TACKLING COMPLEX DISEASES IN THE POST GENOMIC ERA: THE CASE OF THE PINE WILT DISEASE

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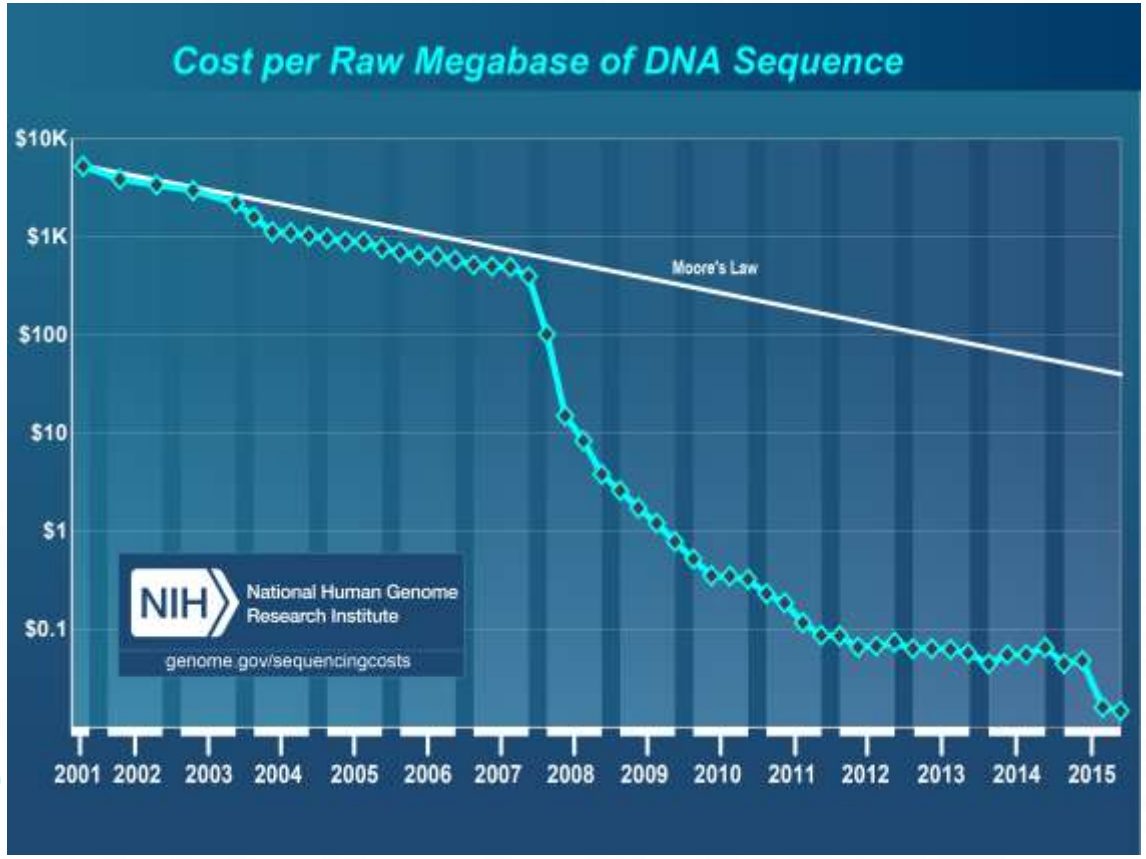
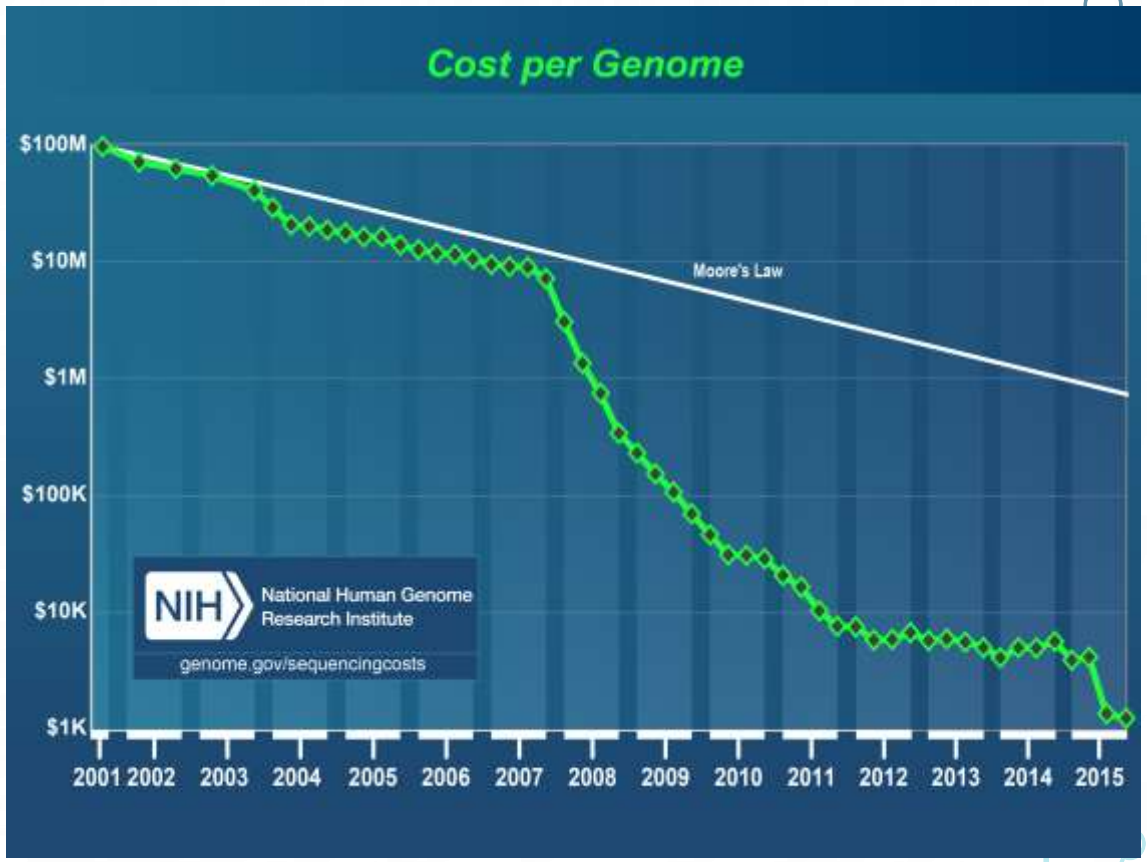
  - BACTERIA
  - NEMATODE
- WHERE ARE WE GOING?

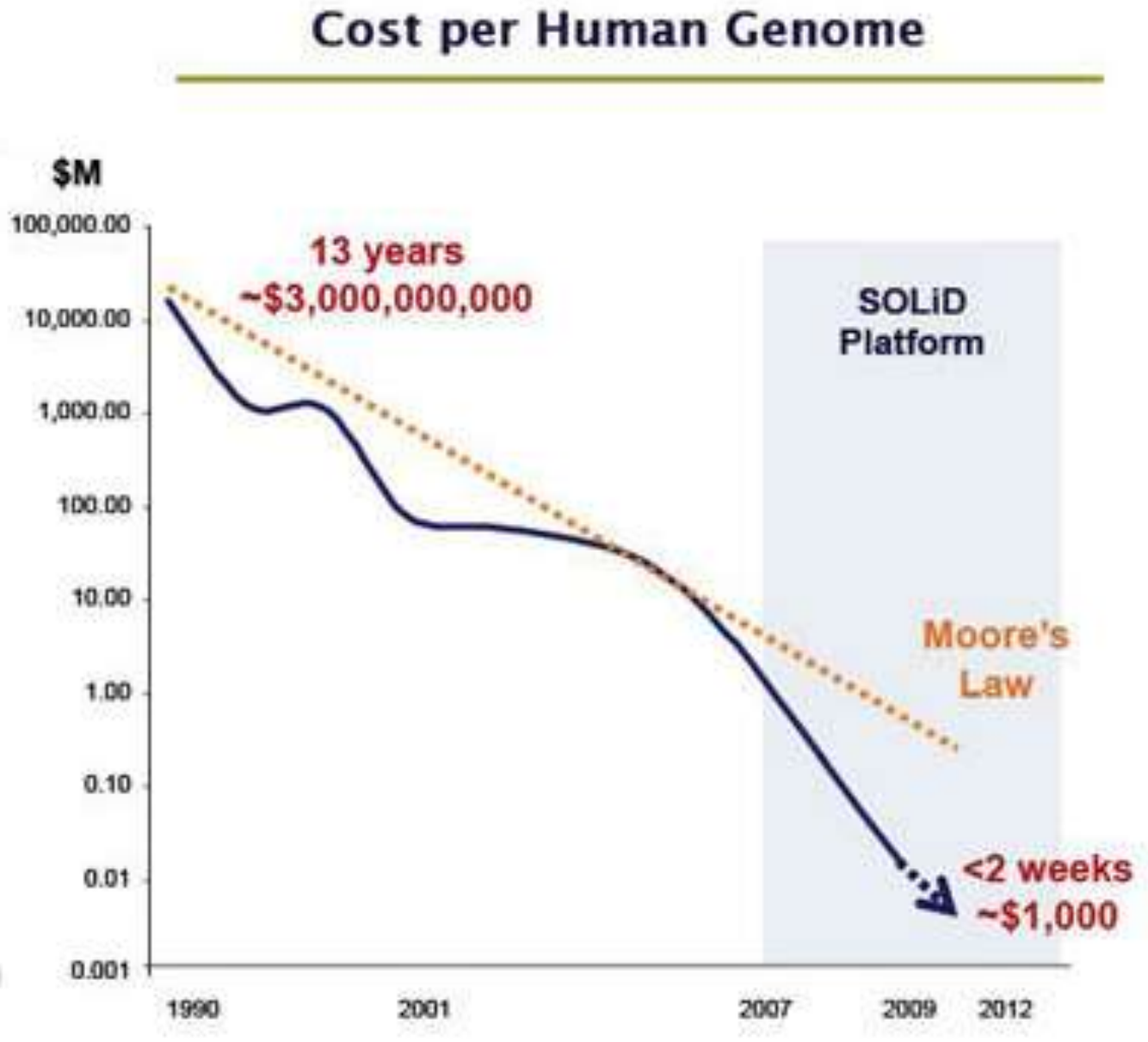
THE EVOLUTION OF COSTS



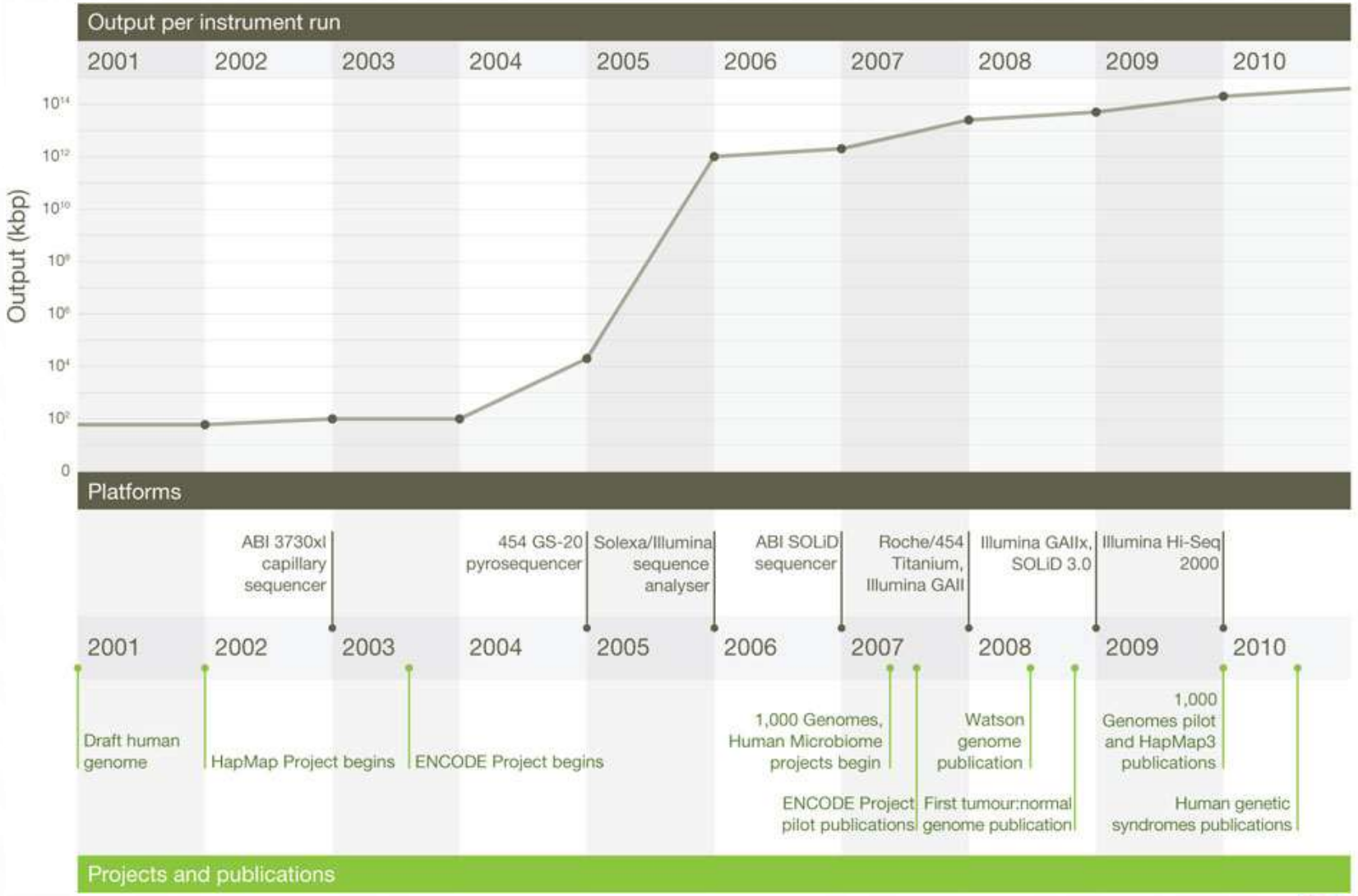


Wetterstrand, NHGRI Genome Sequencing Program (GSP). Accessed September 16th **2016**

