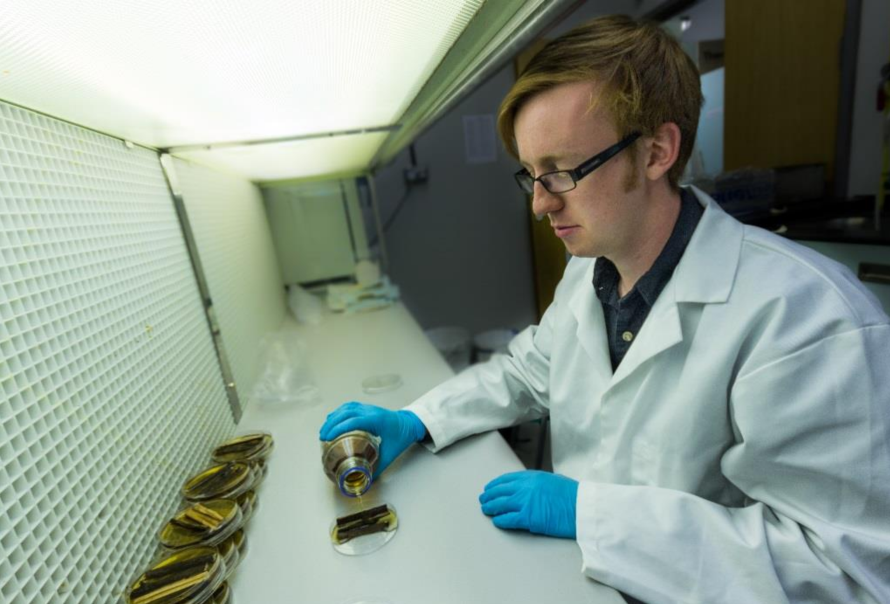
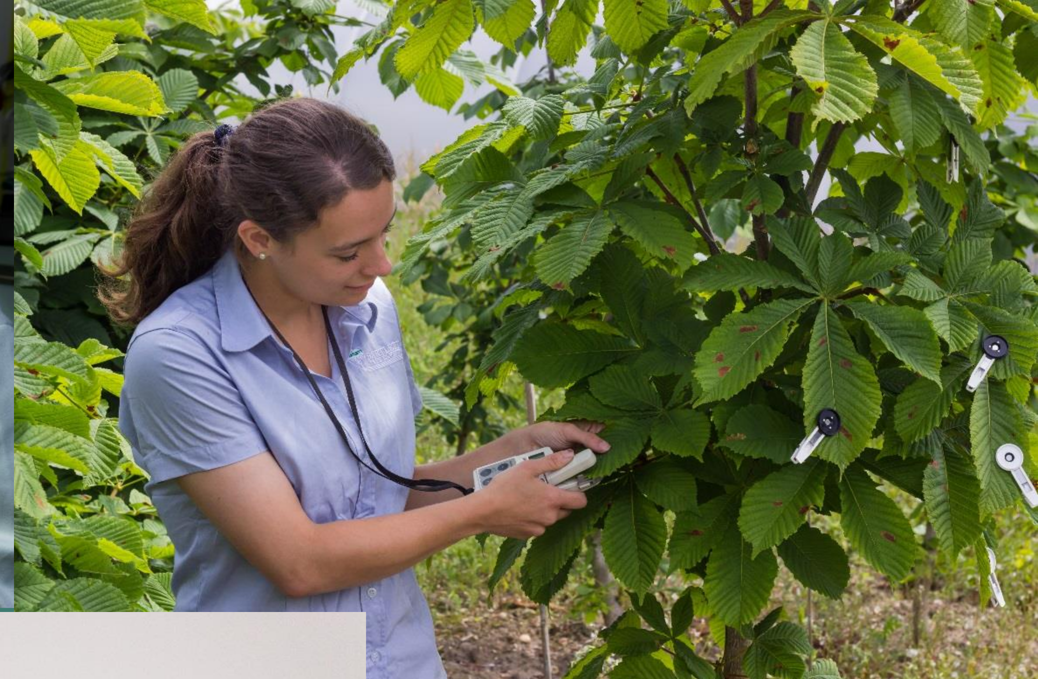
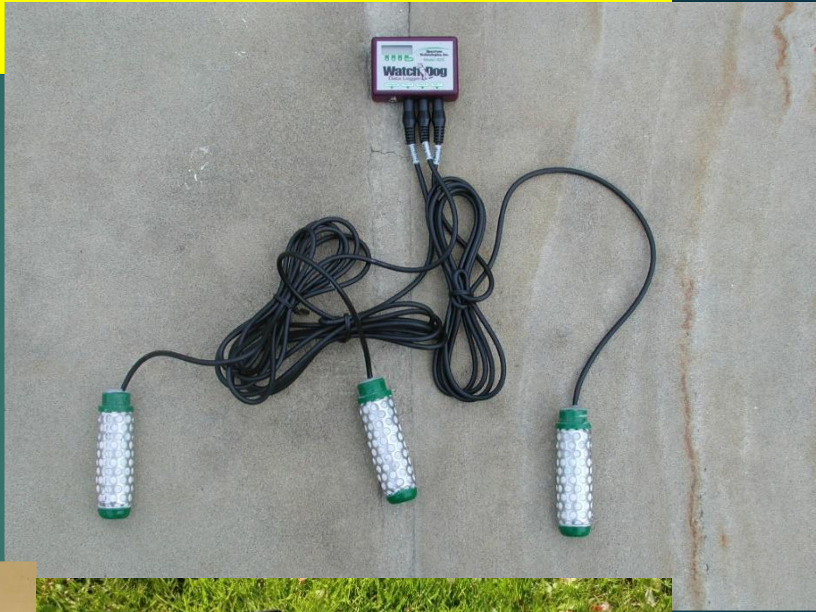
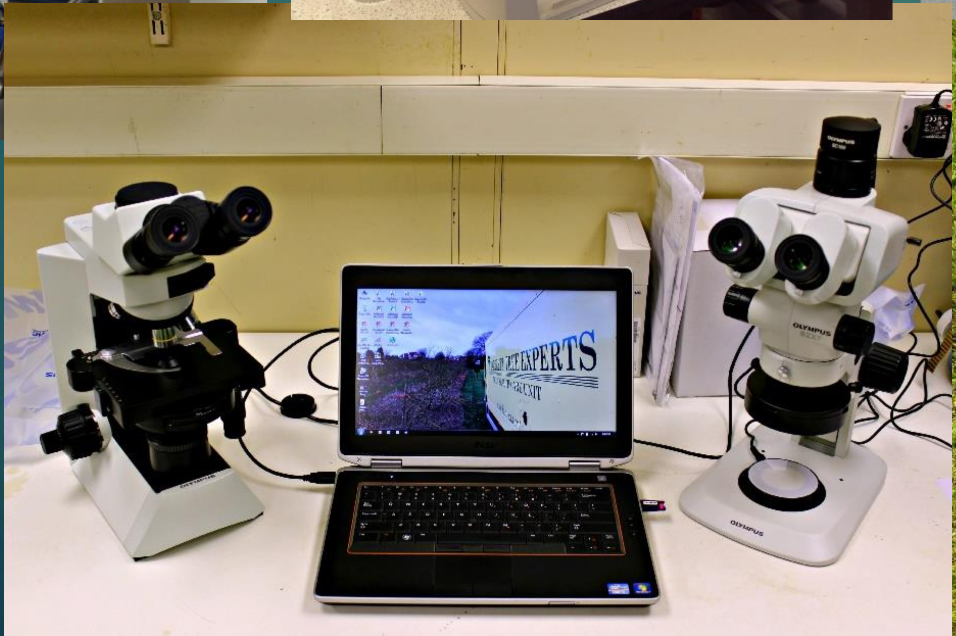
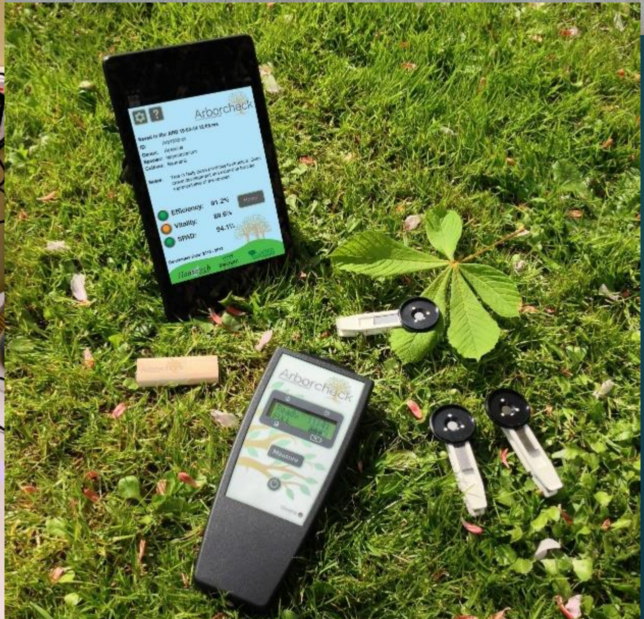
Pest and Disease Update

Dr G Percival: Bartlett Tree Research Laboratory


WatchDog

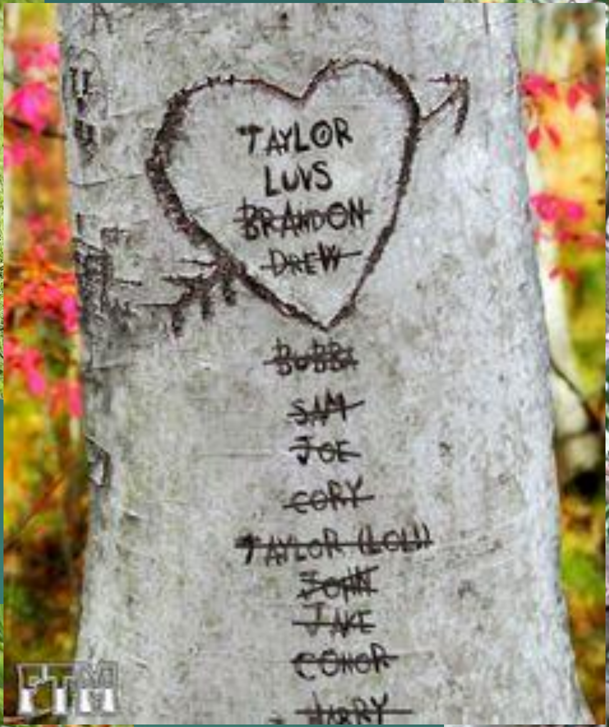

OLYMPUS
EXPERTS
OLYMPUS


Arborcheck
Arborcheck

Wound Treatments

- **Generally considered a BAD thing to do.**
- **However, all wound treatment research was predominantly done in the mid-late 1980's i.e. 25-30 years ago.**
- **Aims of our research were to evaluate a range of treatments on wound formation after trees (English oak, apple) were wounded.**
- **Stimulation of wound closure would be beneficial to reduce wound canker diseases.**

Wound Treatments



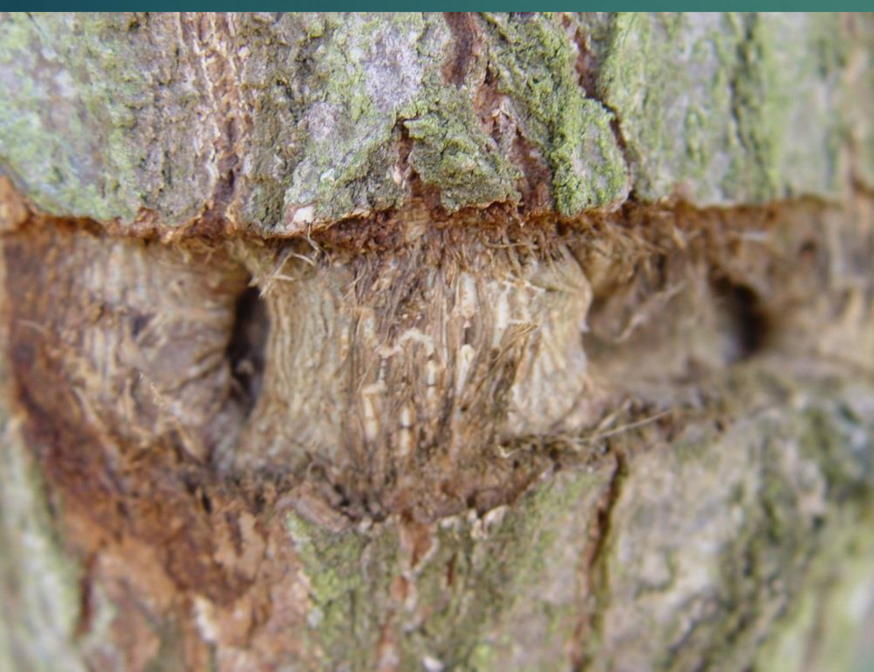
Wound Treatments

Wound pathogens - Cankers

Wound Treatments

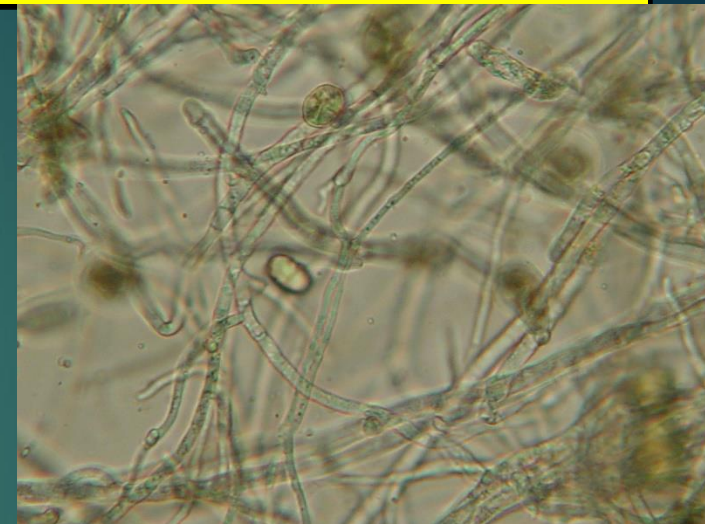
Wound Treatments

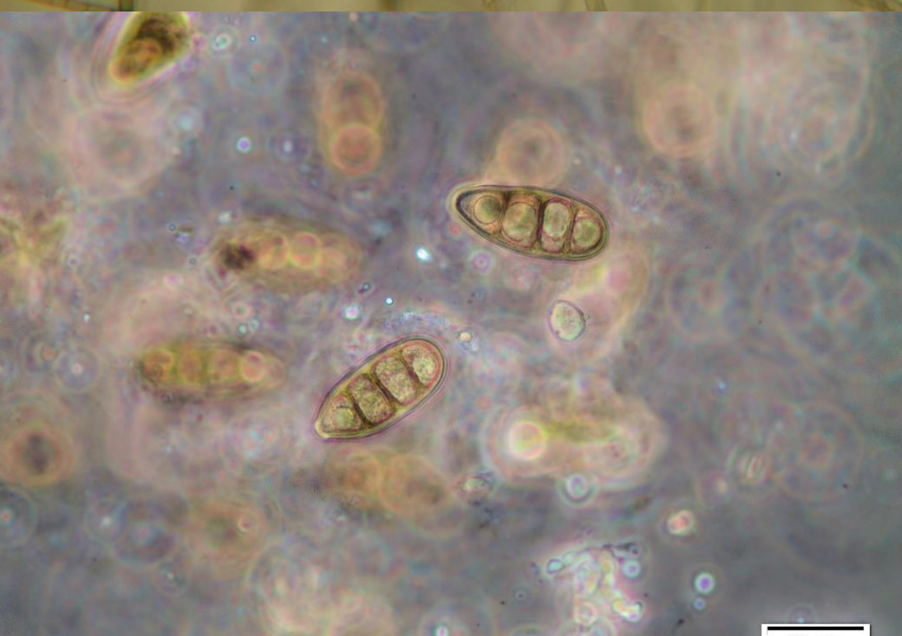
Some species respond well to wounding

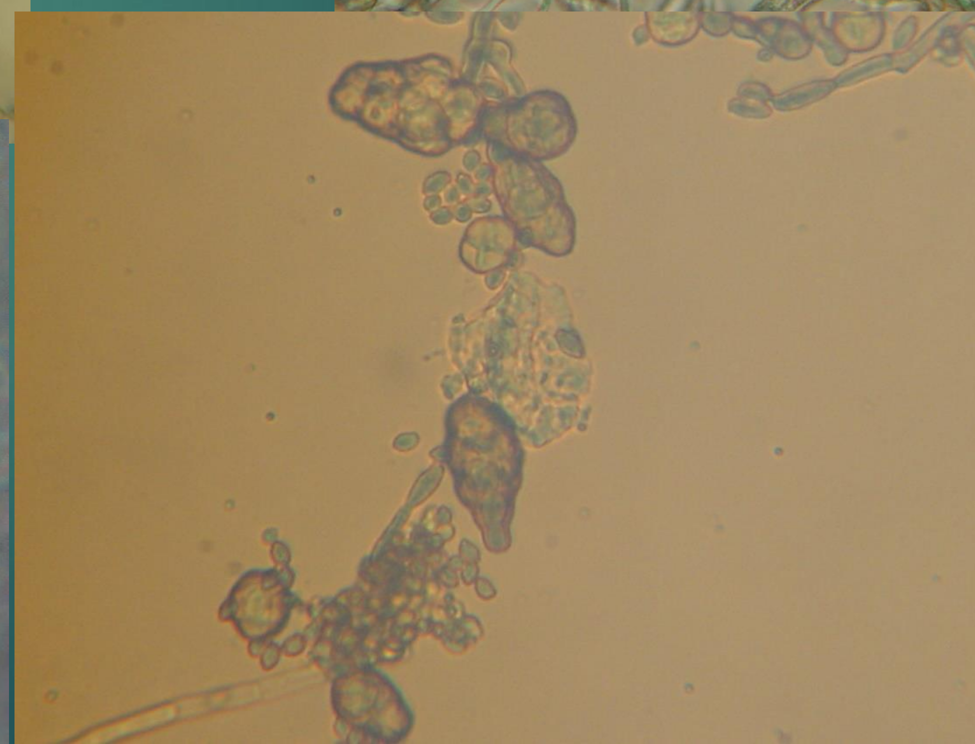


Re-Isolation of Wound Cankers

Identify using spores morphology







Treatment	% Wound Area Callused after 12 months	Presence of wound pathogens
Control	23.0	+
Water sprayed	21.0ns	+
MaxiCrop (Biostimulant)	24.0ns	+
Compost tea	32.0ns	+
Trichoderma	38.0*	-
Serenade (Bacillus spp)	35.0*	-
Penconazole (Fungicide)	38.0*	-
Phosphite	43.0*	+
Soil	45.0*	-

The Take Home Message

Perhaps time to start using/looking at wound dressings again?

An Interesting Read

- HortiWeek (2017): UK research points to bacterial cause of acute oak decline
- The 16-strong team of researchers from Forest Research, Rothamsted Research, Bangor University and the University of Liverpool tested the hypothesis that AOD lesions are caused by "a polymicrobial complex".

  Basically Acute Oak Decline (AOD) is a complex disease syndrome of native oak.
- We call this a pathodome


(4) Length 1.62 µm
(2) Length 1.61 µm
(3) Length 2.04 µm
10 µm

- So does a fungal pathodome exist when we see this?

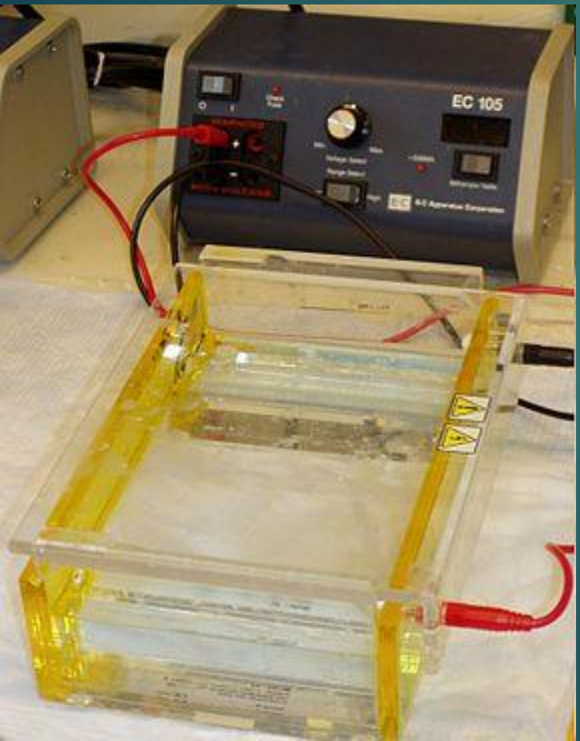
Or this?

