. ailantifolia (=*sieboldiana*) (Japan – Sakhalin)
Dying
J. major (North America)
Dying
J. regia (Central Asia - Europe)
Dead

Historical data: much mortality in *Juglans* spp. And *Carya* spp. New plantations.

Local *J. mandshurica* and hybrid *J. mandschurica x regia* are fine !

Surveys in Russian Far East 2010-2011

Ussuriysk – *Juglans* spp.

Many factors?

Surveys in Russian Far East 2010-2011

Other interesting cases

Pinus contorta

•All planted trees died at the age of 50-60 years

•Unknown reason

Forestry images

Surveys in Russian Far East 2010-2011

Other interesting cases

Pinus banksiana

•All planted trees died at the age of 20-30 years

•Last dead one examined:

- *Pissodes* sp.
- Cerambycidae
- Cryphalus piceus (Scolytidae)

Surveys in Russian Far East 2010-2011

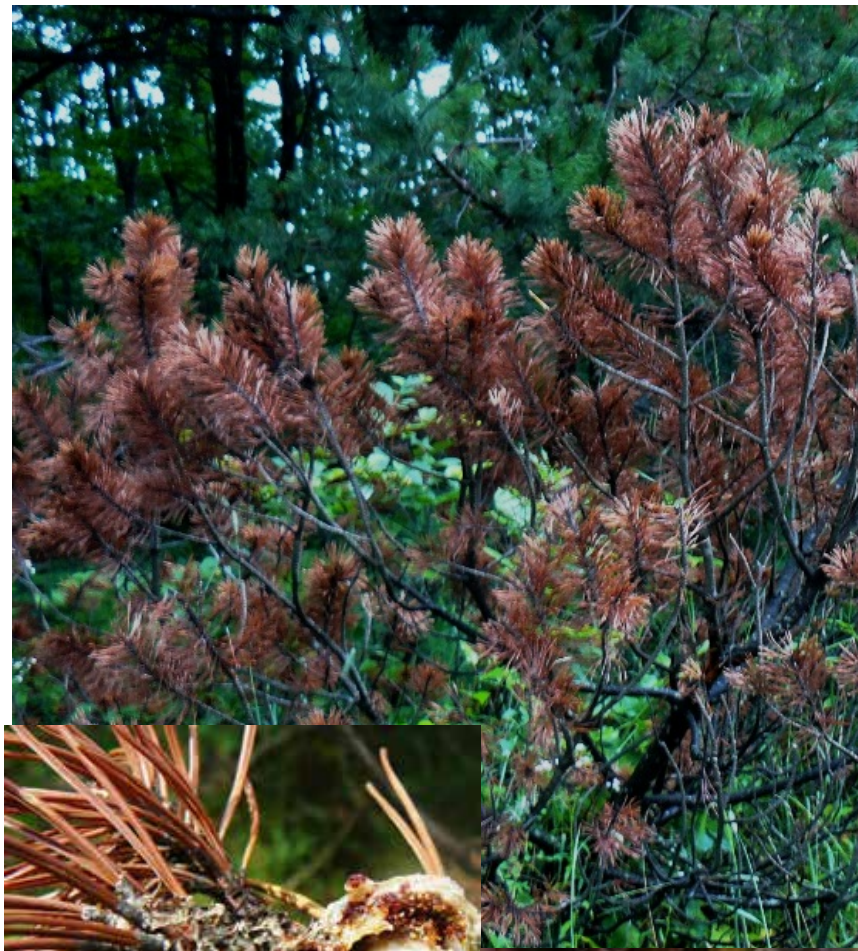
Other interesting cases

Pinus mugo

- Several 40 year-old plants dying, also in Siberia

- *Tomicus minor* found mining in Siberia

- Other bark and wood boring beetles and aphids present



Inspection of European trees and shrubs in Russian Arboreta

Conclusions

Arboreta are good tools to identify future forest pests (*Fraxinus*, *Juglans*, *Acer tataricum*, …)

But …

Punctual surveys from abroad have severe limits

- Expensive
- Often badly timed
- Lethal pests and seedling pests are easily missed

Sentinel tree experiments in China

5 trials planned (E,W, N, S, C)- 2 trials realized

Beijing suburban area
Nursery zone
Continental conditions
May 2007

Fuyang, nr. Hangzhou
Forest area
Warm and humid climate
May 2008 & May 2009

Long-term survey funded by several EU projects:
ALARM (2008- 2009)
PRATIQUE (2008- 2012)
ISEFOR (2011- 2014)

7 species of broadleaved and conifers but no pines (forbidden !)

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

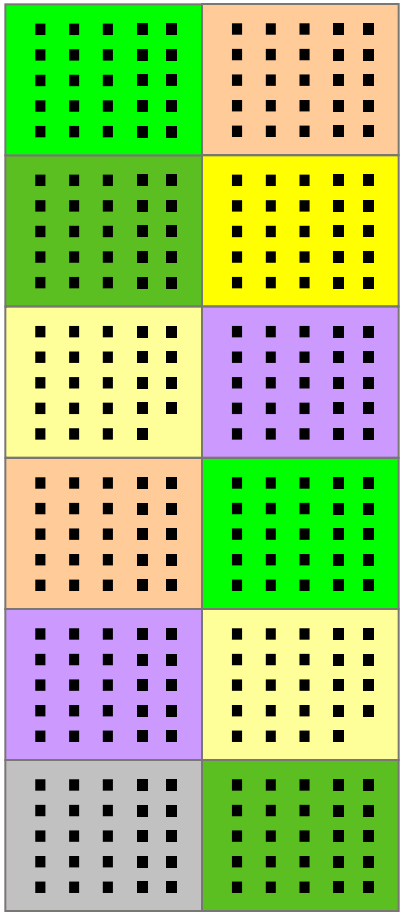
100 seedling per species,
Initial height: 1m-1,5m

Beijing
4 spp

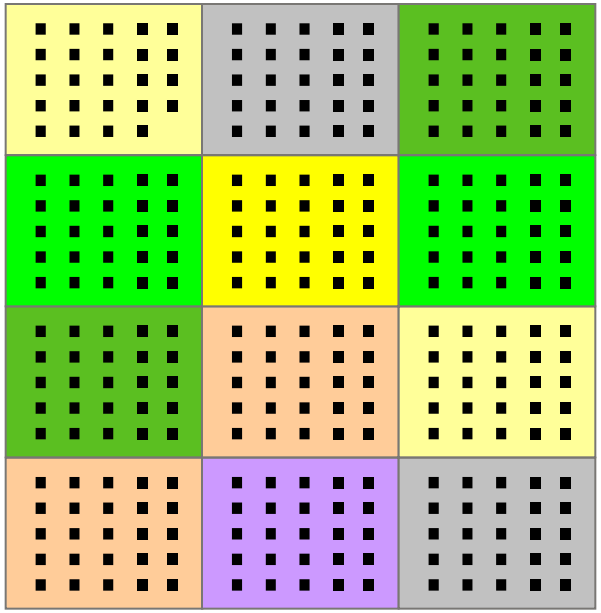
Fuyang
7 spp

Experimental design for statistic analysis of colonization by insects and pathogens

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



Fuyang



Random planting of blocks of 25 seedlings

All seedlings individually tagged

Survey of seedling colonization

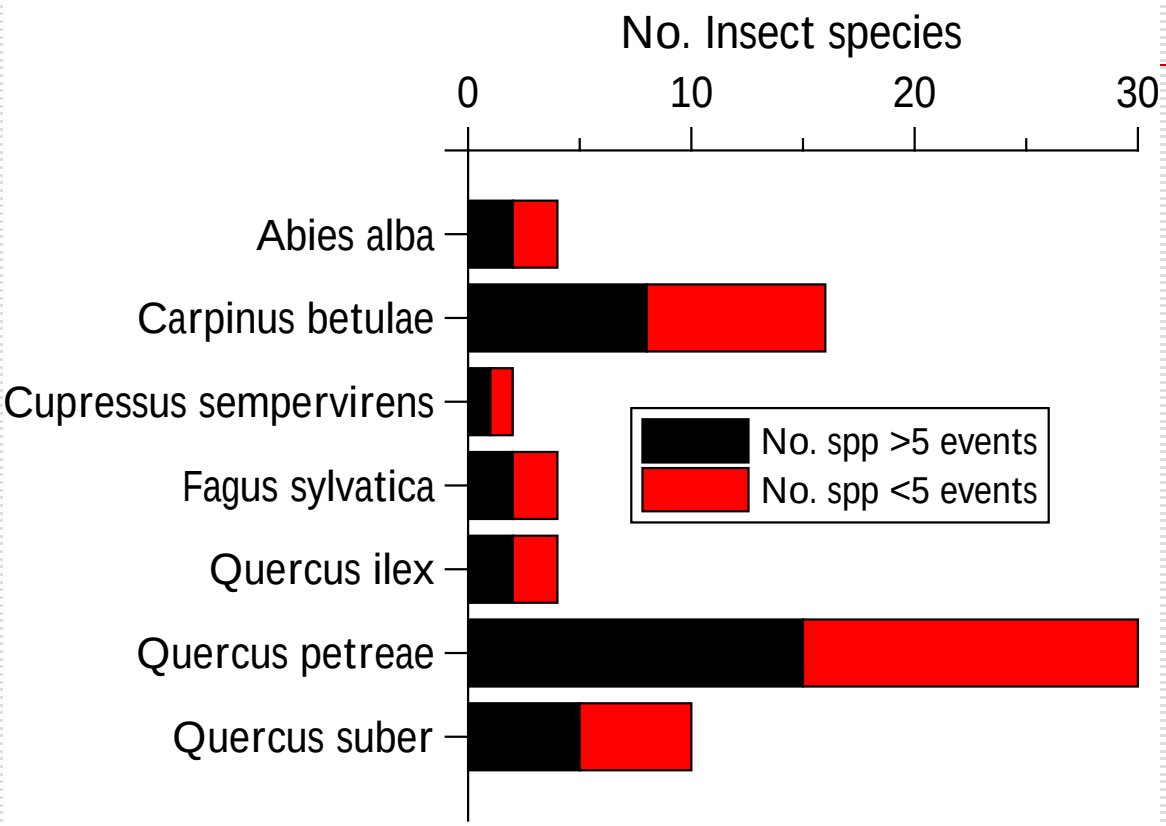
•Survey of all seedlings every 2 weeks May to Oct

• Visual + beating/ net

• Damage quantitatively assessed per seedling (leaves, buds, branches and trunk)

- •Definition of damage types
- •Adults collected, put in alcohol 95 for tentative identification and further DNA barcoding
- •Insect larvae: half in alcohol 95 (barcoding), half reared *in situ* to adults in cages
- •Insect damage without larva/ adult: photo & referred to a damage type
- •Fungi damage, collected for rearing and identification in China
- •Seedling obviously dead, cut and reared at lab

Species with > 5 colonization events
39 species



Quercus petreae significantly more colonized

List of 39 potential threats to European trees

A first dataset of 39 potential Asian pests of selected woody plants not yet introduced into Europe

Species	Order	Family	European host (insect stage)	Native Host
***Aeschyntelus sparsus* Blote**	Hemiptera	Pentatomidae	Quercus (A)	field crops
***Altica cirsicola* Ohno**	Coleoptera	Chrysomelidae	Quercus (A), Cupressus (A)	Thistles
***Basilepta fulvipes* (Motschulsky)**	Coleoptera	Eumolpidae	Quercus petreae (A)	Alnus, Populus
***Calomycterus obconicus* Chao**	Coleoptera	Curculionidae	Quercus (A)	Polyphagous
***Cletus tenuis* Kiritshenko**	Hemiptera	Coreidae	Carpinus (A)	Legume trees
***Compsapoderus continentalis* Legalov**	Coleoptera	Curculionidae	Quercus petreae (A), Carpinus (A)	?
***Dolycoris baccarum* L.**	Hemiptera	Pentatomidae	Carpinus (A)	field crops
***Echinocnemus squameus* Billberg**	Coleoptera	Curculionidae	Quercus petreae (A)	Rice
***Eysarcoris guttiger* Thunberg**	Hemiptera	Pentatomidae	Carpinus (A)	field crops
***Holotrichia diomphalia* Bates**	Coleoptera	Scarabaeidae	Quercus (L/A), Carpinus (L/ A), Abies (L), Fagus (L), Cupressus (L)	Azadirachta, Prosopis, Ziziphus, Populus
***Holotrichia titanus* Reitter**	Coleoptera	Scarabaeidae	Quercus (L/A), Carpinus (L/ A), Abies (L), Fagus (L), Cupressus (L)	Azadirachta, Prosopis, Ziziphus, Populus
***Holotrichia trichophora* Fairm.**	Coleoptera	Scarabaeidae	Quercus (L/A), Carpinus (L/ A), Abies (L), Fagus (L), Cupressus (L)	?
Pteroma nr. pendula	Lepidoptera	Psychidae	Quercus (L), Carpinus (L)	?
***Lema coronata* Baly**	Coleoptera	Chrysomelidae	Quercus petreae (A)	Commelina communis
***Mimela splendens* (Hope)**	Coleoptera	Rutelidae	Quercus petreae (A)	

Next steps:

- Confirm impact under quarantine conditions
- Check survival during transport
- Check establishement possibilities (weather, ...)

The top species

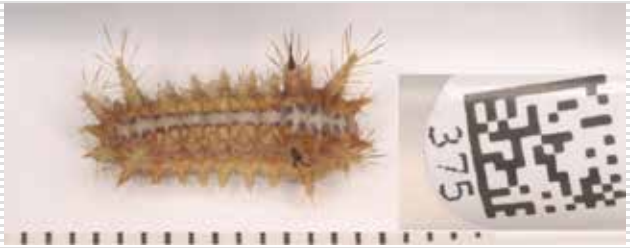
Holotrichia titanis
Holotrichia diomphalia

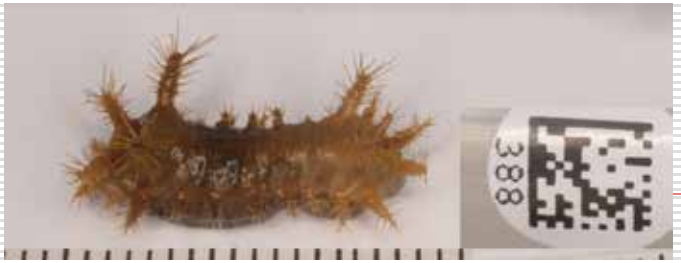
Compsapoderus continentalis

Pteroma nr. pendula (positive tests under quarantine conditions)

Taxonomic identification is a major problem

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





Sytematic barcoding of all morphospecies

- All the sampled larvae genetically analyzed (COI mtDNA barcode gene + nuclear ITSs)
- Tentative match with genetic databases (GeneBank and others)
- Allow to identify 6 more species with > 99% match
- Good for moths and sawflies, much less for other groups less sequenced
- Supply Q-databases (QBoL): a tool for phytosanitary inspectors

Tussock moth larva tracked to *Olene* sp.