THE EVOLUTION OF SPEED



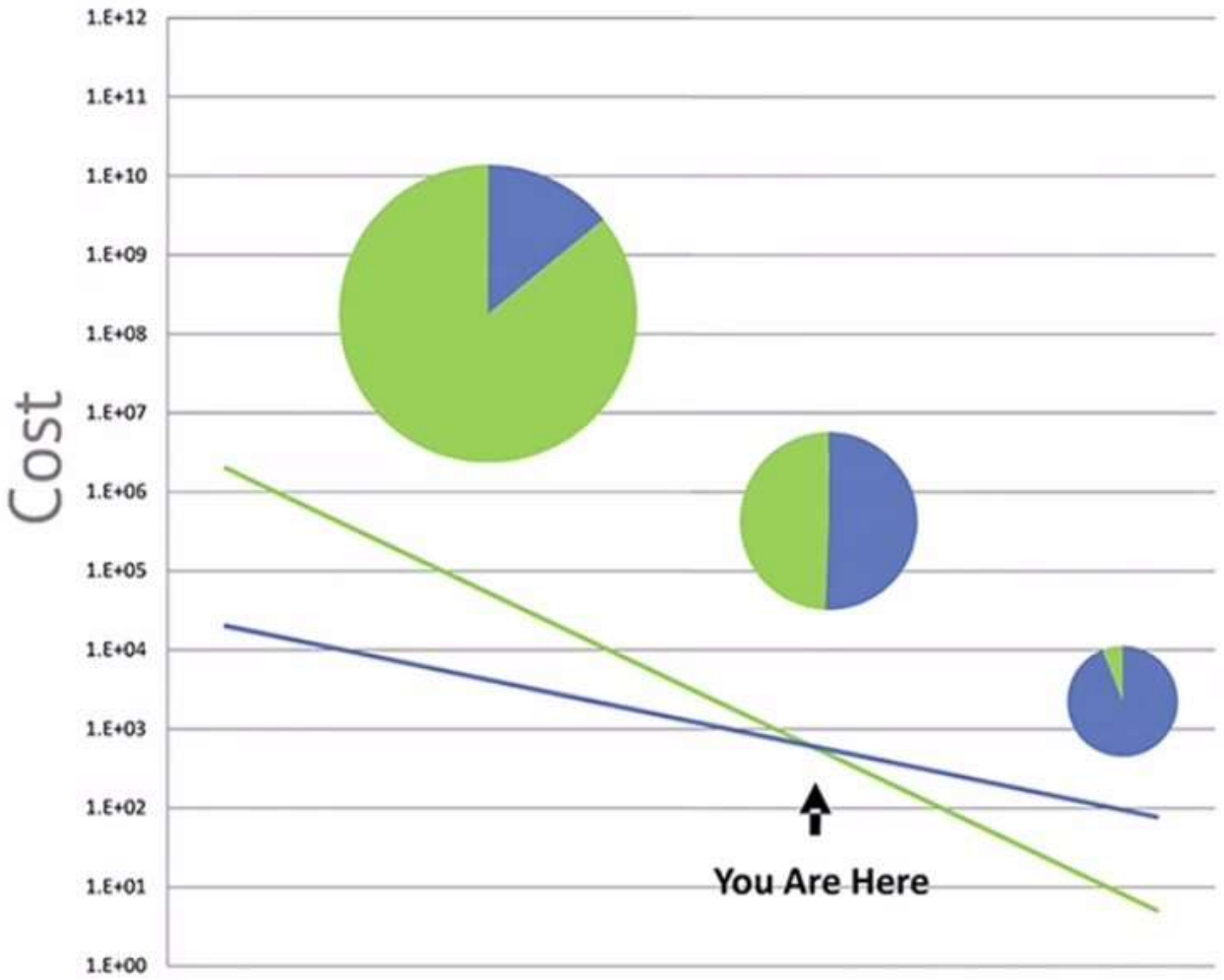
Changes in instrument capacity, major sequencing projects



ER Mardis. *Nature* **470**, 198-203

nature

DNA Sequencing Economics

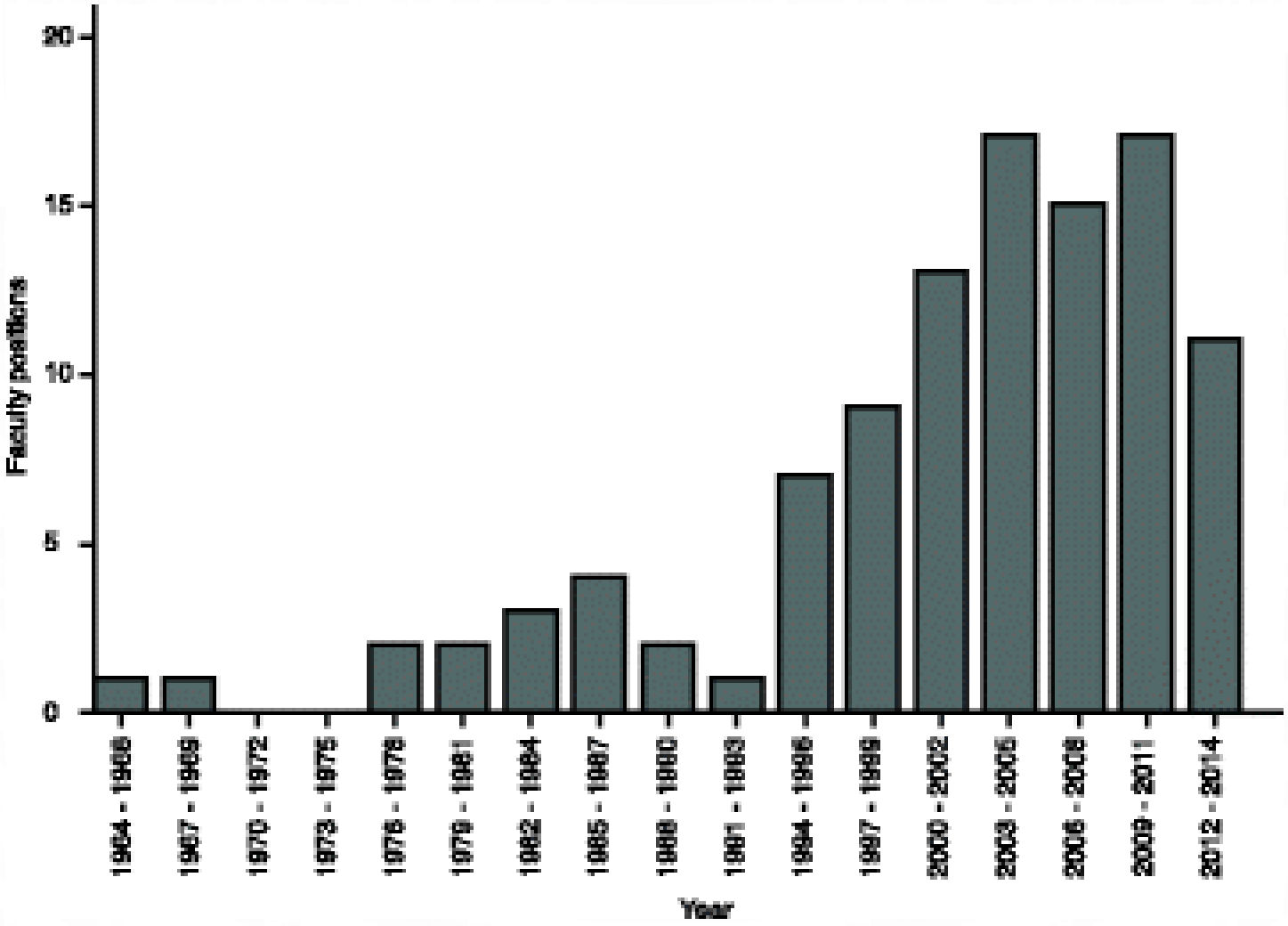


Sequencing cost dropping ~5x per year

Informatics cost dropping ~2x per year

INFORMATICS IS NOW THE BOTTLENECK

FACULTY POSITIONS FOR BIOINFORMATICIANS



Muir et al. Genome Biology (**2016**) 17:53

THE RAREST SPECIES ON EARTH

Amur Leopard

Sumatran Rhinoceros

Western Lowland Gorilla

Philippine Crocodile

Sumatran Orangutan

The Bioinformatician

WHAT WE KNEW PRE-GENOMICS

PINE WILT DISEASE

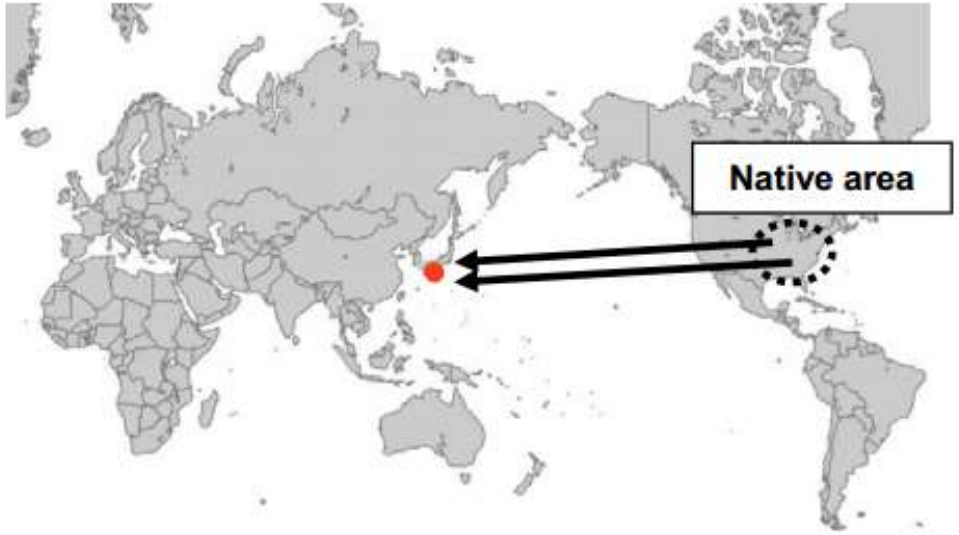
THE PINE WILT DISEASE

- *Pinus pinaster*
- *Bursaphelenchus xylophilus*


Jornal de Notícias
Nacional
Governo autoriza despesa de 1,5 milhões para controlar nemátodo da madeira do pinheiro
LUSA 05/09/2013 - 18:35
Autorização é dada na zona tampão do território continental durante os anos de 2013 a 2015.
PAÍS
MUNDO
RTP NOTÍCIAS
PARTILHE NO FACEBOOK 0
Nemátodo da made
afecta empresas nacion
Antena 1
09/05/2008
INCÊNDIOS E PRAGA SÃO PROBLEMAS DIZ PRESIDENTE DA ASSOCIAÇÃO DAS INDÚSTRIAS DE MADEIRA
Nemátodo do pinheiro pode obrigar a corte dois milhões de árvores
LUSA 20/05/2011 - 20:48