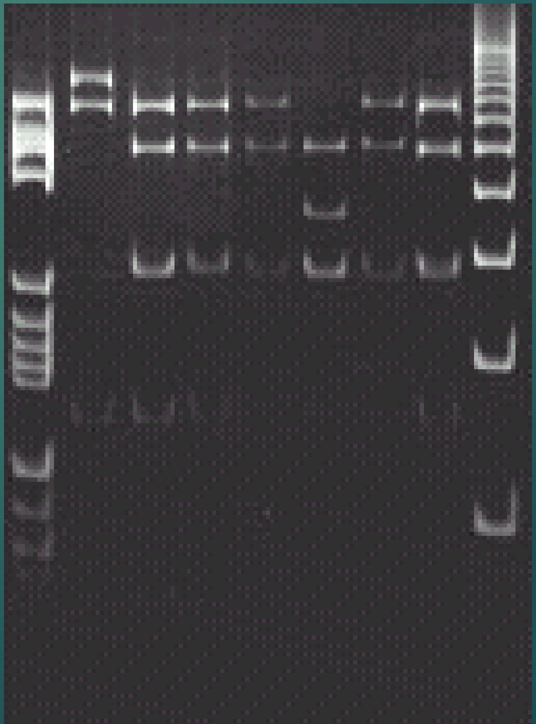
Sample from sound and
“rotten” interface

take 4 samples

Do lots of geeky molecular stuff!

- PCR and gel electrophoresis etc.,





CSI: Tree

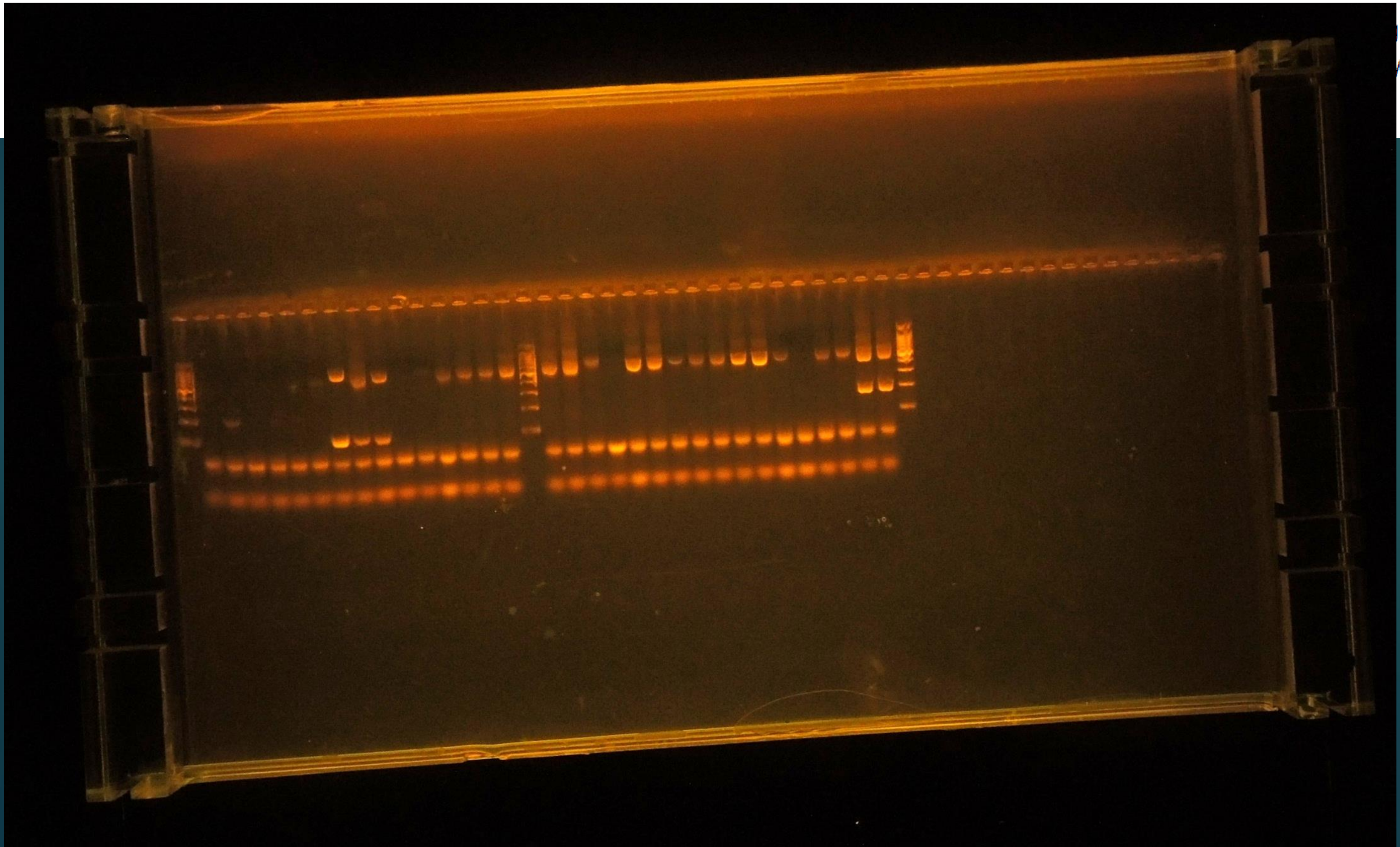
ORIGINAL ARTICLE

A multiplex PCR-based method for the detection and early identification of wood rotting fungi in standing trees

F. Guglielmo[1], S.E. Bergemann[2], P. Gonthier[1], G. Nicolotti[1] and M. Garbelotto[2]

liasco , Italy

, California, USA



Possible Test Results:

Nothing

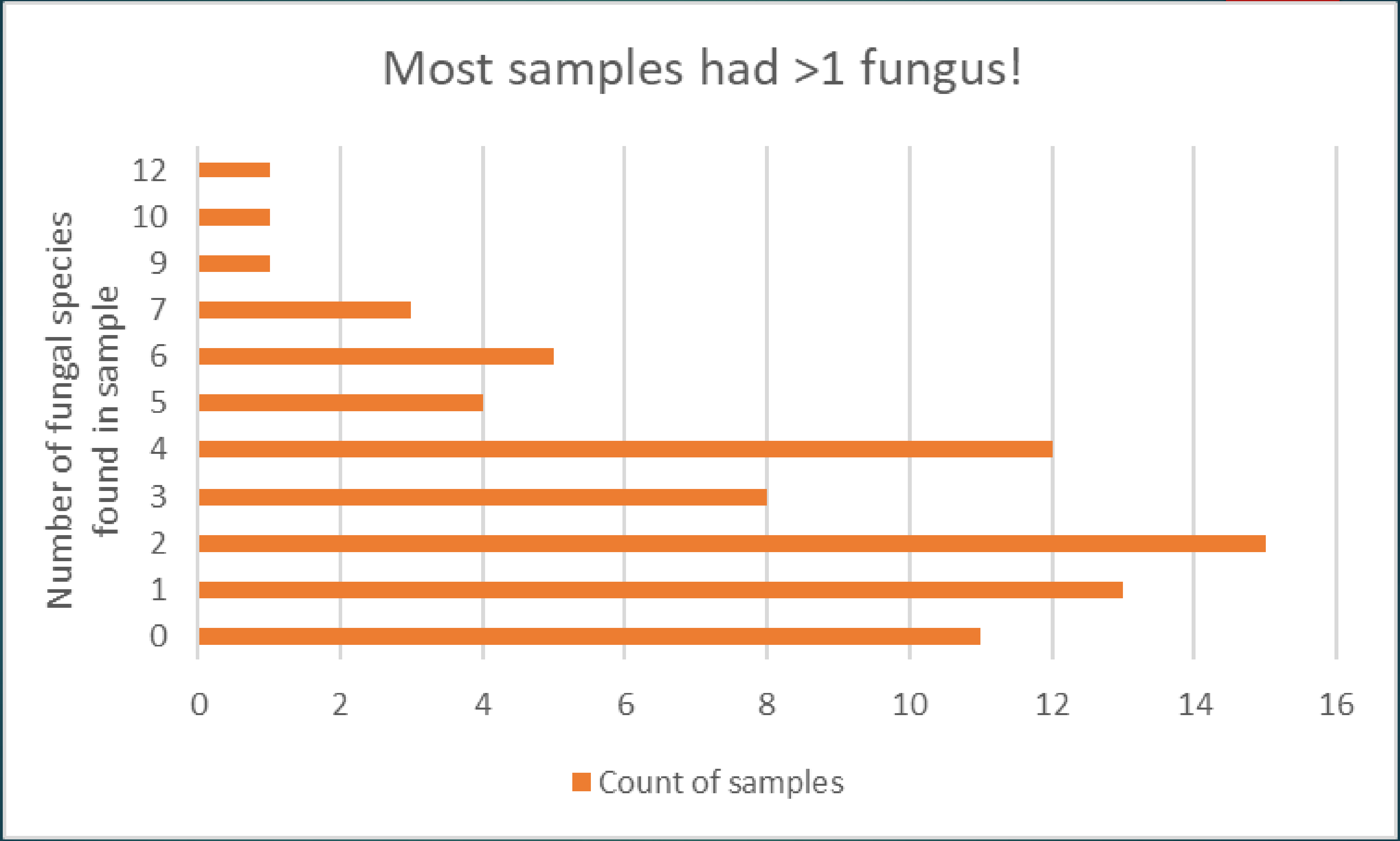
one of the 21 species

Some other fungus

Targets

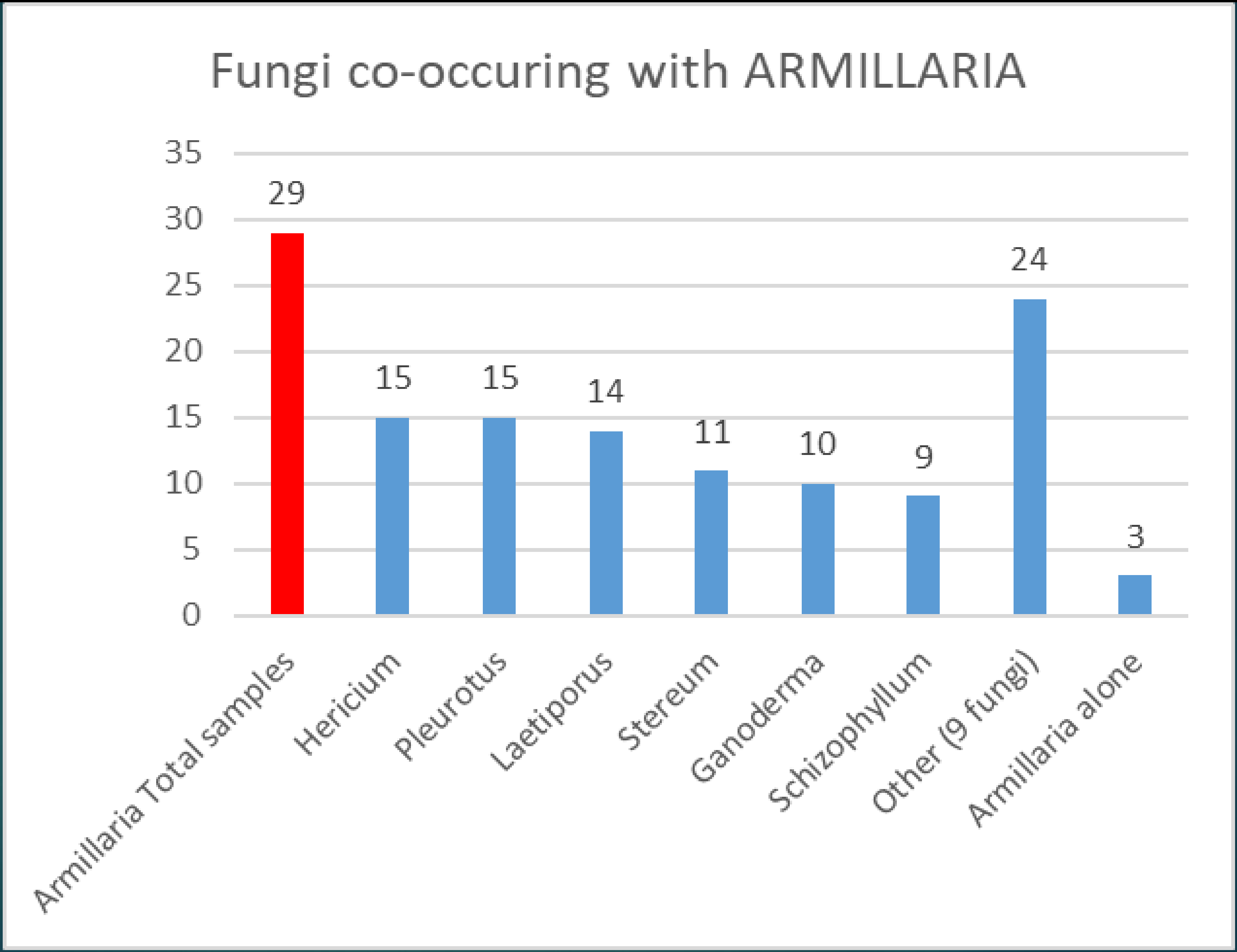
1. Fungal DNA
2. *Armillaria* spp.
3. *Fomitiporia* (*P. punctatus, P. robustus*)
4. *Fuscoporia* (*P. contiguous, P. gilvus, P. torulosus*)
5. *Ganoderma* spp.
6. *Ganoderma adspersum*
7. *Ganoderma applanatum*
8. *Ganoderma lucidum* (Eu)
9. *Ganoderma resinaceum*
10. *Hericium* spp.
11. *Inocutis* (*I. dryophilus*)
12. *Kretzschmaria deusta*
13. *Inonotus dryadeus*
14. *Inonotus* s.s. (*I. andersonii, I. hispidus, I. obliquus*)
15. *Inonotus/Phellinus* spp.
16. *Laetiporus* spp.
17. *Perenniporia fraxinea*
18. *Phellinus* s.s. (*P. igniarius, P. lundelii, P. tremulae, P. tuberculosus*)
19. *Pleurotus* spp.
20. *Schizophyllum* spp.
21. *Stereum* spp.
22. *Trametes* spp.

The most surprising result…

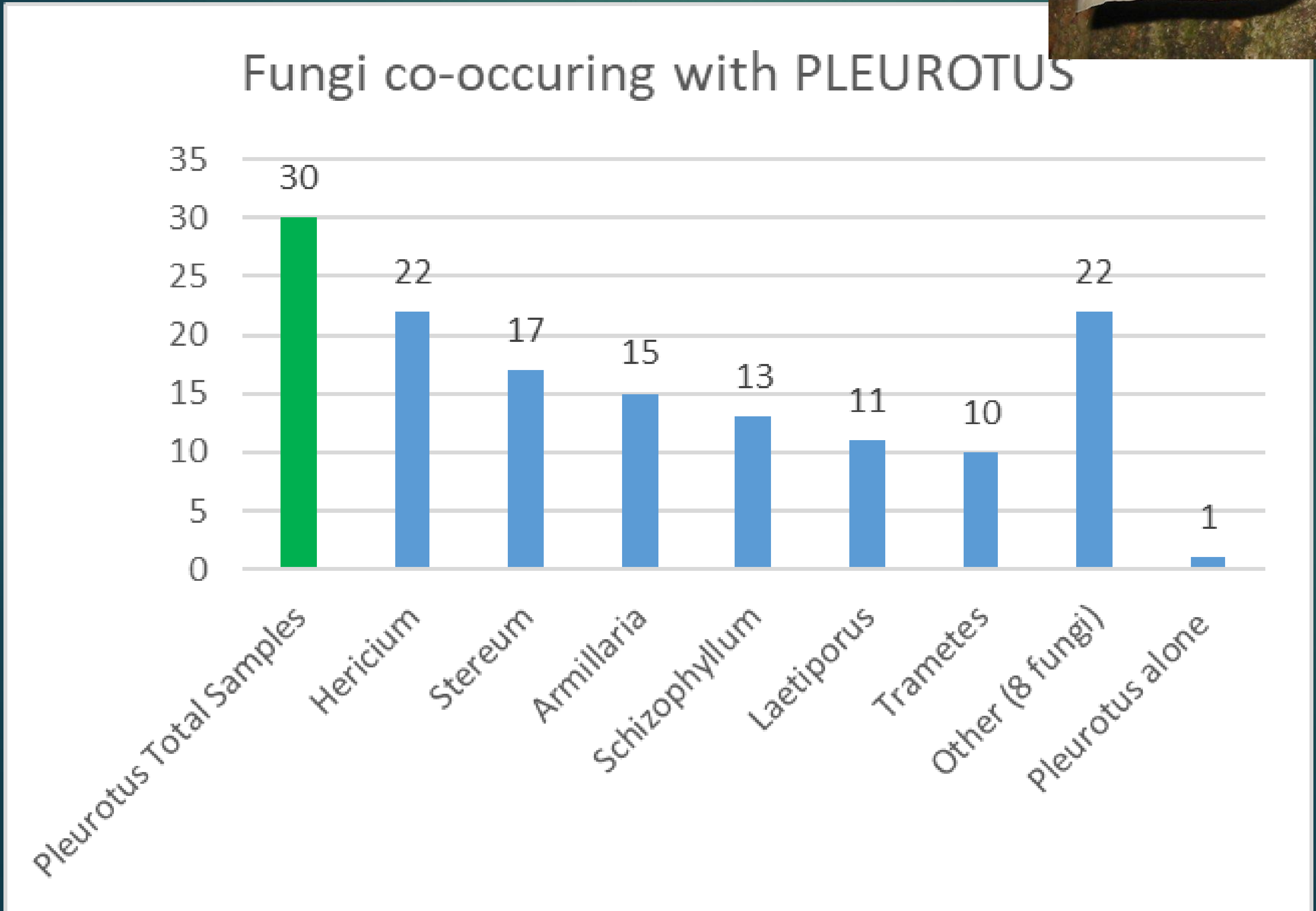


Decay fungi like company!

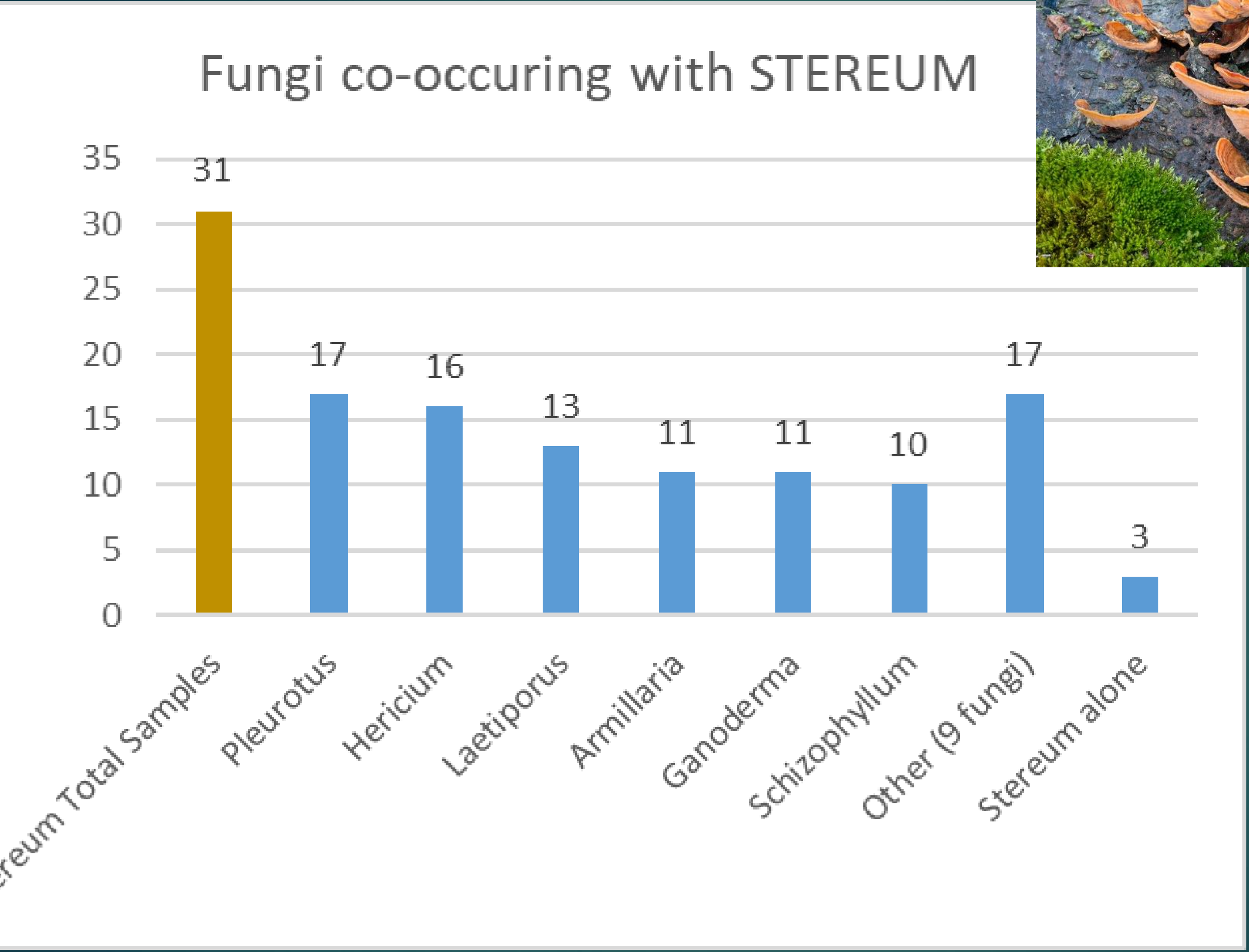
Decay fungi like company



Decay fungi like company



Decay fungi like company



So what dos this mean?