Psychidae tracked to *Pteroma nr. pendula*

Most switched from field crops, few from broadleaved trees

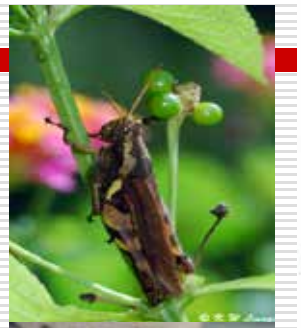
among yet identified species but many leps missing

Xenocatantops brachycerus
Host: *Ligustrum*

Lema diversa
Host: *Commelina communis*

Basilepta fulvipes,
Host: *Alnus, Populus*

Echinocnemus squameus
Host: rice



Holotrichia (3spp.)
Host: *Azadirachta*, *Prosopis, Ziziphus, Populus*

Lema coronata
Host: *Commelina communis*

Cletus tenuis
Host: legume trees

Calomycterus obconicus
polyphagous

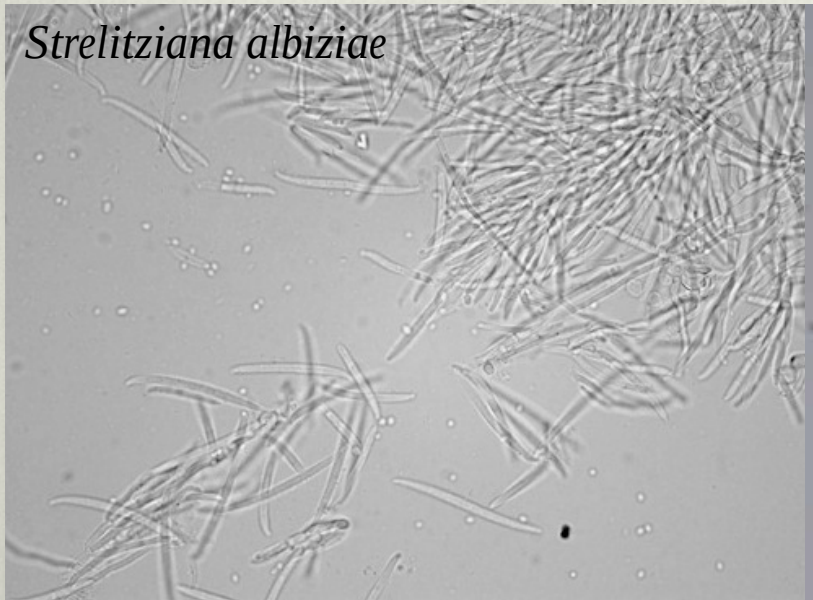
Pathogens detection

- Assessment and description of signs and symptoms

- Morphological assessment of fungal isolates

- Molecular analysis:Metagenomics using 454- next generation sequencing (NGS) technology

Results
Morphological analysis of cultures



Results- NGS

- 19.752 reads after the quality filtering, retained for further analyses
- A total of 127 MOTU

13 fungal species never reported in Europe

N°	OTUs	Genera/species	*Quercus petreae*	*Quercus suber*	*Carpinus betulae*	*Cupressus sempreviren s*	*Quercus ilex*	Geographical distribution
1	117	*Ascomycota sp. MLM4*						China
2	88	*Cf. Ramichloridium /Mycospherella-unbcertain species*						South Korea/China
3	48	*Cryptococcus sp. GT-309*						China
4	91	*Cryptococcus sp.3*						China
5	4	*Erysiphe quercicola*						Japan
6	77	*Lalaria sp. JS-42*						Korea
7	102	*Leptosphaeria sp. South-west0034*						China
8	38	*Mycosphaerella sp. Ponipodef 06*						China
9	83	*Sporobolomyces symmetricus*						China
10	25	*Strelitziana sp.1*						South Africa- Australia- China
11	96	*Strelitziana sp.2*						South Africa- Australia- China
12	110	*Xylaria/Nigrospora-uncertain species*						China/Thailand
13	27	*Xylariales sp. JP150*						Japan

Comparison of the two methods:

	Arboreta	Sentinel trees
Logistics	- “Simple”	- Complicated
No. of plant species	- Many	- Few
Statistics	- Poor	- Robust
Weaknesses	- No seedling pests	- No mature tree pests
		- Mostly foliage pests
	- Lethal pests difficult to assess	- Travel and plantation stress

→ **Complementary methods**

Comparison of the two methods:

	Arboreta	Sentinel trees
Logistics	- "Simple"	- Complicated
No. of plant species	- Many	- Few
Statistics	- Poor	- Robust
Weaknesses	- No seedling pests	- No mature tree pests
		- Mostly foliage pests
	- Lethal pests difficult to assess	- Travel and plantation stress

→ **Both require strong local links !**

Comparison of the two methods:

	Arboreta	Sentinel trees
Logistics	- "Simple"	- Complicated
No. of plant species	- Many	- Few
Statistics	- Poor	- Robust
Weaknesses	- No seedling pests	- No mature tree pests
		- Mostly foliage pests
	- Lethal pests difficult to assess	- Travel and plantation stress

→ **Arboreta network!**

New Project

EUPHRESCO II

Establishing the basis for an International Plant Sentinel Network (IPSN) as an early-warning system for future pest threats

Coordinator: Food and Environment Research Agency (UK)
Partners: Julius Kühn Institute (D); Plant Protection Service (NL), Università degli Studi della Tuscia (IT)
Subcontractors: CABI; Botanic Gardens Conservation International, Forest Research (UK)

Contact: Charles Lane (FERA): charles.lane@fera.gsi.gov.uk

New Project

Objective

Develop a community of botanic gardens and arboreta around the world that will use ‘sentinel’ plants to provide early warning of new and emerging tree and plant pests and diseases

New Project

Research products

1. Prototype and future-proofed databases and a website
2. Platforms for providing diagnostic advice or support
3. A network of scientists from countries willing to cooperate
4. Consistent methods for trials and collection of experiences
5. Protocols, examples of best practice, training material, etc.
6. Bilateral and multi-lateral partnerships and exchanges of information
7. A framework for a long-term IPSN, including options for future self-sustainability or future resourcing.

Sentinel nurseries of native trees used for exportation

CABI China and CH: Hongmei Li, M. Kenis and René Eschen

INRA: Alain Roques, Annie Yart

Zhejiang A & F University, Lin'an, China: Jiang-tin Fan

Dibaf University of Tuscia, Viterbo: Andrea vannini, Anna Maria Vettraino

ISEFOR

Background

- Pest risk analyses are increasingly moving from single species PRA to Pathway/commodity RA (CRA)

- CRA are based on the identification of the most likely pests associated to a commodity, on which a PRA will be made (but the list of pests is based on literature only)

- Other advantages to know what is associated with newly imported commodities:
  - Phytosanitary inspectors need to know what types of pests can be expected on a given commodity
  - Importing countries need to know for which pests they should develop surveillance programme and detection methods

Objectives of the native sentinel nurseries

- To plant and survey commonly imported plants in the area of exportation (in this case China) to obtain lists of potential pests that can be expected on these imported commodities

- Allow EU member states and EPPO to carry out new pest and pathway risk analyses and develop new surveillance and detection techniques accordingly

- Test whether literature surveys are sufficient to identify potential pests hosted by imported plant species

Two sentinel nurseries

Beijing suburban area

Continental conditions

Fuyang, nr. Hangzhou
Forest area
Warm and humid climate

Plant species

Five woody species commonly sent from China to Europe:

Acer palmatum

Ilex cornuta

Buxus microphyllus

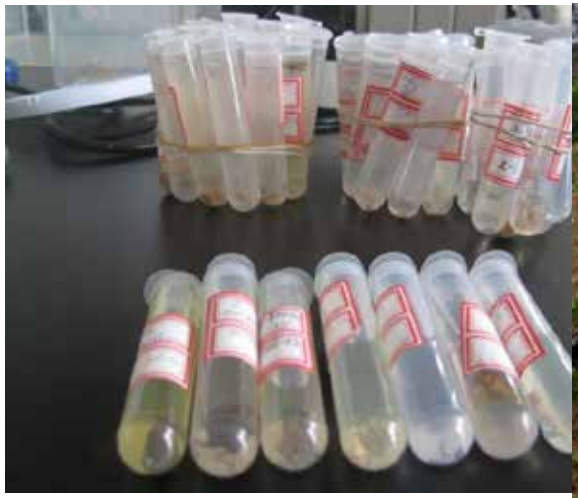
Fraxinus chinensis

Zelkovia schneideriana

Setting up nursery in April 2012

Methods for insect surveys

- For each species: 5 blocks of 20 individuals each = 500 trees
- Inspection every 2 weeks from April to October: Visual + beating
- Damage assessment
- Collection of specimens (alcohol), keeping adults and larvae, rearing larvae
- Uproot if dead seedling (+ inspection)



Comparison with literature

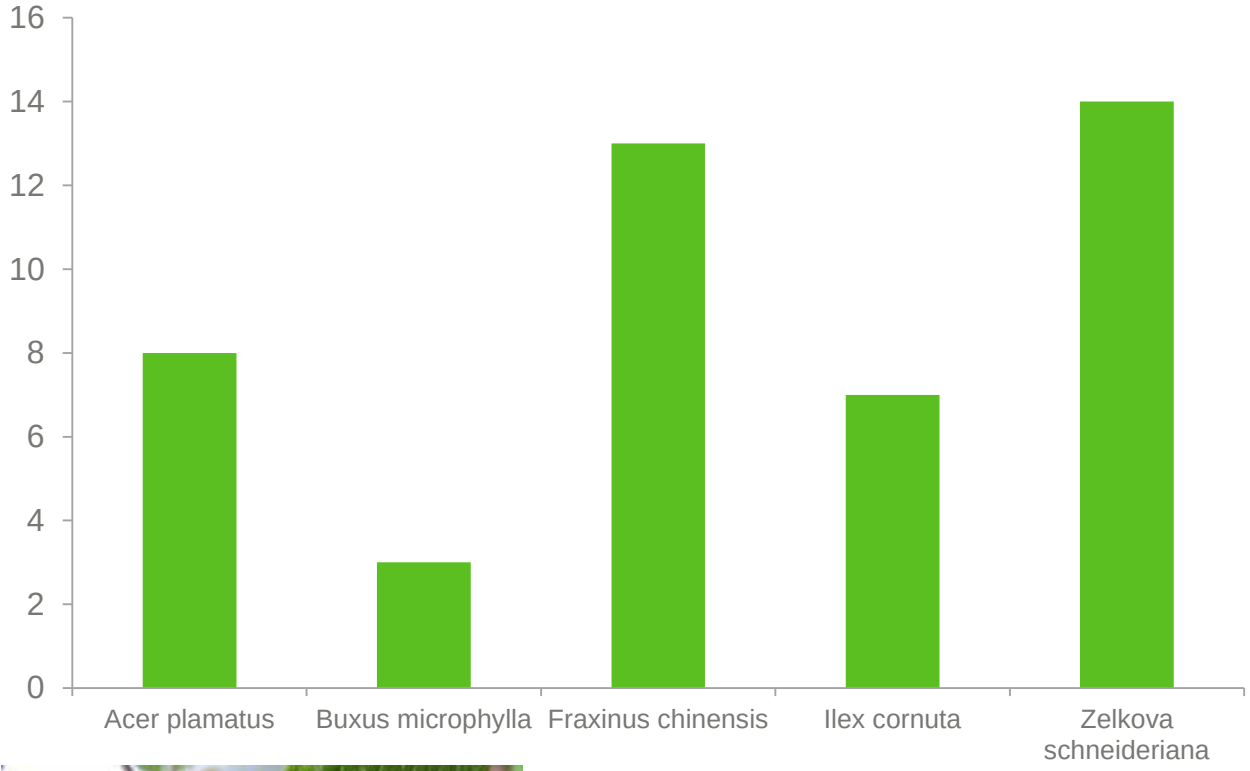
The list of insects and pathogens obtained though the sentinel nursery survey is compared with what would be found compiling:

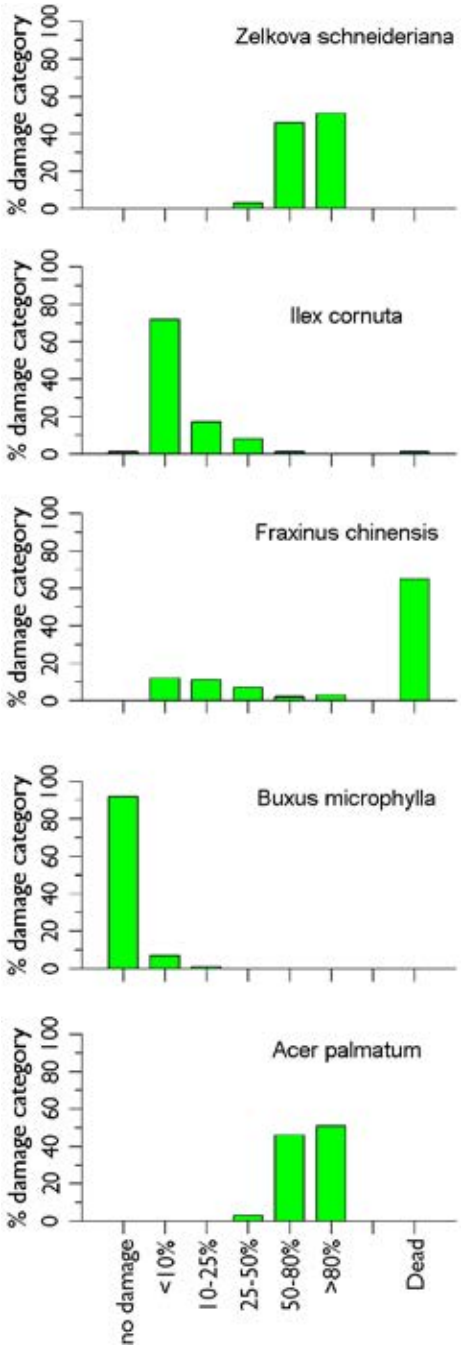
1. The Chinese literature
2. The International literature

Main question: are potentially damaging species missed in literature surveys?

Halyomorpha picus Fahricius	*Lepyrus japonicus* Roelofs
Metonnymia glandulosa Tbhunberg	*Tamnaspis nankinea* Pic
Cryptotympana atrata Fahricius	*Sinoxylon japonicus* Lesne
Oncotympana maculaticollis Motschulsky	*Zamacra excavata* Dyar
Phenacoccus fraxInus Tang	*Latoia consocia* Walker
Lycorma delicatula (White)	*Cnidocampa flawescens* Walker
Drosicha corpulenta Kuwana	*Thosea sinensis* Walker
DrosYcha corpuienta Kuwana	*Parasa consocia* Walker
Rhopalosiphum nymphaeae Linnaeus	*Parasa hilarata* Staudinger
Tettigella Viridis Linnaeus	*Hyphantria cunea* Drury
Prociphinus fraxini Fahricius	*Stilpnotia salicis* Linnaeus
Batocera horsfieldi (Hope)	*Holcocerus Insularis* Staudinge
Mesosa myops Dalman	*Holcocerus vicarious* Walke
Potosia brevitarsis Lewis	*Holcocerus orientalis* Gaede
Agrilus planipennis Fairmaire	*Zeuzera coffeae* Nietner
Holotrichia trichophora Fahricius	*Cossus cossus* Linnaeus
Dyscerus cribripennis Matsumura *et* Kono	*Psilogramma menephron* (Grame
	Macrorbya fraxina Zhow et Hua

Sentinel nurseries in China





Sentinel nurseries in China

Some remarkable results:

Huge damage by *Cydalima perspectalis* on *Buxus*

Sentinel nurseries in China

Some remarkable results:

The introduction of *C. perspectalis* could have been easily predicted considering:

(1) The high prevalence of these pests in test nurseries

(2) the dramatic increase of box tree importations in the last years (900x in 2006-2010 in the NL)

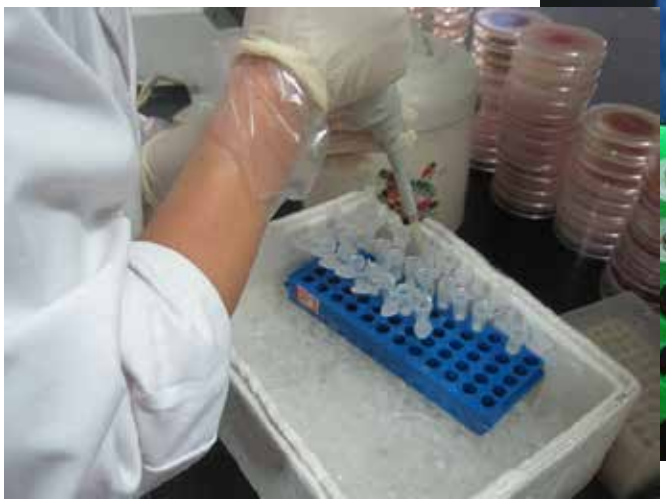
(3) The innocuous life forms likely to be present on shipped plants