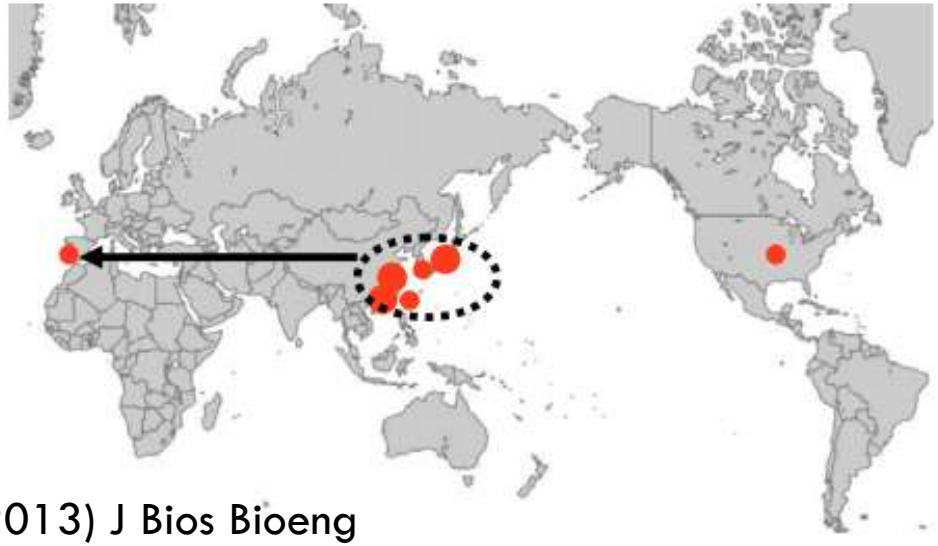
HISTORY OF DISEASE SPREAD

1905



The late 1970s - 1980s

The late 1990s



The late 2000s

Shinya et al (2013) J Bios Bioeng

THE PINE WILT DISEASE

+

=

PWD

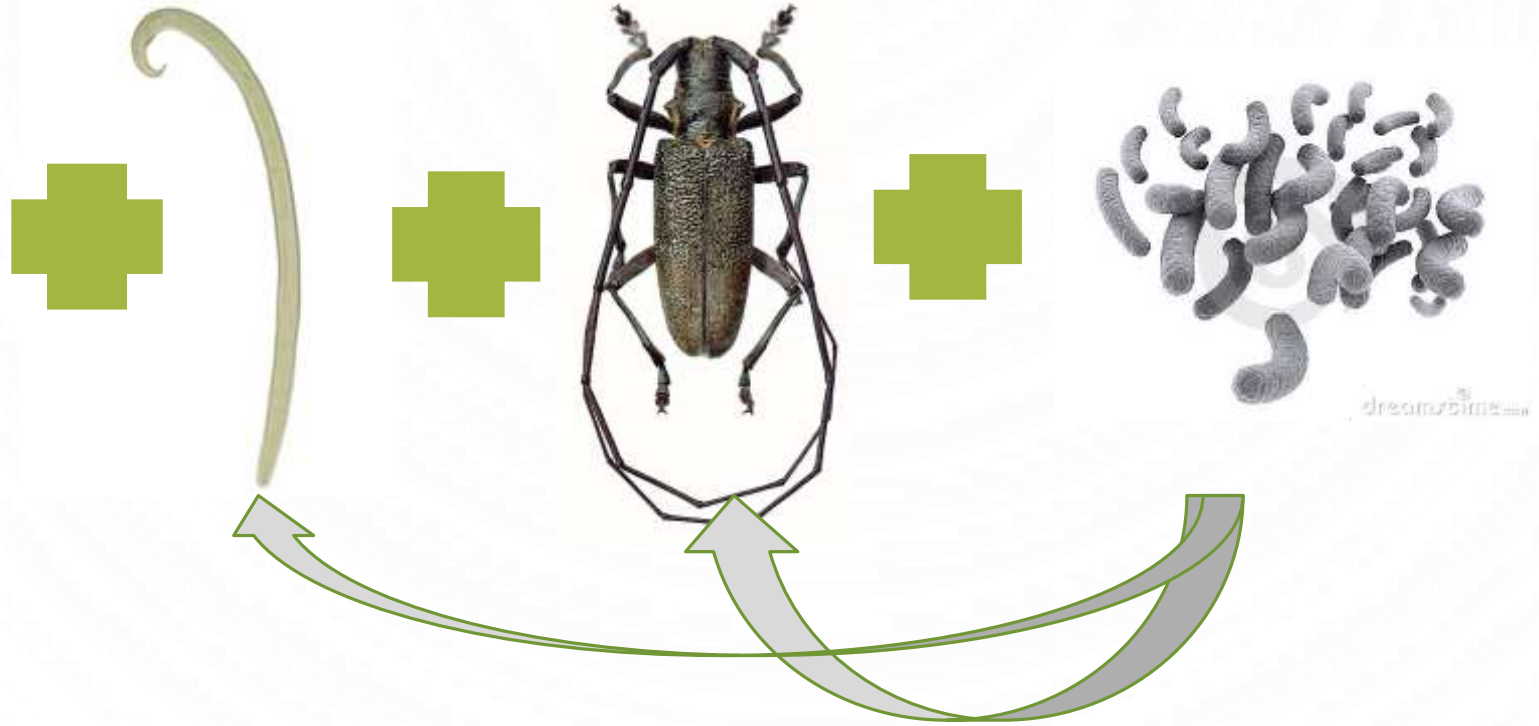
THE PINE WILT DISEASE

+

+

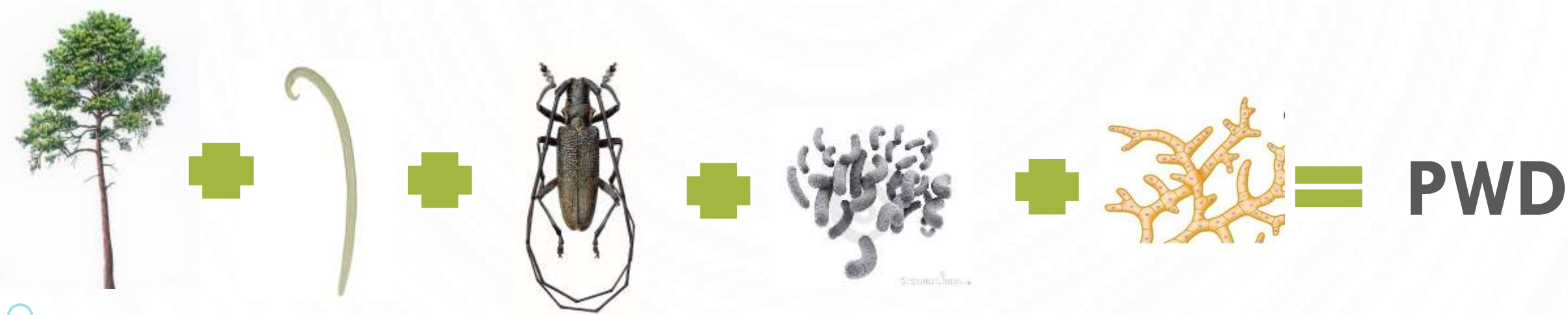
= PWD

THE PINE WILT DISEASE



PWD

THE PINE WILT DISEASE

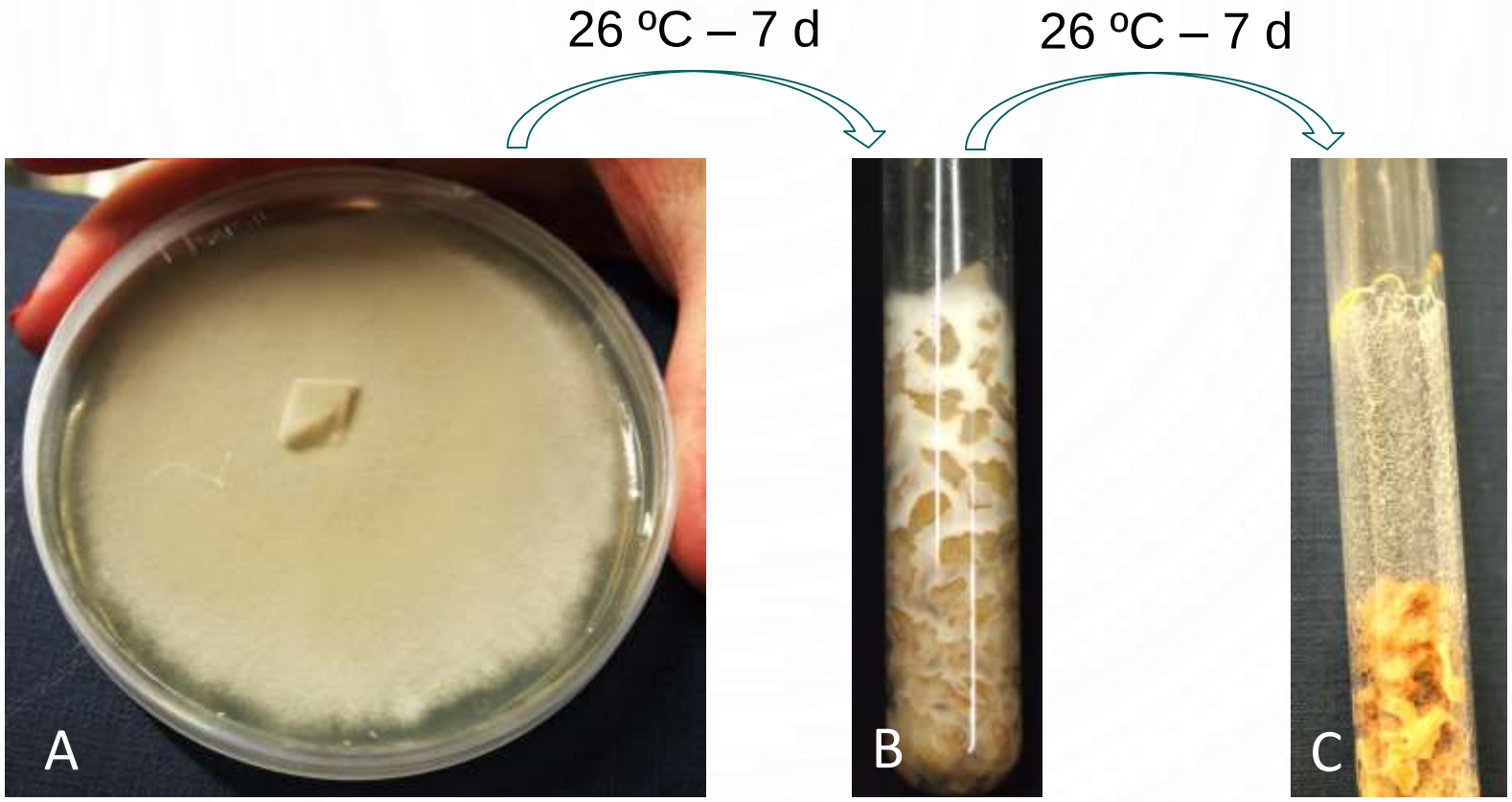


WHAT WE KNOW PRE GENOMICS?

- Experimental inoculations;
- Histochemical observations;
- Ecological surveys;
- Empirical data on differential susceptibilities

Limited knowledge of the molecular basis of the interactions between the host, pathogen, and its vector.

Nematode culture



A) *Botrytis cinerea* cultured in PDA; B) *Botrytis cinerea* cultured in barley grains; C) *Bursaphelenchus xylophilus* strain HF.

TREE INOCULATION

SCORING FOR DISEASE SEVERITY

		Early phase		Developing phase	
	Stage	**1**	**2**	**3**	**4**
External	Symptom	None →		Discoloration of old needles	Discoloration of young needles → Death
	Oleo-resinosis	Normal →	Decreasing →	None	
Pine wood nematode		Low population →		Propagation	Extensive propagation
Time (weeks) Example of *Pinus thunbergii*		1	2	3	4 and beyond

Zhao *et al.*, 2008

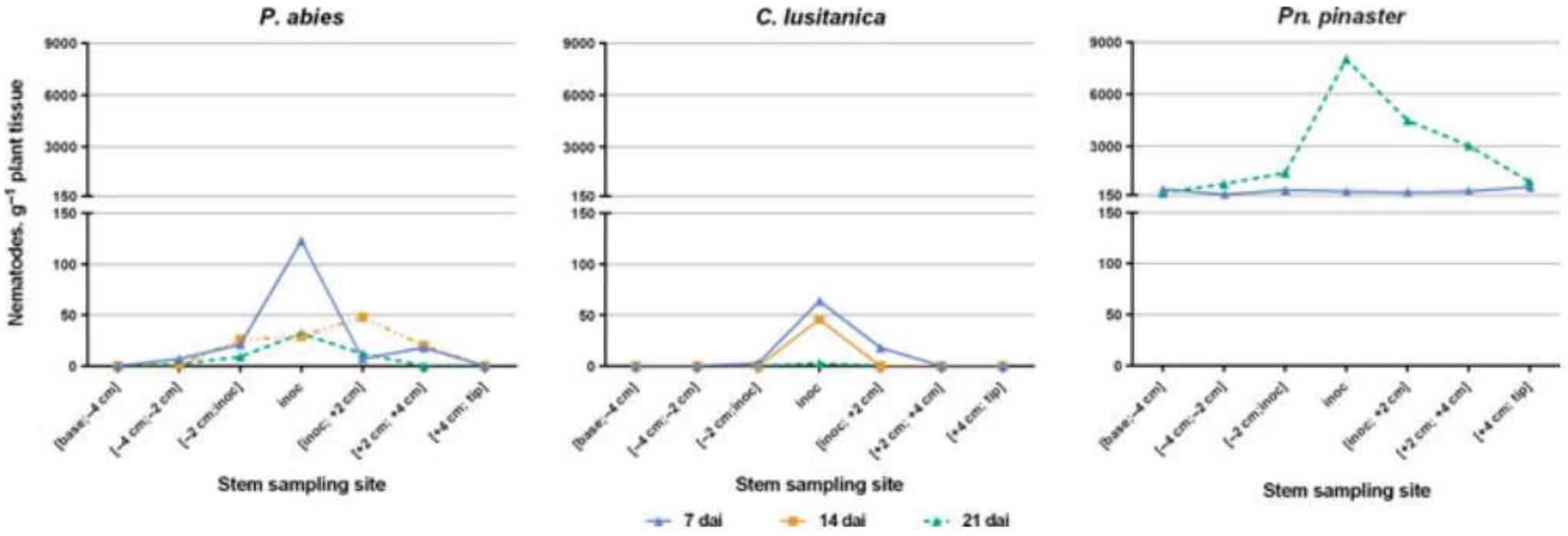
WHAT WE KNEW PRE GENOMICS?

Treatment conditions	Incubation time (d) T_0	T_{10}	T_{20}
PP H_2O	I	I	II
PP A	I	II	III
PP HF	I	III	IV
PPi H_2O	I	I	I
PPi A	I	II	II
PPi HF	I	I	II
Pni H_2O	I	I	I
Pni A	I	II	II
Pni HF	I	II	II
Psy H_2O	I	I	II
Psy A	I	II	II
Psy HF	I	II	III

I, Healthy plant; II, partial needle discolouration; III, partial needle discolouration, necrosis and reduction in the resin production; IV, total discolouration, necrosis and seedling death.

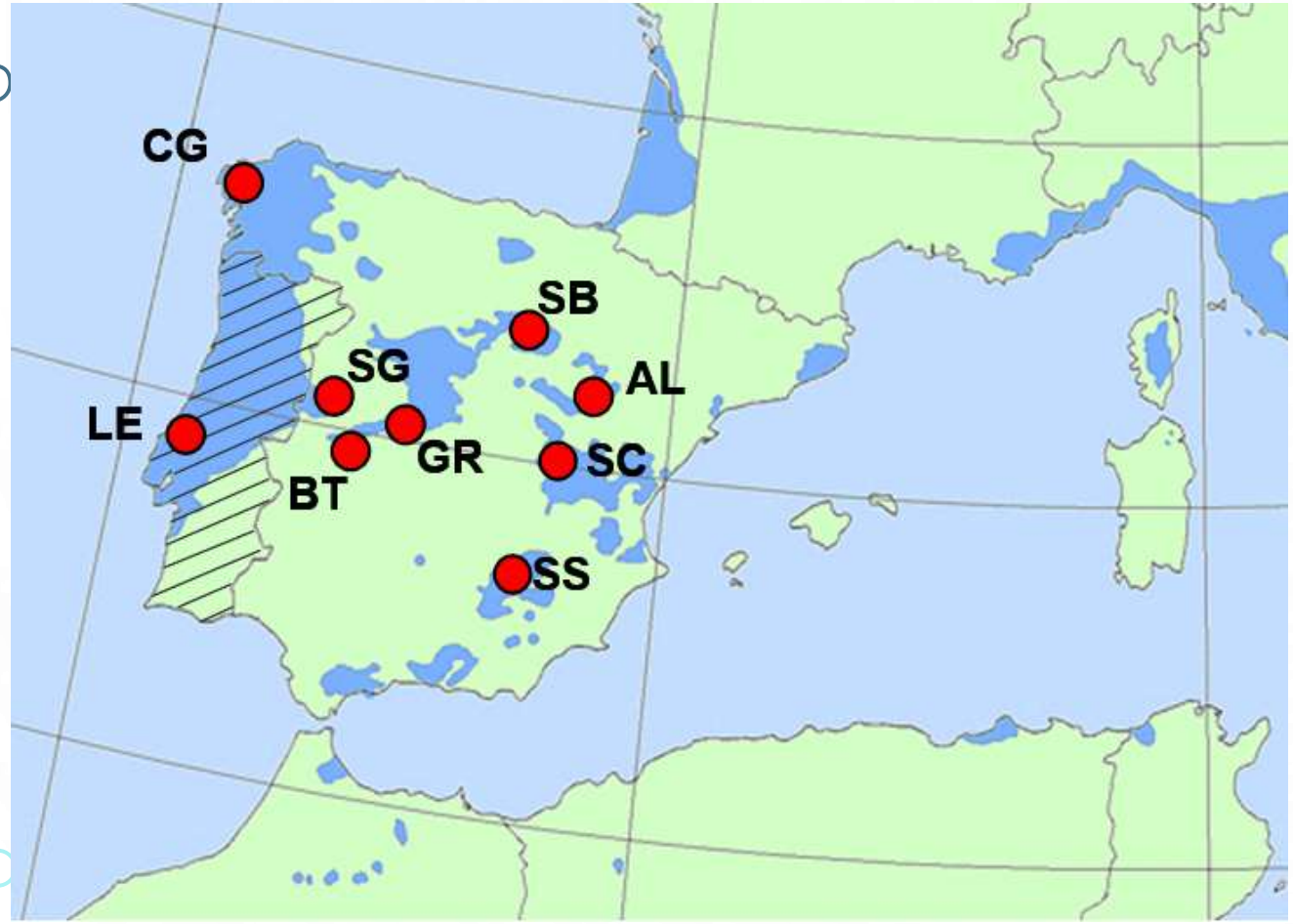
Franco et al. (2011) Plant Gen Res. Util. 9: 272-275

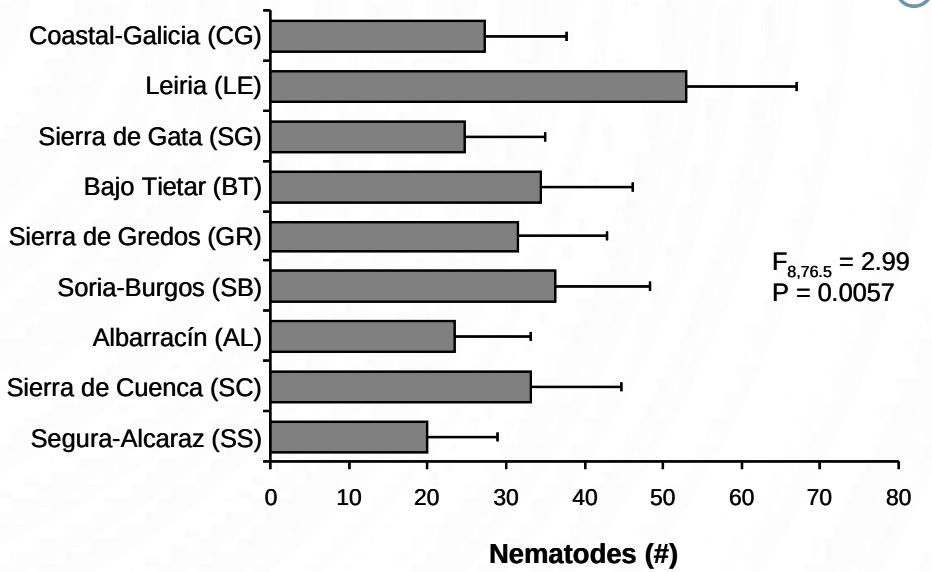
WHAT WE KNEW PRE GENOMICS?