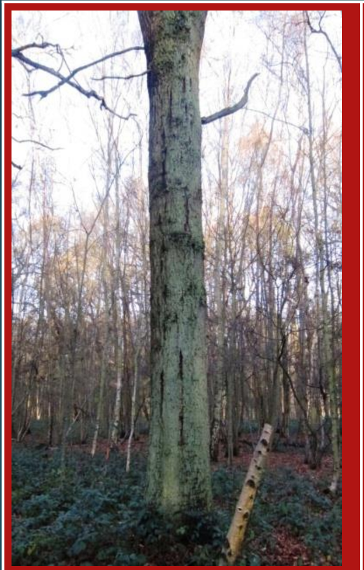
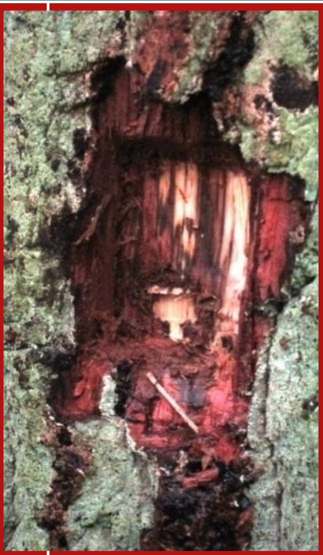
To be honest I don’t really know?

Fungi don't follow rules to exhibit a decay form (white/brown rot?)
i.e. that simple descriptors of wood decomposition do not necessarily reflect the diversity in decay strategies exhibited by fungi?

Wood is a complex substrate in a complex environment, consequently, evolution of decay mechanisms will be complex as well?

Riley, R., *et al.* (2014). **Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi**. *Proceedings of the National Academy of Sciences*, 2014;
DOI: 10.1073/pnas.1400592111

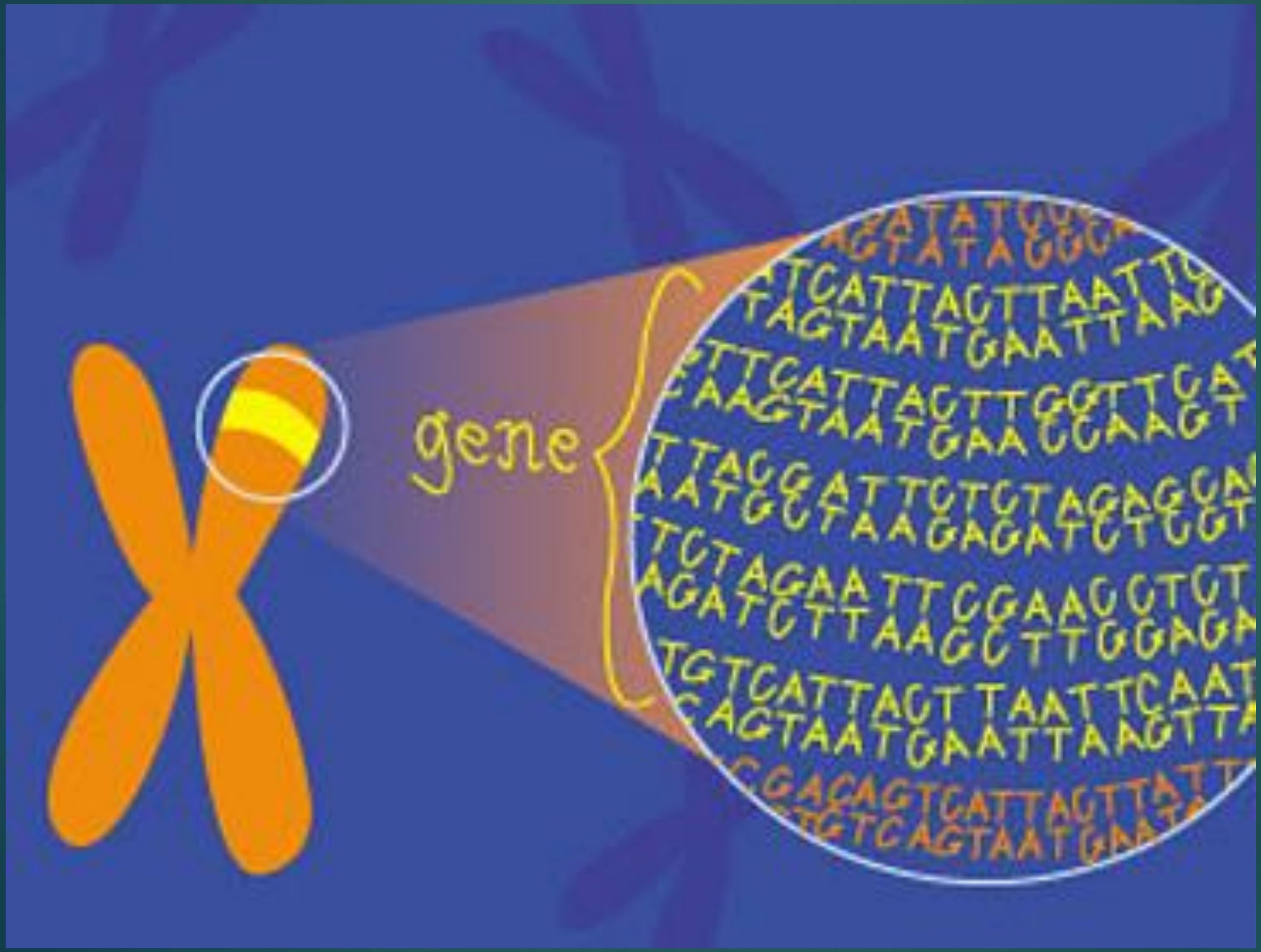
Acute Oak Decline.

				
Overview of stem of tree	**Individual canker**	**Sample of outer bark**	**Sample of inner bark**	**Sample of sapwood**




Why?

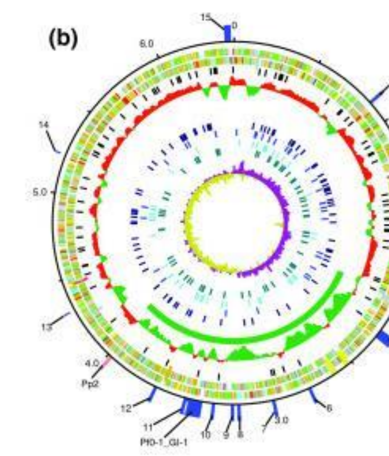
Genetics?



A Different Idea

- Endophytes are organisms, often fungi and bacteria, that live between/within plant cells without causing disease.
- Evidence exists that the endophytic populations within a tree can be the difference between disease resilance and susceptibility (Soliman et al., Plant Biology 2013, Vol 13 pp 93-99).

Isolation of endophytic fungi from asymptomatic and symptomatic trees

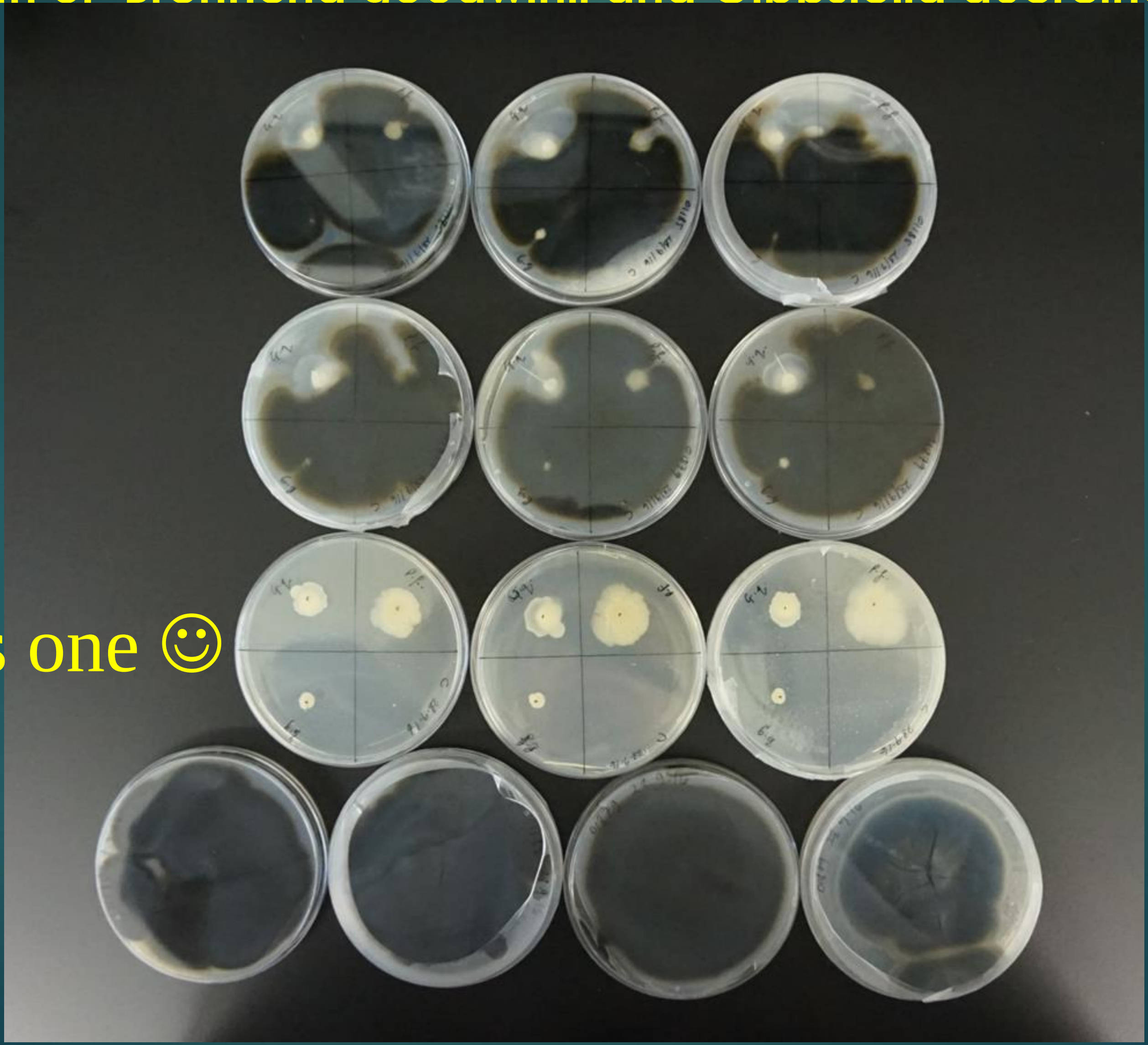


- Samples were sterilised before growing on a number of different media to establish culturable endophytic fungi.
- DNA was then extracted and PCR sequencing of ITS1 and ITS4 region.
- Processing of data still in progress.
- Aim to establish if there is an antagonistic fungi within non symptomatic trees that will reduce growth of AOD bacterial complex.

Endophytic fungi from asymptomatic tree

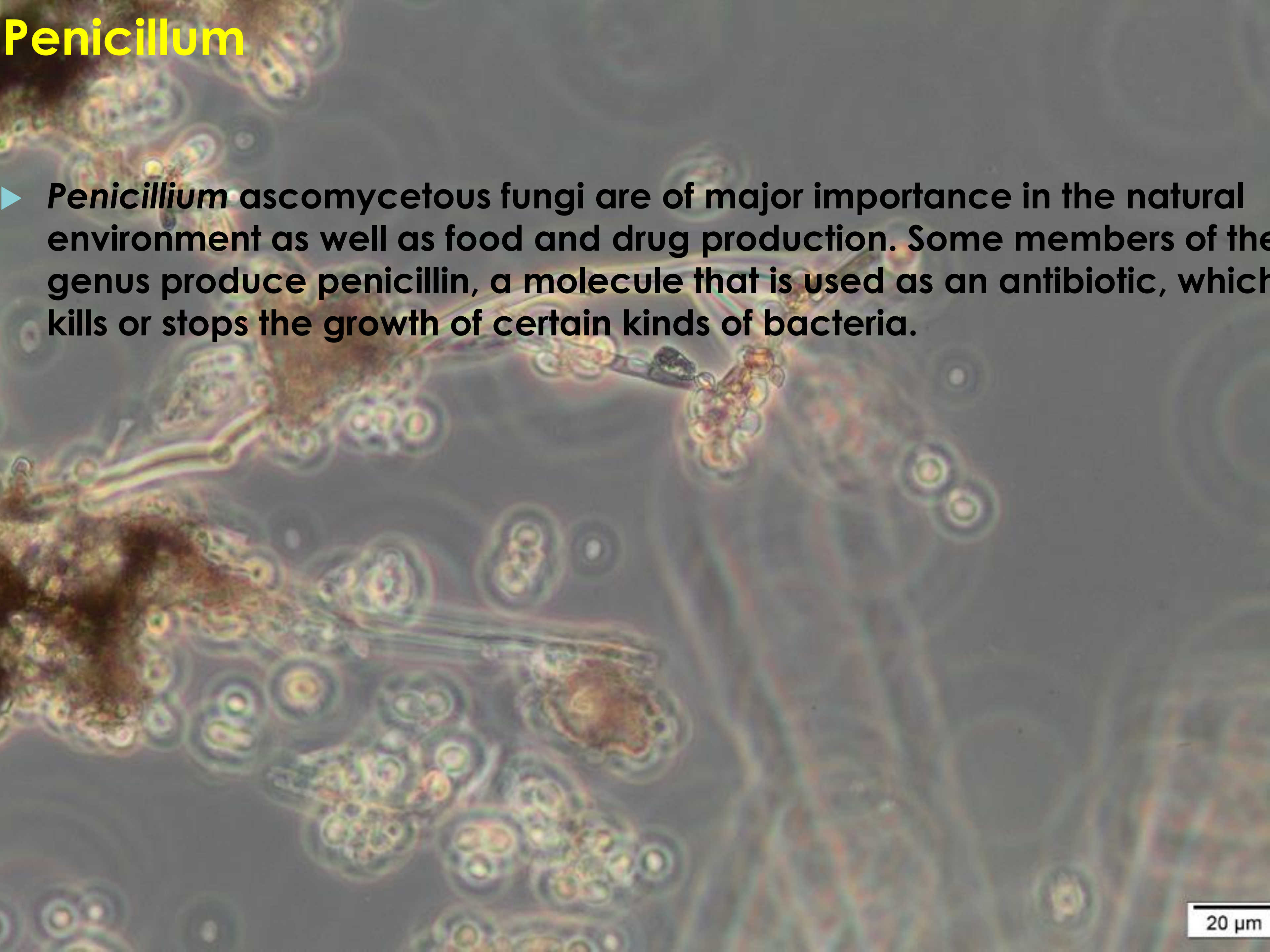
Tree	No. of samples sequenced	No. of samples not sequenced	No. of genus	No. of species	Ident.1	Ident. 2	Ident. 3	Ident. 4	Ident. 5	Ident. 6
473	5	1	1	3	Penicillium aeneum	Penicillium aeneum	Penicillium citreosulfuratum	Penicillium miczynskii	-	-
912	6	0	3	3	Penicillium brevicompactum	Penicillium brevicompactum	Botryosphaeria stevensii	Botryosphaeria stevensii	Botryosphaeria stevensii	Davidiella macrospora
913	6	2	3	3	Botryosphaeria stevensii	Botryosphaeria stevensii	Leotiomycetes sp	Cladosporium sp.	-	-
474	5	0	2	3	Botryosphaeria stevensii	Botryosphaeria stevensii	Botryosphaeria stevensii	Penicillium aurantiacobrunneum	Penicillium sp.	-
915	6	1	2	4	Penicillium aeneum	Penicillium aeneum	Penicillium manginii	Penicillium aurantiacobrunneum	Leotiomycetes sp.	-
916	6	1	3	5	Penicillium citrinum	Penicillium aeneum	Penicillium cravenianum	Talaromyces sp.	Aspergillus versicolor	-
917	3	0	3	3	Uncultured Phialocephala	Ascomycota sp.	Penicillium citreosulfuratum	-	-	-
918	5	0	2	2	Botryosphaeria stevensii	Botryosphaeria stevensii	Penicillium citrinum	Botryosphaeria stevensii	Trichoderma koningii	
919	4	1	2	3	Penicillium verhagenii	Leotiomycetes sp.	Penicillium sp.	-	-	-
920	5	1	3	3	Botryosphaeria stevensii	Botryosphaeria stevensii	Cladosporium sp.	Penicillium sp	-	-

From initial antagonistic assays (- on going work), one of the endophytic fungi (*Penicillum* species) appears to inhibit the growth of Brenneria goodwinii and Gibbsiella quercinecans.



This one ☺

Penicillum

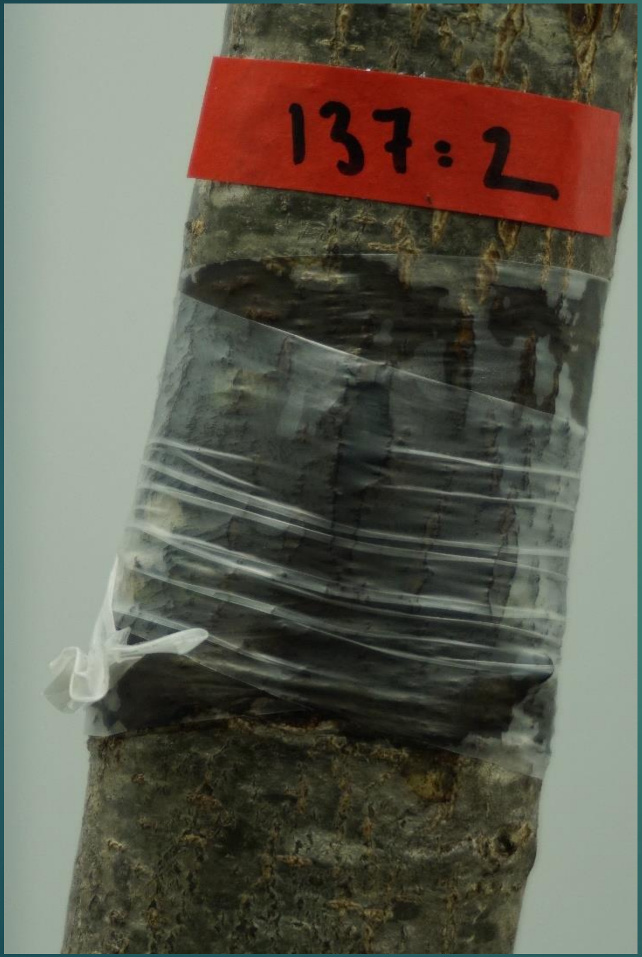


- *Penicillium* ascomycetous fungi are of major importance in the natural environment as well as food and drug production. Some members of the genus produce penicillin, a molecule that is used as an antibiotic, which kills or stops the growth of certain kinds of bacteria.