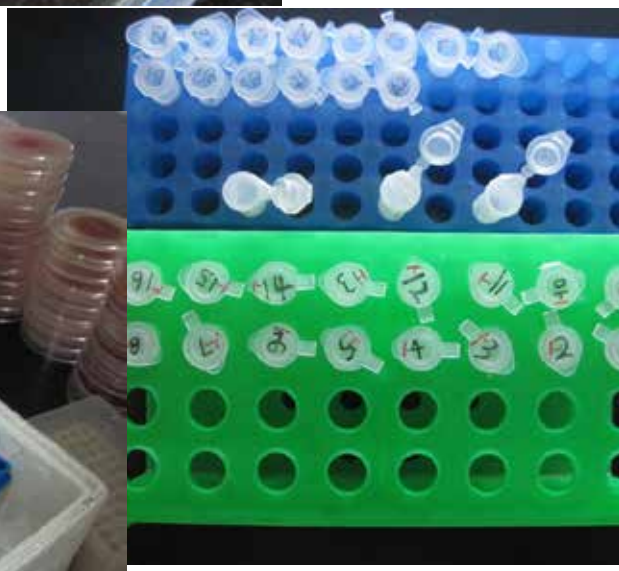
Sentinel nurseries in China

Some remarkable results:

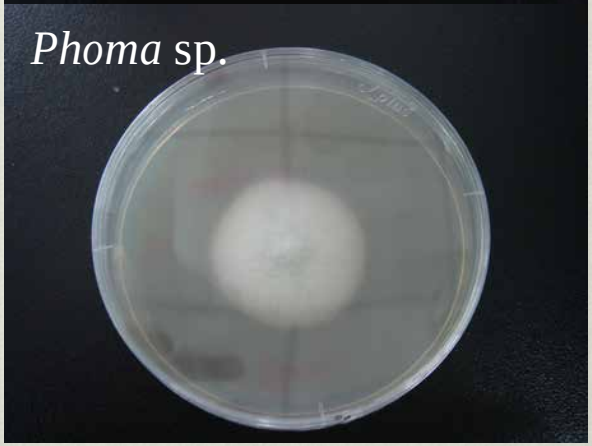
Methods for pathogen surveys

- For each species: 5 blocks of 20 individuals each = 500 trees
- Inspection every 2 weeks from April to October: Visual
- Morphological assessments
- 3x / year: Extract DNA from damaged leaves
- DNA extracts sent to pathologists
- Also asymptomatic collections





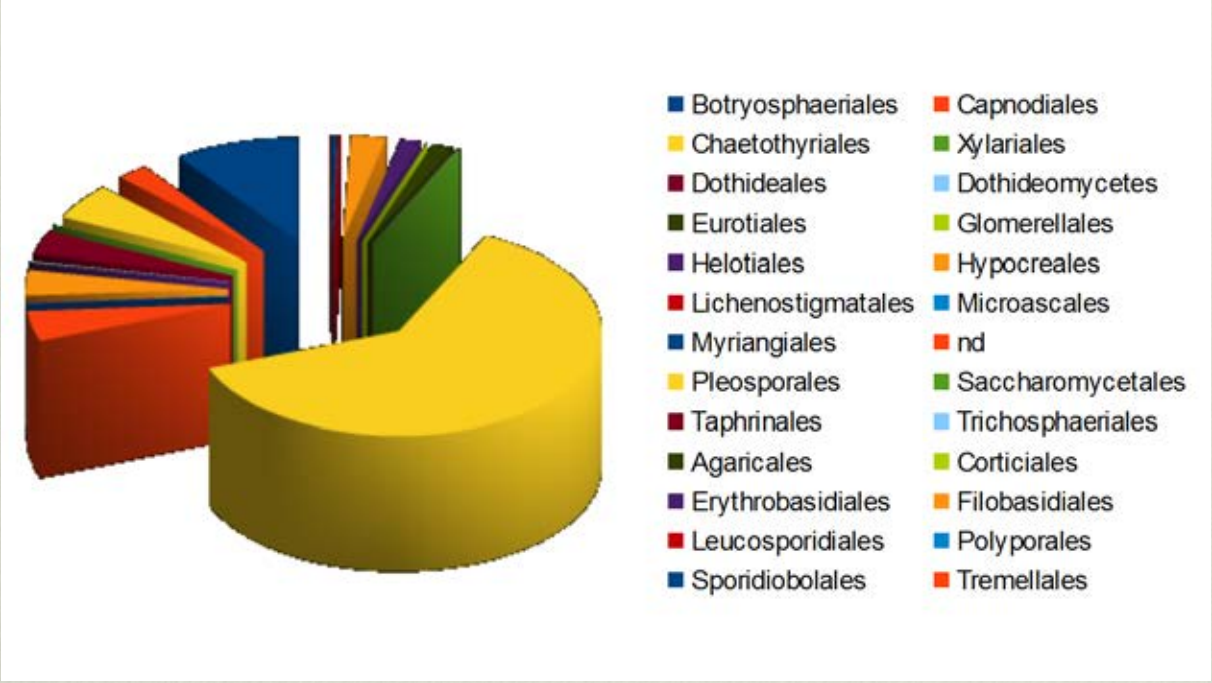
Biological detection



Acer palmatum, Fraxinus chinensis , Ilex conrnuta , Zelkova schneideriana , Buxus microphylla

NGS statistics

- 35.839 reads after trimming
- Minimum 93, maximum 6.549 per sample
- A total of 148 Operational Taxonomic Units

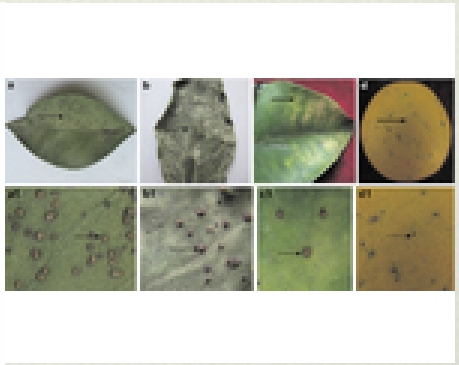


- 25 fungal orders

Acer palmatum, Fraxinus chinensis , Ilex conrnuta , Zelkova schneideriana , Buxus microphylla

Buxus microphylla

Phyllosticta citrichinaensis



Foliar pathogen of *Citrus* in China

Fraxinus chinensis

Strelitziana mali

Zhang, Rong, Hanli Yang, Guangyu Sun, Huanyu Li, Jieli Zhuang, Xiaoru Zhai & Mark L. Gleason. *Strelitziana mali*, a new species causing sooty blotch on apple fruit***. Mycotaxon 110:*** 477–485. 2009.

Thank you for your attention

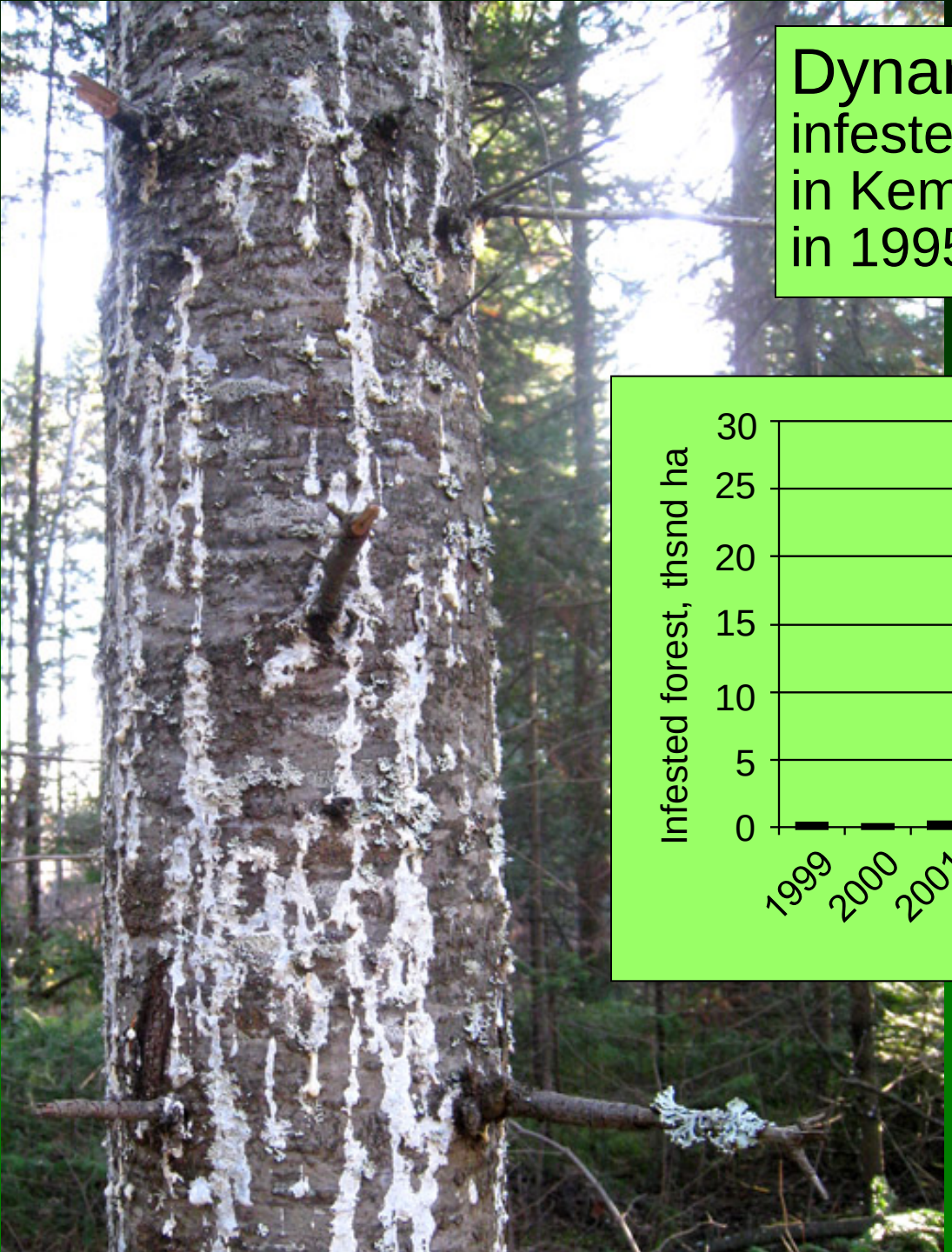
Four-eyed fir bark beetle and associated fungus - an aggressive tandem in fir forests of Siberia and European part of Russia

Baranchikov Yuri

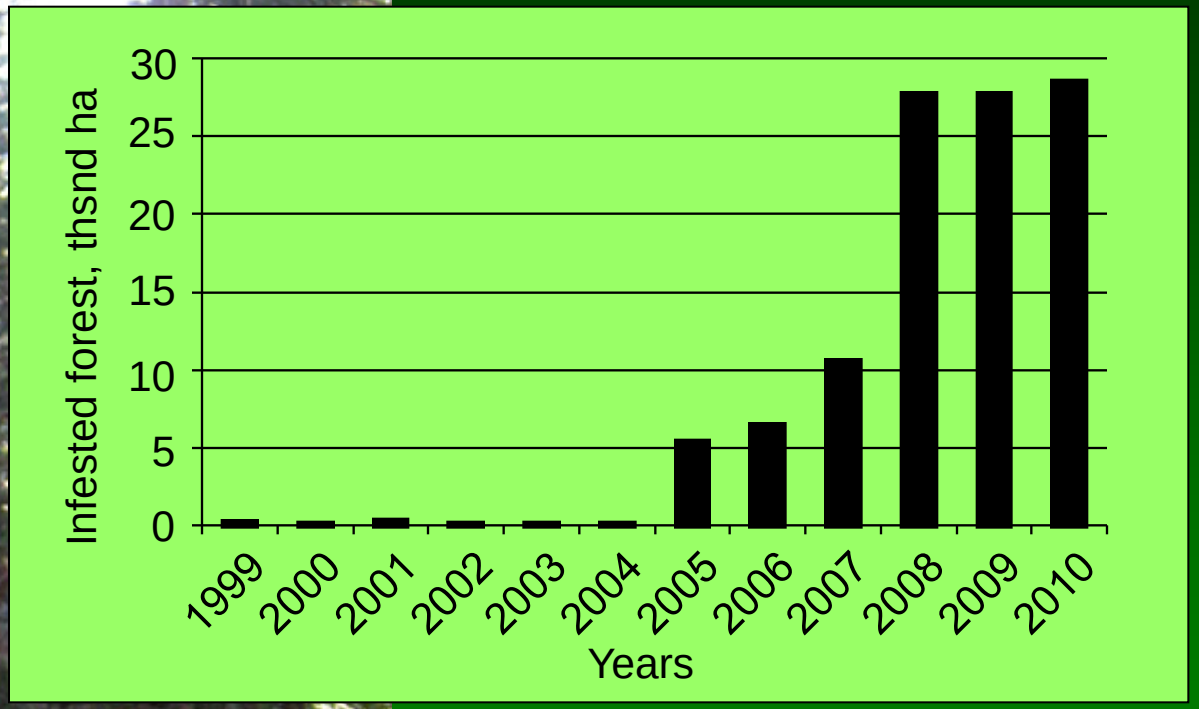
V.N.Sukachev Institute of Forest
Siberian Branch
Russian Academy of Science,
Krasnoyarsk, RUSSIA

Bark beetles of genus *Polygraphus* have eyes, divided by the basis of antenna, they are called four-eyed bark beetles.
Common name of *P. proximus* - Four-eyed Fir Bark Beetle
or **FFBB**

FFBB distribution and outbreaks

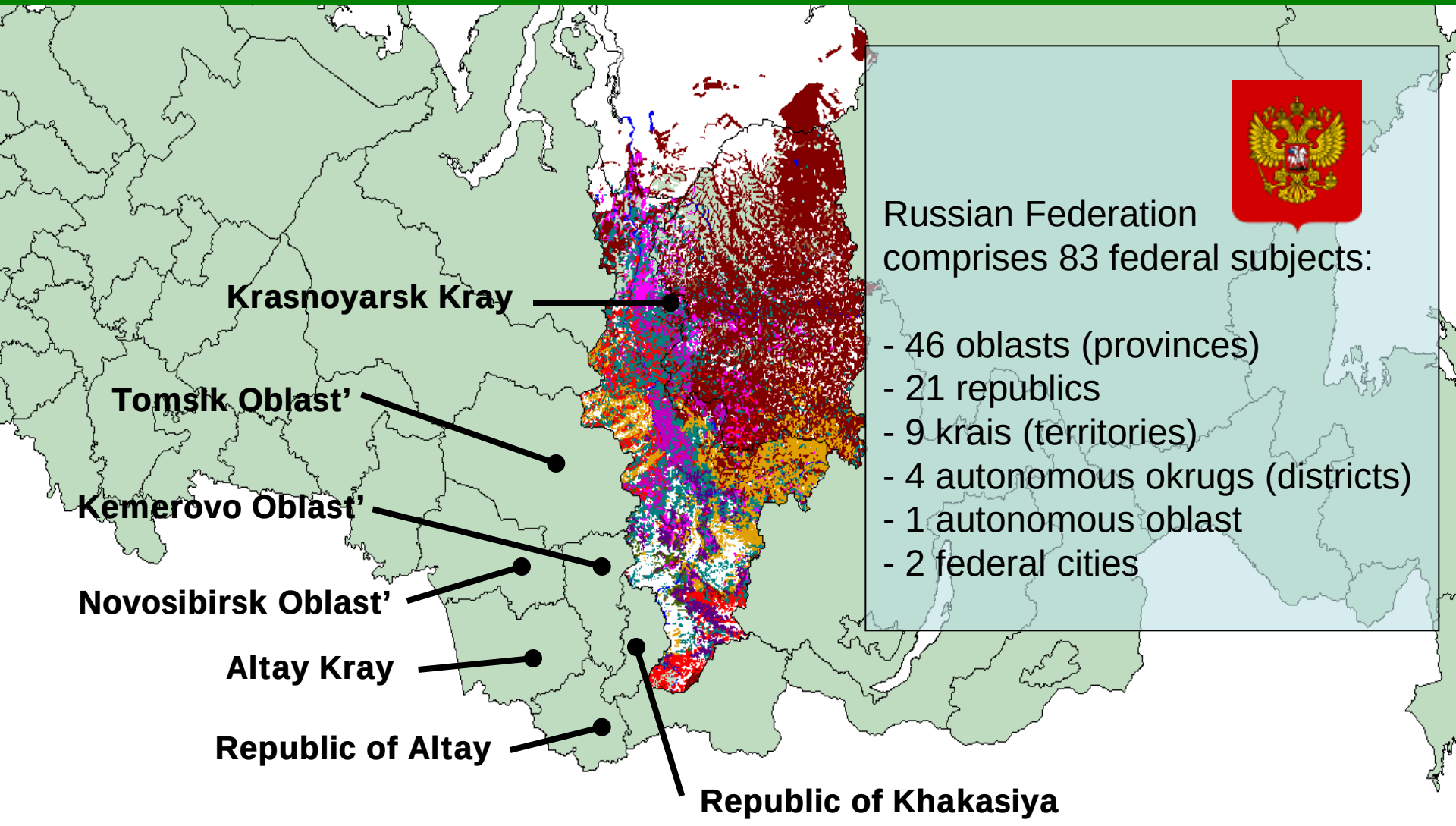


Dynamics of forest area infested by FFBB in Kemerovo Oblast in 1995-2010



Source:
Russian Center of Forest Protection, 2004, 2010.