Silva et al (2013) Plant Pathol

INTRA SPECIFIC VARIABILITY

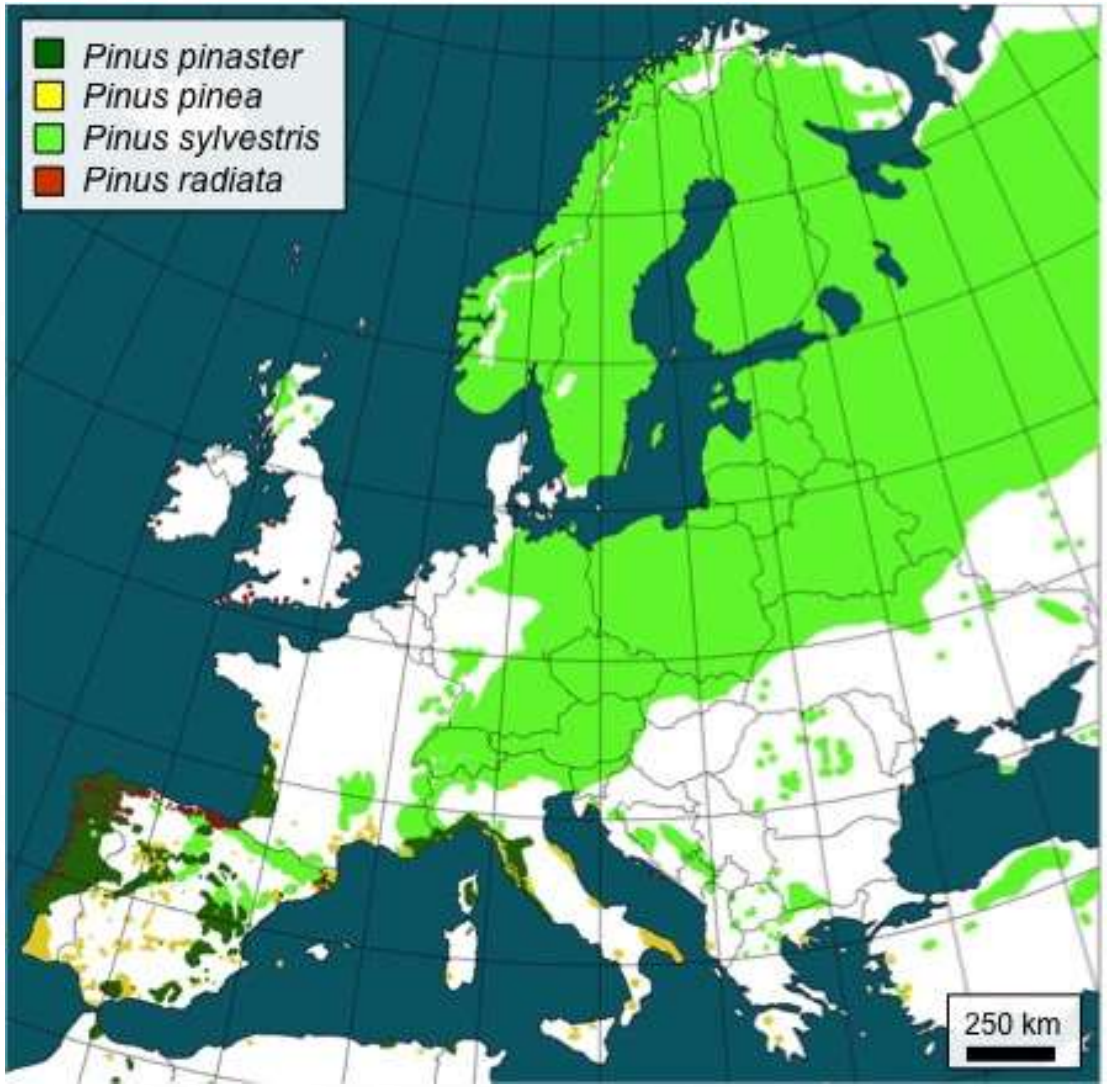




Leiria provenance has the highest susceptibility

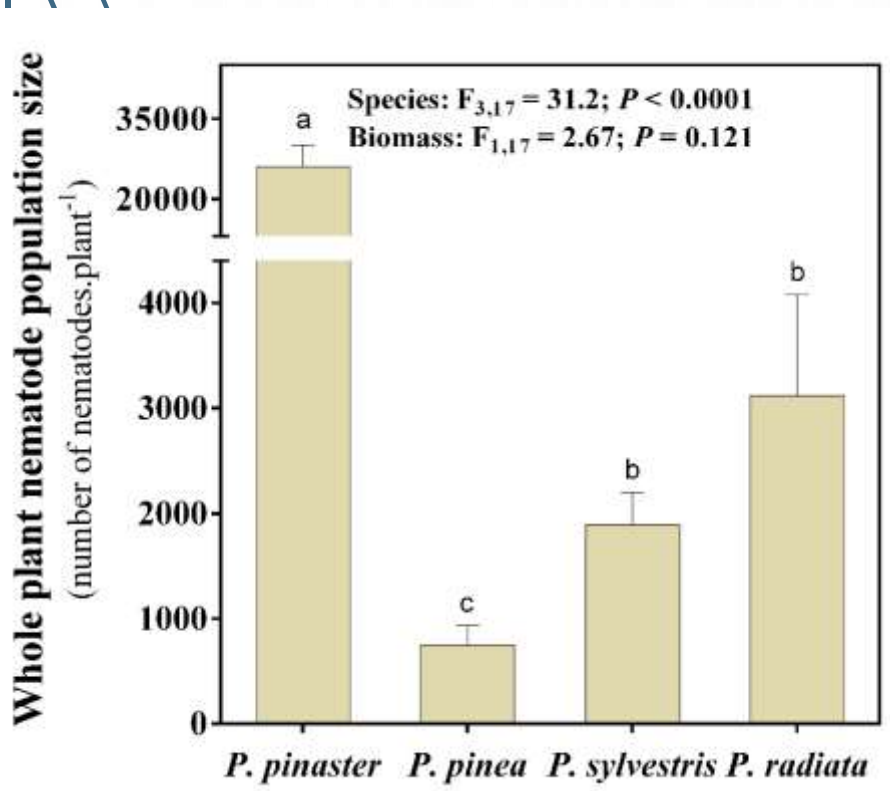
Zas et al. (2014) **Trees**

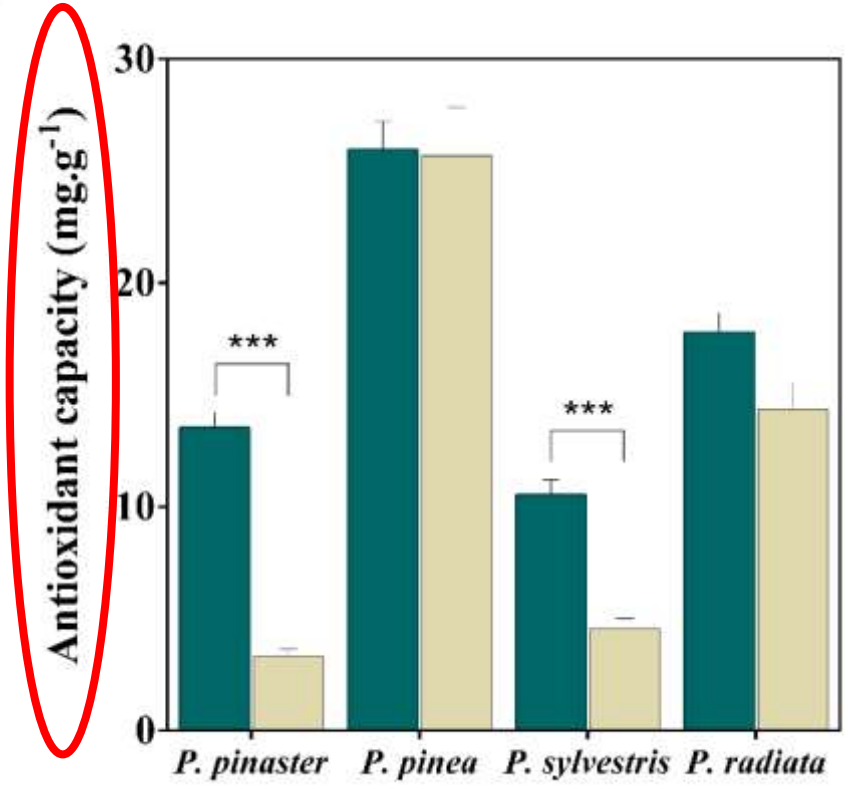
INTER SPECIFIC VARIABILITY

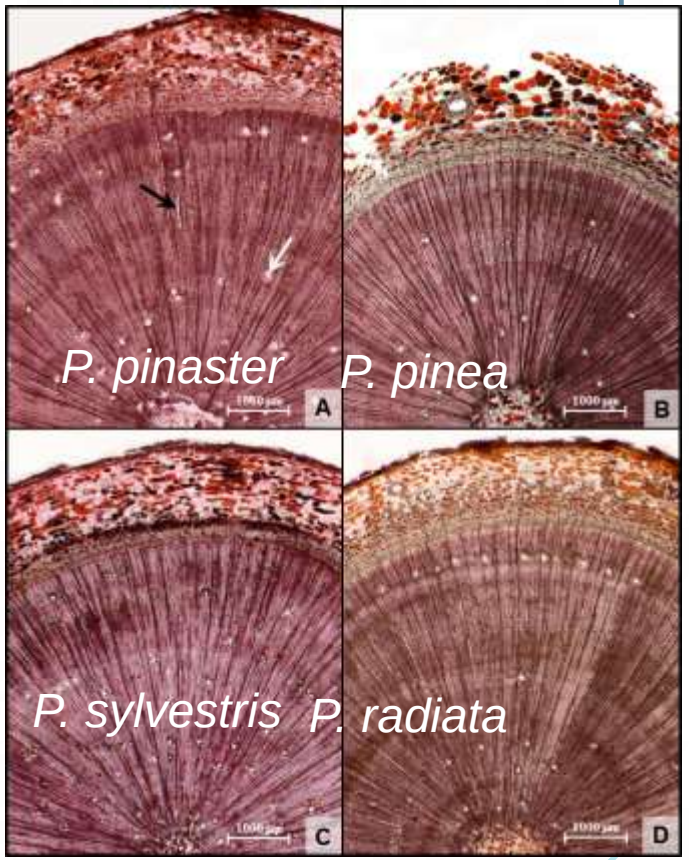


Silva et al. (2014) **Tree Physiology** .

INTER SPECIFIC VARIABILITY







Anatomy

Biochemistry

Silva et al. (2014) **Tree Physiology**

HOW IS GENOMICS HELPING: EXAMPLES

FROM A PLANT PERSPECTIVE

SEARCHING FOR RESISTANCE GENES

P. pinea	
Putative clavata-like receptor	
Putative protein belonging to Class-II DAHP synthetase family	Plant defense
Possible s-adenosyl methionine synthetase 2	
Likely copper resistance protein	
mRNA up-regulated during drought stress	Oxidative stress
Probable RNA recognition motif	Drought resistance
Sm-like protein	
Protein similar to one belonging to DUF231 *Arabidopsis* proteins	
NifU-like protein	

Santos et al. (2012) Eur J Plant Pathol 132: 407-418

SEARCHING FOR RESISTANCE GENES

Searching for resistance genes to *Bursaphelenchus xylophilus* using high throughput screening

Santos *et al.*

BioMed Central

Santos *et al. BMC Genomics* 2012, **13**:599
http://www.biomedcentral.com/1471-2164/13/599