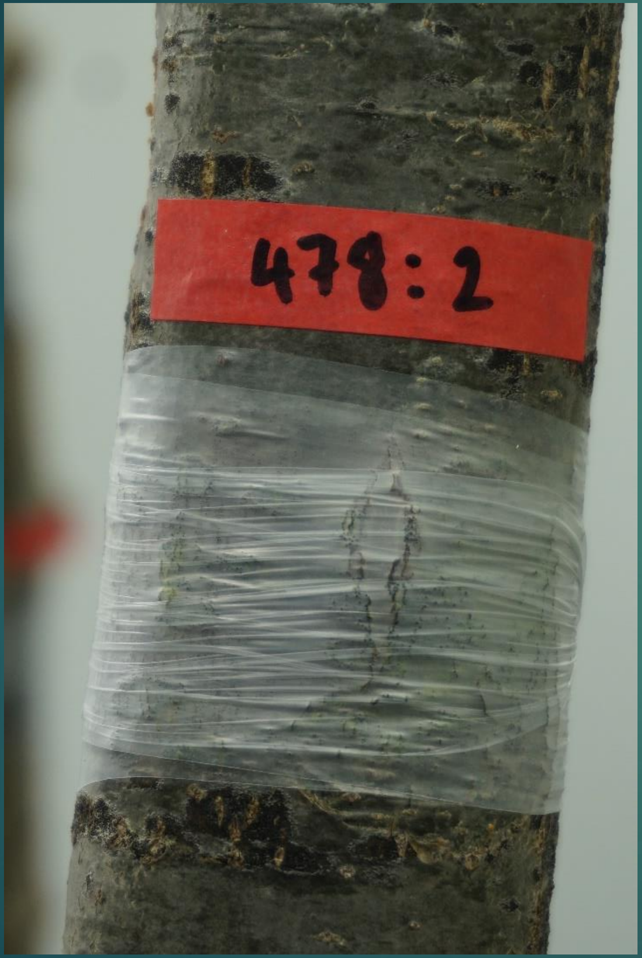
Inoculation within Controlled Environmental Room

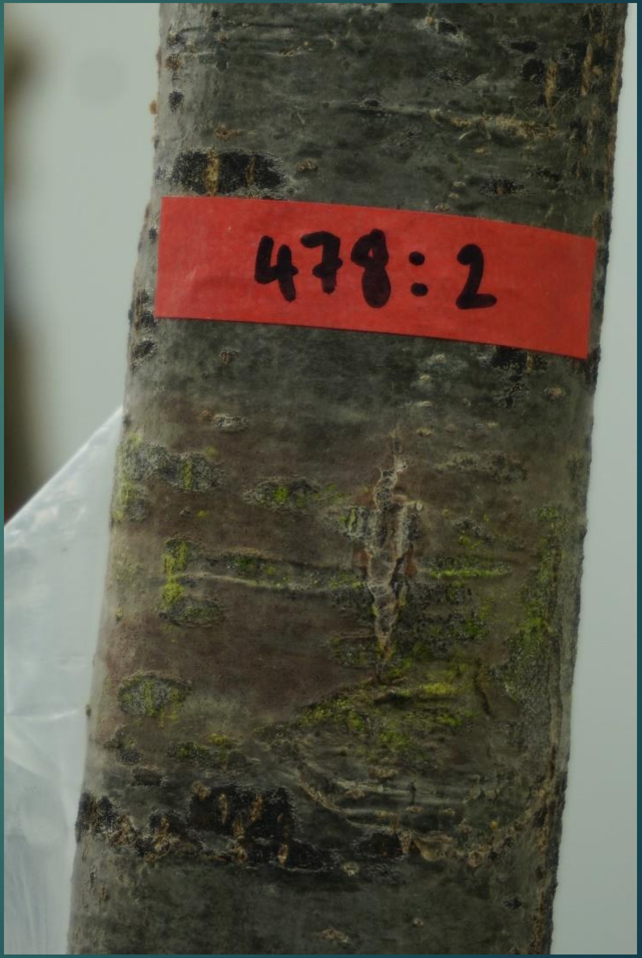
Bite - Blade for Infusion in Trees

Bleed Symptom on inoculated trees (AOD only)

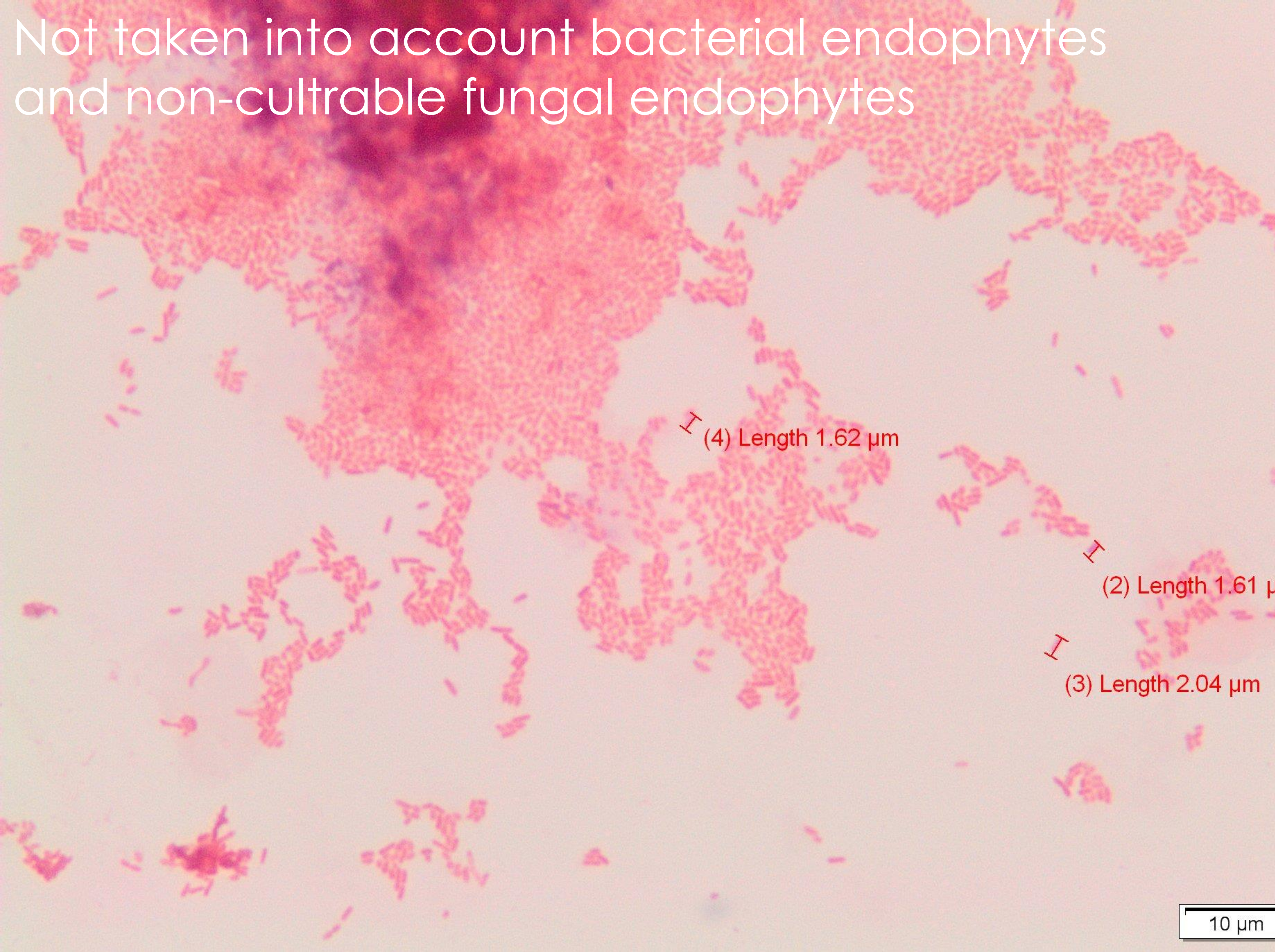


Bleed Symptom on inoculated trees (AOD + Endophytes)





Not taken into account bacterial endophytes and non-cultrable fungal endophytes



Endophytic Bacteria

Pseudomonas

- Three antagonistic strains identified.
- Most antagonistic = up to 50% growth reduction

Bacillus

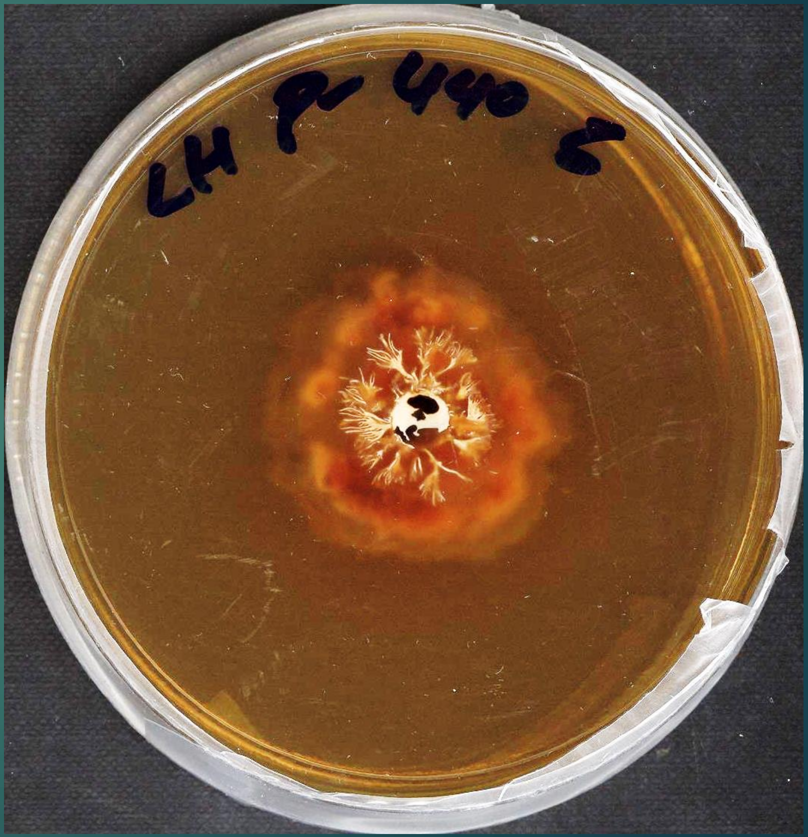
- One strain = 40% growth reduction
- To test combination with *Pseudomonas*

Bacillus + Pseudomonas vs Armillaria

Untreated

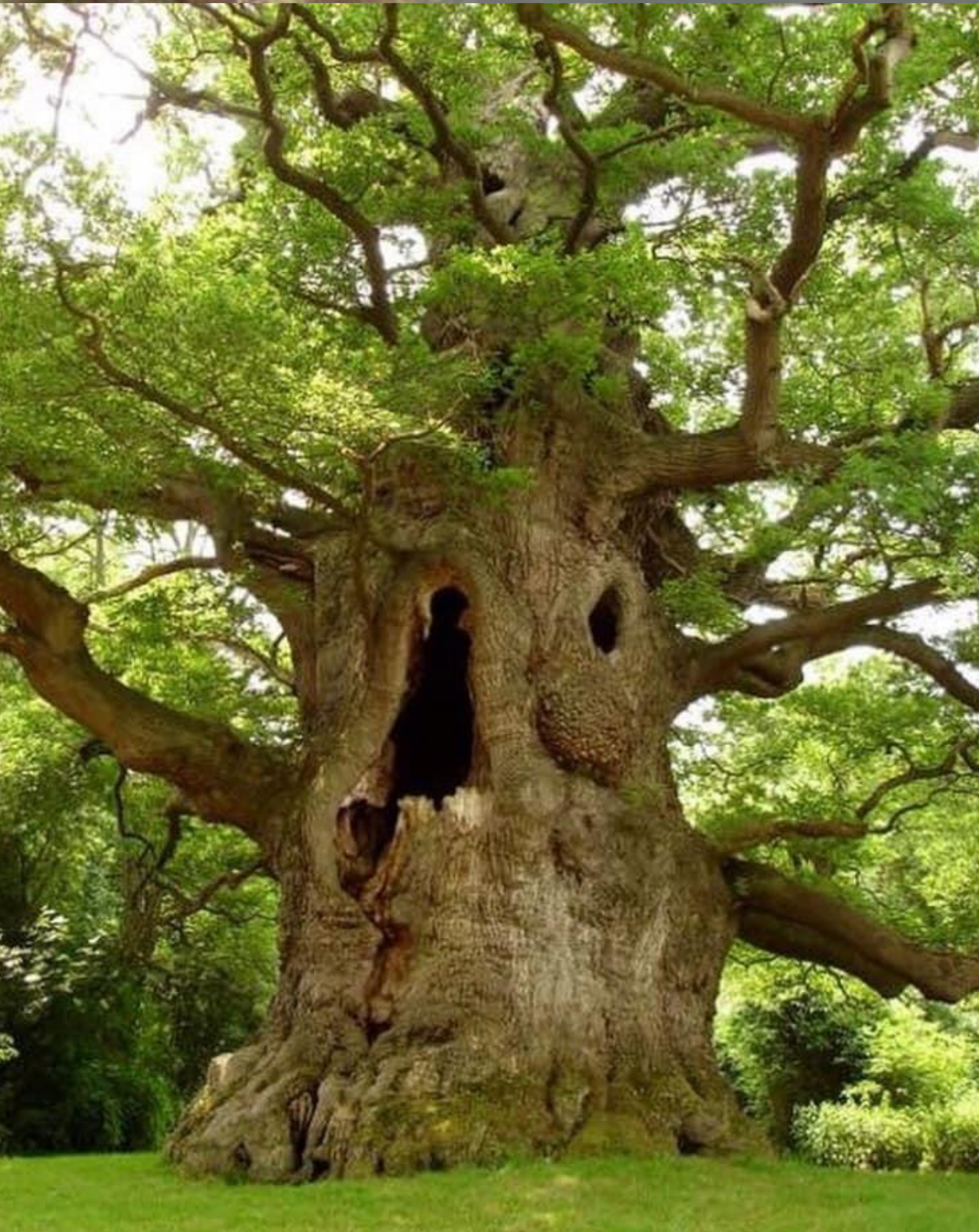
Treated

= up to 90% inhibition

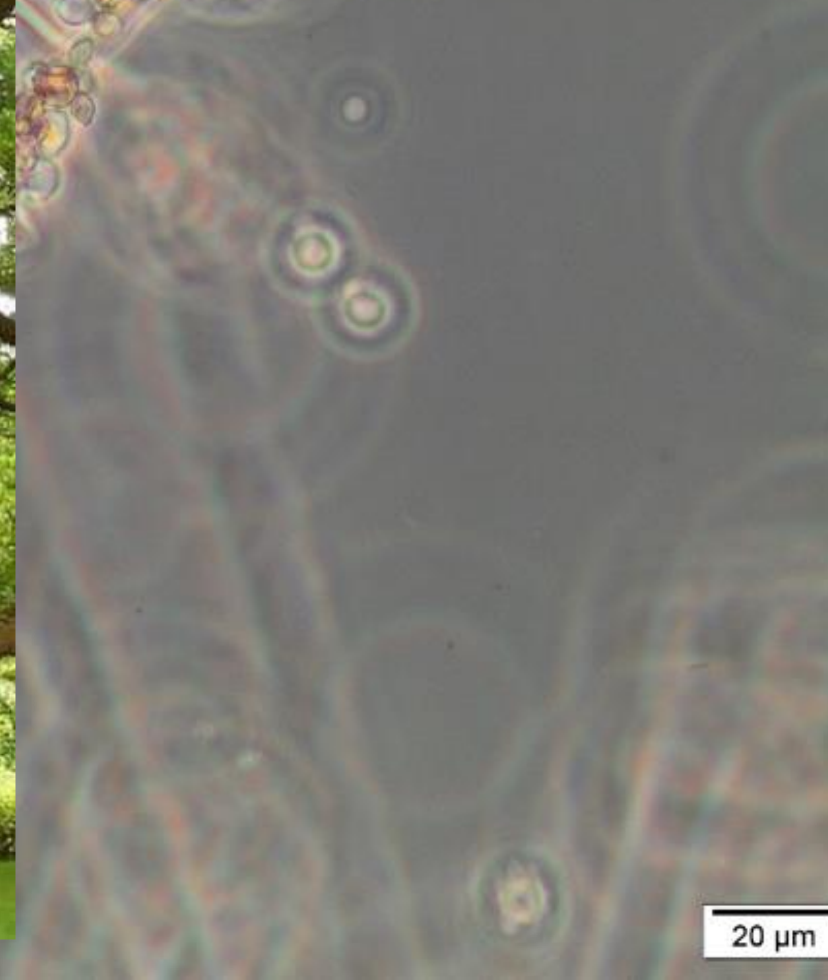


The “Take Home” Message

Trees not showing any symptoms of AOD were found to contain a range of fungal and bacterial species which appear to confer protection against AOD and Armillaria which have no detrimental effect on tree health.



"Majesty" The Fredville Oak, Kent,

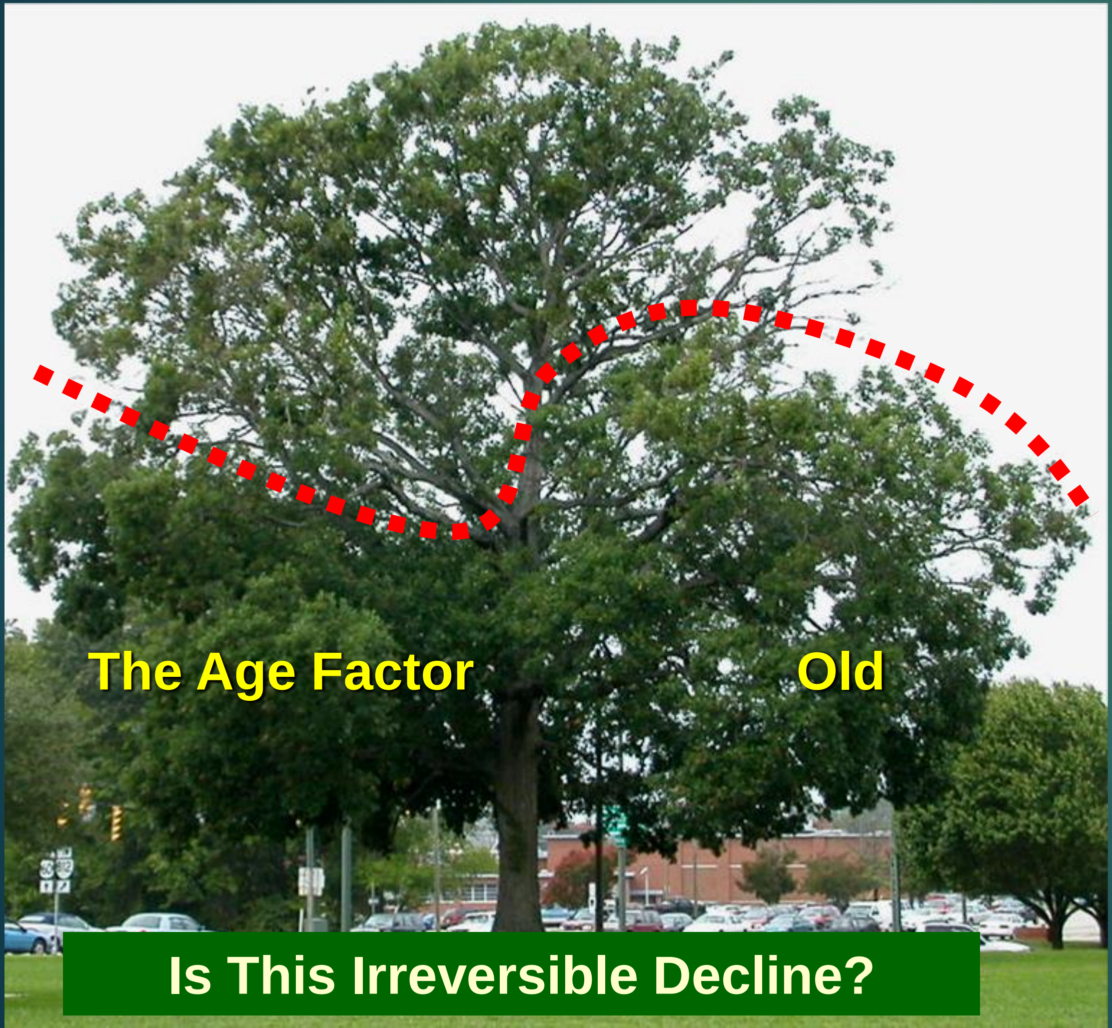


Drought

During the summer of 2018 the UK experienced a prolonged drought period identified as one of the hottest summers on record when daytime temperatures in parts of the country consistently exceeded 30°C.

Of greater concern is that climatic models indicate the UK will become a warmer drier country over the next twenty years with mean summer temperatures estimated to increase by 1-2ºC by the 2050s, and summer rainfall predicted to decrease by *ca*. 13%.