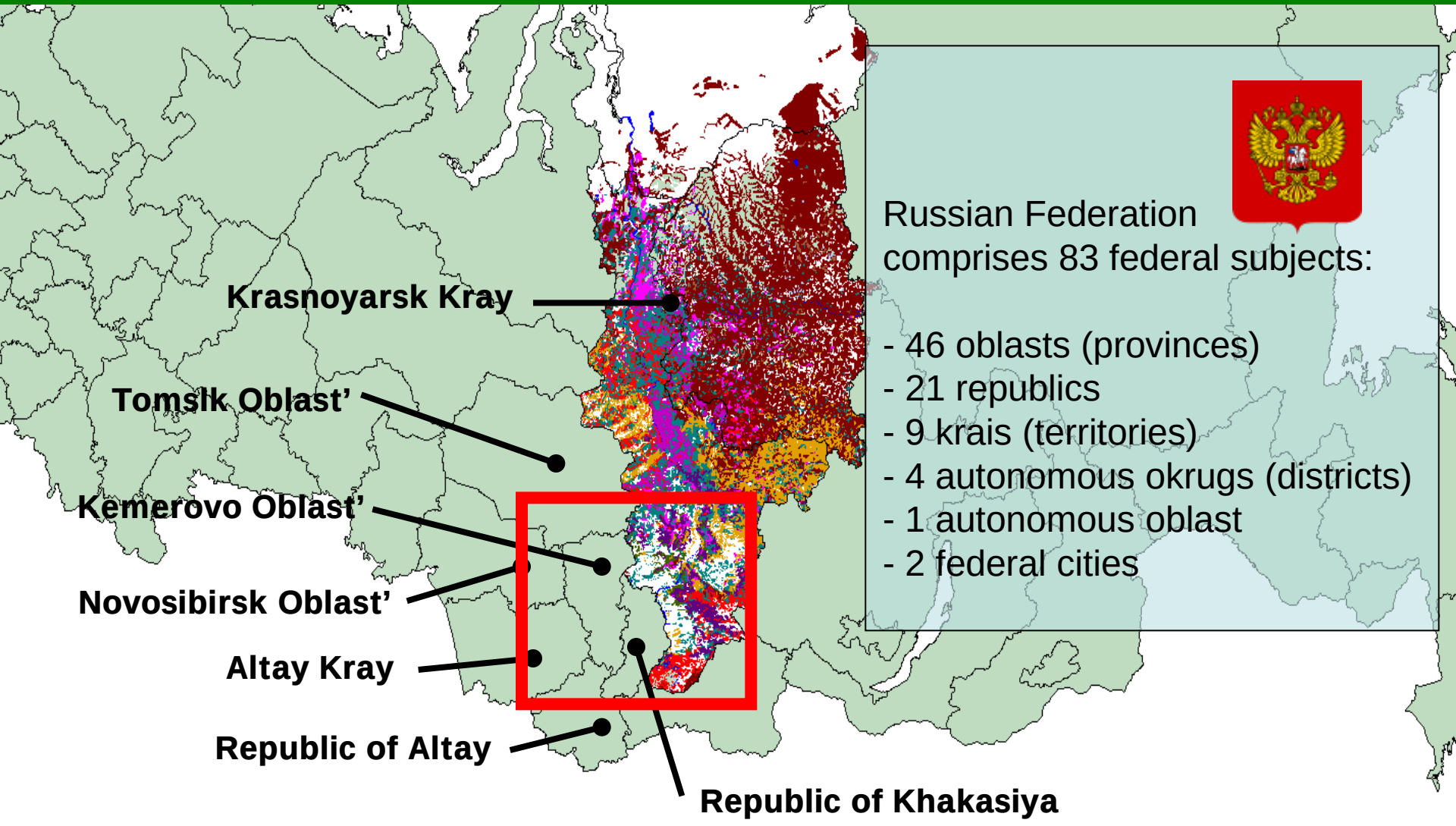
Russian Federation: administrative terminology



Russian Federation comprises 83 federal subjects:

- 46 oblasts (provinces)
- 21 republics
- 9 krais (territories)
- 4 autonomous okrugs (districts)
- 1 autonomous oblast
- 2 federal cities

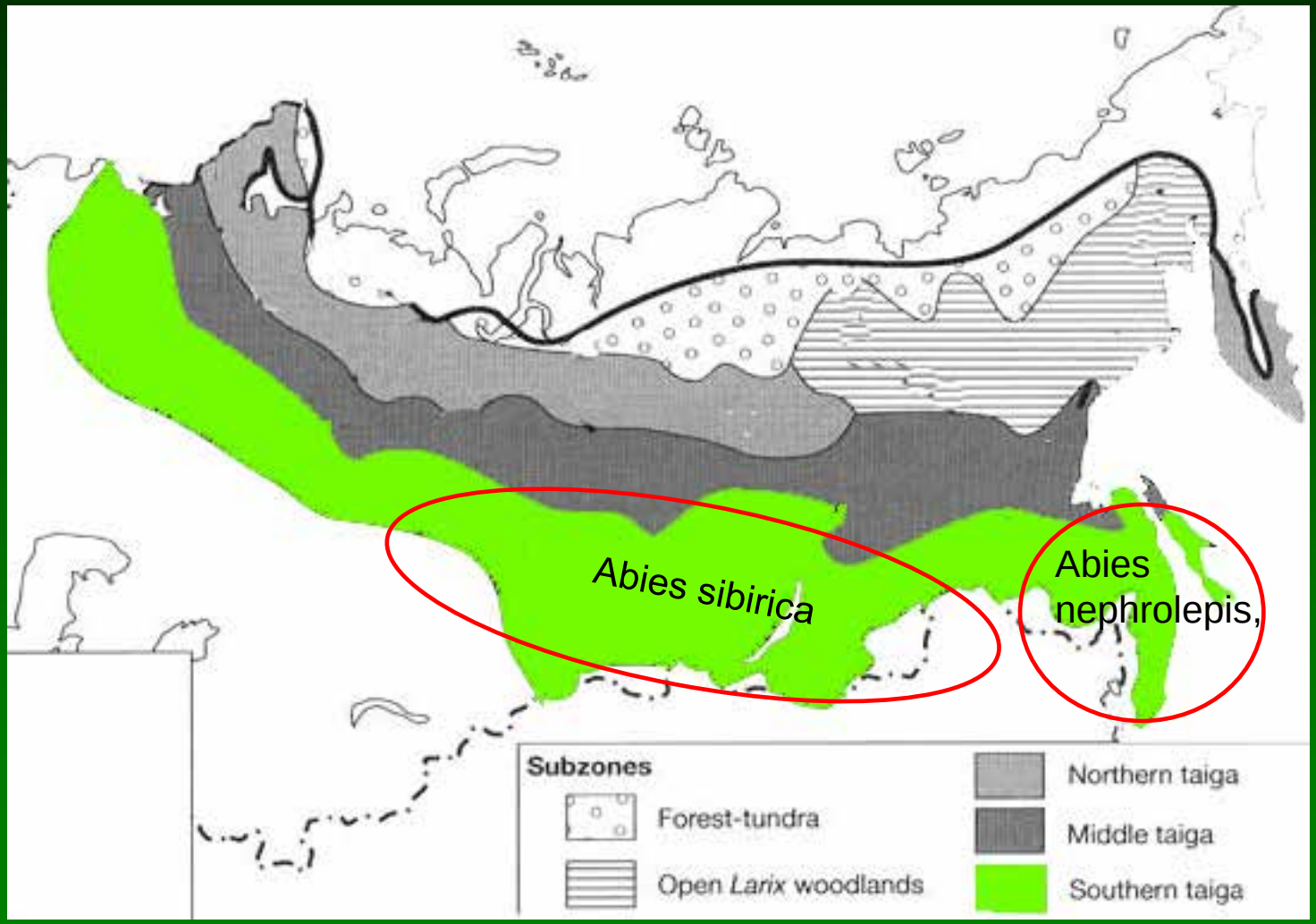
Russian Federation: outbreak area of *Polygraphus proximus*



Area of registered outbreaks of *Polygraphus poximus* in Southern Siberia (left), projected on the map of Europe (right) (Google Earth, same scale)

Area of registered outbreaks of *Polygraphus poximus* in Southern Siberia (left), projected on the map of USA (right)
(Google Earth, same scale)

Southern taiga subzone



South Siberian fir taiga

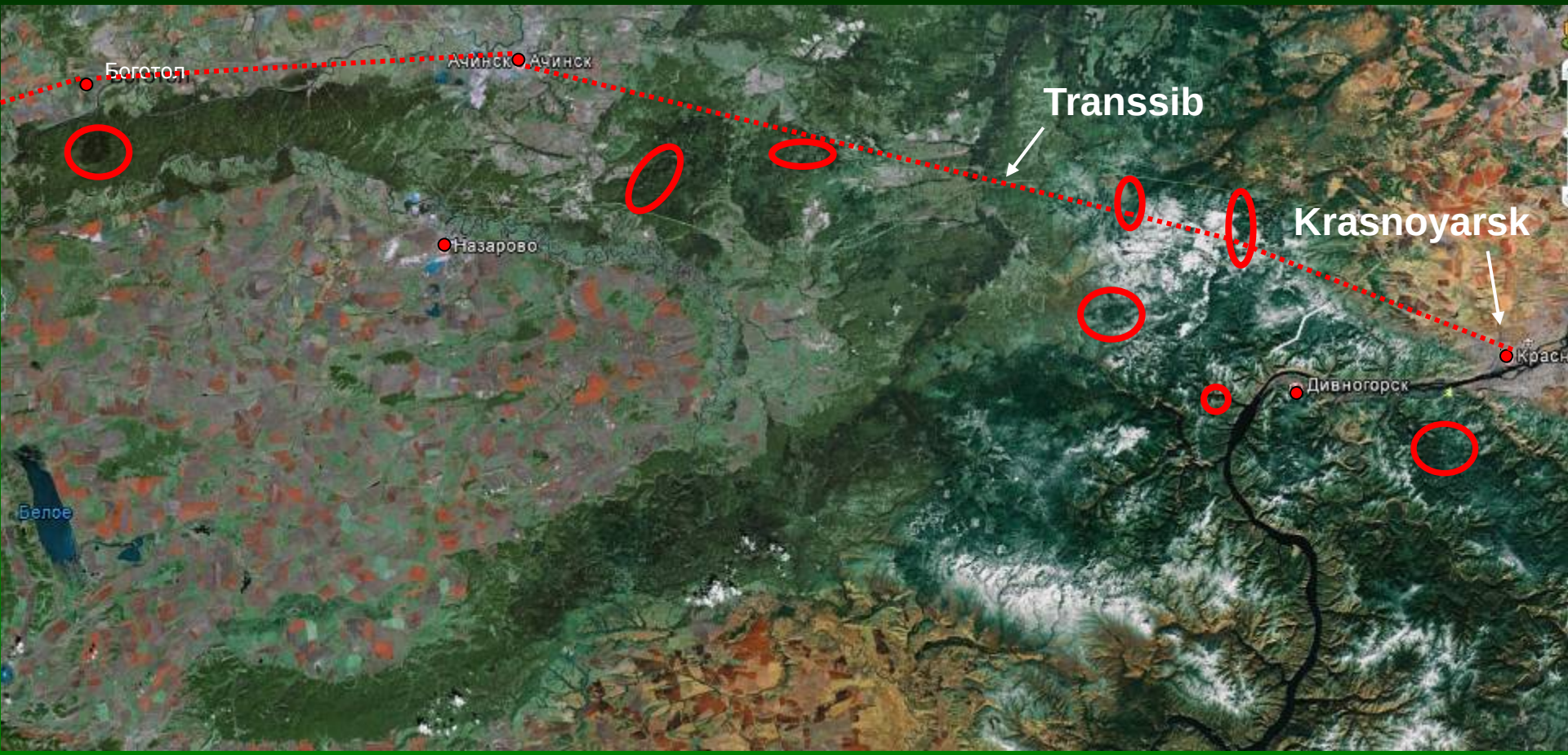
Siberian moth - *Dendrolimus sibiricus* Tchetvrk. –
a main pest of coniferous forests of Asian part of Russia

Fir sawyer beetle *Monochamus urussovi Fischer (Coleoptera: Cerambycidae)*

Leptographium sibirica Jacobs & Wingfield

Jacobs, K., Wingfield, M.J., Pashenova, N.V. & Vetrova, V.P. (2001).
A new *Leptographium* species from Russia. Mycological Research 104, 1524-1529.

Areas of *Polygraphus proximus* outbreaks in Krasnoyarsk Kray



«Crying firs»- first sign of massive beetle attack

During first attacks of beetles healthy fir covers them with resin and kills.