SEARCHING FOR RESISTANCE GENES

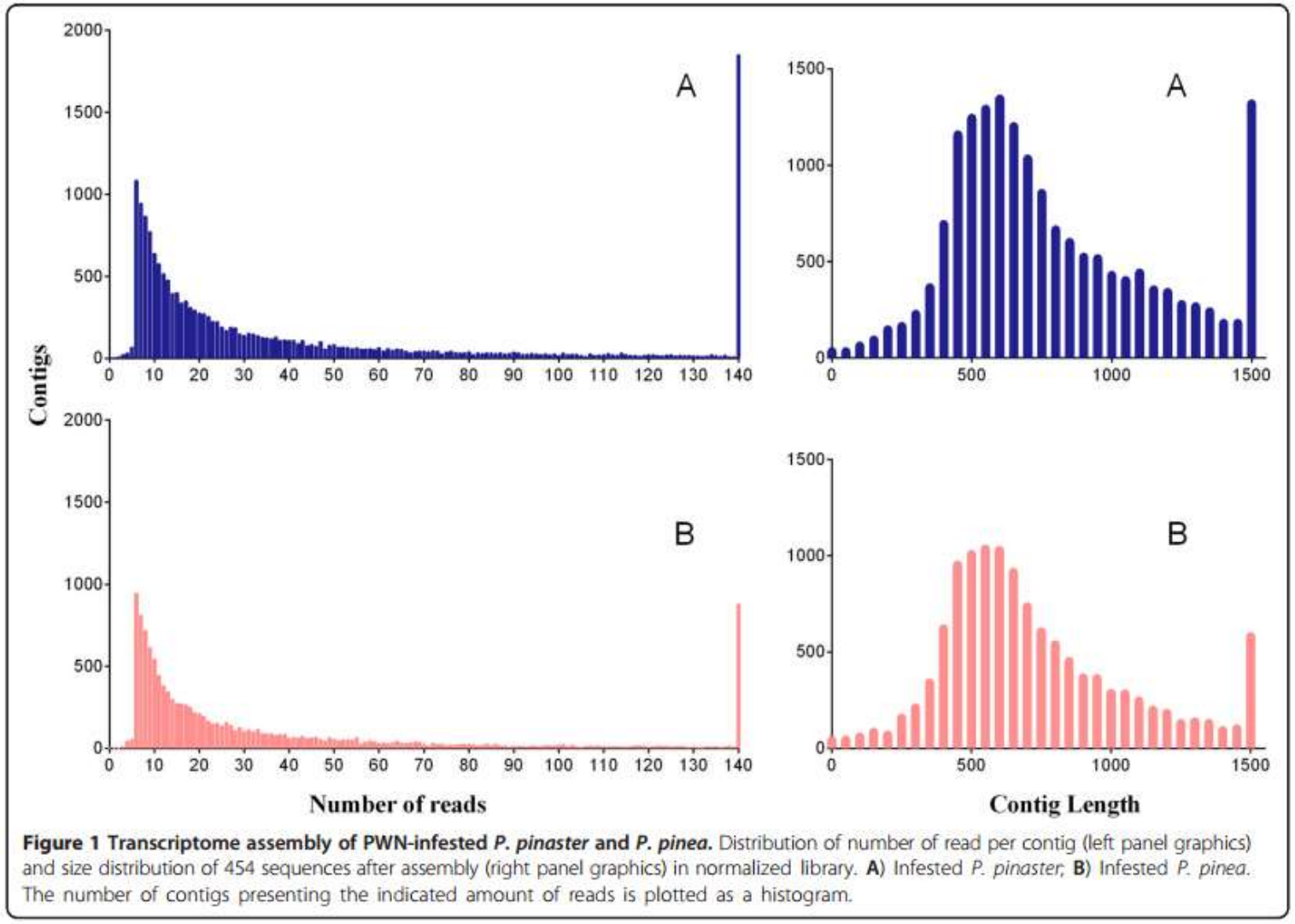
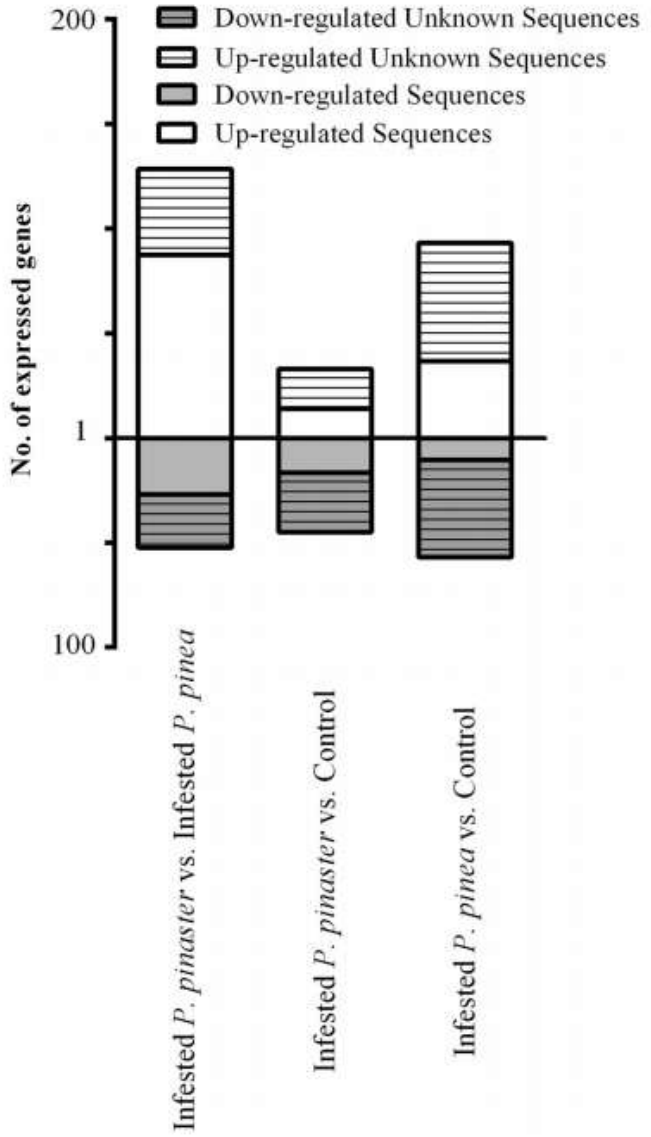
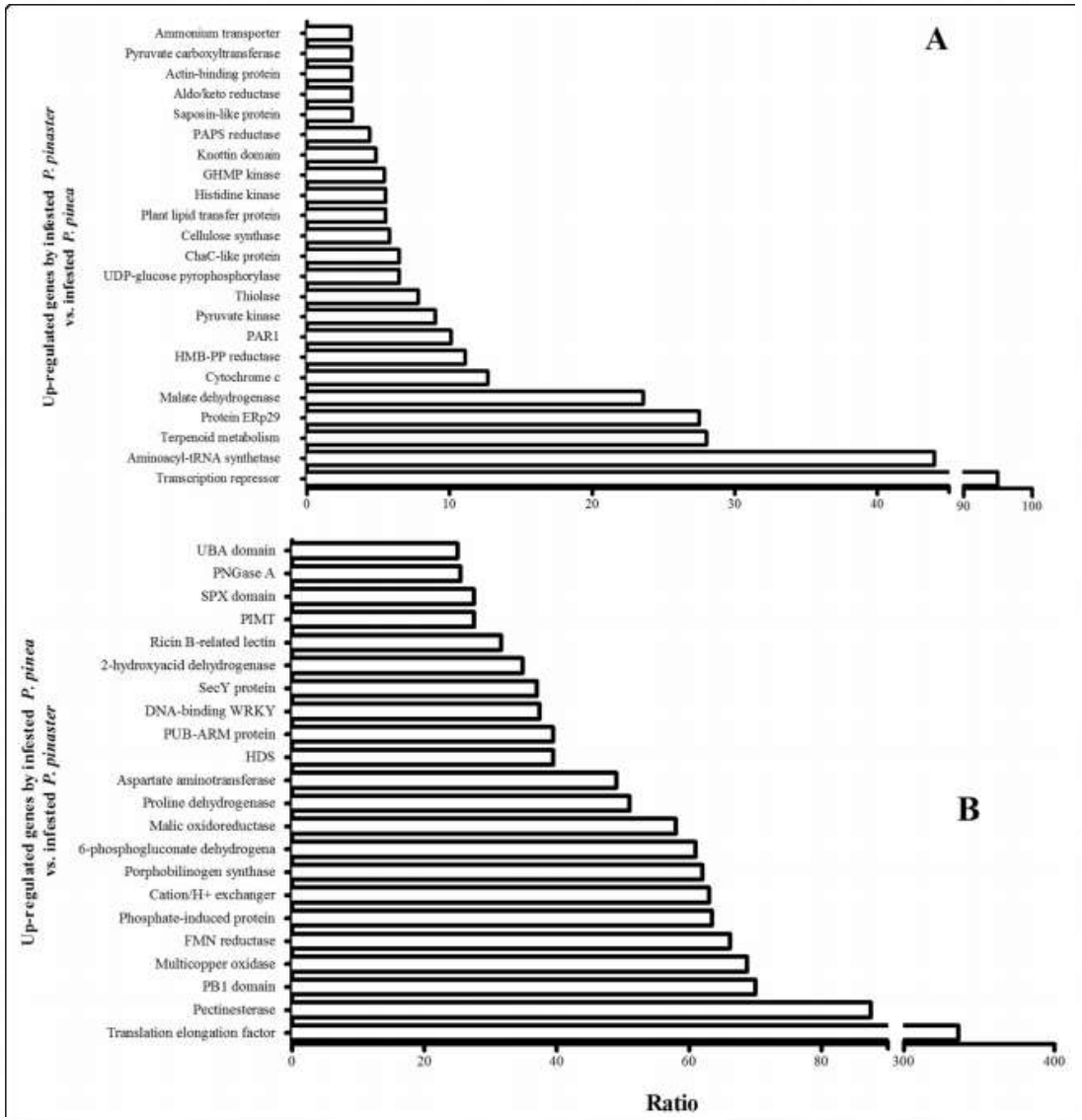


Figure 1 Transcriptome assembly of PWN-infested *P. pinaster* and *P. pinea*. Distribution of number of read per contig (left panel graphics) and size distribution of 454 sequences after assembly (right panel graphics) in normalized library. **A)** Infested *P. pinaster*; **B)** Infested *P. pinea*. The number of contigs presenting the indicated amount of reads is plotted as a histogram.

Satntos et al. (2012) BMC Genomics

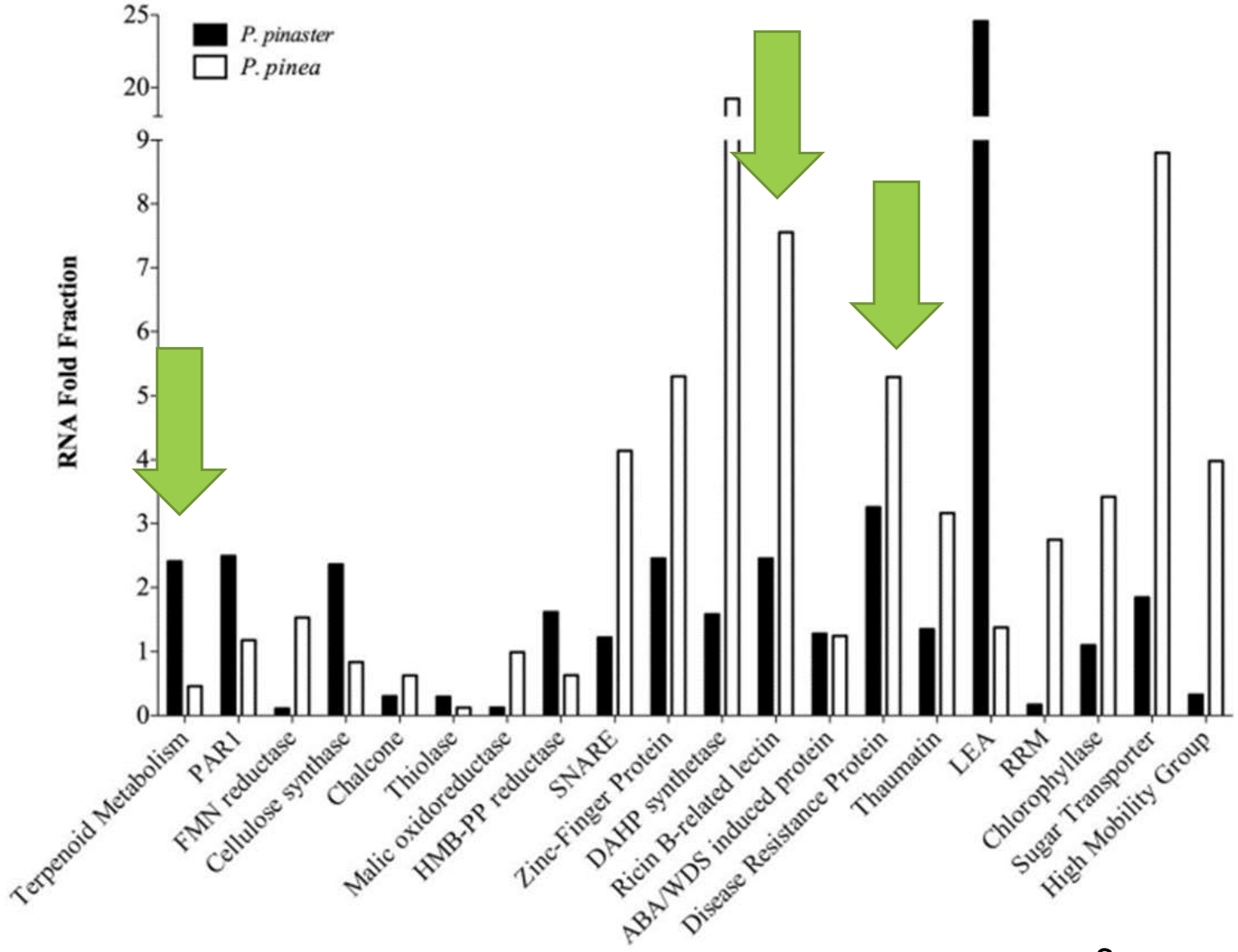
SEARCHING FOR RESISTANCE GENES





Santos et al. (2012) BMC Genomics

SEARCHING FOR RESISTANCE GENES



Santos et al. (2012) BMC Genomics

SEARCHING FOR RESISTANCE GENES

Table 3 General gene function and correspondent genes found between the differentially expressed data

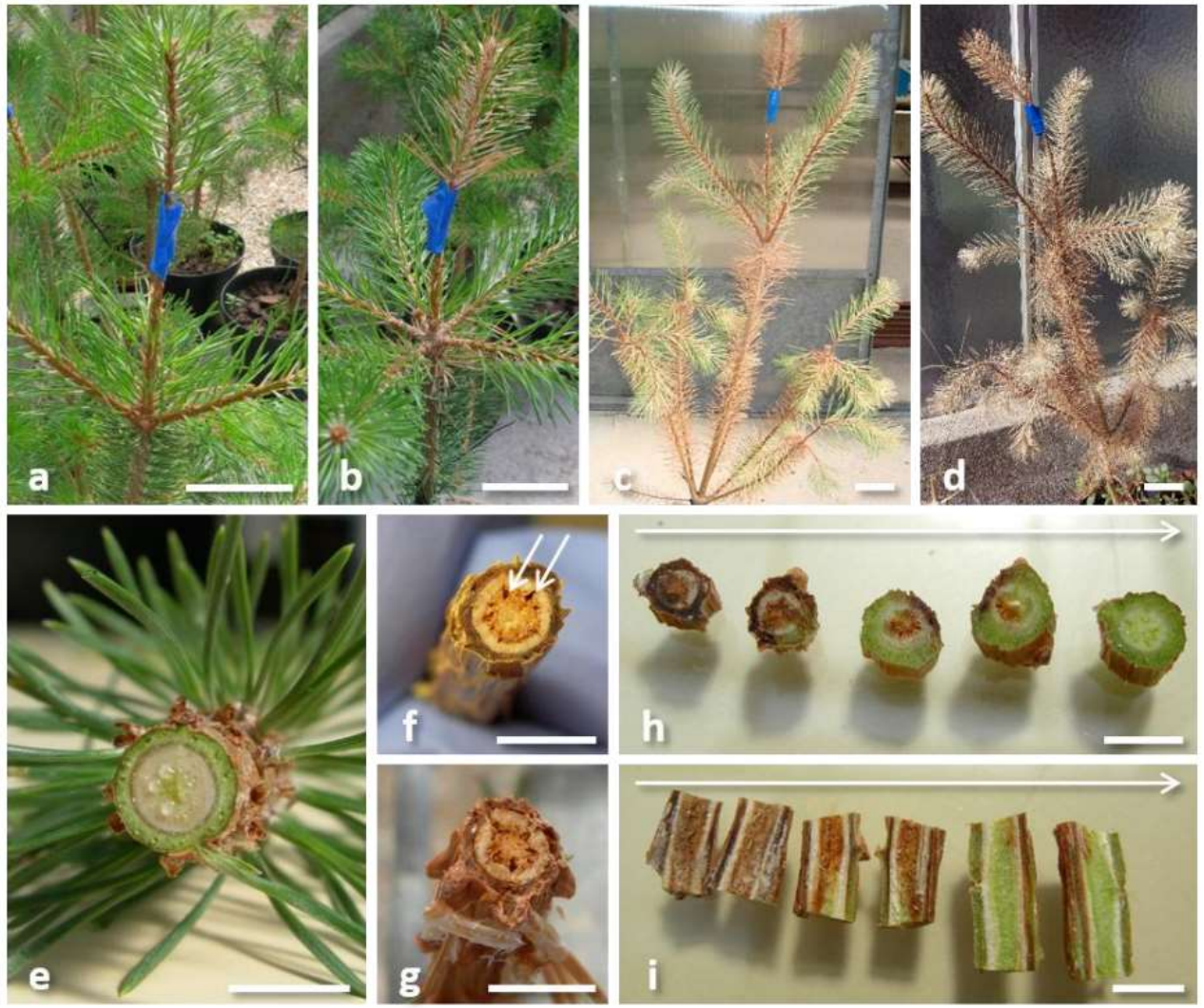
General function	Genes	References
Oxidative stress	Aldo/keto reductase	33
	Multicopper oxidase	45
	2-hydroxyacid dehydrogenase	48
	6-phosphogluconate dehydrogenase	46
	PB1	47
	Cytochrome c	32
	FMN reductase	32
	Malic enzyme	49
	Proline dehydrogenase	50
Defense-related	Sugar related proteins	38, 39, 40
	PAPS reductase	42
	PAR1	36
	Plant Lipid Transfer Protein	37
	Saposin-like	43
	Pectinesterase	52, 53
	PUB-ARM protein	54, 55
	WRKY protein	25, 56
	UBA domain	57
Transcription factors	aminoacyl-tRNA synthetase	25
	ERp29 protein	26
	Translation elongation factor	51
Secondary metabolites production	HMB-PP reductase	30
	HDS	58

1. *P. pinaster* + *P. pinea*: **defense-related genes**
2. *P. pinaster:* higher abundance of genes related to transcriptional regulation, **terpenoid secondary metabolism** and pathogen attack.
3. *P. pinea:* higher abundance of genes related to **oxidative stress** and higher levels of expression in general of stress responsive genes.