Drought-Related Decline? Affects The Old And The Young



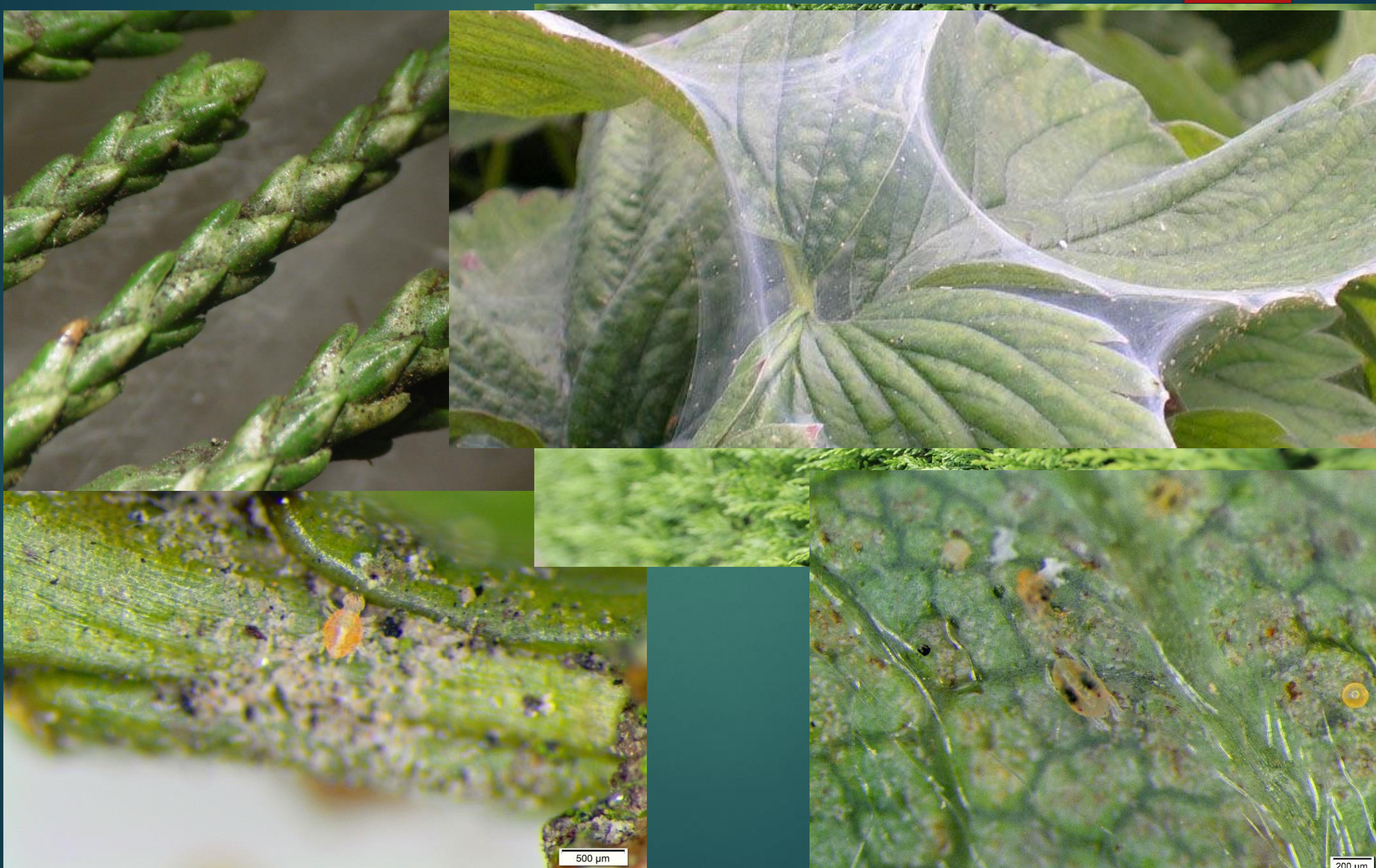
DIEBACK AND THINNING OF CROWN

Please note that drought symptoms may not appear until one or two years later (as reserves are depleted)

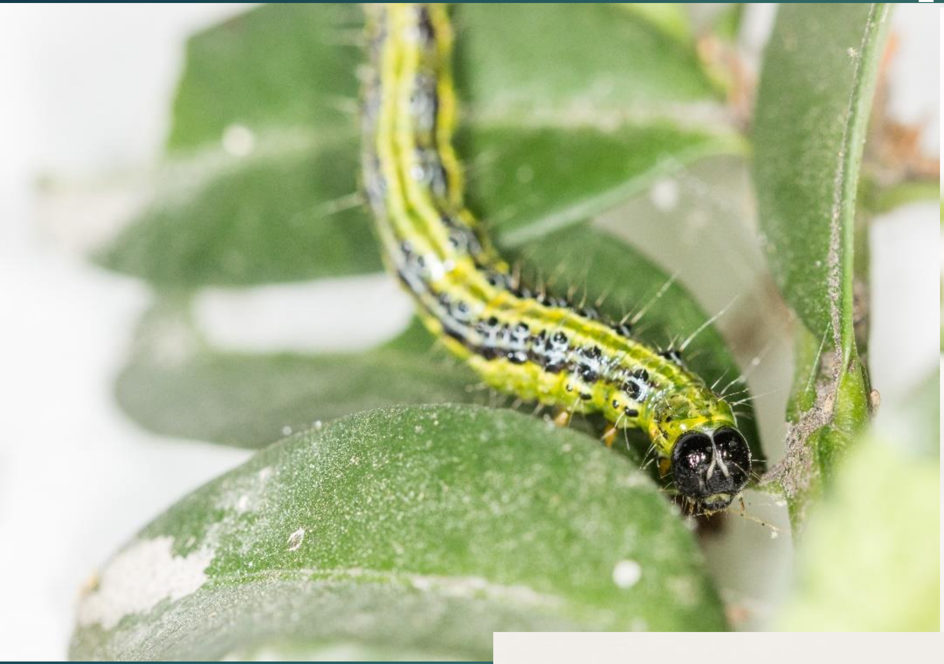
The scarier part is that effects may last for years – up to a decade!

Drought Induced Pest and Diseases Severity

Mites



Box Tree Caterpillar



OPM


000-73-193
FAGACEAE
1413
"TURKEY OAK"
QUERCUS
CERRIS
S.EUROPE & W.ASIA

Boring Beetles






Honey Fungus

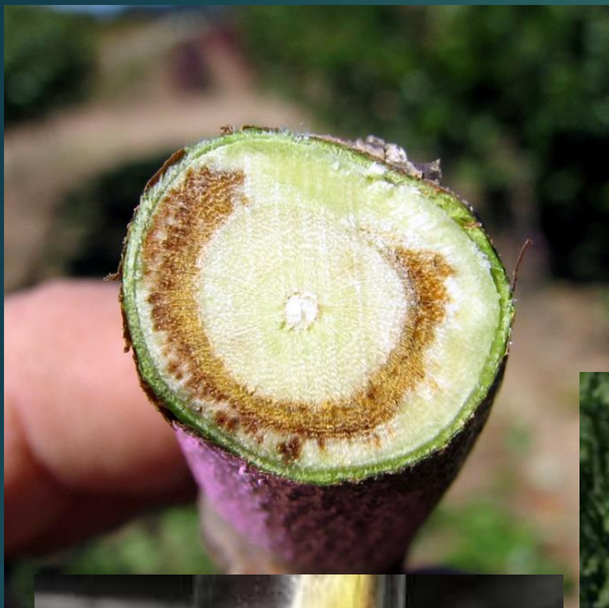
Massaria

AOD

Twig Cankers




Vascular Wilt



Massaria

Ash Die-Back

It is important to emphasise that these problems will NOT go away.

Incidence and severity will only get worse

Nature will NOT magically adapt

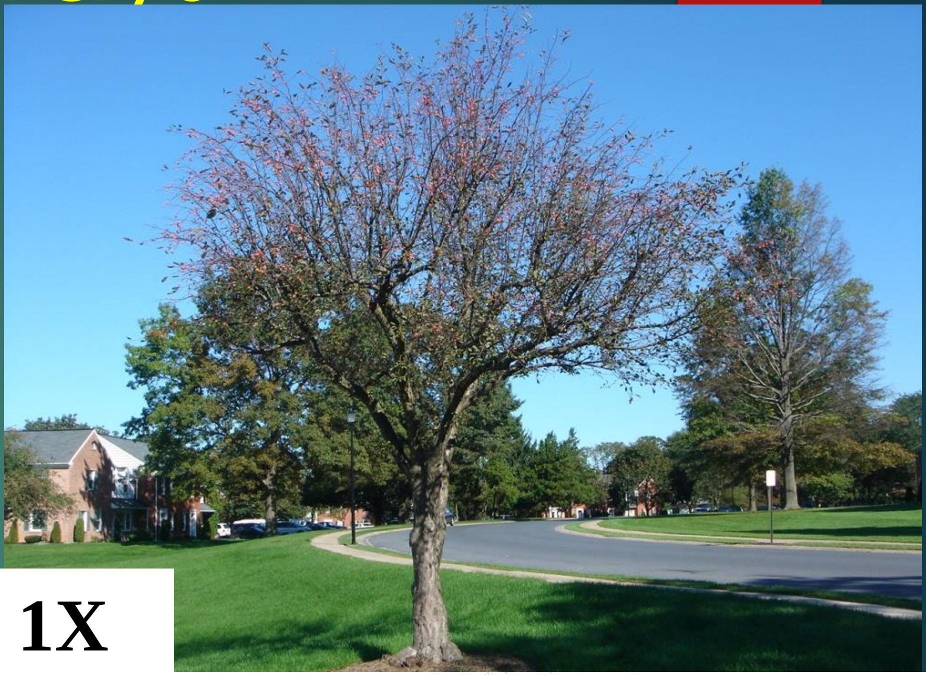
Pests and Diseases over the past 10-12 years

Ever Increasing Threats

- Conventional control relies heavily on fungicide/insecticide applications.
- Increased pest/disease tolerance.
- Failure of many products to adequately control problems once a tree is infected and
- Increased legislative restrictions regarding the use and application of chemicals means new techniques of disease control are now of environmental and economic importance.

Fungicide Sprays

Control



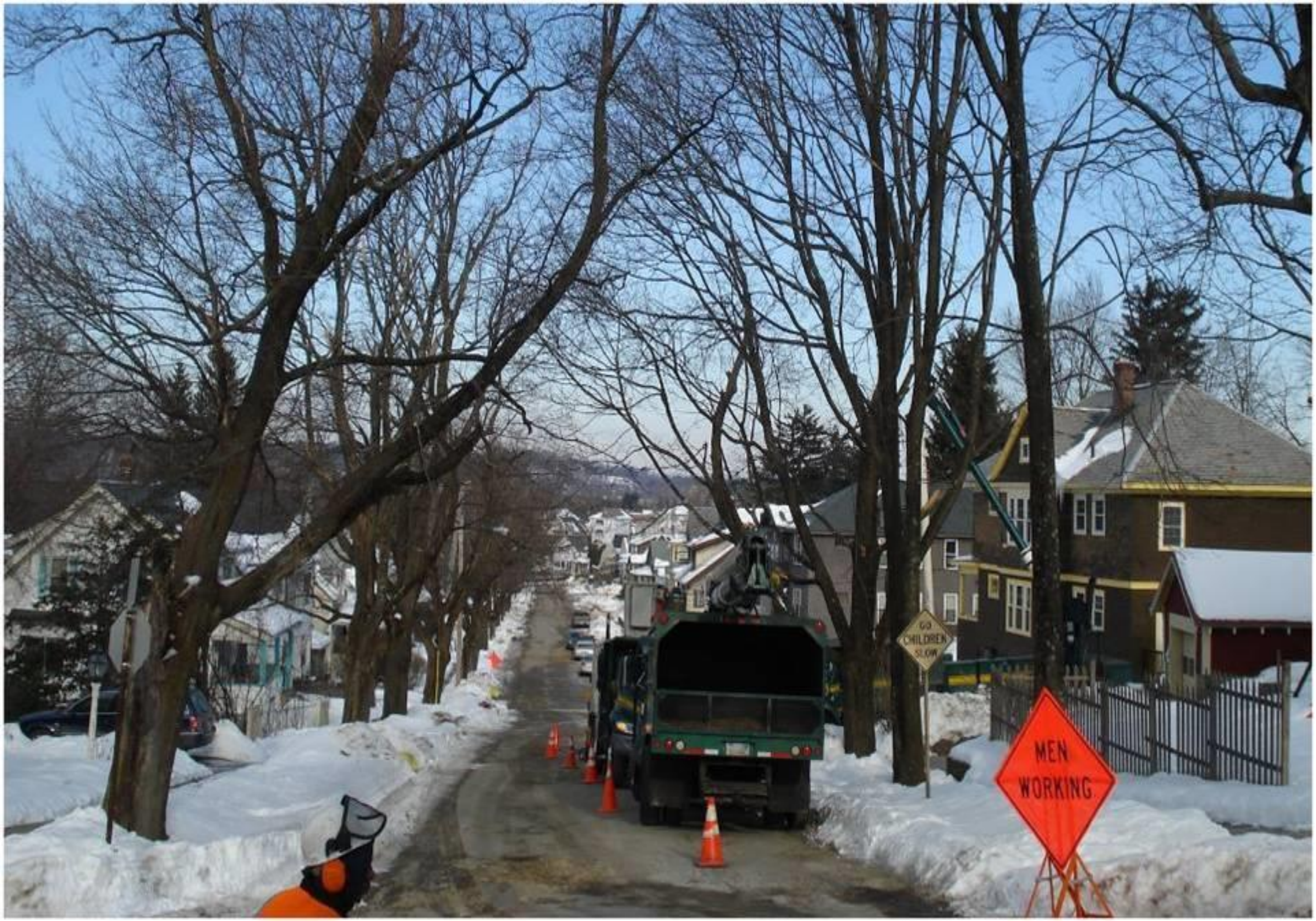
1X

2X

3X

BARTLETT
BARTLETT TREE EXPERTS
SCIENTIFIC TREE CARE SINCE 1907

Options?



Tree Removal? History has shown this option is not viable

P.ramorum 3,000,000 larch trees felled in Somerset

Disease damage: An example from the US: Xylella Leaf Scorch

- 'Bloodgood' London Plane
- 2000 Trees Planted 1989-90
- 1993 Leaf Scorch Confirmed

1996 Tree Mortality Begins

2001: Infection Rate-75%
Mortality Rate- 25%

Induced Resistance

Western medicine dictates that preventation of infectious diseases (typhoid, diphtheria, measles, hepatitis, small pox) is primarily via vaccination. In such circumstances the human body is injected with a weakened strain of a disease. This in turn stimulates the body to produce antibodies against that disease which in turn confers immunity. Importantly a “one-off” vaccination can confer immunity many years (at least 10) and in some cases an entire life time.

Can we use these vaccination principles for trees?

The answer is yes. Vaccinating plants against diseases is not a new concept; the idea of inducing resistance in plants was recognised in the early 20th century when heat or cold treated *Botrytis cinerea* (grey mould) when exposed to *Begonia* plants instead of causing infection as expected, resulted in the plants developing resistance.

We call this concept Induced Resistance (IR)

Studies have found that IR to be effective in controlling:

Fire blight (*Erwinia carotovora*)

Phytophthora root rot.

Powdery mildew (*Sphaerotheca pannosa* var. *rosa*, *Phyllactinia* sp and *Uncinula necator*)

Wilt disease of spruce (*Ceratocystis polonica*)

Importantly, the level of control achieved was comparable with currently used synthetic fungicides and a "one-off" vaccination provided growing season protection.

Developments in plant protection technology have led to the formulation of a range of commercially available IR agents.

Messenger (a.i. Harpin protein) in the US.

Bion (BTH) in Europe.

Agri-Fos (a.i. Potassium phosphite) in Australia and the US .

Rigel (a.i. Salicylic acid analog) in the UK

Oryzemate (a.i. Probenazole) Japan.

- A small but significant step.
- Trees responses can be induced by applying products as a root drench! (Percival G.C and Banks J M (2015). *Arboricultural Journal*: 37(1): 7-20
- Applying products via the roots opens up opportunities to manage tree pests and diseases .

- So what soil amendments can we use to vaccinate trees?

Willow mulch


No mulch

Willow mulch

Biochar: A highly purified form of charcoal

Sequestering 'biochar' in soil, which makes soil darker in colour, is a robust way to store carbon.

Slide courtesy Drs D Zwart/K Fite

Phytophthora Management

Vinca and Gardenia inoculated with *Phytophthora*

Control Compost Biochar

Phosphite

Chitin: Natural Polymer

Apple scab trial site

Aims of this study are to investigate the efficacy of these four IR agents singly and in combination on managing *Phytophthora* and *Armillaria spp* (honey fungus).

i.e. Biochar
Biochar + Chitin
Biochar + Willow Mulch
Biochar + Phosphites
Biochar + Chitin + Willow Mulch etc.

Biochar, Mulch, Chitin are waste products.

Honey Fungus (*Armillaria* spp)

Pot Experiment

Experiments used containerized stock of Privet (*Ligustrum ovalifolium*) sensitive to *Armillaria* diseases.

Research Plan

Trees were potted up into 10 litre containers (40% general potting compost; 50% John Innes No 2; 10% wood chip) amended as follows:
Chitin (1% by volume)
Potassium phosphite (20 ml per litre water; 500ml per 10 litre pot).
Willow Mulch (5 cm deep)
Biochar (5% by volume)
A combination of the above

In addition a comparative evaluation of a conventional fungicide (Scotts Octave (a.i. prochloraz) and Subdue (Metalaxyl) used within the UK for *Armillaria* and *Phytophthora* control respectively was conducted:

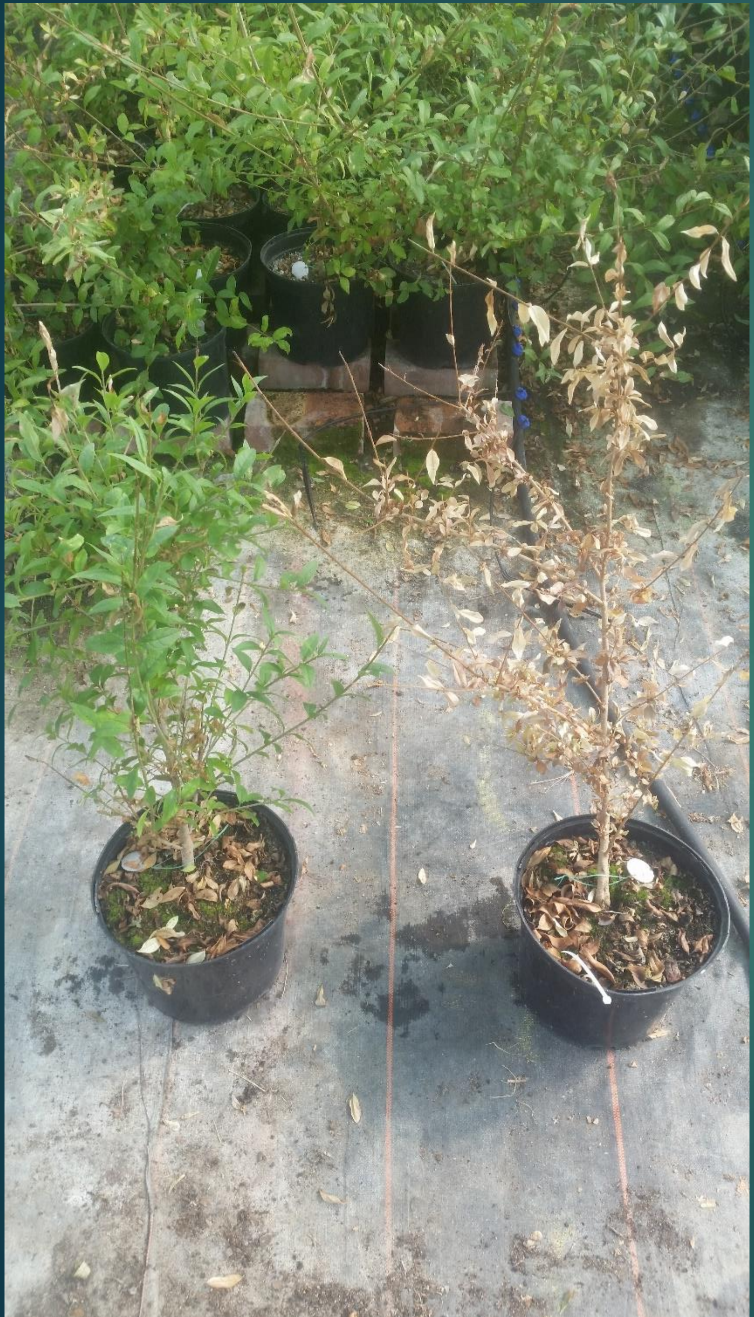
Research Plan

Two weeks later pure cultures of *A. mellea* (6 agar plates) were then added to a liquidizer containing 2 litres sterile distilled water and 150 ml of *A. mellea* slurry added to each pot

Trees were placed in a unheated Polytunnel and supplemented with drip irrigation

Symptoms of infection began to develop on controls *circa* 3 months later. Treatment effects were quantified at month 9 after inoculation.