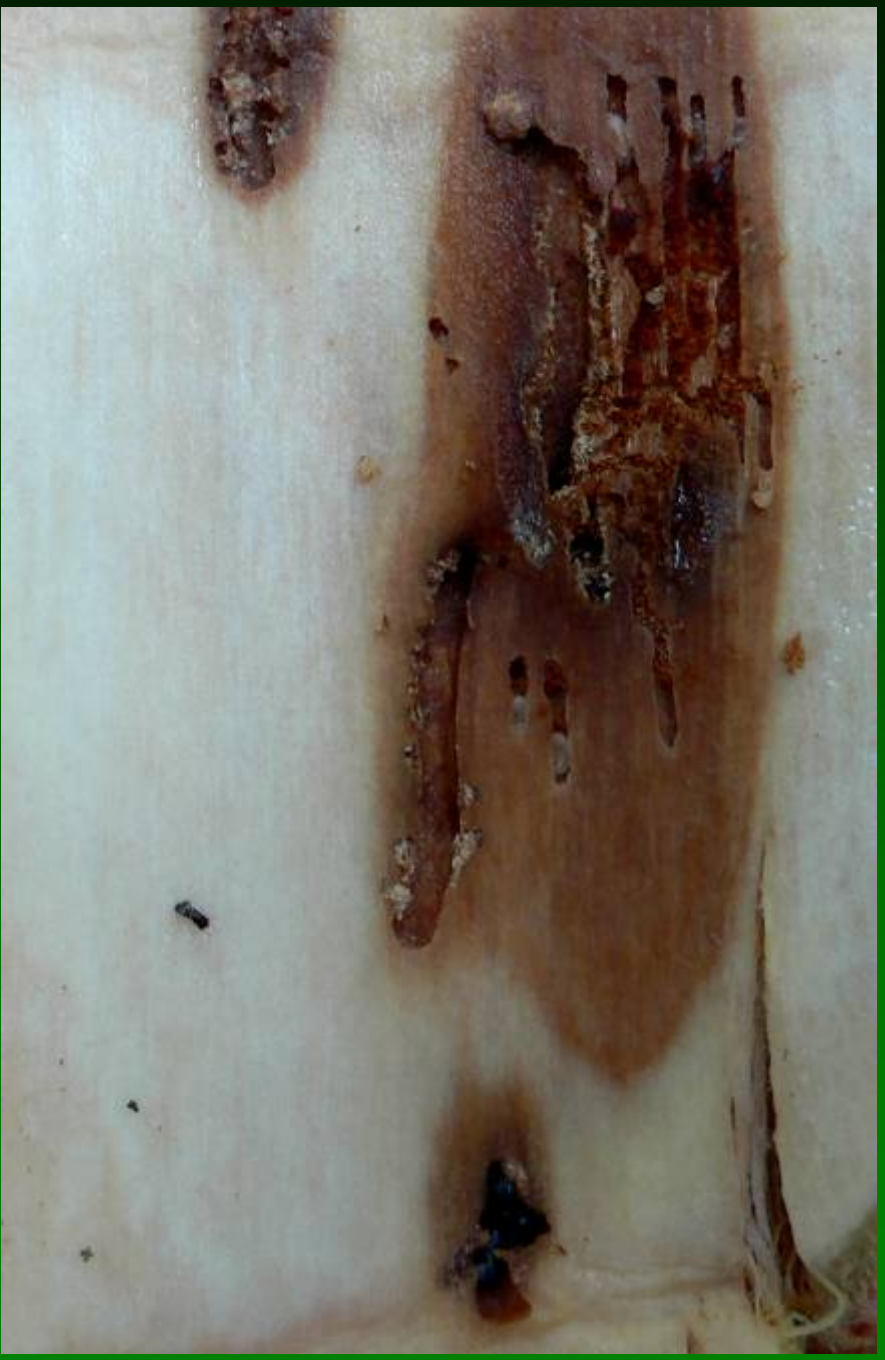
Photo: S.Astapenko

Fungus

The mechanical damage by the beetle to the phloem does not exceed 3-4 mm^2, but in absolute majority of cases the beetle inoculates fungus into the phloem. It will end with phloem necrosis of 10-20 mm in diameter or even more before the healthy tree will stop the fungus and isolate it with vivid red-brown layer of periderma.

On the second year firs can not resist beetle attack with resin flow, the new necroses connect with the old ones. The tree loose resistance to the fungus and periderma can not be formed.
Finely an exclusive rings of the dead tissue will be produced in few places over the stem…

Photo: Y. Baranchikov



Fungus

… and beetles will succeed in constructing galleries.

Polygraphus proximus on Siberian fir

1-2 year of attack

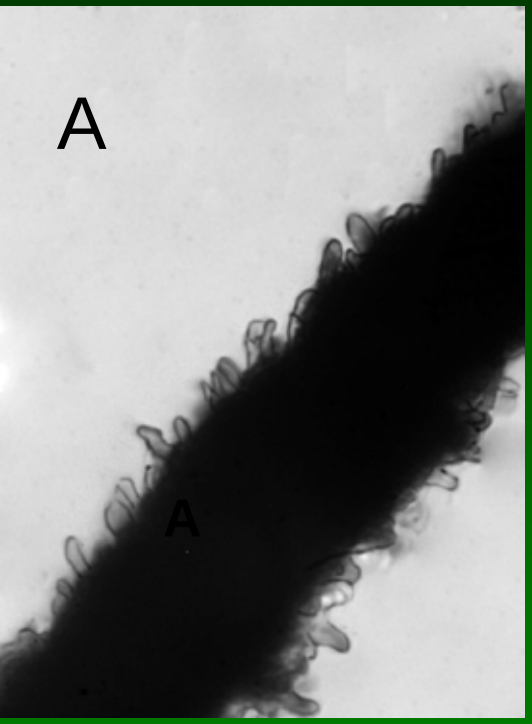
3d year of attack, August

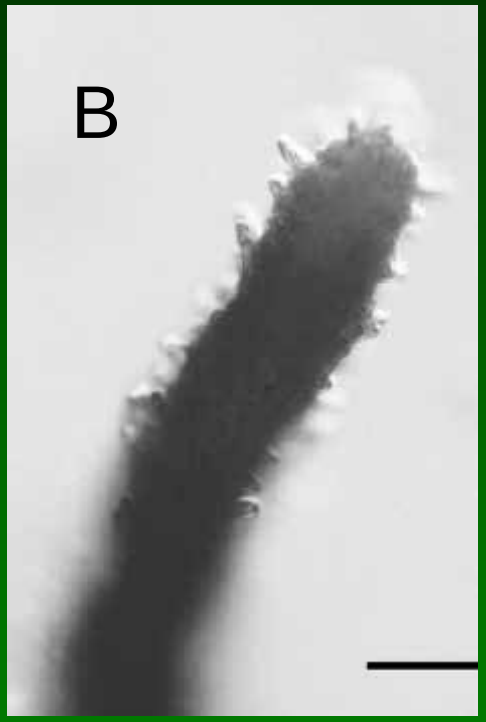
Comparison of some cultural and morphological characters of *Ophiostoma sp. A* and *Ophiostoma aoshimae*

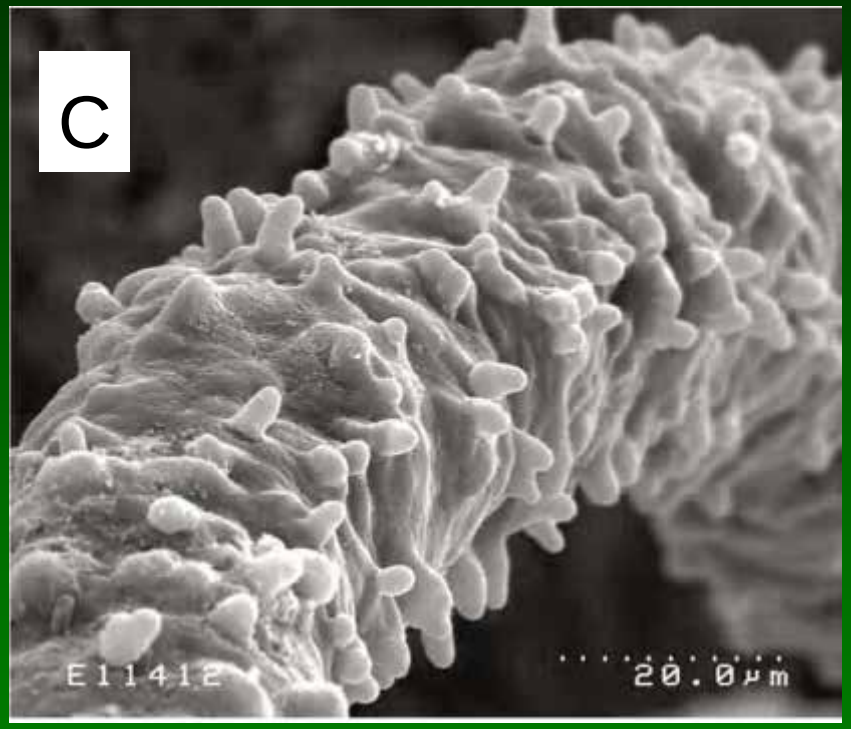
Characters	Fungi	
	Ophiostoma sp. A	*O. aoshimae**
Perithecial base width (µm)	165 – 319	155 – 275
Perithecial neck		
length (µm)	429 – 1055	300 – 820
base width (µm)	33 – 55	45 – 80
tip width (µm)	22 – 44	20 – 50
Ostiolar hyphae	Absent	Absent
Projections on the surface of the neck	Present	Present
Ascospores		
size (µm)	2,7-4,3 x 1,2-2,0	2,5-4,5 x 1,2-2,4
shape	Oblong	Ellipsoid to oblong
Conidial state	*Leptographium*-like	Not found
Color of colony grown on MEA	Dark braun	Braun to dark olive

Fungus

Ophiostoma sp. A *vs Ophiostoma aoshimae* micromorphology





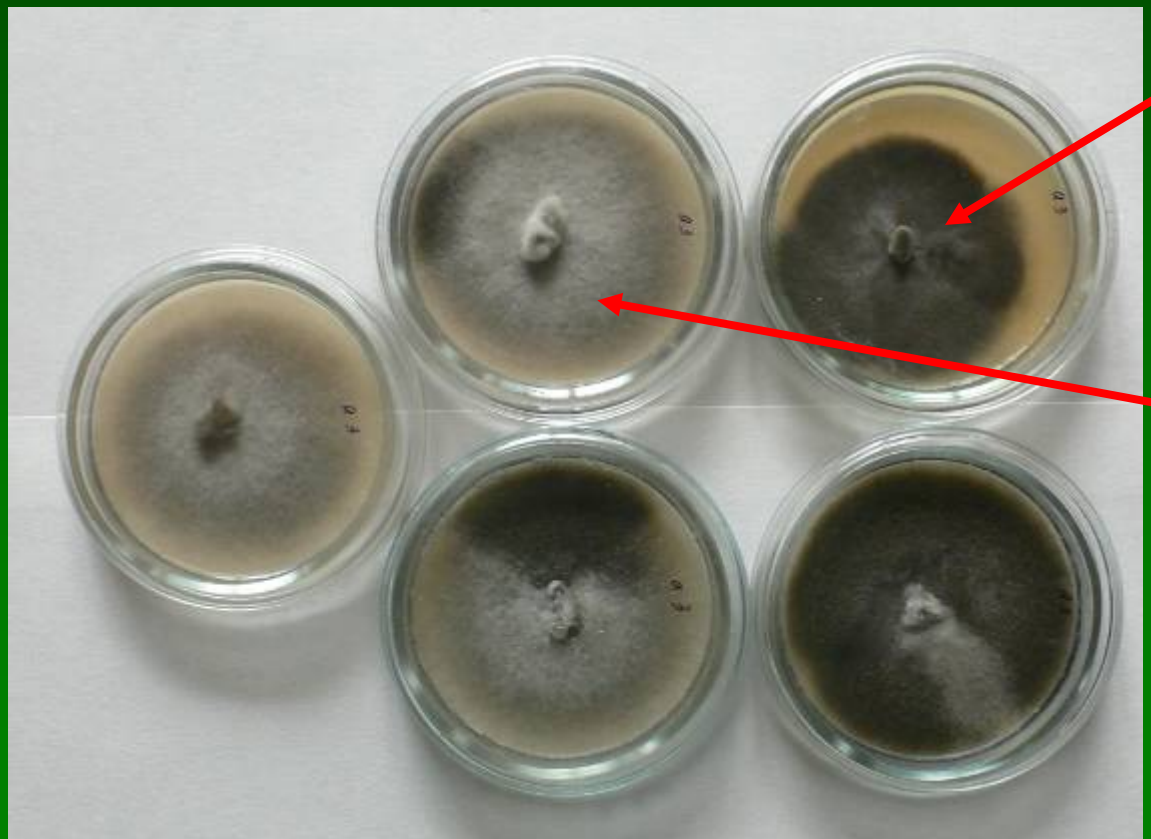


Upper part of peritecium neck:
A - *Ophiostoma sp.* A (Siberia);
D, C - *O.aoshimae* (from Ohtaka et al., 2006)

Polymorphism *of Ophiostoma sp.* A

Ophiostoma sp. A typical “wild” culture

Ophiostoma sp. A laboratory generations



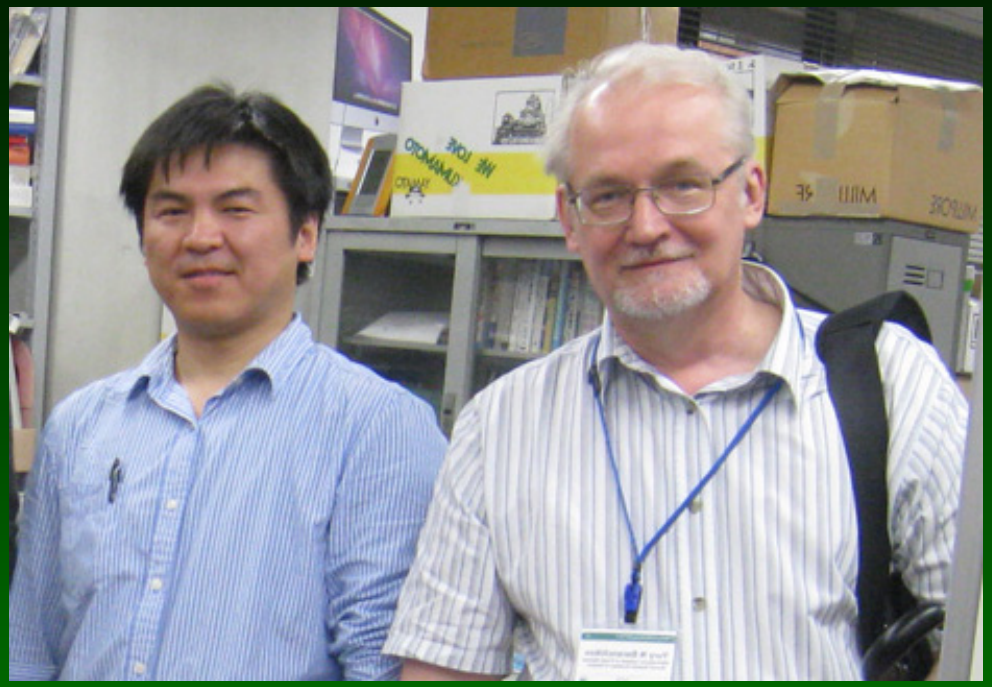
Ophiostoma sp. A4

Ophiostoma sp. A7

In May 2012 Dr. Stephen Woodward, (University of Aberdeen, UK) and his students ran PCA analyses of our fungus isolates and proved that Ophiostoma A4 and A7 belonged to the same species - *Ophiostoma aoshimae*.

http://ftp.dna.affrc.go.jp/pub/dna_all/G/U1/34/16/GU134162/GU134162

Dr. H. Masuya.
Forestry & Forest Products Research Institute,
Tsukuba, Ibaraki, Japan.
15 июня 2012 г.



Masuya H., Yamaoka Y., Wingfield M.J. Ophiostomatoid fungi and their associations with bark beetles in Japan // Ophiostomatoid Fungi: Expanding Frontiers (Seifert KA, Wingfield MJ, eds). – Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. – CBS Biodiversity Series 12. 2013. – P. 79-91.