Santos et al. (2012) BMC Genomics

LIPIDOMICS OF PWD

- Oleoresins: seals wounds
- Time-course analysis essential oils of *P. halepensis, P. pinaster*, *P. pinea* and *P. sylvestris*
- EO chemotypes for *P. pinaster*, *P. halepensis* and *P. sylvestris*
- *P. pinea* showed homogenous EO composition.
- Increase of sesquiterpenes and diterpenic compounds in *P. pinea* and *P. halepensis*, comparatively to healthy whole plants EOs.



Rodrigues et al. (2016) Chem. & Biod. *(in press)*

HOW IS GENOMICS HELPING: EXAMPLES

FROM A BACTERIAL PERSPECTIVE

M. galloprovincialis and *M. alternatus microbiome*

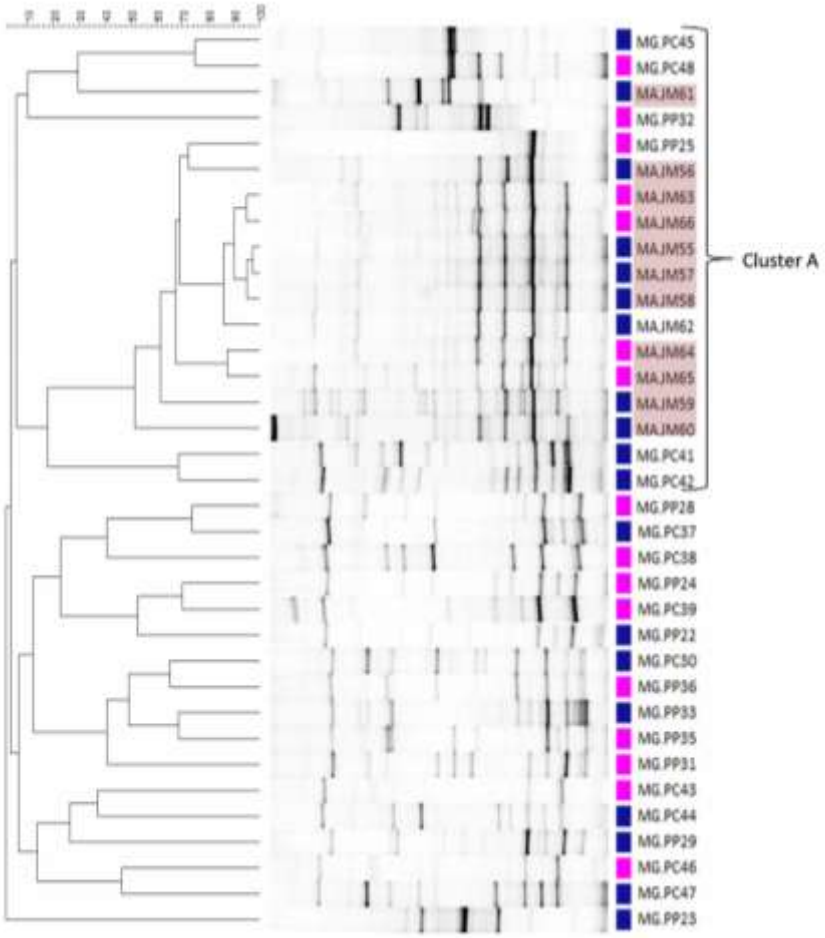
Species	Location (Country)	Nr Insects	*B. xylophilus* carriers	Nr females	Nr males
Monochamus alternatus	Hikobe (Japan)	12	11	4	8
Monochamus galloprovincialis	Mortágua (Portugal)	12	0	7	5
	Comporta (Portugal)	11	0	5	6
Total	3 (2)	35	11	16	19

- **23 from Portugal**
- **22 from Japan**

Alves et al., (**2016**) Sci. Reports

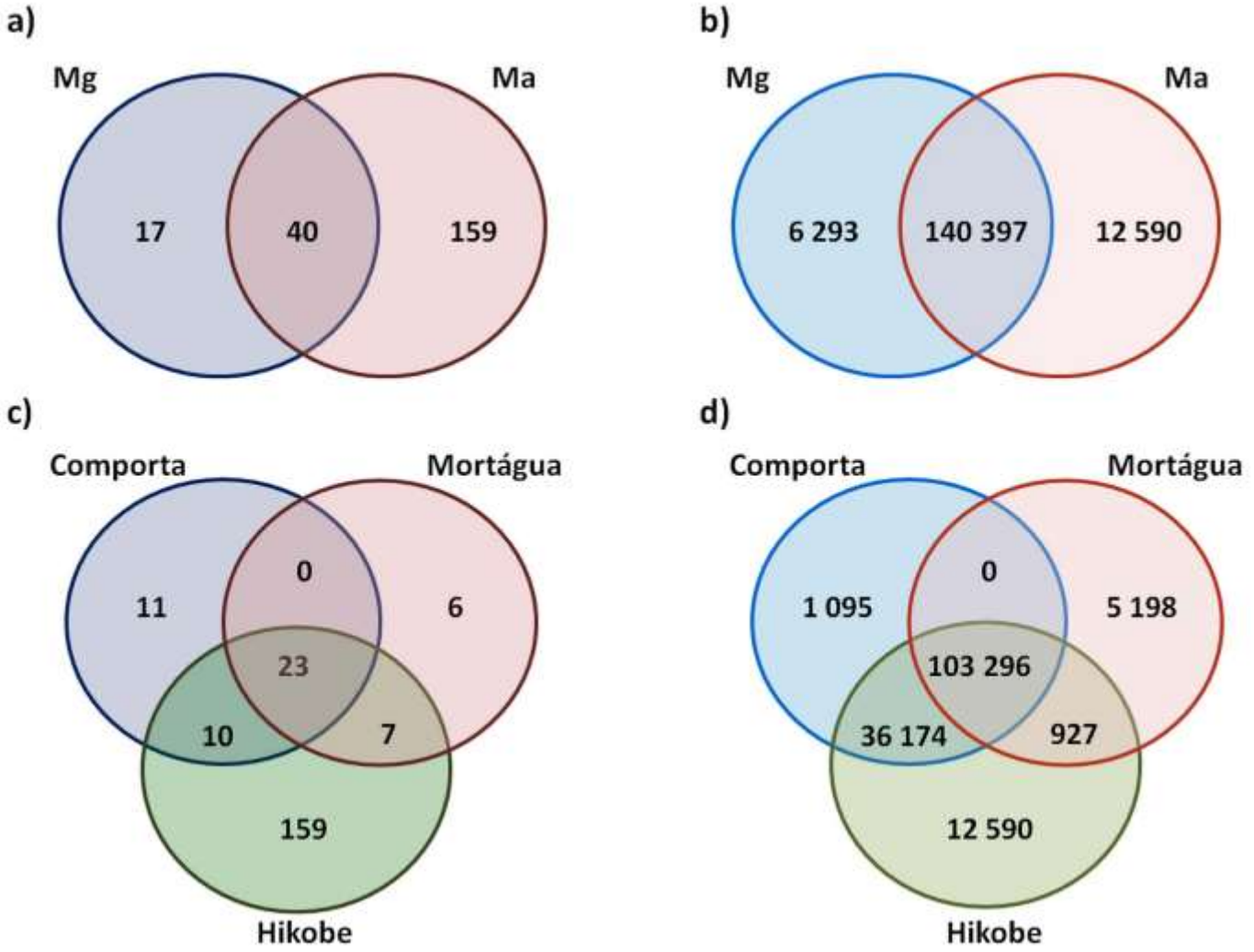
M. galloprovincialis and *M. alternatus microbiome*

DGGE



Alves et al., (2016) Sci. Reports

PYROSEQUENCING



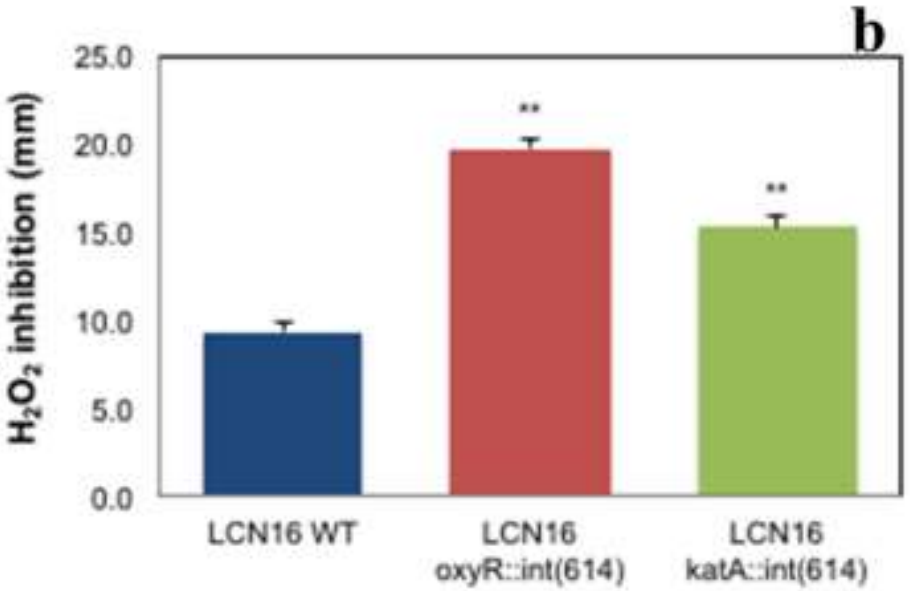
M. galloprovincialis and *M. alternatus microbiome*

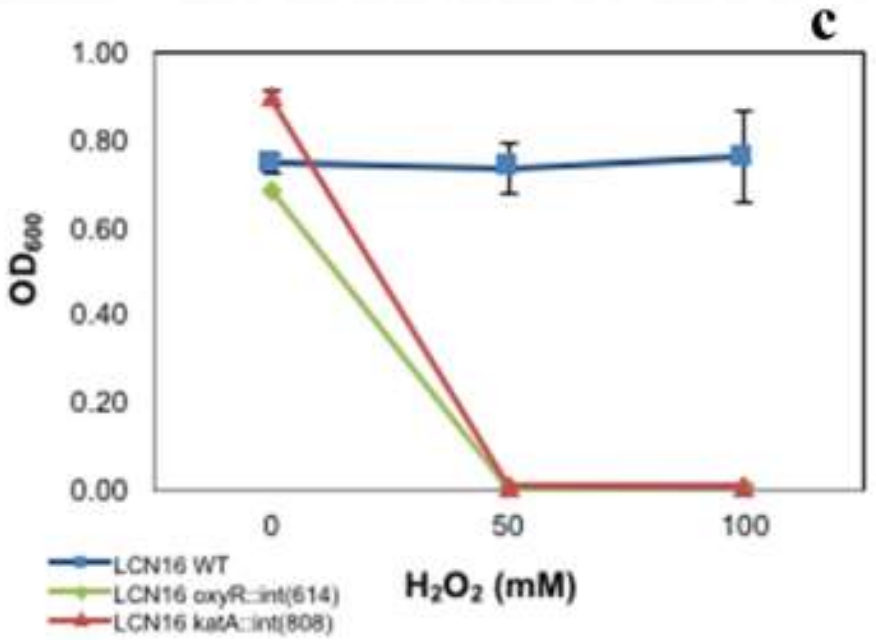
- *Monochamus* tracheae microbiome: species-specific, independent of gender and location.
- Several bacterial groups common to found in *P. pinaster* and *B. xylophilus*. Chicken or egg?
- Involved in processes of detoxification: helping tree invasion?
- Elaboration of bio-control strategies?

Alves et al., (2016) Sci. Reports

GENOME OF SERRATIA SP. LCN16

- Bacteria present in virulent *B. xylophilus*
- Provides resistance to oxidative stress
- LCN16 mutants: sensitive to H_2O_2
- Mutants failed to protect the PWN from H_2O_2-stress exposure.