Prochloraz Control

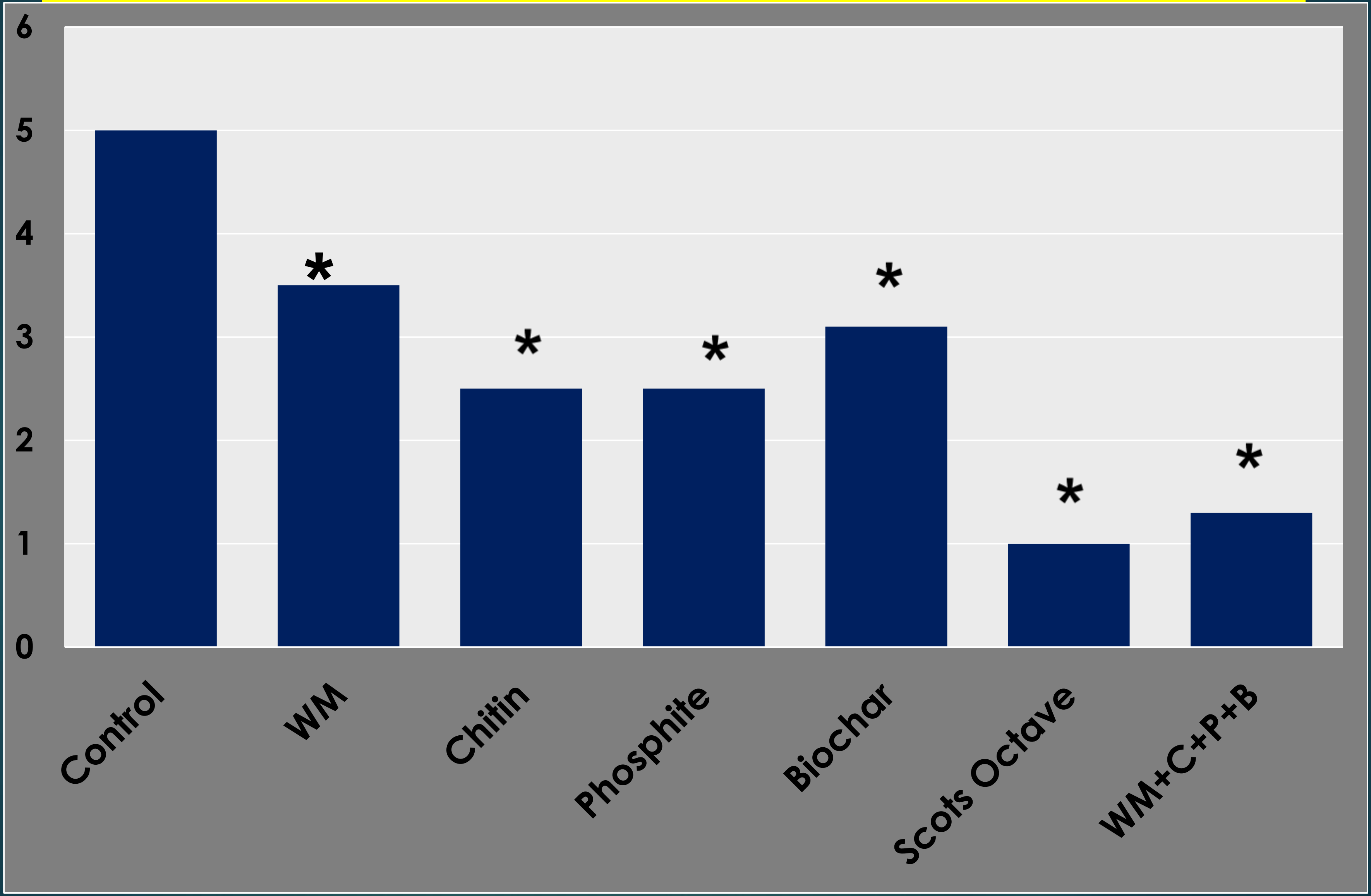


PP + Chitin Control

Potassium phosphite

Chitin Control

HF Severity at month 9 after inoculation



Alterations in host plant physiology

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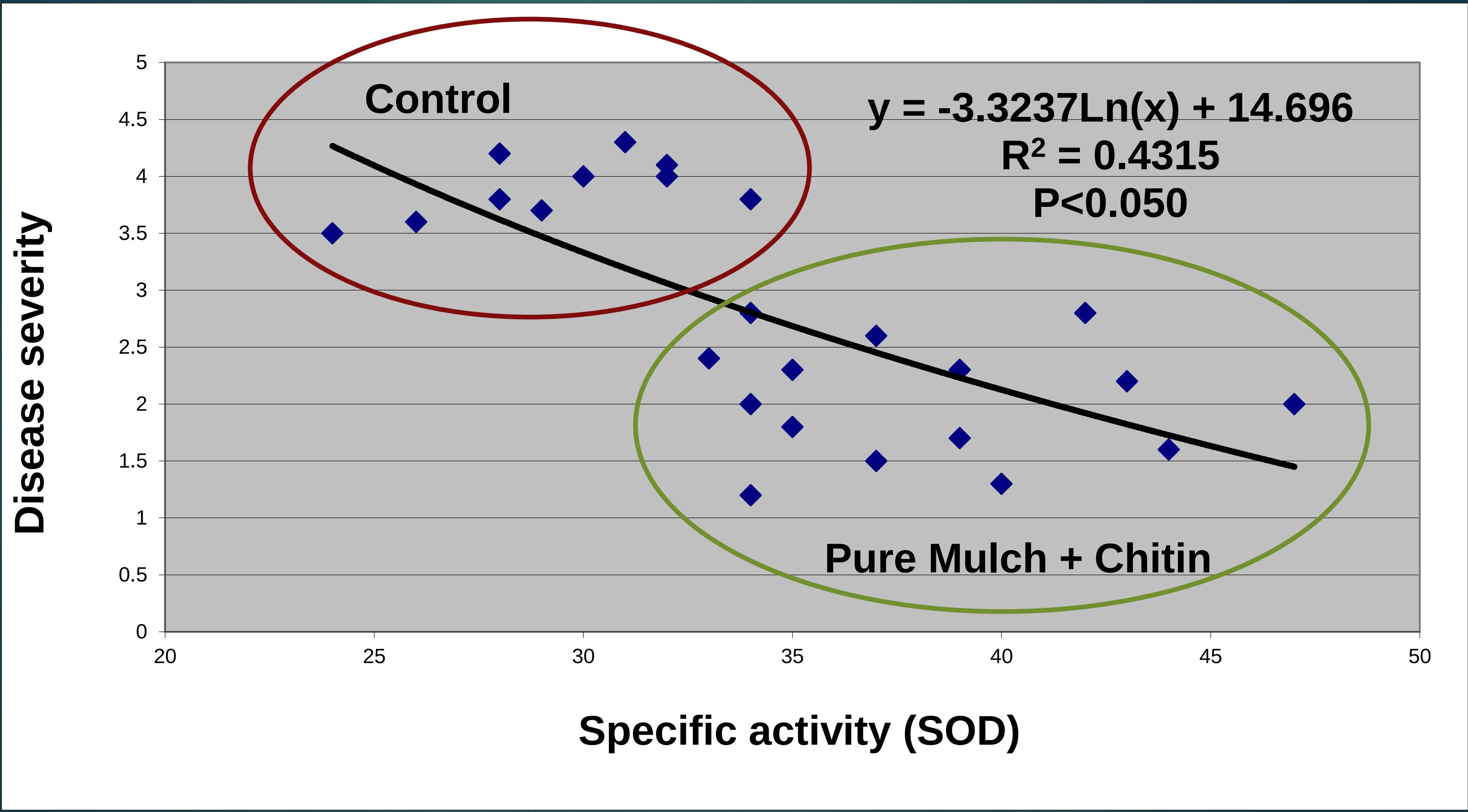
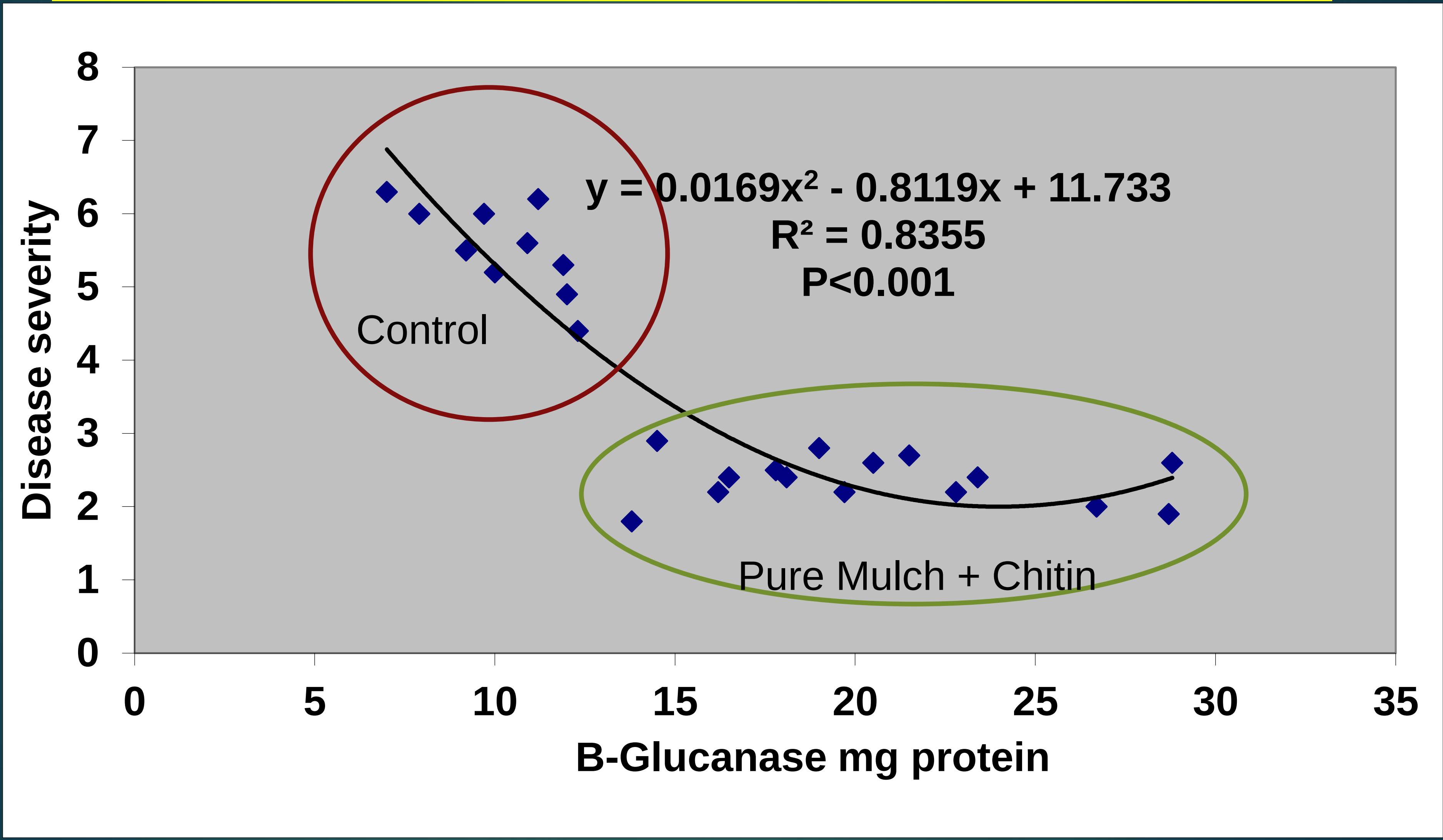
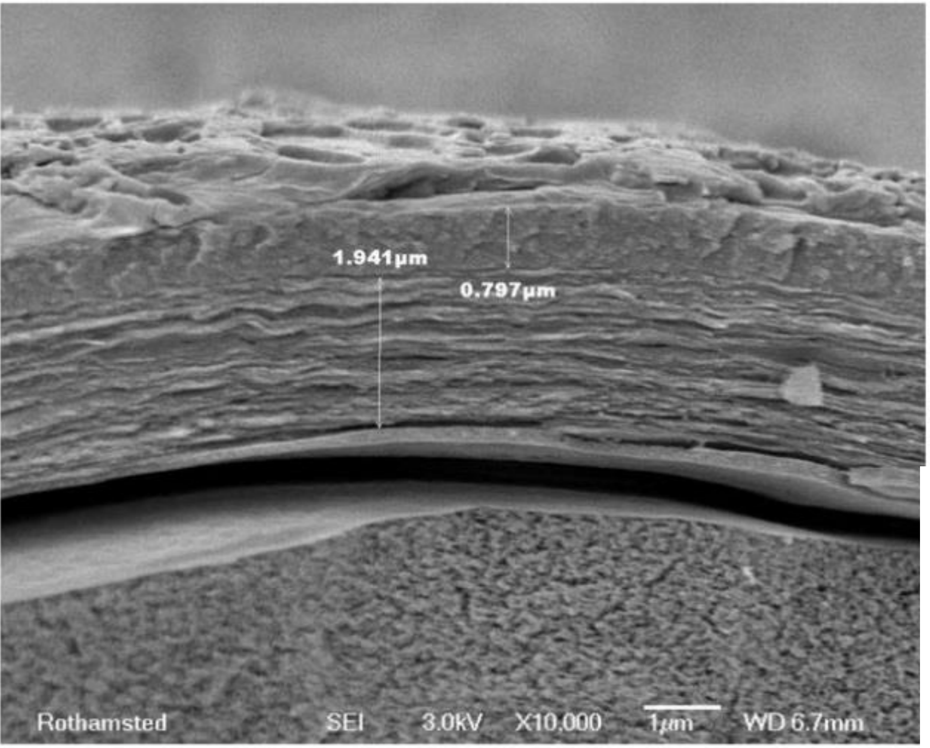
Specific activity of defensive root enzymatic activity Vs ARMILLARIA severity



Specific activity of defensive root enzymatic activity Vs ARMILLARIA severity



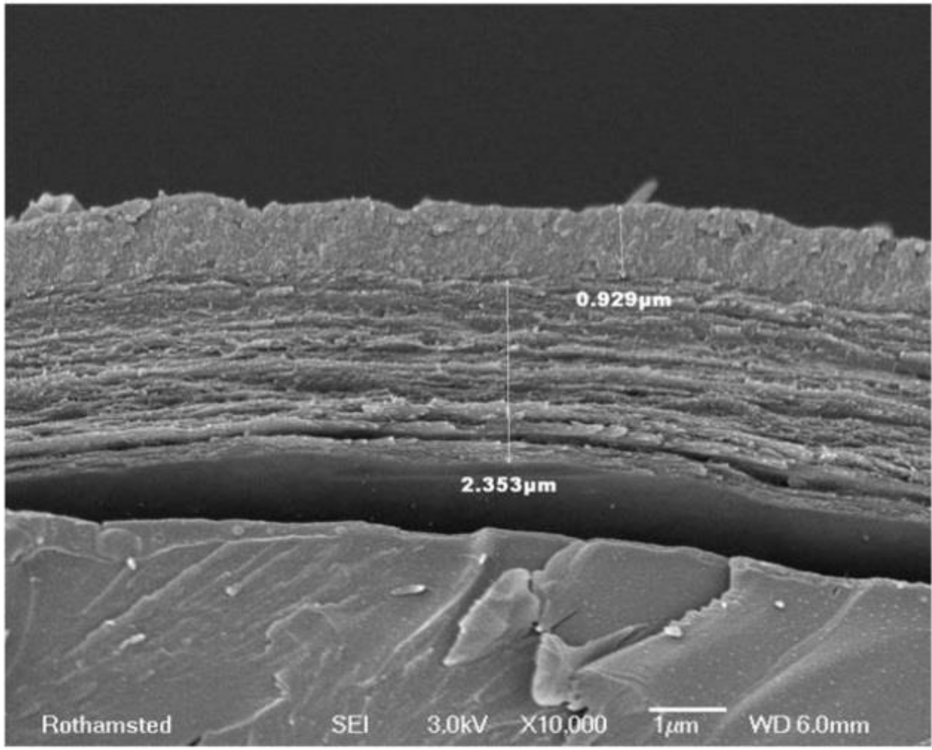
Thicker leaves



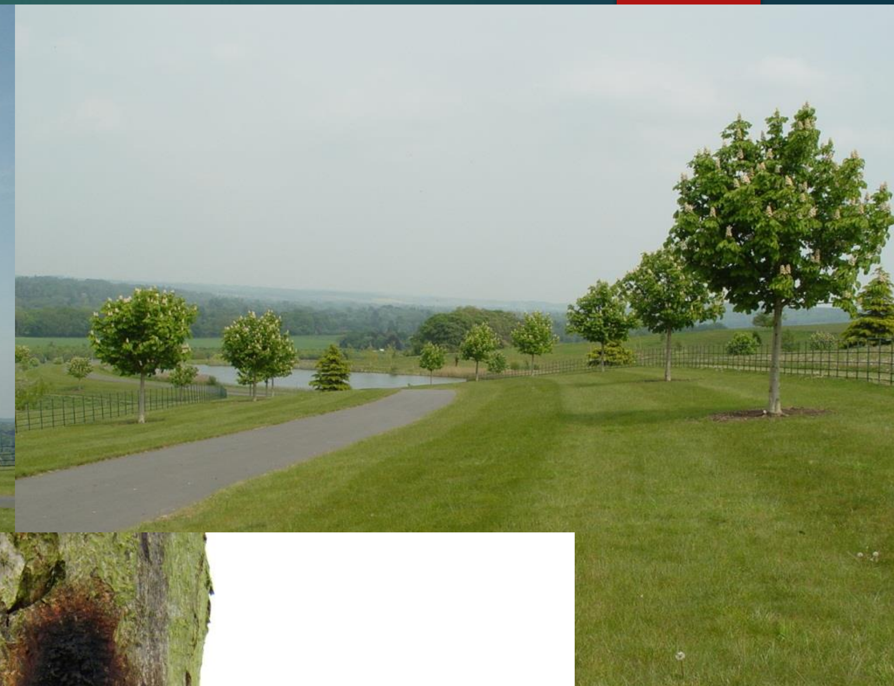
treatment 0.25% fracture 050.tif

Control

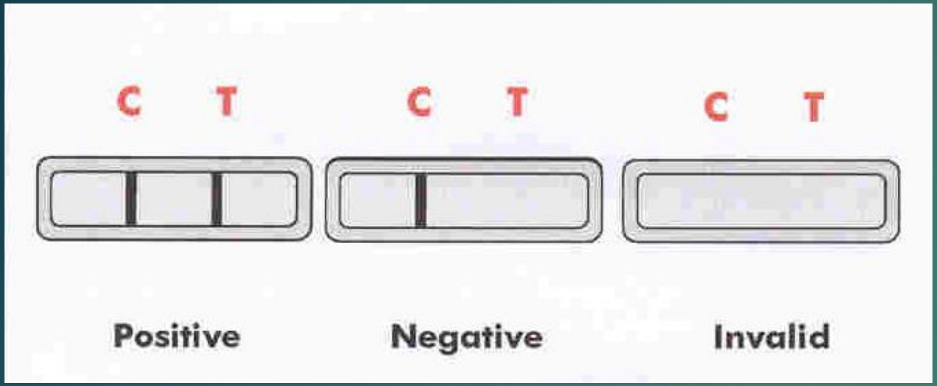
Biochar + Chitin



Trial Site – Reading University



Result Interpretation



Any colouration for the test line is considered a positive result

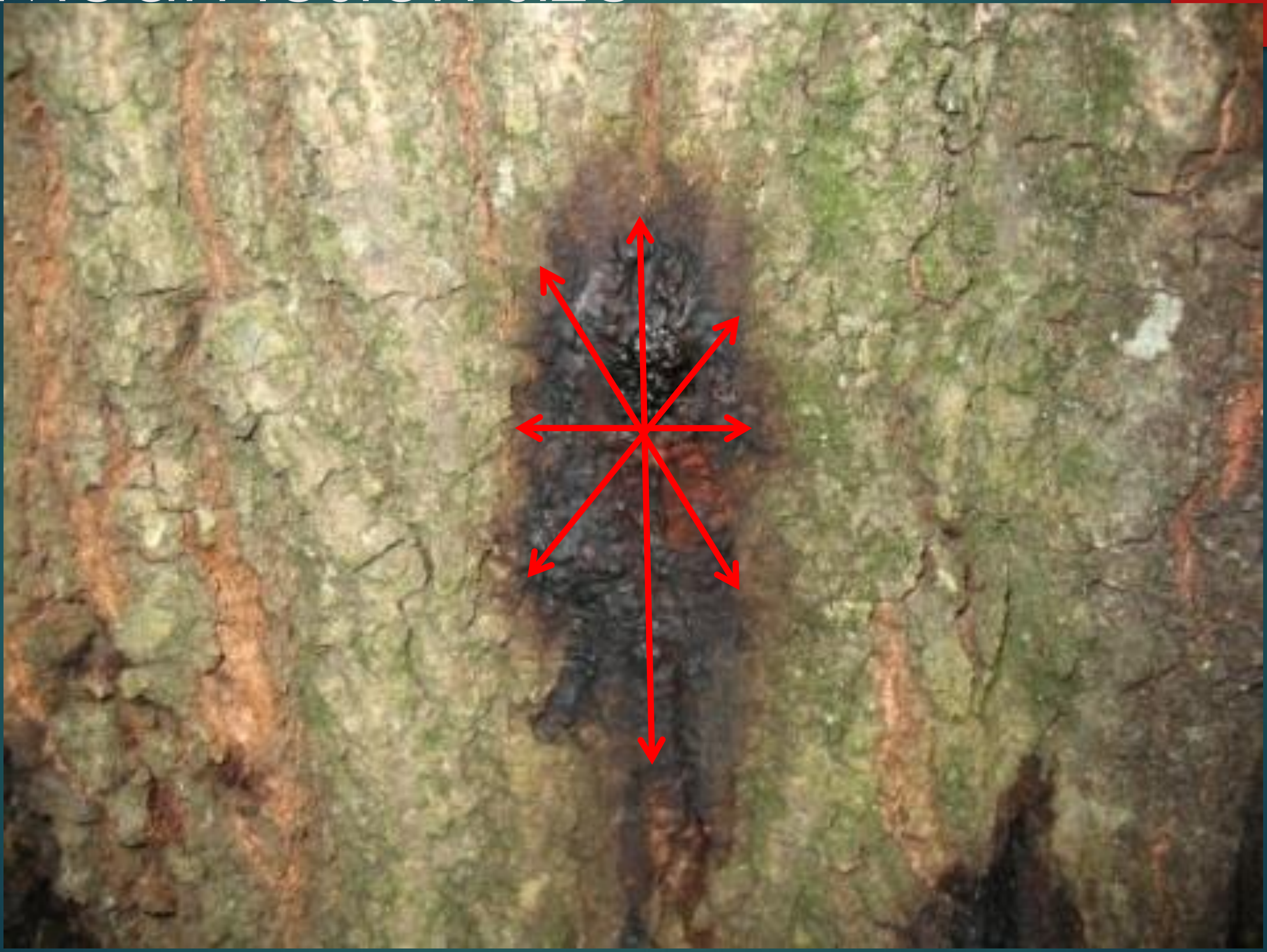
Materials and Methods

Only trees where a positive result for *Phytophthora* was obtained were used for experimental purposes.