Grosmannia aoshimae (Masuya & Yamaoka), 2006

Micromorphology of *Grosmannia aoshimae* (Ohtaka et Masuya) Masuya et Yamaoka

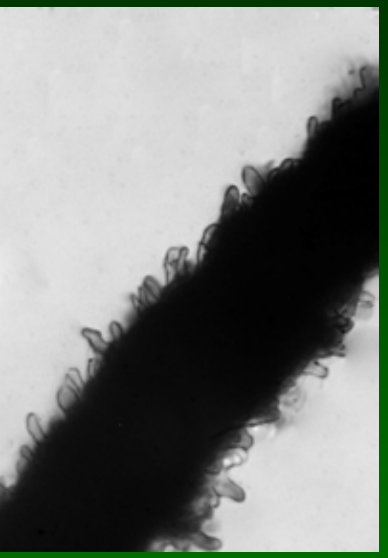


Фото Н.Пашеновой

Upper part of perithecium neck with numerous very specific projections

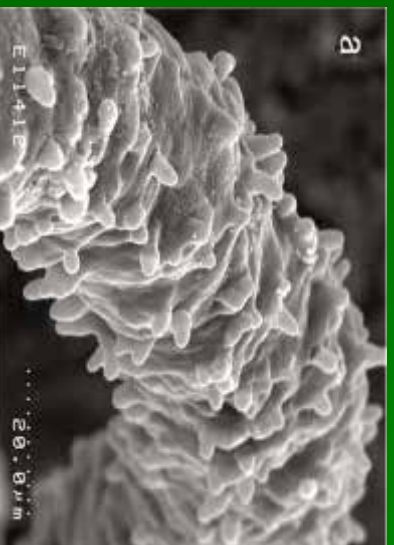
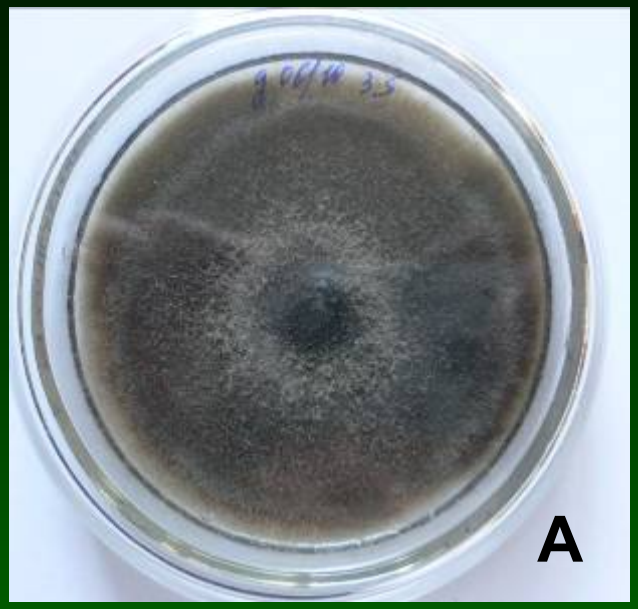


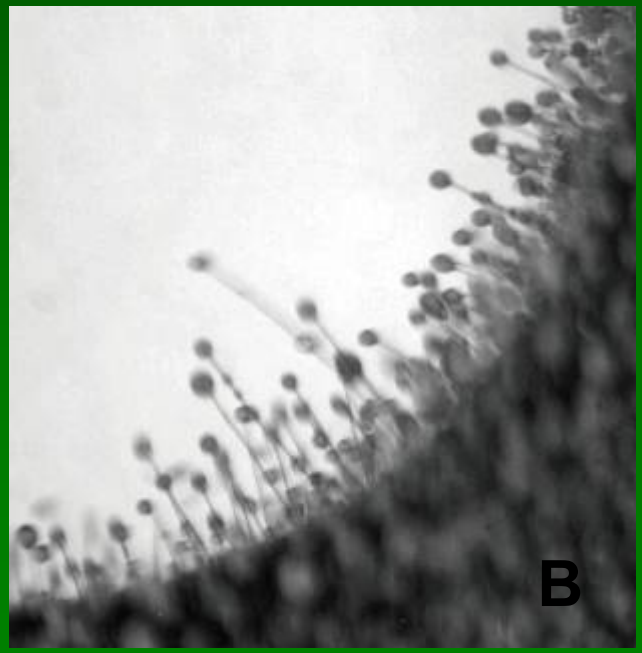
Фото: Ohtaka et al., 2006

Commonly anamorths are absent. If they are present, they have *Leptographium*-type conidiophores.

Фото Н.Пашеновой

Leptographium sibirica colony (A) and its conidiophores (B,C) on agar





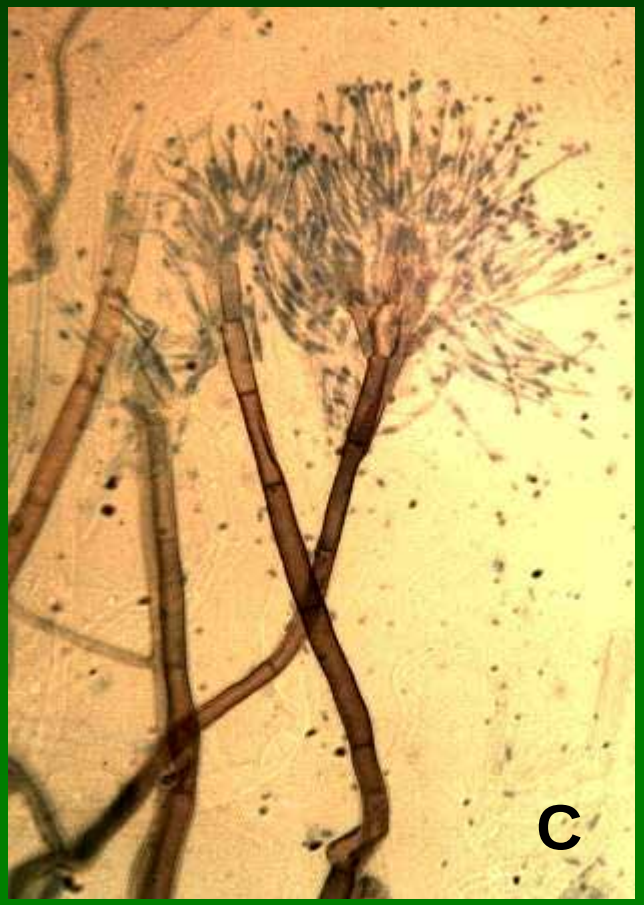


Фото Н.В.Пашеновой

Hypothesis to test

- is *Abies sibirica* – a new host for FFBB in Siberia – more susceptible to *Grosmannia aoshimae*, than FFBBs' native host – *A.nephrolepis* ?

- is *G.aoshimae* more pathogenic to Siberian fir, than local Siberian fungi ?

Host-plant susceptibility to invasive fungus

To compare aggressiveness of *G.aoshimae* for its native host – *Abies nephrolepis* and a new host – *A.sibirica*, we inoculated stems of mature fir trees in Krasnoyarsk in Khabarovsk with wild cultures of *Grosmannia aoshimae* from Siberia and from the Far East.

We also used different amount of fungus – a piece of infested agar of 6 mm and 0,7 mm in diameter.

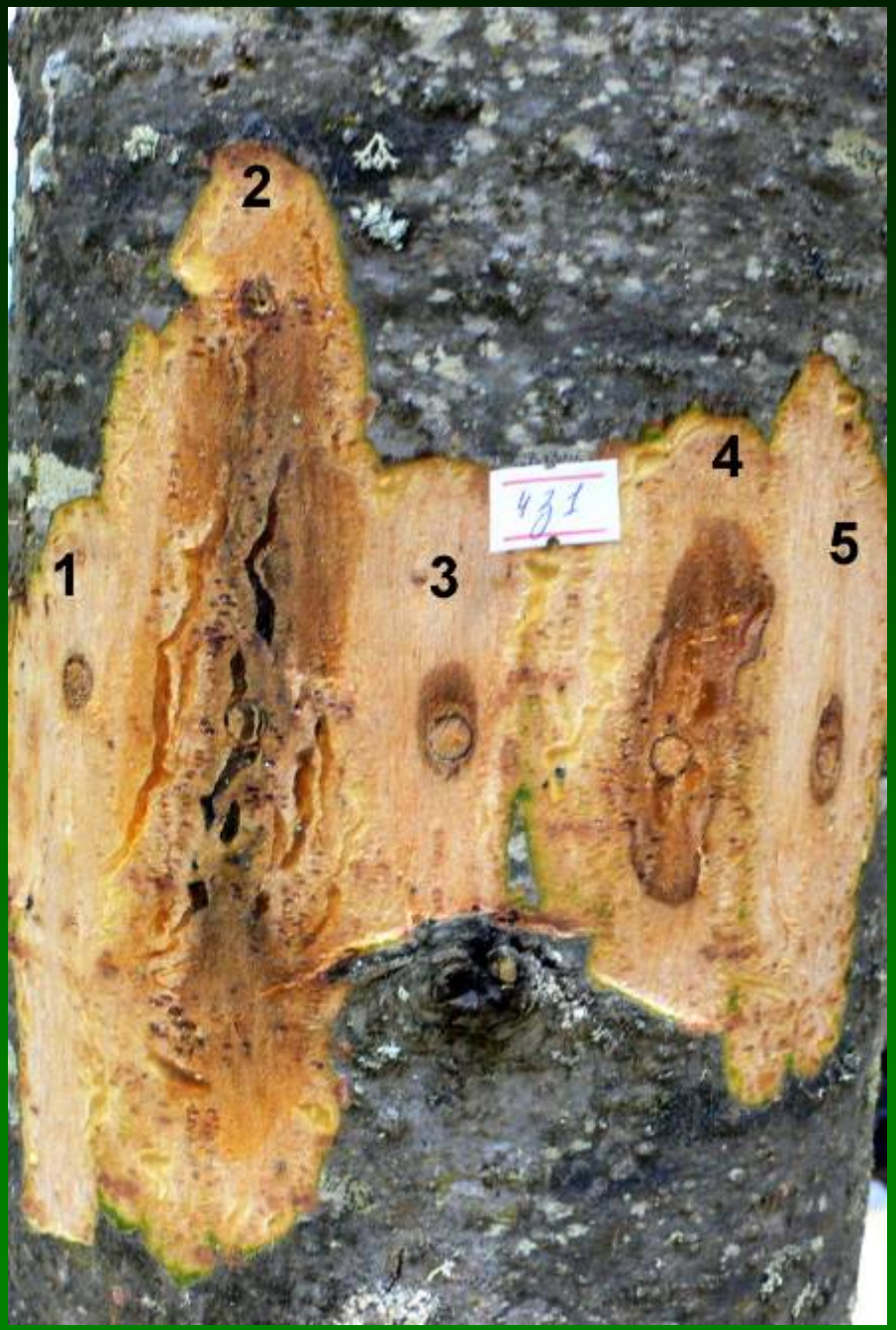
Host-plant susceptibility to invasive fungus

Size of necrotic lesions in the *Abies sibirica* inner bark

four weeks after artificial inoculation with the *P. proximus* associated fungi

Inoculum (code of isolate)	Necrotic lesions (mm)*	
	length	width
Grosmannia aoshimae (A4)	125,6 a	28,0 a
Grosmannia aoshimae (A7)	43,8 b	16,8 b
Ophiostoma sp. B (B1)	16,6 c	12,0 c
Ophiostoma sp. B (B3)	18,8 c	10,0 c
Leptographium sibirica (L1)	46,8 b	23,2 a
Leptographium sibirica (L2)	41,0 b	20,0 ab
Graphiun sp. (G7)	14,2 cd	11,0 c
Graphiun sp. (G8)	17,6 c	12,8 c
Control	**11,5 d**	**11,6 c**

* Values in a column (mean, n=5) followed by the same letter are not significantly different (P > 0,05).



The necrotic lesions formed in the *Abies sibirica* inner bark, 4 weeks after inoculations

Inoculums:

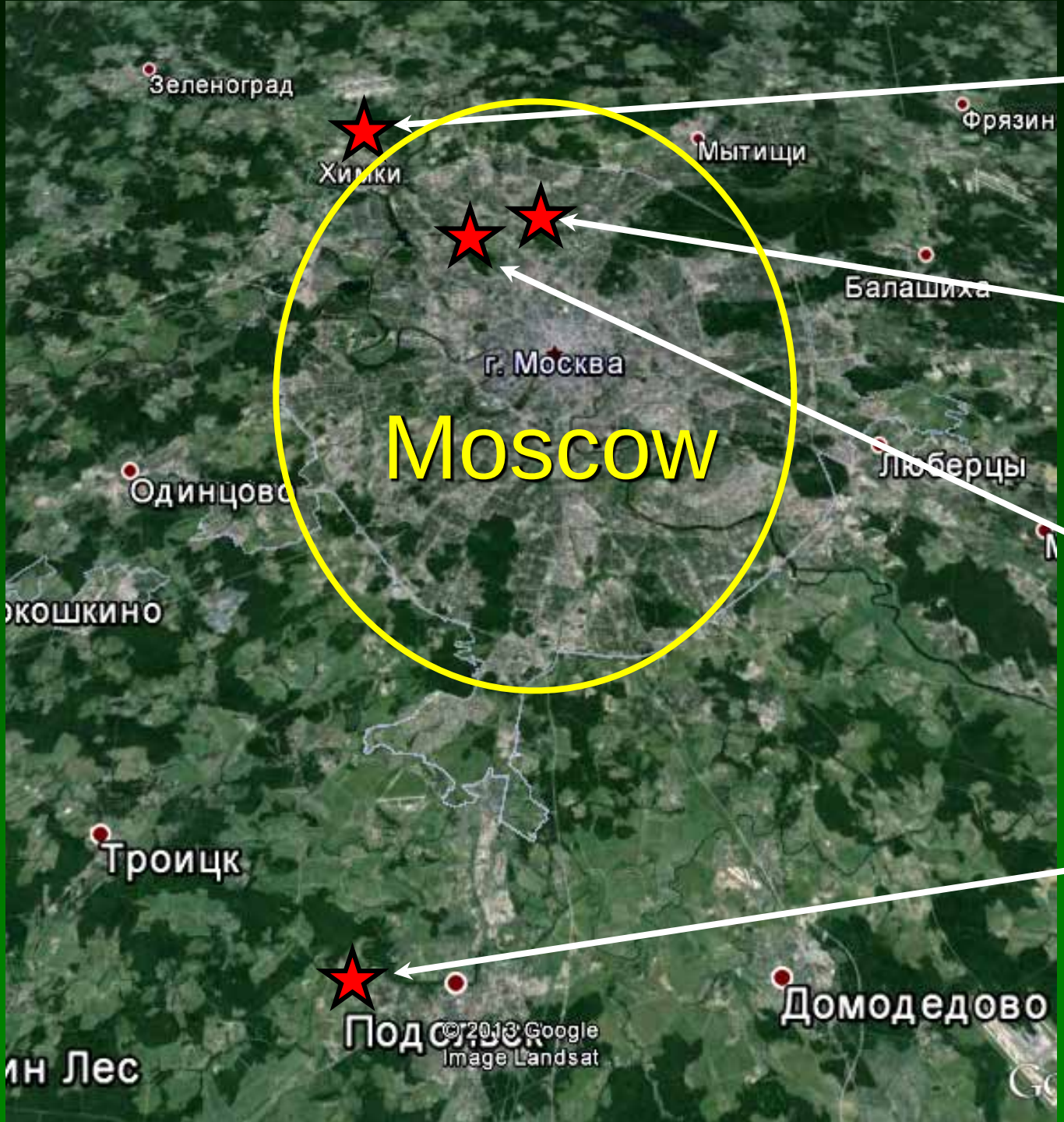
1 – control (mechanical wounding without inoculation),
2 - *Grosmannia aoshimae*,
3 - *Ophiostoma sp.* B,
4 - *Leptographium sibirica.*,
5 – *Graphium sp.*

Photo by N.Pashenova

Hypothesis to test

Yes - is *Abies sibirica* – a new host for FFBB in Siberia – more susceptible to *Grosmannia aoshimae*, than FFBBs' native host – *A.nephrolepis* ?

Yes - is *G.aoshimae* more pathogenic to Siberian fir, than local Siberian fungi ?



Khimki

Botanical Garden RAS

Park of Timiryasev Academy

Podolsk

Source: VNIILM, 2013

Summary (1 of 3)

Polygraphus proximus, a four-eyed fir bark beetle – Far Eastern invasive and aggressive pest of Siberian fir. It's populations formed long lasting outbreaks in the fir stands in Southern Siberia.

Summary (2 of 3)

Beetle's success in a large extent is connected with lack of Siberian fir resistance to a blue stain fungus *Grosmannia aoshimae*, associated with the invasive bark beetle.

Summary (3 of 3)

The main problem nowadays is in the possibility of forming of some new insect-fungi associations when introduced invasive fungus will be transported by indigenous wood boring insects. This can be a real disaster for South Siberian fir stands.