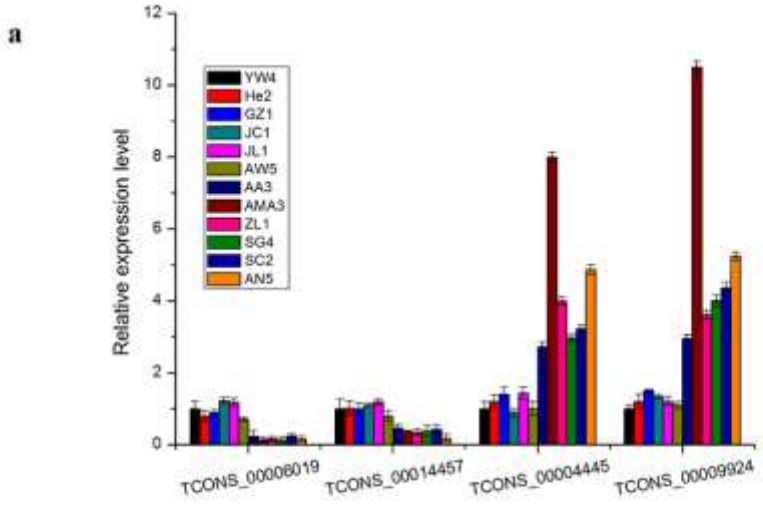
Vicente et al. BMC Genomics (2016) 17:301

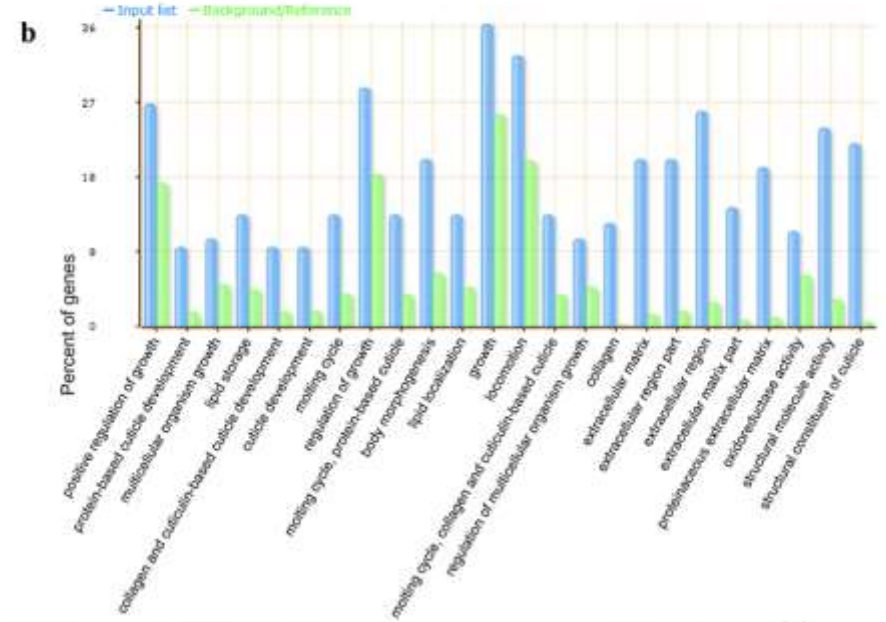
HOW IS GENOMICS HELPING: EXAMPLES

FROM A NEMATODE PERSPECTIVE

MOLECULAR VARIATIONS *B. xylophilus* WITH DIFFERENT VIRULENCES

- Transcriptome and genome sequences of 3 strongly virulent +1weakly virulent strain.
- Changes in 238 transcripts and 84 exons including pectate lyase
- 117 SNPs were identified as potential genetic markers
- Help diagnose nematode sp. with diff. virulence and facilitate disease control





Ding X. et al. (**2016**) PLoS ONE 11(5): e0156040.

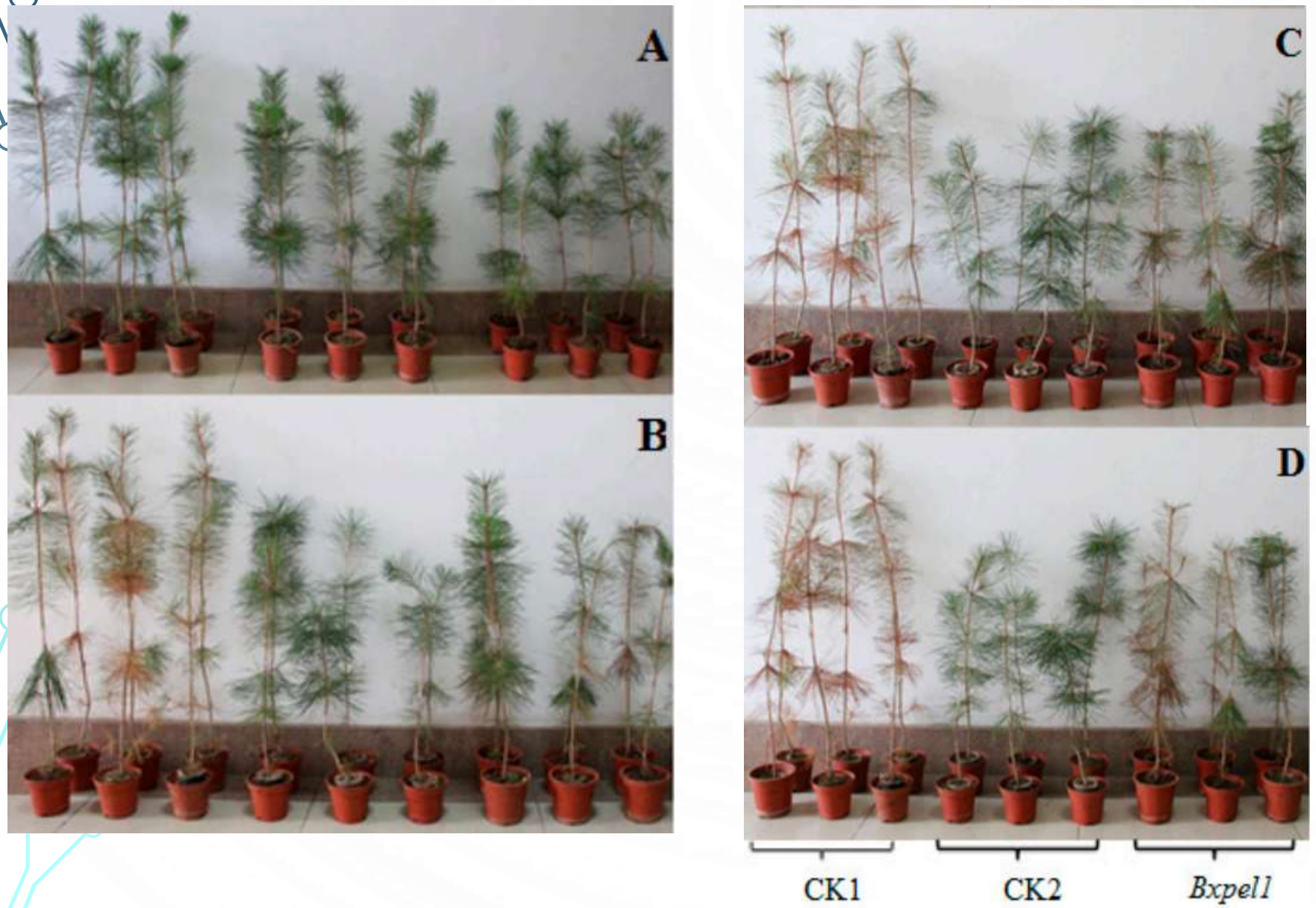
THE ROLE OF THE NEMATODE PECTATE LYASE

- **Pectate lyase**: essential for successful invasion of their host plants
- RNAi pectate lyase 1 gene in *B. xylophilus* (*Bxpel1*).
- Quantity of *B. xylophilus* was significantly reduced after treatment with dsRNAi
- *Bxpel1* dsRNAi reduced the migration speed and reproduction of *B. xylophilus*
- *Bxpel1* is a significant pathogenic factor in the PWD process

Int. J. Mol. Sci. **2016,** *17*, 125 6 of 14

Wilting symptoms

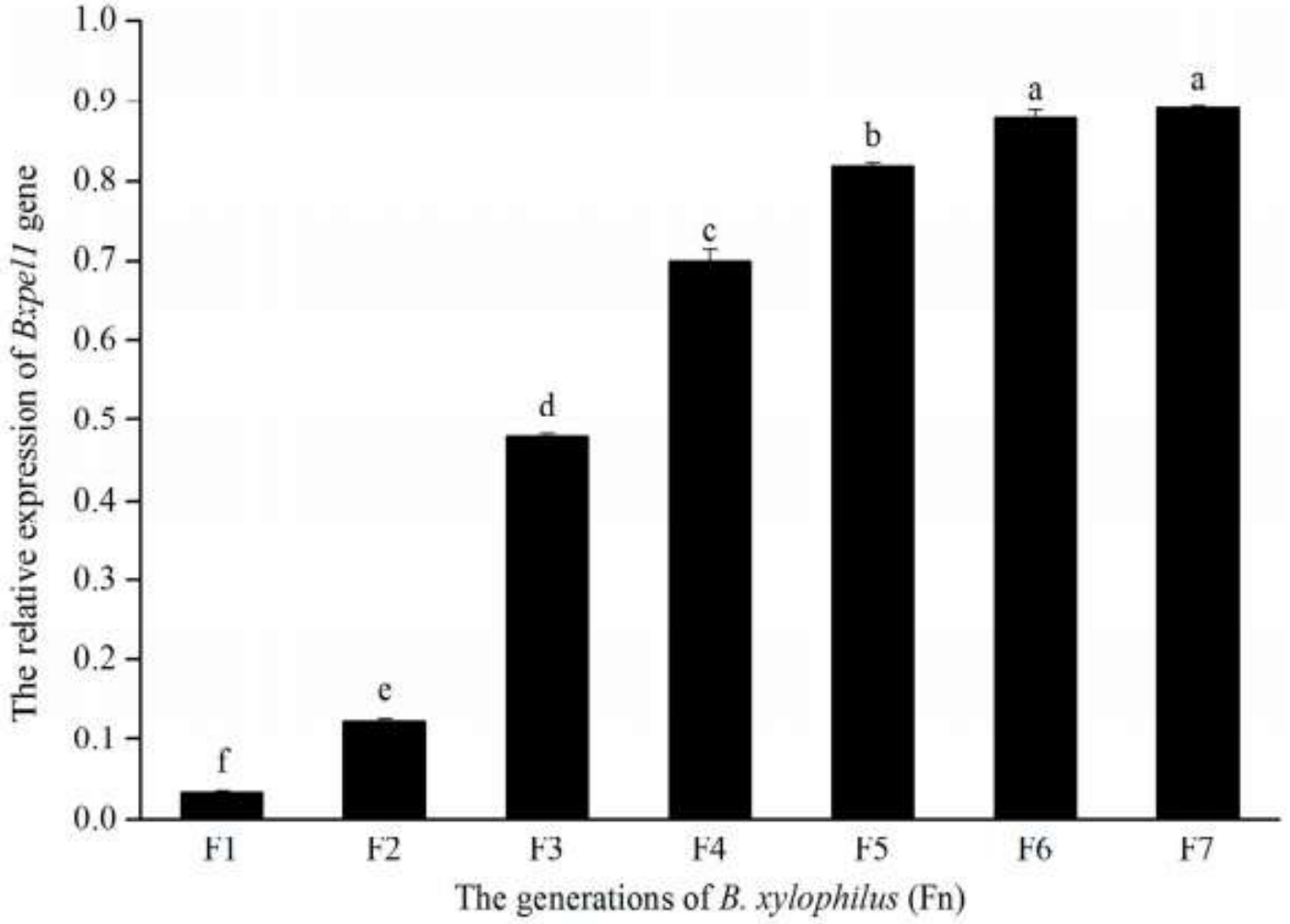
THE ROLE OF NEMATODE PECTATE LYASE



A-1 day
B-10 days
C-20 days
D-30 days

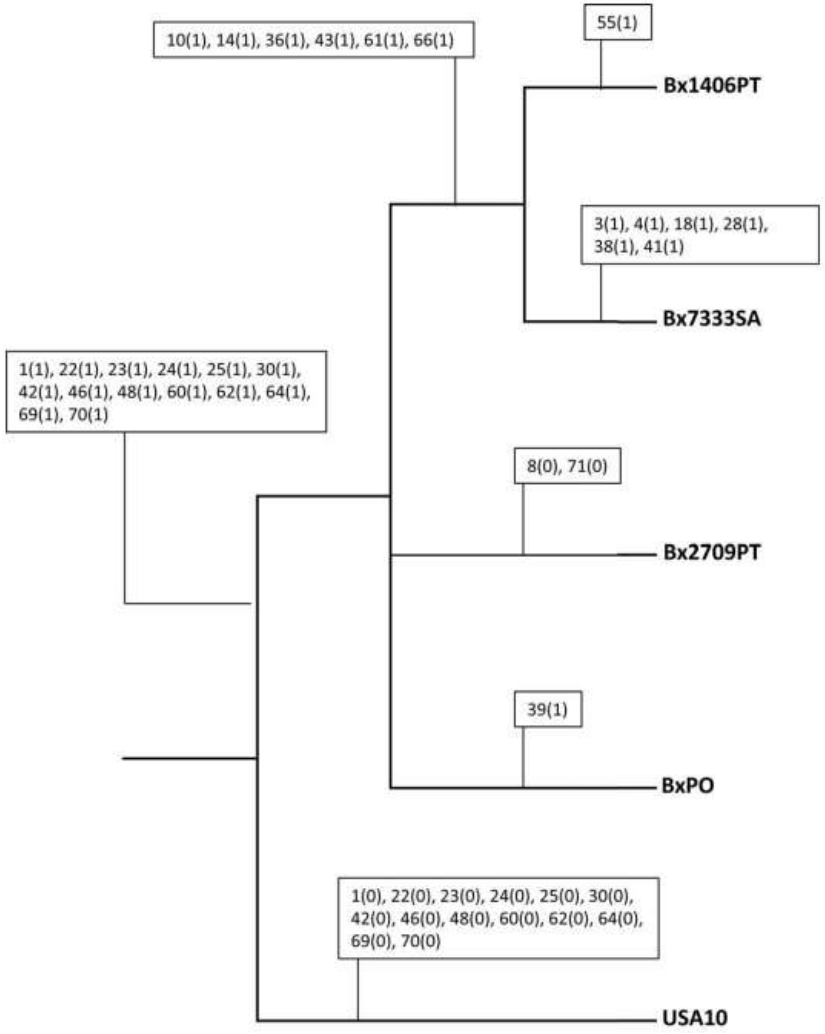
CK1-control solution
CK2-ddH2O
Bxel1-dsRNA

THE ROLE OF NEMATODE PECTATE LYASE



PROTEIN MARKERS OF *BURSAPHELENCHUS XYLOPHILUS*

- 4 Iberian, 1 American population
- Quantitative proteomics (iTRAQ)
- 2860 proteins
- 30 proteins unique markers for the populations or groups
- Potential for development of diagnostic tools



Ciordia et al. **2016**, *Proteomics 16*, 1006–1014

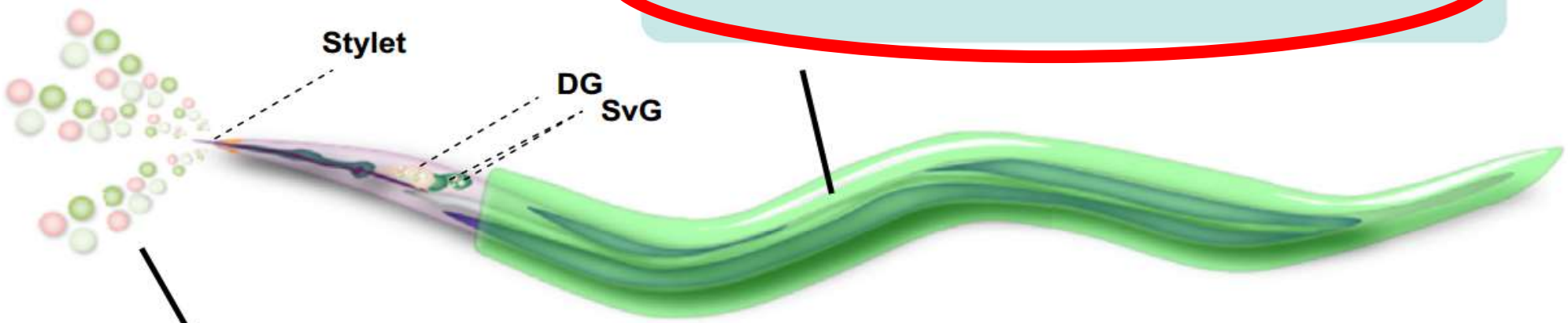
PATHOGENICITY PROTEINS FROM *B. xylophilus*

Putative role in infection	Molecular function	References
Tolerance	Catalase	Shinya et al. (submitted)
	Glutathione peroxidase	Shinya et al. (submitted)
	Superoxide dismutase	Shinya et al. (submitted)
	Peroxiredoxin	Li et al. (89)
	Glutathione s-transferase	Shinya et al. (4, submitted)
	Protease inhibitor	Shinya et al. (submitted)
Migration	Beta-1,3-endoglucanase	Kikuchi et al. (56)
	Beta-1,4-endoglucanase	Kikuchi et al. (20), Zhang et al. (22)
	Pectate lyase	Kikuchi et al. (21)
	Expansin-like protein	Kikuchi et al. (57)
	Protease	Shinya et al. (submitted)
Disturbance	Thaumatin-like protein	Shinya et al. (submitted)
	Protease inhibitor	Shinya et al. (submitted)

Shinya et al., 2013 J Biosc Bioeng.