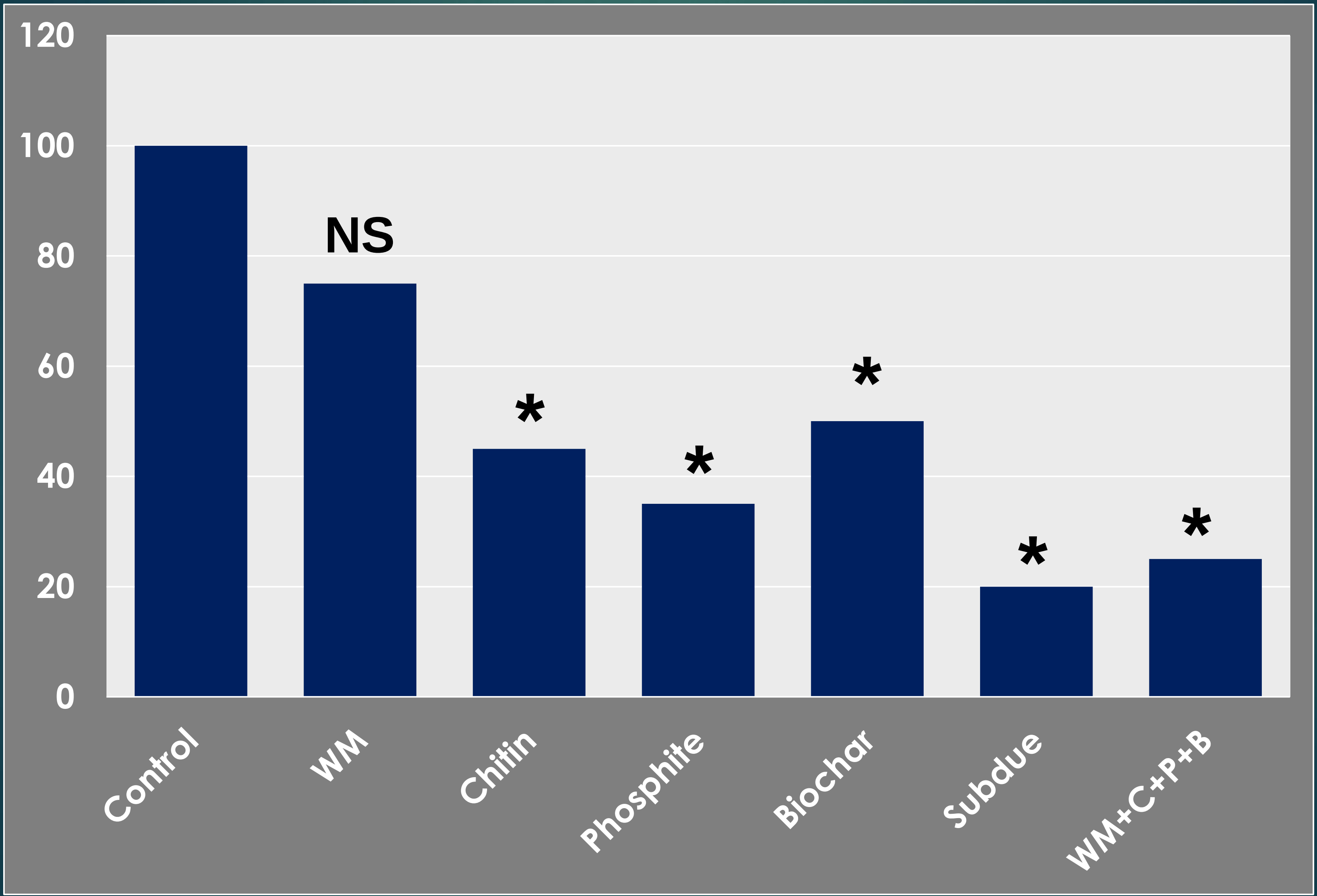
Vertical Mulching

- Trees were subjected to soil core removal (7.5 cm wide, 30 cm deep; at 50 cm spacings under the tree crown dripline.
- Core holes left behind re-filled with 50% general potting compost; 50% John Innes No 2 amended as follows:
- Chitin (1% by volume)
- Potassium phosphite (20 ml per litre water; 100ml per hole).
- Willow Mulch (5 cm deep)
- Biochar (5% by volume)
- A combination of the above.

Mean lesion size



Phytophthora lesion canker at month 12 after treatment – Horse chestnut



Summary

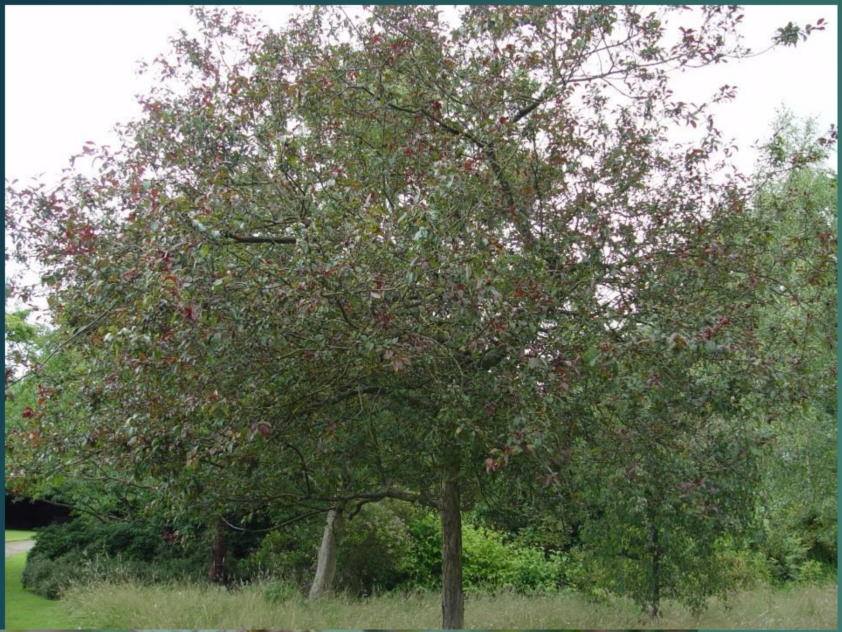
- Use of biochar, pure mulch, chitin and phosphites:

- 1. Cause enhancement of defensive enzymes in leaves and roots (SOD, Peroxidase, Beta Glucanase)
- 2. Leaves become thicker and more lignified.
- 3. Highly likely many more defence enzymes/metabolites enhanced that we haven’t analysed for.
- 4. 15-20% reduction in growth

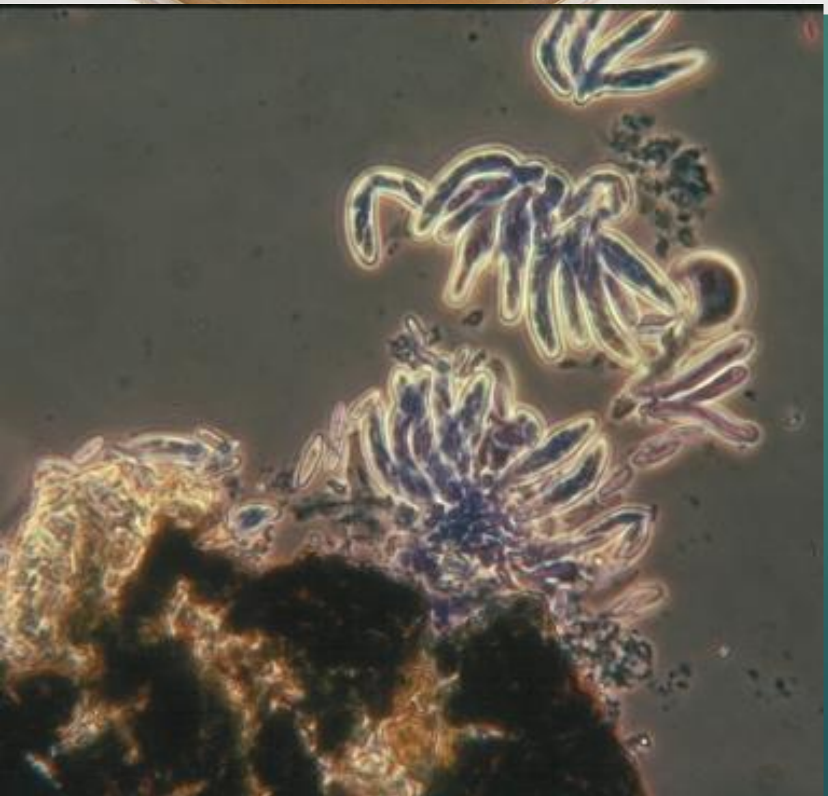
Conclusions

- All IR agents resulted in a reduction in canker size over a growing season. Greatest reductions occurred following combinations of IR agents.
- Reductions in Armillaria and Phytophthora we equal to those obtained using conventional plant protection products.
- Pot and Field results indicate that combinations of IR agents can help in the management of Phytophthora and Armillaria diseases.

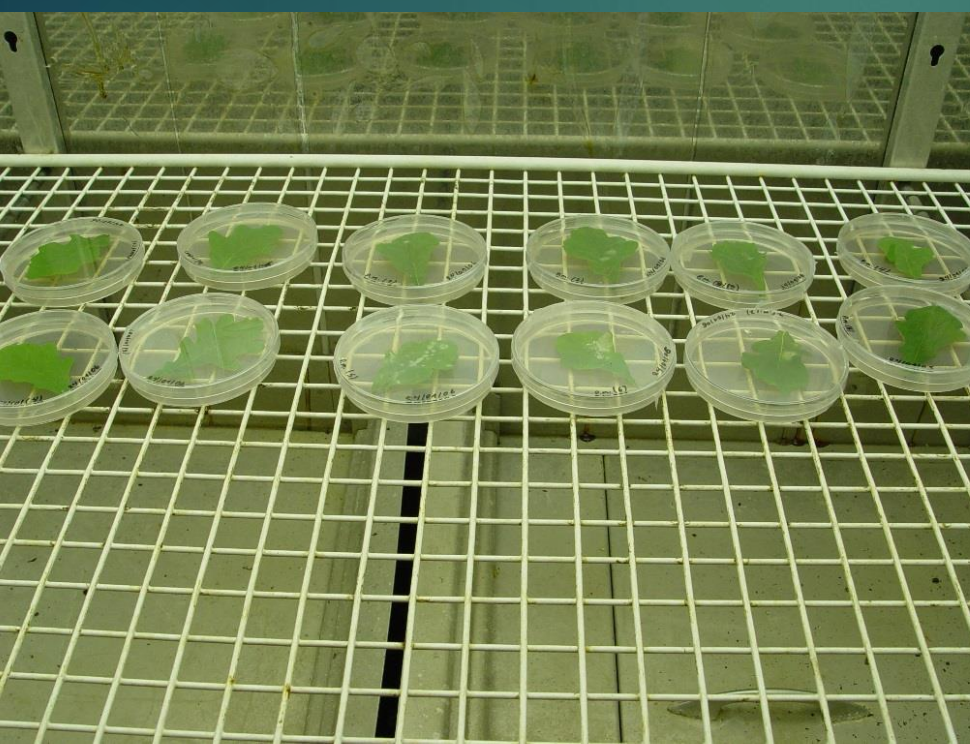
Detached leaf bioassay (Yepes and Aldwinckle (1993) Plant Science. 93:211-216



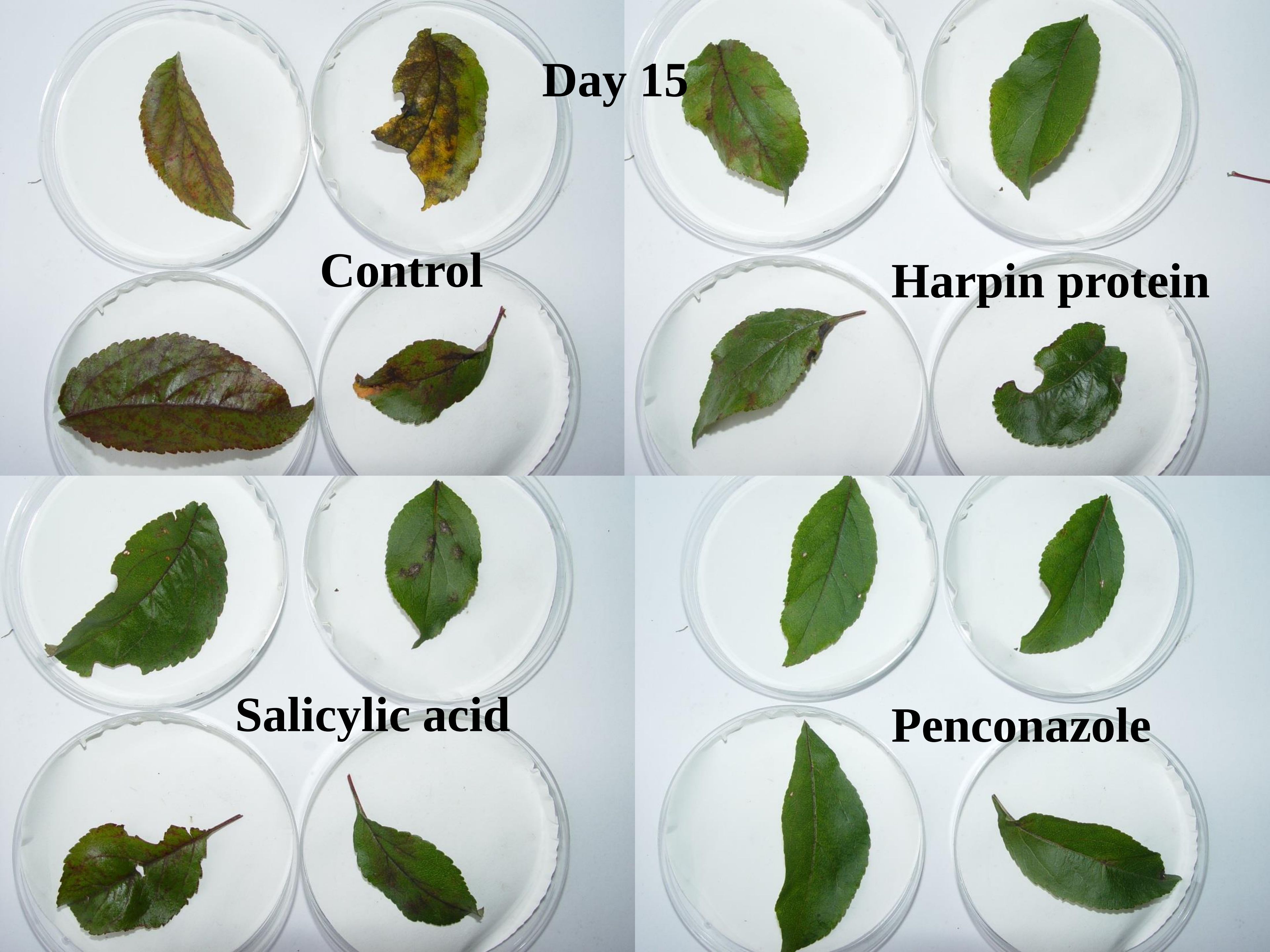
Detach healthy leaves (i.e. no signs of scab development) of a susceptible *Malus* cultivar (cv. Floribunda) and surface sterilize (Tween 20).



Pippette a spore suspension (10^6 ml) from cultures of apple scab onto the detached leaf previously treated with a IR agent.



- Incubate the detached leaf (19°C (66°F), 16h photoperiod).


Day 15
Control
Harpin protein
Salicylic acid
Penconazole

Chitin – What is it?

- 2nd most widespread natural polymer
- Forms structure of:
  - Fungi cell walls
  - Insect exoskeletons
  - <u>Crustacean exoskeletons</u>
- Insoluble!
  - Derivatives soluble… and more effective?

COMMERCIAL CHITIN/CHITOSAN PRODUCTS

TRIAL SITE

Street Planting. London UK

Heavy pear scab infection

CHITIN/CHITOSAN PRODUCTS

1 = CRAB MEAL 0.75KG PER TREE BASED ON MRR 0.5-1.0KG PER 2.5CM TRUNK DIAMETER

2 = PURE CHITIN (120G SQ M) 360 G PER TREE APPLIED

3 = PURE CHITOSAN (120G SQ M) 360 G PER TREE APPLIED

4 = LIQUID CHITOSAN 1ML INTO 5 LITRES. APPLY 1 X 3 WEEKS AND THEN 1 PER MONTH

5 = HORTI FEEDS 0.8G PER LITRE. RATE APPLIED = 2.0G PER TREE. APPLY EVERY 2 WEEKS

6= TOPAS (PENCONAZOLE)