Acknowledgements

Special thanks to:

-Steve Woodward and Eleni Seasou (University of Aberdeen, UK) for fungus' PCA;
- Alex Blinov & Kirill Ust'yantsev (Institute of Genetics, Novosibirsk, Russia) for bark beetle PCA analysis;
- Katerina Tselikh (Zoological Institute RASc., St.Petersburg) for parasite determination;
- Yuri Gninenko (Pushkino); Galina Yurchenko (Khabarovsk), Svetlana Krivets & Ivan Kerchev (Tomsk) and Katerina Chilahsayeva (Moscow) for beetle collections.

Work was supported by the Russian Fund for Fundamental Research

and

FP7 *ISEFOR* project.

A.Khomutinnikov

Fir forest

An artist occasionally chose for his painting this colorful group of firs in a bound of an outbreak area of *Polygrahus proximus*, thinking this was a typical view of Siberian fir forest.

Thank you for attention!

Ash decline in Europe

Michelle Cleary
Sveriges lantbruksuniversite

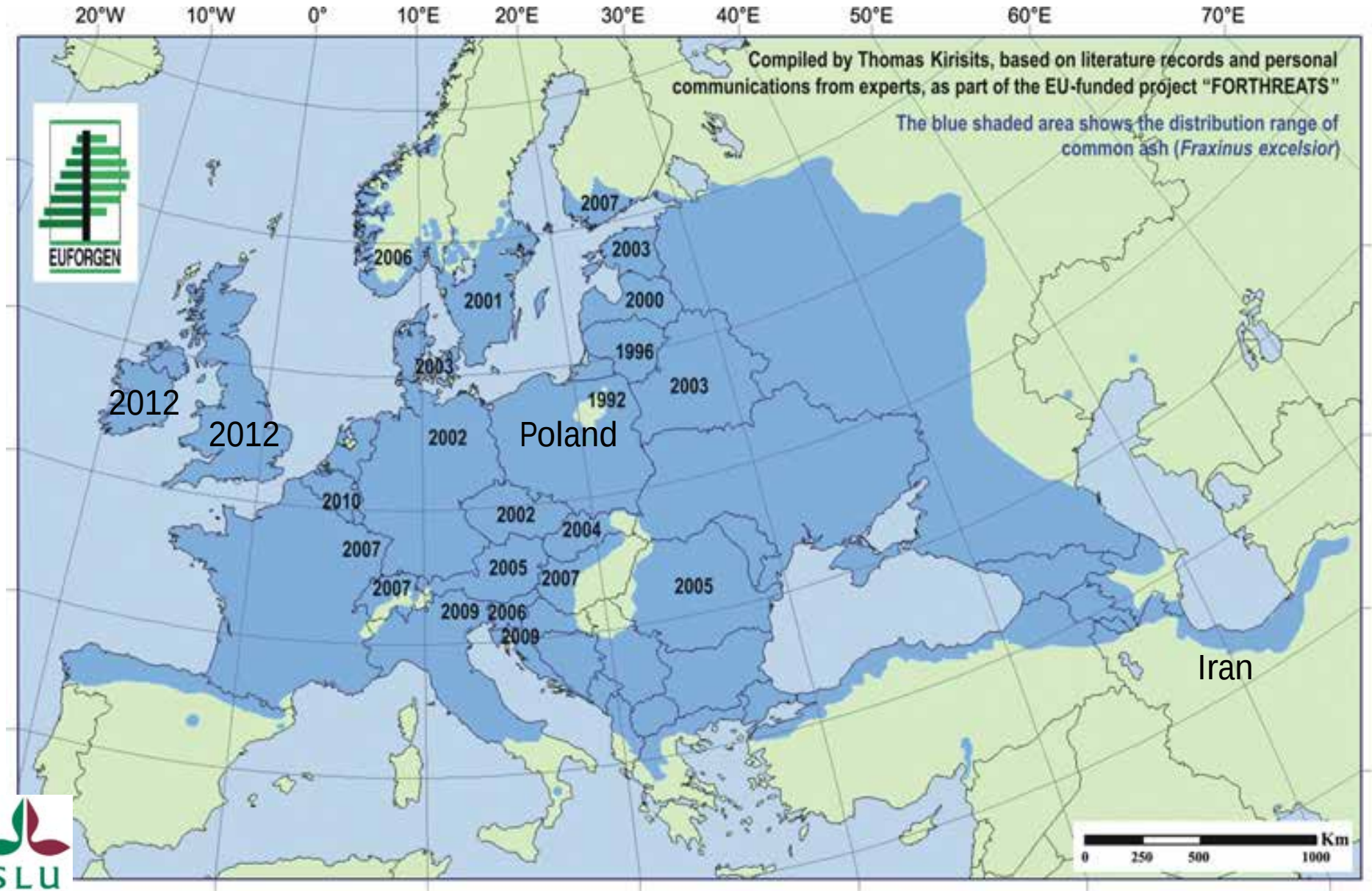
Dušan Jurc, Lucio Montecchi
Slovenian Forestry Institute

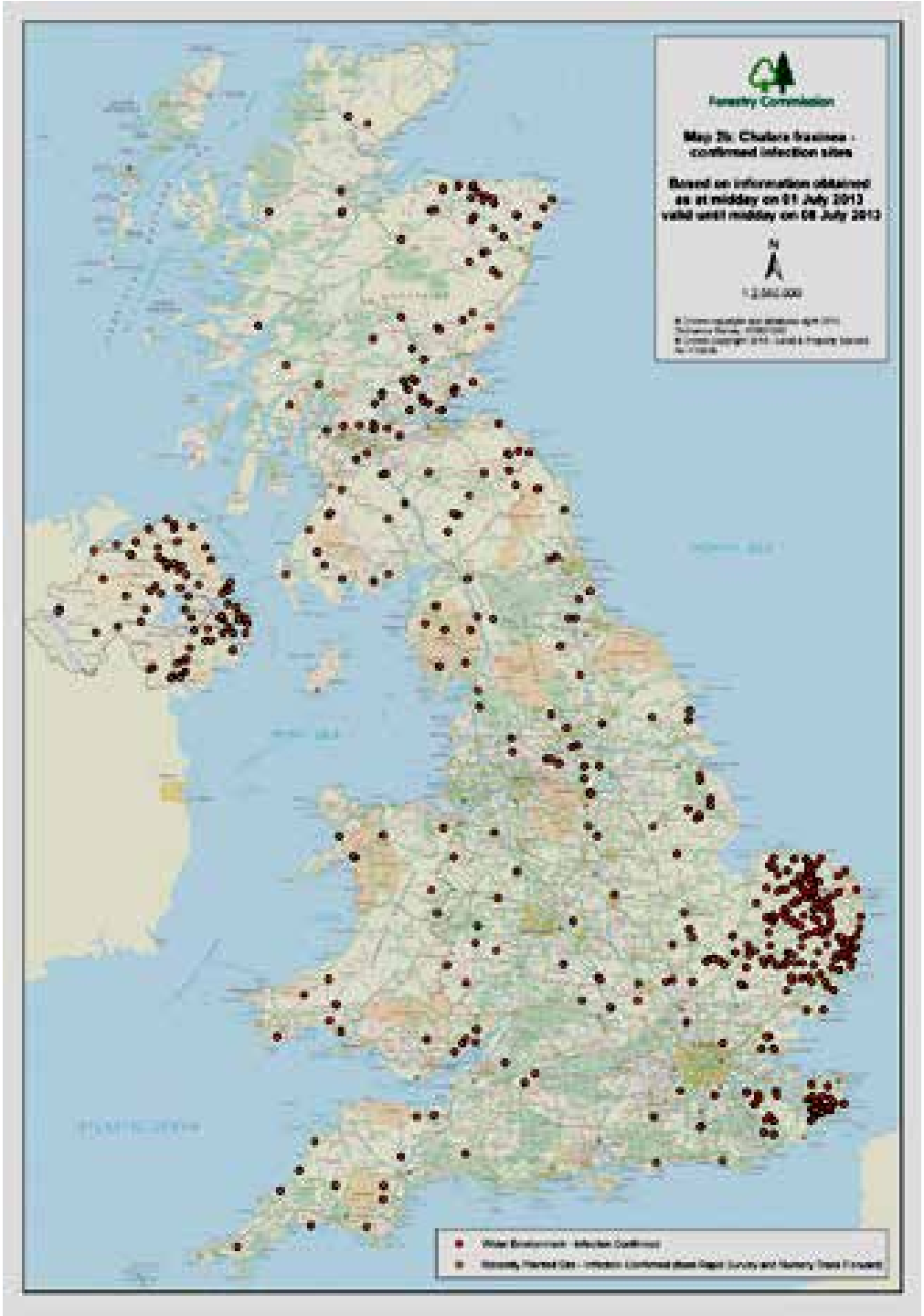
Eric Allen
Natural Resources Canada

IFQRG-11
Qingdao, China
Oct 28 - Nov 1, 2013

Distribution range of *Fraxinus excelsior* (in blue) and the year of the first observation of symptoms of ash dieback in each country

(Timmermann et al. 2011)




Forestry Commission
May 29: Chalara fraxinea - confirmed infection sites
Based on information obtained as at midday on 01 July 2013 valid until midday on 08 July 2013
N
1:2,540,000

Causal agent of ash dieback

- In 2006, the causal agent was described as *Chalara fraxinea* (Kowalski 2006)
- Initial taxonomic studies concerning *C. fraxinea* revealed its perfect state was the ascomycete *Hymenoschyphus albidus*, a common saprotrophic fungus and harmless decomposer of ash leaves and petioles; known in Europe since 1851 (Kowalski & Holdenrieder 2009)
- More recent molecular studies of the teleomorph, however, indicated evidence for the existence of 2 morphologically very similar taxa; *H. albidus* & a new spp, *H. pseudoalbidus* (Queloz et al. 2010)
- A population study including isolates of *H. pseudoalbidus* from diseased trees in Sweden, Denmark, Poland, Austria and Hungary, shows that it is an outcrossing species with limited geographic genetic structure (Bengtsson et al. 2012), which is compatible with a scenario of rapid spread of an aggressive race of the fungus over Europe.

Apothecia of *H. pseudoalbidus*

Symptoms

Bark cankers;
Twig, shoot, branch dieback

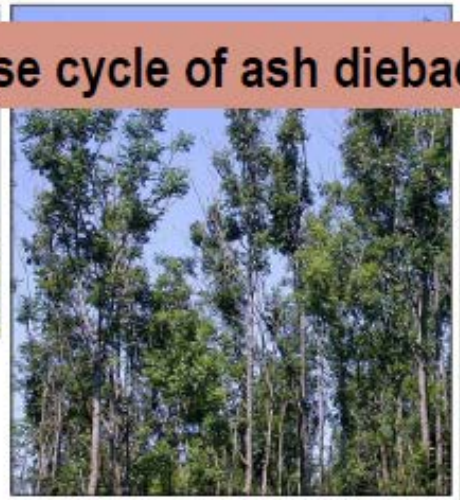
Necrotic lesions on leaves/petioles

Wilting of leaves/new shoots;
adventitious branching

Discoloration of xylem

Hypothetical disease cycle of ash dieback

from spring onwards)



Sexual fruiting bodies predominantly on leaf rachises from the previous year

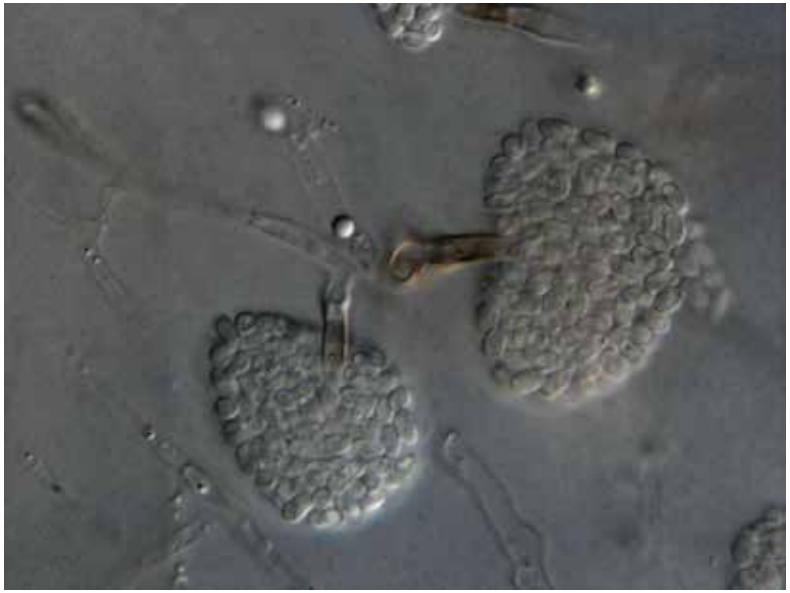
Necrotic lesions on shoots and twigs visible from late summer onwards, but mostly later, **symptom progression continues** also in autumn and winter

Release of ascospores (late spring and summer)

Leaf infections (most conspicuous from August onwards), **(direct infection of shoots?)**

T. Kirisits, 2013 FRAXBACK

Sexual state: *Hymenoscyphus pseudoalbidus*

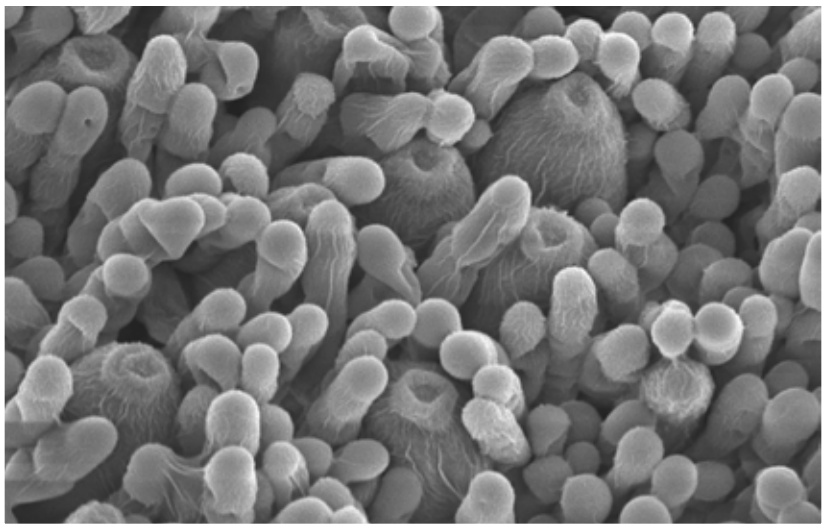


Asexual state: *Chalara fraxinea*

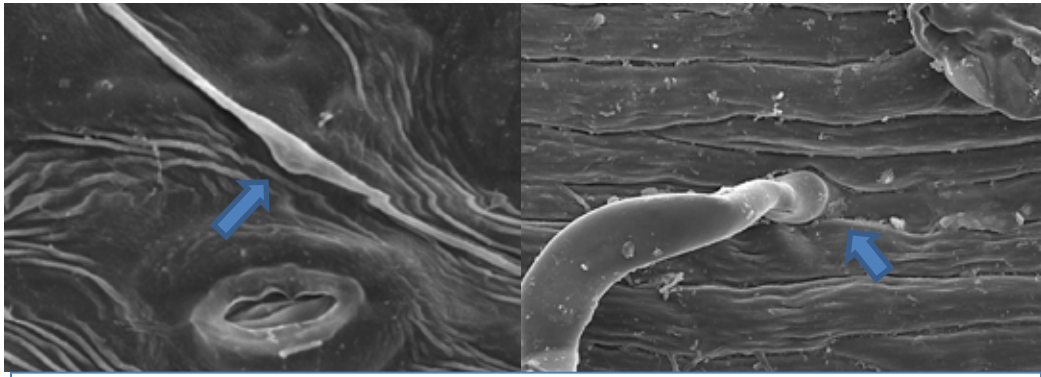
Early infection stages of *H. pseudoalbidus* on *F. excelsior*