Trial site (Sept 2017) 7-8 months after treatment
ST. JAMES CLOSE


Pure Chitin


Liquid Chitosan
Control


Penconazole


September 2017
ST. JAMES CLOSE

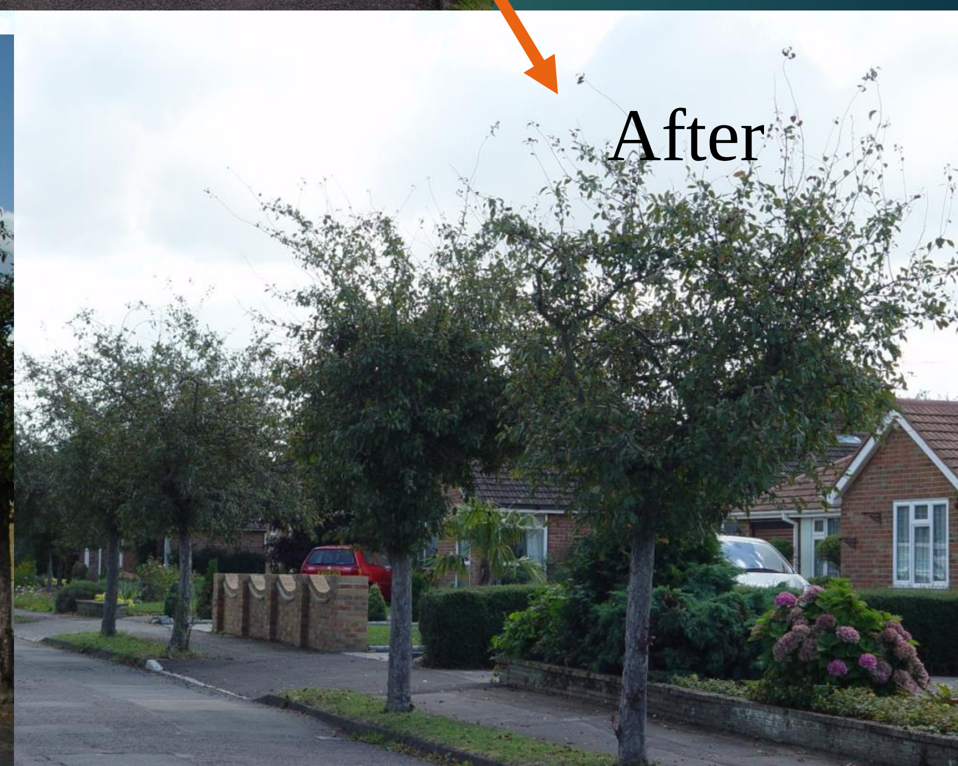

Penconazole
Pure Chitosan


Crab Meal


Pure Chitin

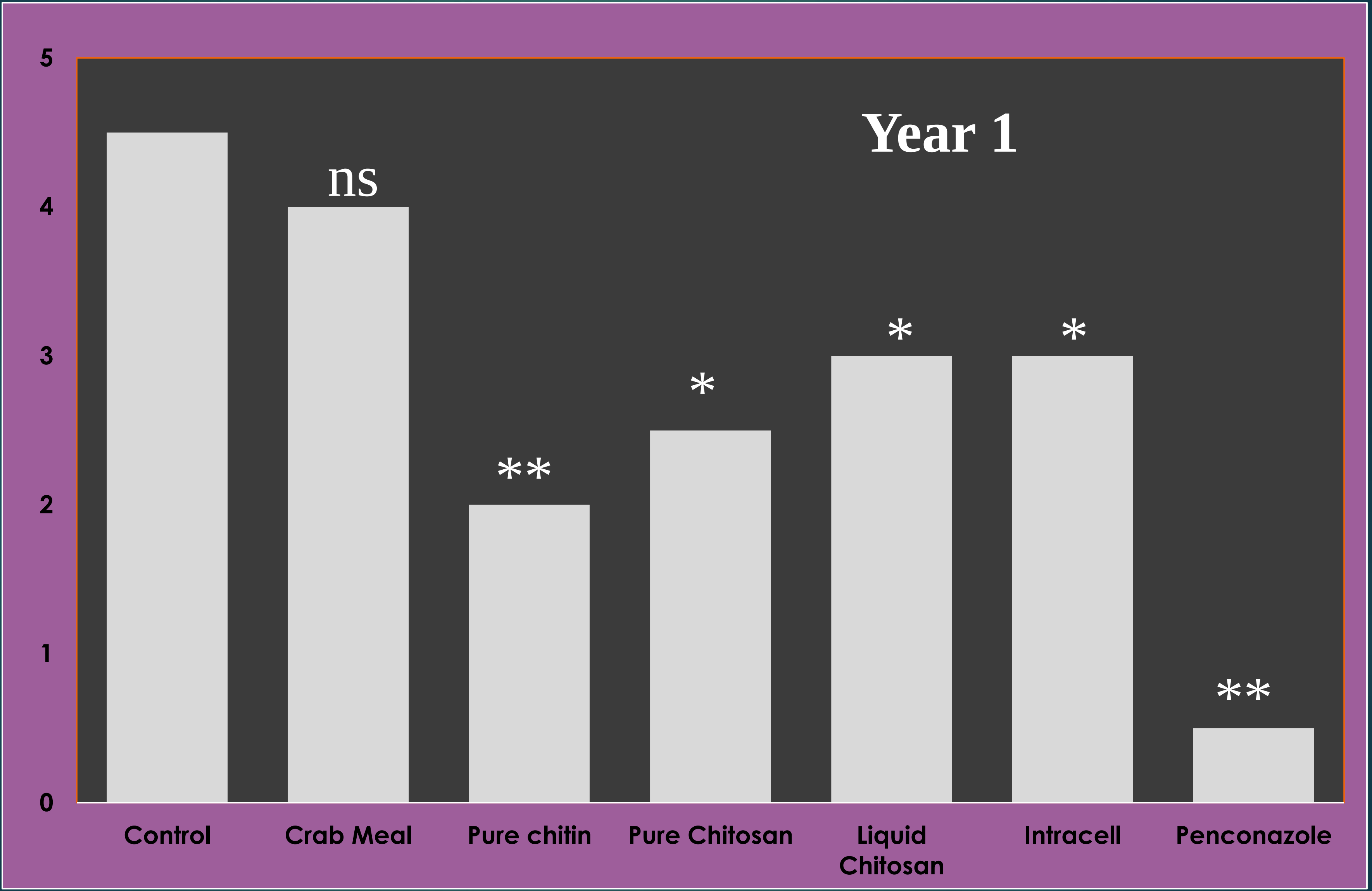

Before


After

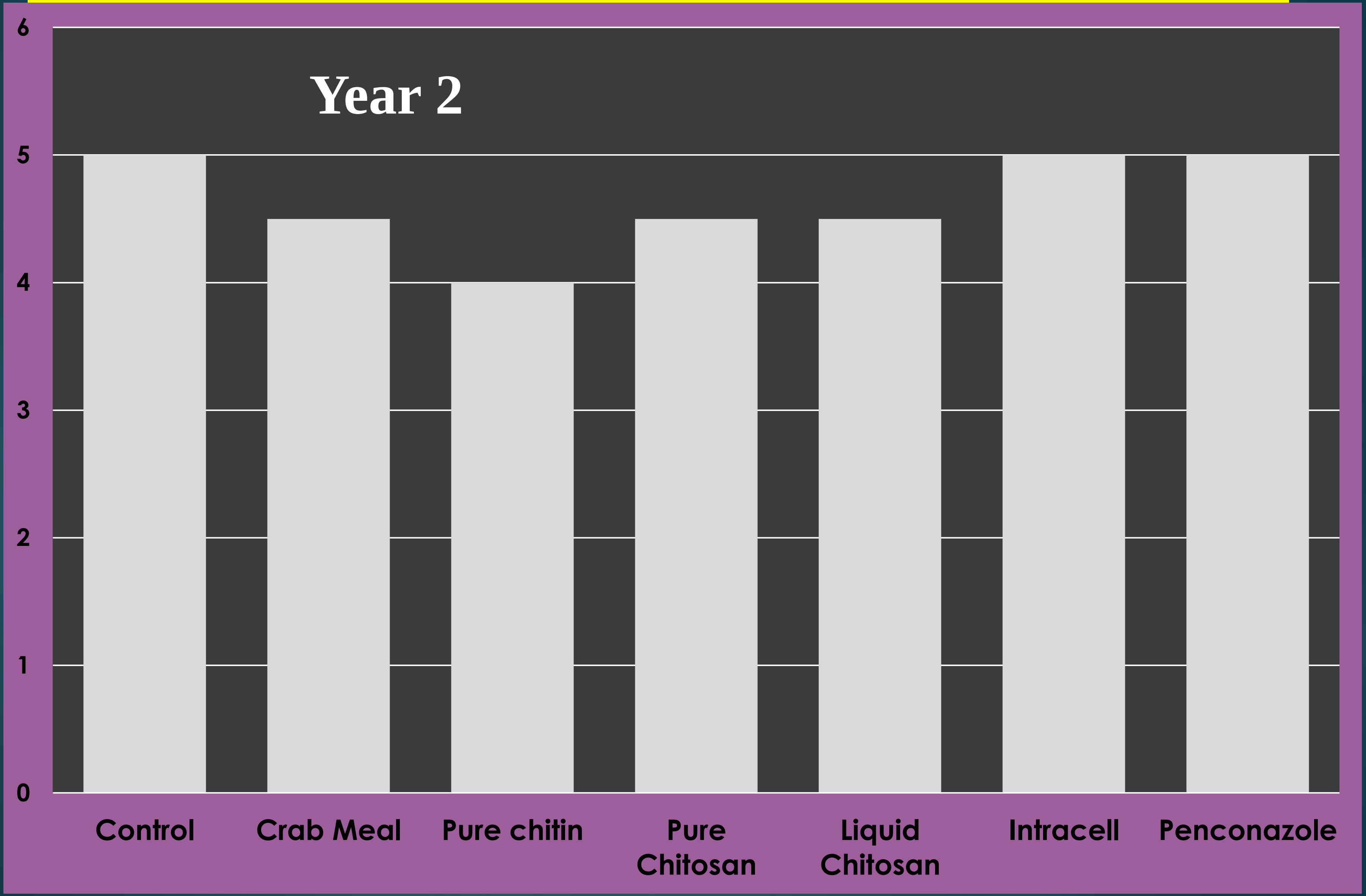

After

Year 2 Sept 2018: No Significant Difference

The influence of chitin based IR agents on apple scab severity



The influence of chitin based IR agents on apple scab severity



Conclusions

A reduction in scab severity was recorded at the end of the first growing season indicating application of chitin as a soil amendment offer potential for scab management.

None of the chitin/chitosan treatments provided any form of control against the fungal pathogen apple scab in the second growing season. this indicates that these products need to be applied annually.

Pure chitin and chitosan resulted in the greatest reduction in scab severity.

A SLIGHTLY DIFFERENT APPROACH

Research findings to date show that all of the ir agents tested are generally less effective than standard synthetic fungicides for pathogen control.

Perhaps a more appropriate role for these ir agents would be in combination with a reduced dose of synthetic fungicide to achieve control comparable or significantly higher than stand-alone applications of fungicides at full dose?

PEAR SCAB TRIAL

IR + Fungicide

IR agent and fungicide treatments were applied at four growth stages or combinations of stages identified as key spraying times for scab control under field conditions, namely:

Bud break (March 11, 2018)
Green cluster (April 2, 2018)
90% petal fall (May 13, 2018)
Early fruitlet (June 6, 2018).

Rigel-g (a.i. salicylic acid): 3ml per litre of water

Signum (a.i. 7% pyraclostrobin + 27% boscalid): 0.9g per litre of water.

Scab Severity Scale - Leaf

Scab Severity Scale - FRUIT

IR + FUNGICIDE COMBINATION

Treatment		
	Leaf Scab Severity	Fruit Scab Severity
Water (control)	3.5d	2.2e
Rigel-G (SA)	1.9bc	1.3cd
Signum FS	1.0ab	0.2ab
Signum FS + SA	0.5a	0.0a
Signum 66% + SA	1.2abc	0.8bc
Signum 33% + SA	2.0c	1.8de

Comparison of Signum + SA applied as four foliar sprays for the control of Pear Scab on *Pyrus communis* 'Williams' Bon Chrétien'

IR + FUNGICIDE COMBINATION

Treatment		
	SPAD	Fruit Yield
Water (control)	26.8a	10.1a
Rigel-G (SA)	33.2ab	11.8abc
Signum FS	42.4cd	13.6bc
Signum FS + SA	44.5d	14.0c
Signum 66% + SA	40.8bcd	12.9bc
Signum 33% + SA	35.0bc	11.5ab

Comparison of Signum + SA applied as four foliar sprays for the control of Pear Scab on *Pyrus communis* 'Williams' Bon Chrétien'

IR + Fungicide

Treatment	Black Spot Leaf Severity	
	2014	2015
Water (control)	3.6c	3.3d
SA	1.5b	1.8c
Topas FS	0.8ab	0.5ab
Topas FS + SA	0.3a	0.0a
Topas 66% + SA	0.8ab	0.7ab
Topas 33% + SA	1.2ab	1.2bc

Comparison of Topas (Penconazole) + Rigel-G (SA) applied as four foliar sprays for the control of black spot (*Diplocarpon rosae*) on Rosa 'The Fairy'

CONCLUSIONS

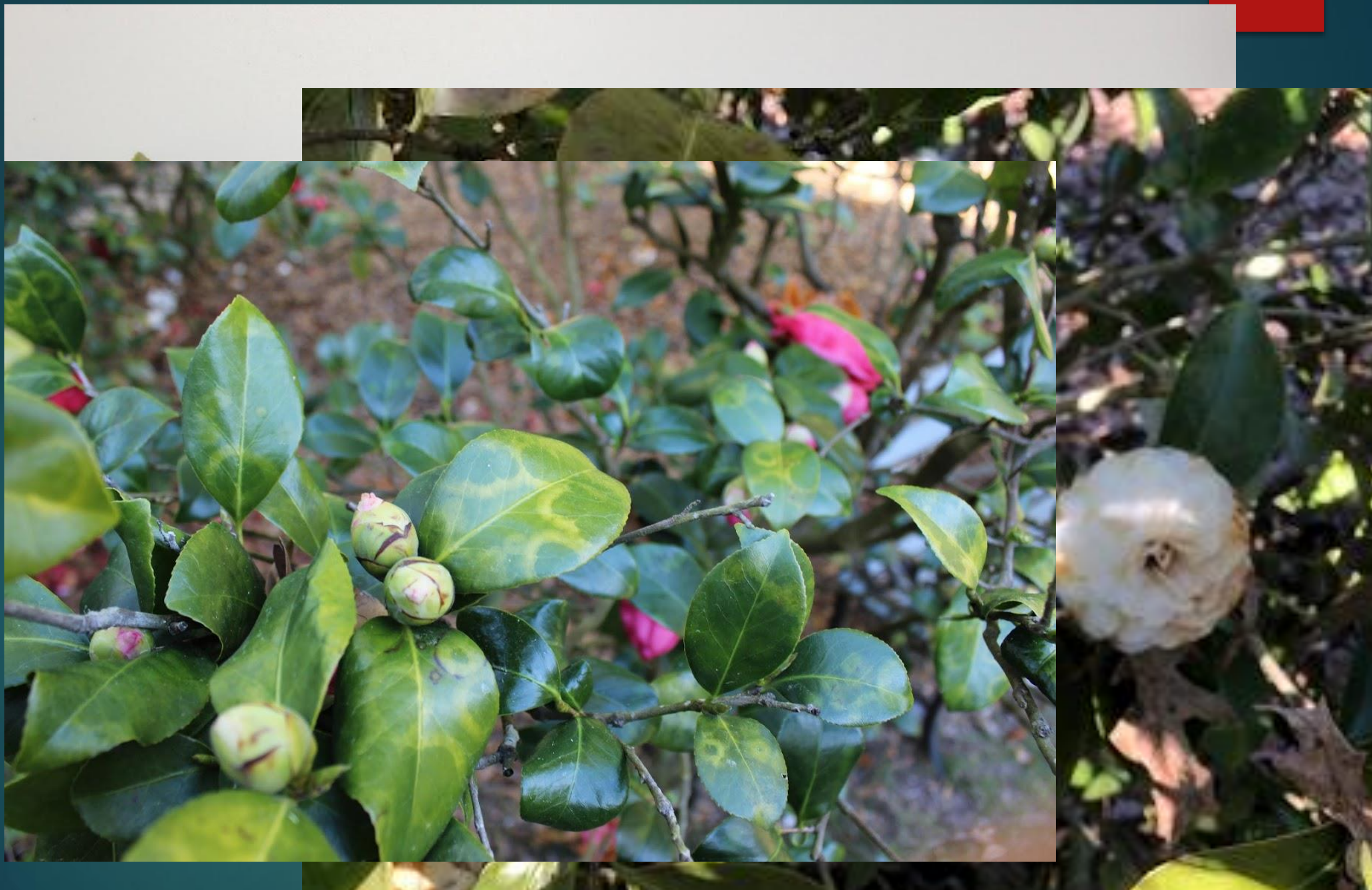
In all pot and field studies to date application of a fungicide at two third strength plus IR agent provided the same degree of pathogen control as a fungicide at full strength.

Application of a fungicide at one third strength plus IR agent provided a reasonable degree of pathogen control but not to the same degree as that of a fungicide applied at full strength.

Combinations of IR agents ongoing

Chitin + Willow Mulch

New Challenges



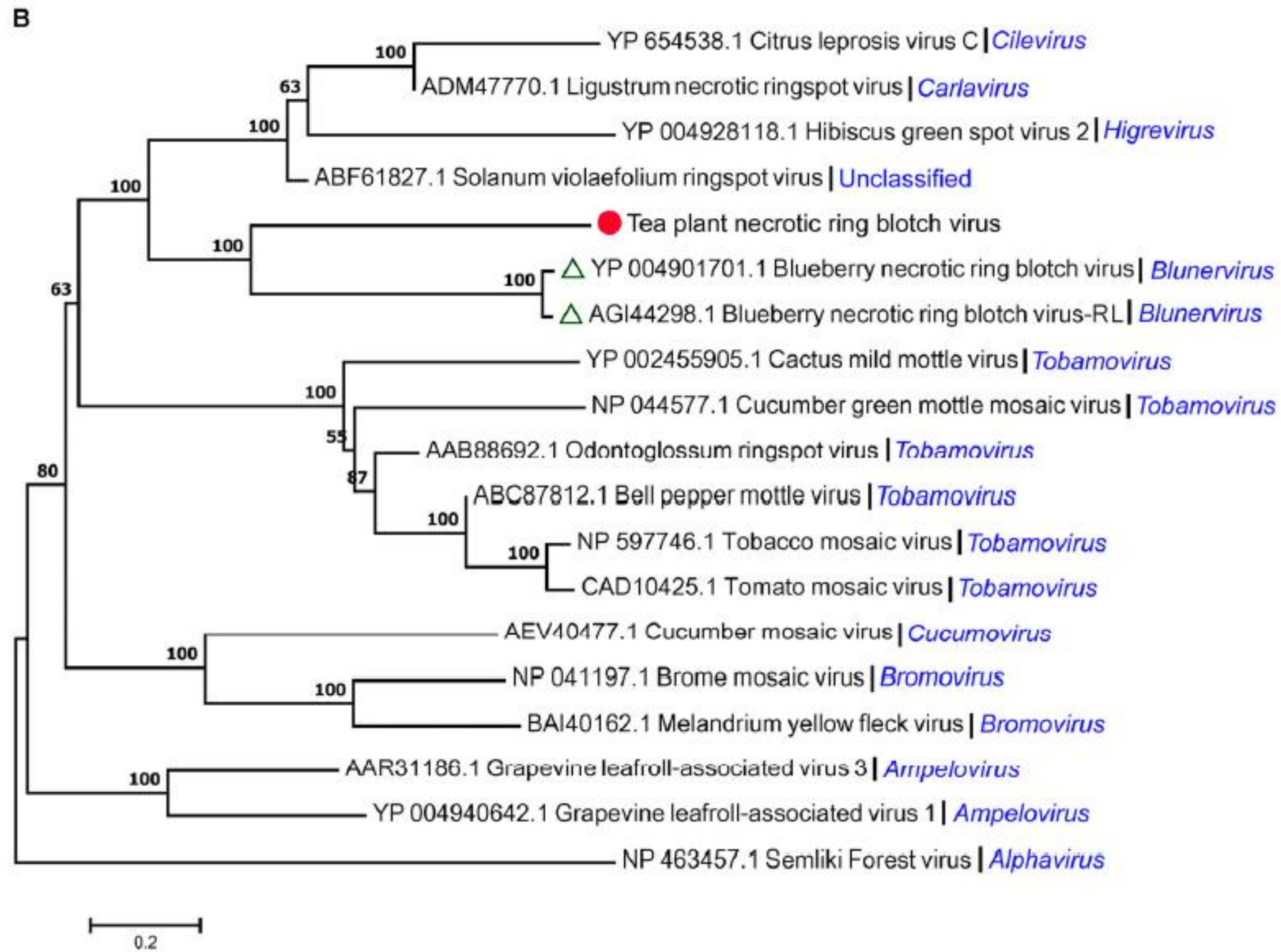
Discovery of Plant Viruses From Tea Plant (*Camellia sinensis* (L.) O. Kuntze) by Metagenomic Sequencing

Xinyuan Hao[1,2], Weifu Zhang[1,2], Fumei Zhao[3], Ying Liu[1,2], Wenjun Qian[1,2], Yuchun Wang[1,2], Lu Wang[1,2], Jianming Zeng[1,2], Yajun Yang[1,2]* and Xinchao Wang[1,2]**

[1] National Center for Tea Improvement, Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, China, [2] Key Laboratory of Tea Biology and Resources Utilization, Ministry of Agriculture, Hangzhou, China, [3] Institute of Plant Protection, Henan Academy of Agricultural Sciences, Zhengzhou, China

Sample Set: 2 camellia samples	Request: Camellia 2019	Account: Martin Lab In House Testing	2019

		PCR							
Sample	**Sample ID**	**Nad 2**	**TPLPV 1**	**TPLPV 2**	**TPLPV 3**	**TPNRBV 1**	**TPNRBV 2**	**TPNRBV 3**	**TPNRBV 4**
Camellia	1	+	-	-	-	-	-	+	-
Camellia	2	+	-	-	-	-	+	+	+


B
YP 654538.1 Citrus leprosis virus C | Cilevirus
ADM47770.1 Ligustrum necrotic ringspot virus | Carlavirus
YP 004928118.1 Hibiscus green spot virus 2 | Higrevirus
ABF61827.1 Solanum violaefolium ringspot virus | Unclassified
Tea plant necrotic ring blotch virus
YP 004901701.1 Blueberry necrotic ring blotch virus | Blunervirus
AGI44298.1 Blueberry necrotic ring blotch virus-RL | Blunervirus
YP 002455905.1 Cactus mild mottle virus | Tobamovirus
NP 044577.1 Cucumber green mottle mosaic virus | Tobamovirus
AAB88692.1 Odontoglossum ringspot virus | Tobamovirus
ABC87812.1 Bell pepper mottle virus | Tobamovirus
NP 597746.1 Tobacco mosaic virus | Tobamovirus
CAD10425.1 Tomato mosaic virus | Tobamovirus
AEV40477.1 Cucumber mosaic virus | Cucumovirus
NP 041197.1 Brome mosaic virus | Bromovirus
BAI40162.1 Melandrium yellow fleck virus | Bromovirus
AAR31186.1 Grapevine leafroll-associated virus 3 | Ampelovirus
YP 004940642.1 Grapevine leafroll-associated virus 1 | Ampelovirus
NP 463457.1 Semliki Forest virus | Alphavirus
100
63
100
100
100
100
63
100
55
87
100
100
80
100
100
100
0.2

Dear tree scientists,
Please Please Please
find a cure for
honey locust. If
you need to cut
my honey locust,
Lila, I will never
be whole again.
Please find a cure
before august
when you need to

cut it down.
you are breaking
childrens hearts.
xoxo

Allegra / Allegra
Dorman.

PS: Please
find a cure
before august.

THE END!