Inoculation of plants with ascospores in moist-chamber system

Symptoms on leaves & petioles



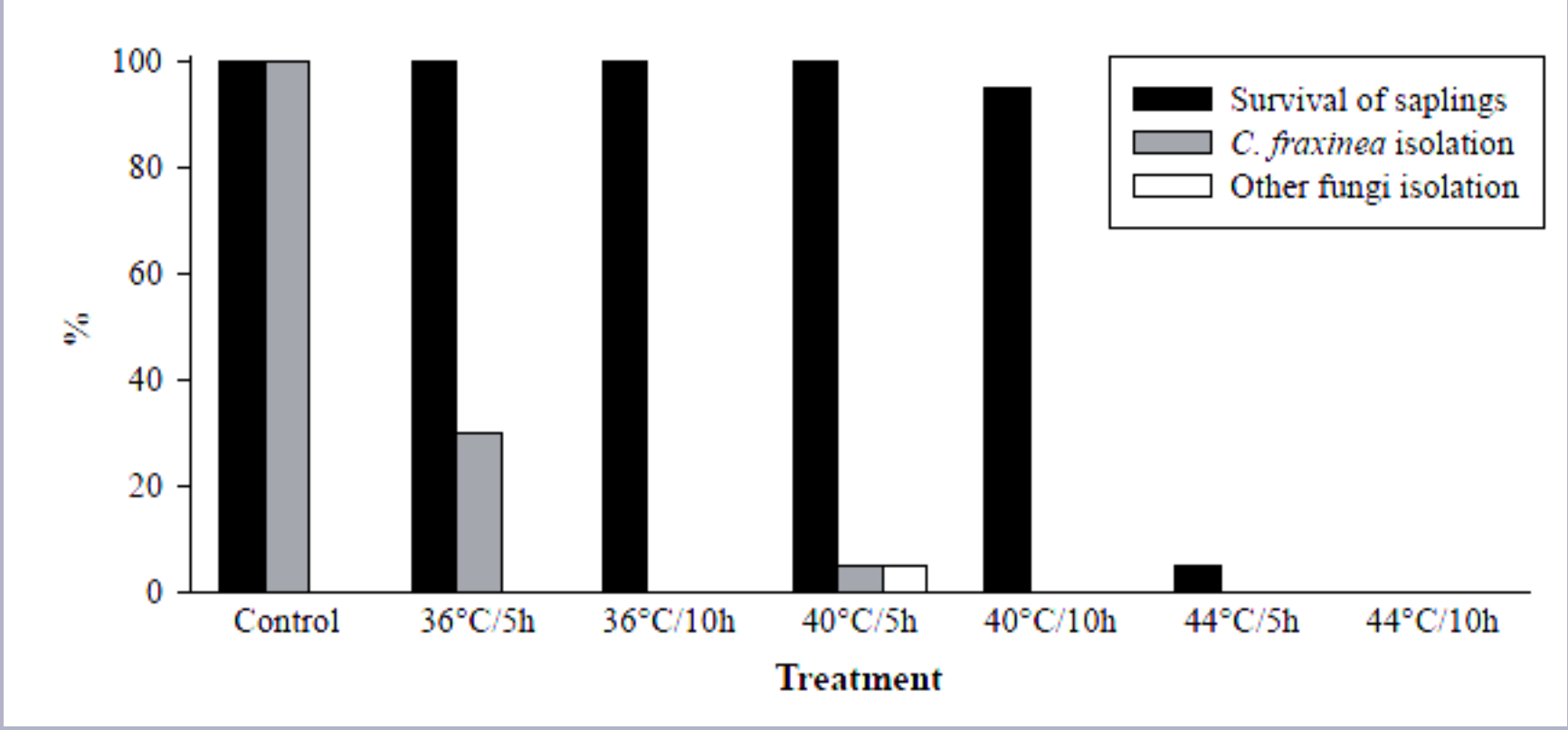
SEM of apothecia of *H. psuedoalbidus* showing mature asci interspersed with cylindrical paraphyses.



Germ tube formation**appressorium** formation and penetration of epidermal cells on BOTH leaves and petioles. PCR with *H. pseudoablidus* specific primers confirmed pathogen presence

Cleary et al. 2013. Light and scanning electron microscopy studies on the infection process of *Fraxinus excelsior* by *Hymenoscyphus pseudoalbidus* (anamorph = *Chalara fraxinea*). Plant Pathology [*in press*]

Heat Treatment



Survival of *Fraxinus* saplings and Chalara fraxinea following hot water treatment

Hauptmann et al. 2013

The search for resistant genotypes of *Fraxinus*

Is Canada protected from *Hymenoscyphus pseudoalbidus*?

Possible pathways:

- natural spread
- plants for planting
- wood
- seed
- handicrafts
- citizen smuggling
- firewood

Fraxinus species in Canada

Native	**White Ash**	**(*Fraxinus americana*)***
	Green – Red Ash	**(*Fraxinus pennsylvanica*)***
	Black Ash	**(*Fraxinus nigra*)***
	Blue Ash	**(*Fraxinus quadrangulata*)**
	Pumpkin Ash	**(*Fraxinus profunda*)**
Non-native urban use	Manna Ash	(*Fraxinus ornus*)*?
	European Ash	(*Fraxinus excelsior*)*
	Raywood Ash	(*Fraxinus oxycarpa*)
	Oregon Ash	(*Fraxinus latifolia*)
	Manchurian Ash	(*Fraxinus mandschurica*)*
	Narrow-leaved Ash	(*Fraxinus angustifolia*)*
	Velvet Ash	(*Fraxinus velutina*)

Canada's regulatory response

Pest Categorization

- meets the definition of a quarantine pest for Canada
- regulatory status under review

Regulated articles:

- rooted nursery stock from Europe (D-08-04 – Plants for planting)
  - *Fraxinus excelsior* and *F. latifolia (*D-01-01 – *P. ramorum)*
- Ash products from the continental USA (D-03-08 - *A. planipennis)*
- Ash wood products from off-continent (D-02-12 – wood products)
- Ash seed (no import permits being issued)
- Firewood D-01-12 (phytosanitary certificate, 56/30)
- Handicrafts (D-02-12 – wood products - RSPM 38, new ISPM)

Lingering questions

- Presence of *H. pseudoalbidus* in seed and wood
  - atypical spore production process??
- Role of conidia
- Treatment options for *H. pseudoalbidus*
  - testing protocols should consider IPPC guidance
- Import risk from areas other than Europe (Japan, Russia)
- Other pathways?

Ash decline in Europe

Michelle Cleary
Sveriges lantbruksuniversite

Dušan Jurc, Lucio Montecchi
Slovenian Forestry Institute

Eric Allen
Natural Resources Canada

IFQRG-11
Qingdao, China
Oct 28 - Nov 1, 2013

Review on the pine wilt disease control

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- Background
- Control of Pine wilt disease
- Perspective

Background

- Pine wilt disease in different countries

  Japan(1905?)，China(1982)，Portugal(1999) .

- Pine wilt disease in China

  Nanjing(1982) , Shenzhen (1988)

- My research field on plant nematode diseases

My research field on plant nematode disease

- Nematode detection by molecular technology
- Molecular interaction between host and pathogenic nematodes
- Plant resistance to its pathogenic nematode
- Nematode disease control in the field

My paper list

（2003年1月--2012年8月31日）

序号	题　　目	发表刊物、时间	刊物级别(IF,分区)
1	Direct PCR-based method for detecting *Bursaphelenchus xylophilus*, the pine wood nematode in wood tissue of *Pinus massoniana*.	*Forest Pathology*, 2011.05 (三区类期刊)	SCI收录 (1.74,Q3)
2	A rapid detection of the pinewood nematode, *Bursaphelenchus xylophilus* in stored *Monochamus alternatus* by rDNA amplification.	*Journal of Applied Entomology*. 2011.02	SCI收录 (1.311, Q4)
3	A New method for viability identification of larvae 3-4 of *Meloidogyne*.	*RSETE*. 2011.06	EI全文检索

My paper list

（2003年1月--2012年8月31日）

序号	题　　目	发表刊物、时间	刊物级别(IF,分区)
4	**A rapid β-Myrcene-attractant assisted wood sampling method for PCR-based detection of *Bursaphelenchus xylophilus* in *Pinus massoniana* wood tissue .**	***RSETE*. 2011.06**	**EI全文检索**
5	**Growth charts of root knots and giant cells caused by *Meloidogyne incognita* in susceptible tomato roots *in vitro***	***RSETE*. 2011.06**	**EI全文检索**
6	**A rapid staining-assisted wood sampling method for PCR-based detection of pine wood nematode *Bursaphelenchus xylophilus* in *Pinus massoniana* wood tissue.**	***Forest Pathology*, 2010.12 (三区类期刊)**	**SCI收录(1.74,Q3)**

My paper list

题　　目	发表刊物、时间	刊物级别(IF,分区)
Cloning arginine kinase gene and its RNAi in *Bursaphenchus xylophilus* causing pine wilt disease.	*European Journal of Plant Pathology* . DOI: 10.1007/s10658-012-0035-0 2012.07 (二区类期刊) 2012(134):521-532	已经被 SCI 收录论文(1.42, Q2)
Proteomic profiles of soluble proteins from esophageal gland in female *Meloidogyne incognita*	*International Journal for Parasitology* , (二区类期刊) 2012.11.http://dx.doi.org/10.1016/j.ijpara.2012.10.008/ 2012，42（13-14）: 1177-83	已经被 SCI 收录 (IF=3.339,Q2)

出版2部林业行业标准 Technical Standards

1. 王新荣(Wang xinrong) 廖金铃 钟填奎 等. 2009. 松褐天牛防治技术规程. LY/T-1866-2009. 北京:中国标准出版社. 书号： 155066·2-19838, 已采纳;

2. 王新荣(Wang xinrong) 廖金铃 钟填奎 等. 2009.松材线虫病疫木清理技术规范. LY/T-1865-2009 . 北京:中国标准出版社. 书号： 155066·2-19867, 已采纳。

ICS 65.020
B 65

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中华人民共和国林业行业标准

LY/T 1866—2009

松褐天牛防治技术规范

Technical standard for controlling pine sawyer
(*Monochamus alternatus* Hope)

2009-06-18 发布　　2009-10-01 实施

国家林业局　发布

前　言

本标准的附录B、附录E、附录F为规范性附录，附录A、附录C、附录D为资料性附录。

本标准由华南农业大学提出。

本标准由国家林业局森林病虫害防治总站归口。

本标准负责起草单位：华南农业大学资源环境学院。

本标准参加起草单位：广州市森林病虫防治检疫站、广东省惠州市神龙松材线虫病综合治理有限公司。

本标准主要起草人：王新荣、廖金铃、钟镇奎、孙思、朱孝伟、陈纪文、贾文慧、孔祥超、李亚东、冯志新。

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中华人民共和国林业行业标准

LY/T 1865—2009

松材线虫病疫木清理技术规范

Technical standard for cleaning up pine trees infested by pine wood nematodes

2009-06-18 发布　　2009-10-01 实施

国家林业局　发布

前　言

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Control of pine wilt disease

Before 2000

- Quarantine .

  Traditional nematode identification with nematode's morphology

- Cleaning up dead pine trees
- Establishment of natural forest safe belt
- Beetle vector control with insecticides
- Accumulating knowledge related to pine wilt disease
- Pine wilt disease control projects

Control of Pine wilt disease

Between 2000 and 2008

- Quarantine .

  Pine wilt nematode detection. PCR. QTPCR

- Cleaning dead pine trees infested by nematodes

  Establishment of Infested wood process factories

- Beetle Vectors control

  Insecticides , biological control , lure trap

Control of pine wilt disease

Between 2000 and 2008

- Injecting both insecticides and nematicides into living pine trees that harbor nematodes and beetles.
- Setting up companies specialized in pine wilt disease control
- Establishing the demonstration sites for controlling pine wilt disease

After 2008

- Announcement of three Technical standards related to pine wilt disease control by Chinese government

- Resistant testing on the various pine species.

- Establishment of new administration system on pine wilt disease control

- Announcement of three **Technical standards related to pine wilt disease control by Chinese government**

  1. Beetle vector lure trap (Guang zhou)(2009)

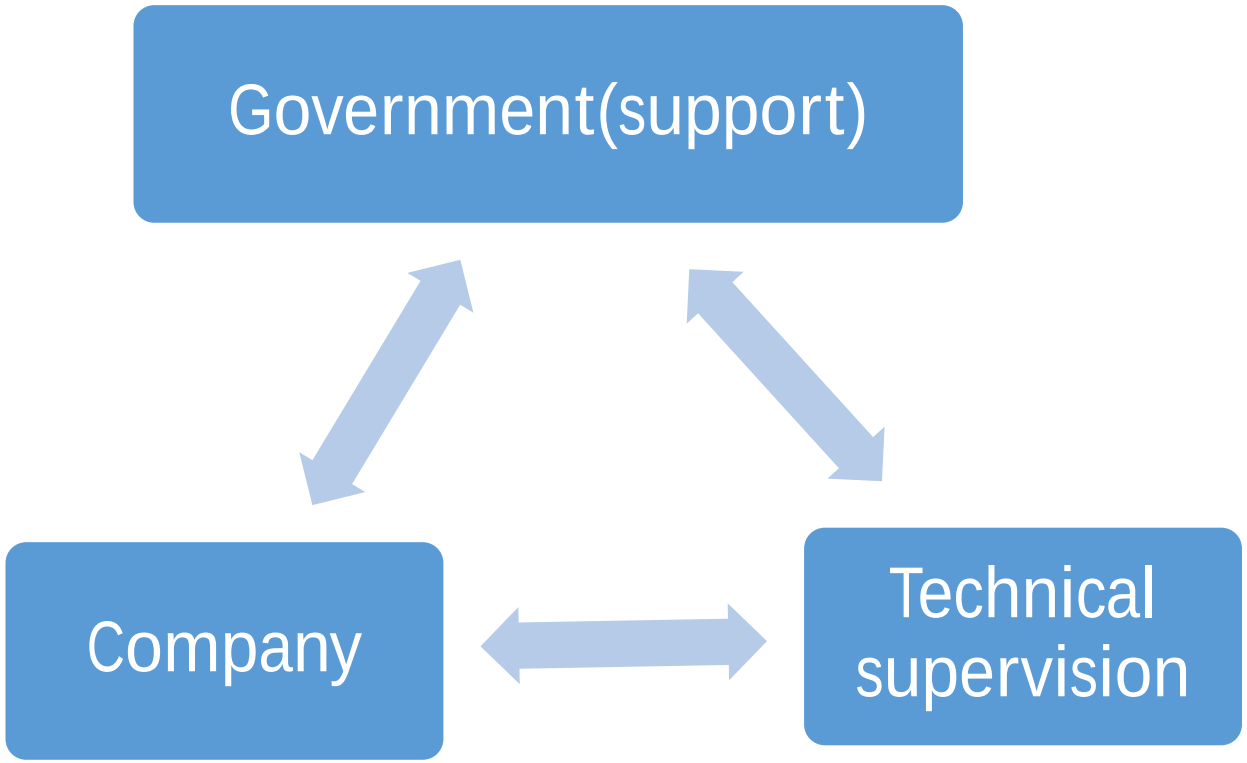
  2. Cleaning up pines trees infested with the pine wilt disease(2009)

  3. Beetle vector control (2009)

  4. Detecting *Bursaphelenchus xylophilus* from Pine sawyer （*Monochamus alternatus* Hope） by PCR amplification(2013)

- **Resistant testing on the various pine species.**

Establishment of new administration system on the pine wilt disease control




2000 - 2010

Perspective

- Training

  Create the confidence level
- Application of new technologies for monitoring pine wilt disease
- Plantation of pine species resistant to pine wilt disease

- Establish the control models for different forest types

Pine trees in city parks; mature pine trees (20-50 years old) ; post mature pine trees (more than 50 years old)


谢谢！
Thank you！