Surface coat proteins

- Anti-oxidant & Detoxifying enzymes



Secreted proteins

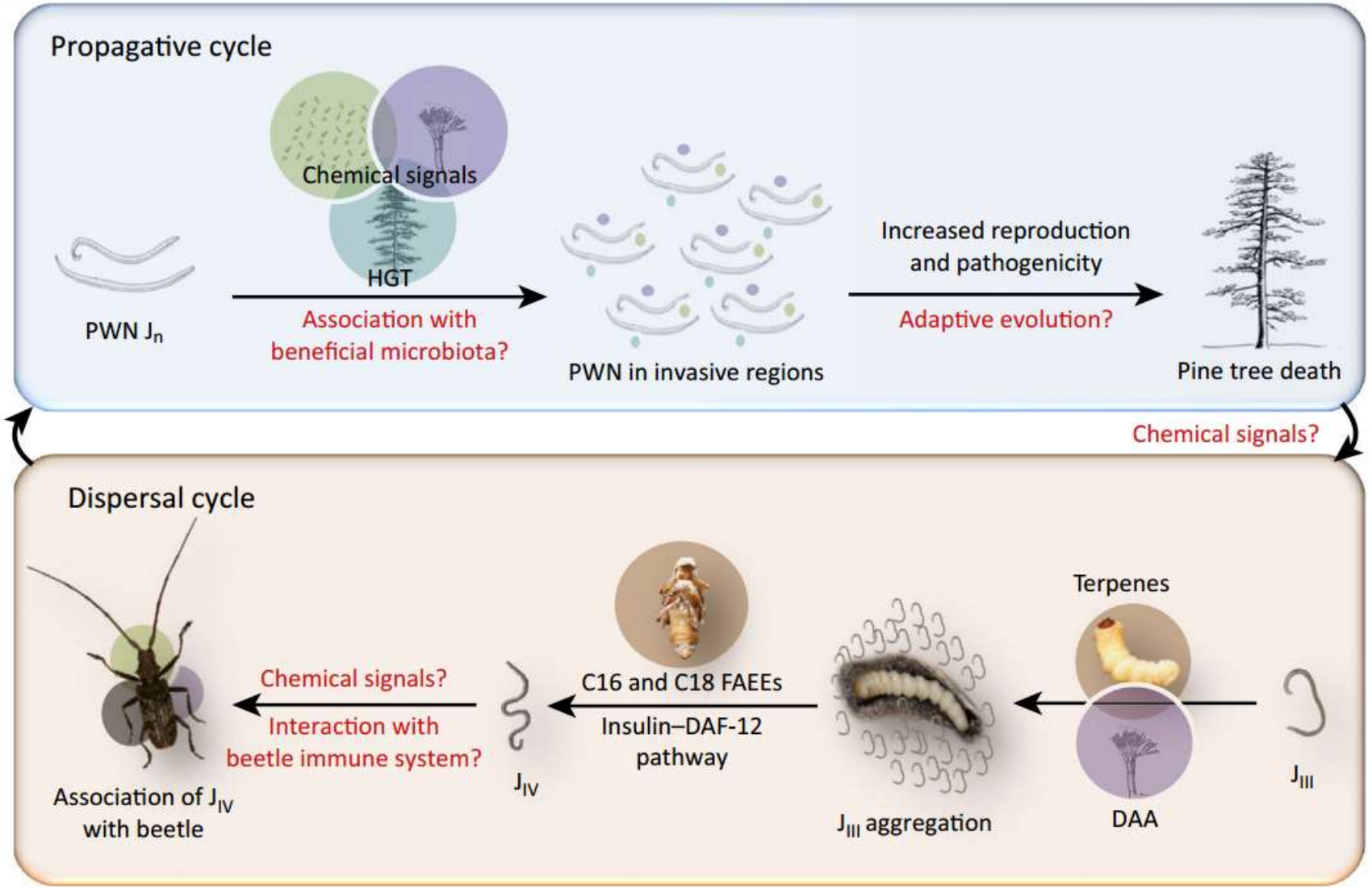
- Proteases & Protease inhibitors
- Cell wall-degrading enzymes
- Anti-oxidant & Detoxifying enzymes
- Host mimicry proteins

Shinya et al (2013) J Bios Bioeng

POST GENOMICS ERA

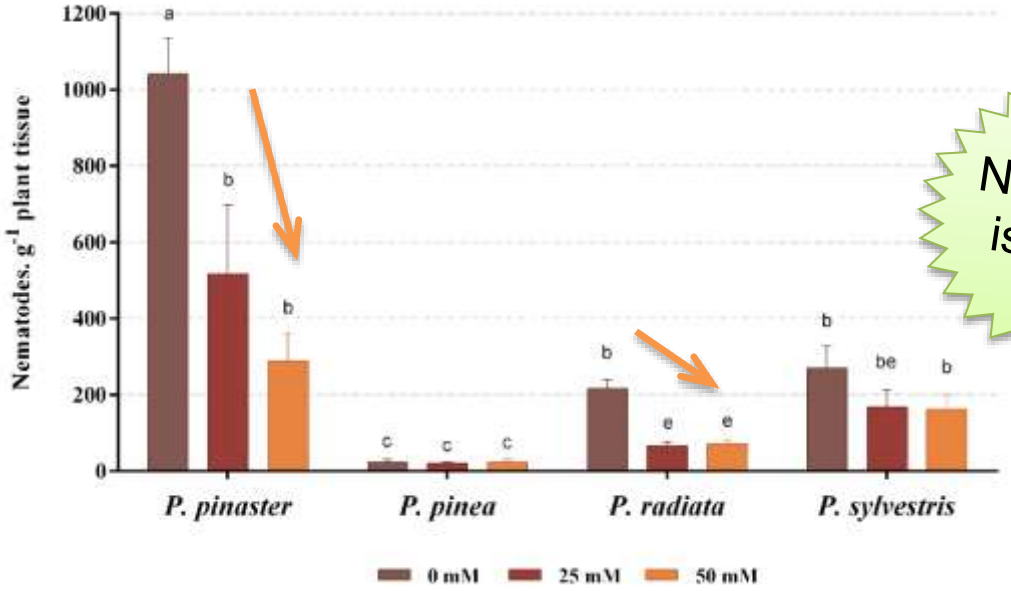
WHERE ARE WE GOING?

MULTISPECIES INTERACTION



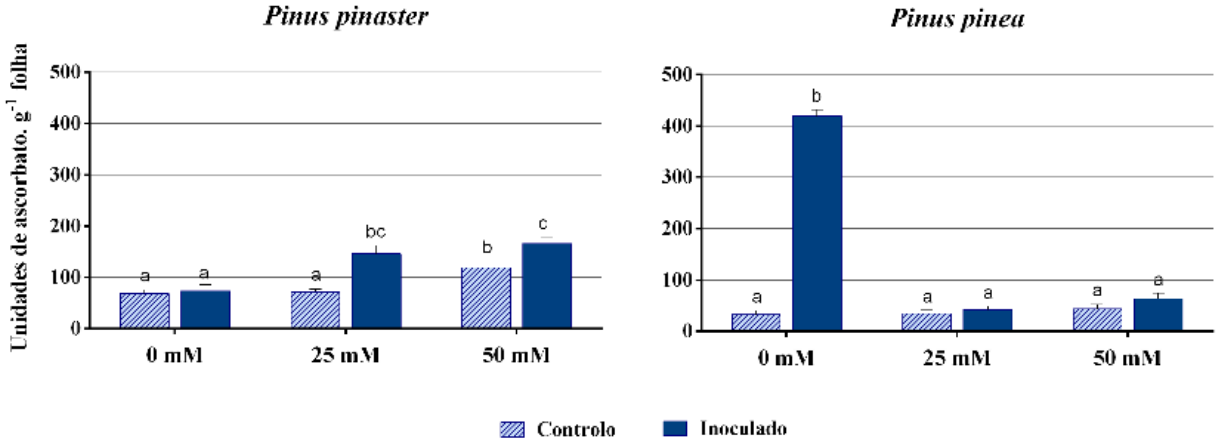
TRENDS in Parasitology

NEW BIOCONTROL TOOLS: MEJA



Nematode # is reduced

Foliar application



Antioxidant defenses enhanced

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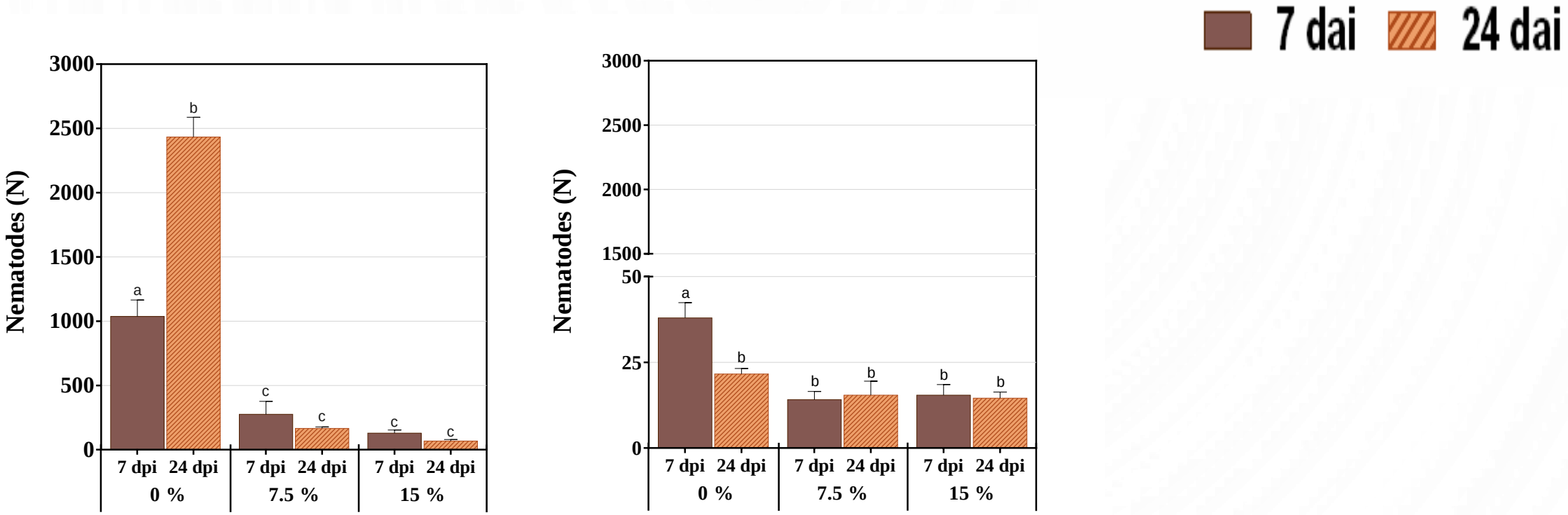
MBG Misión Biológica de Galicia

NEW BIOCONTROL TOOLS: CHITOSAN



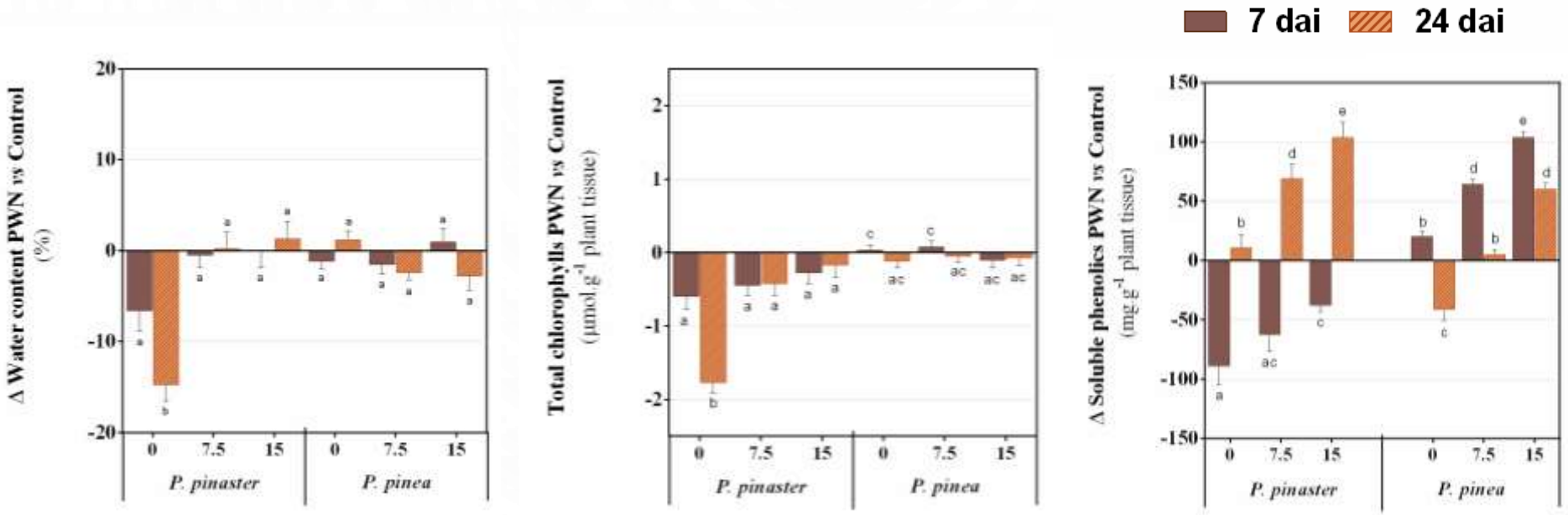
P. pinaster

Biofertilizer reduced PWN number

P. pinea

shows signs of high tolerance to PWD

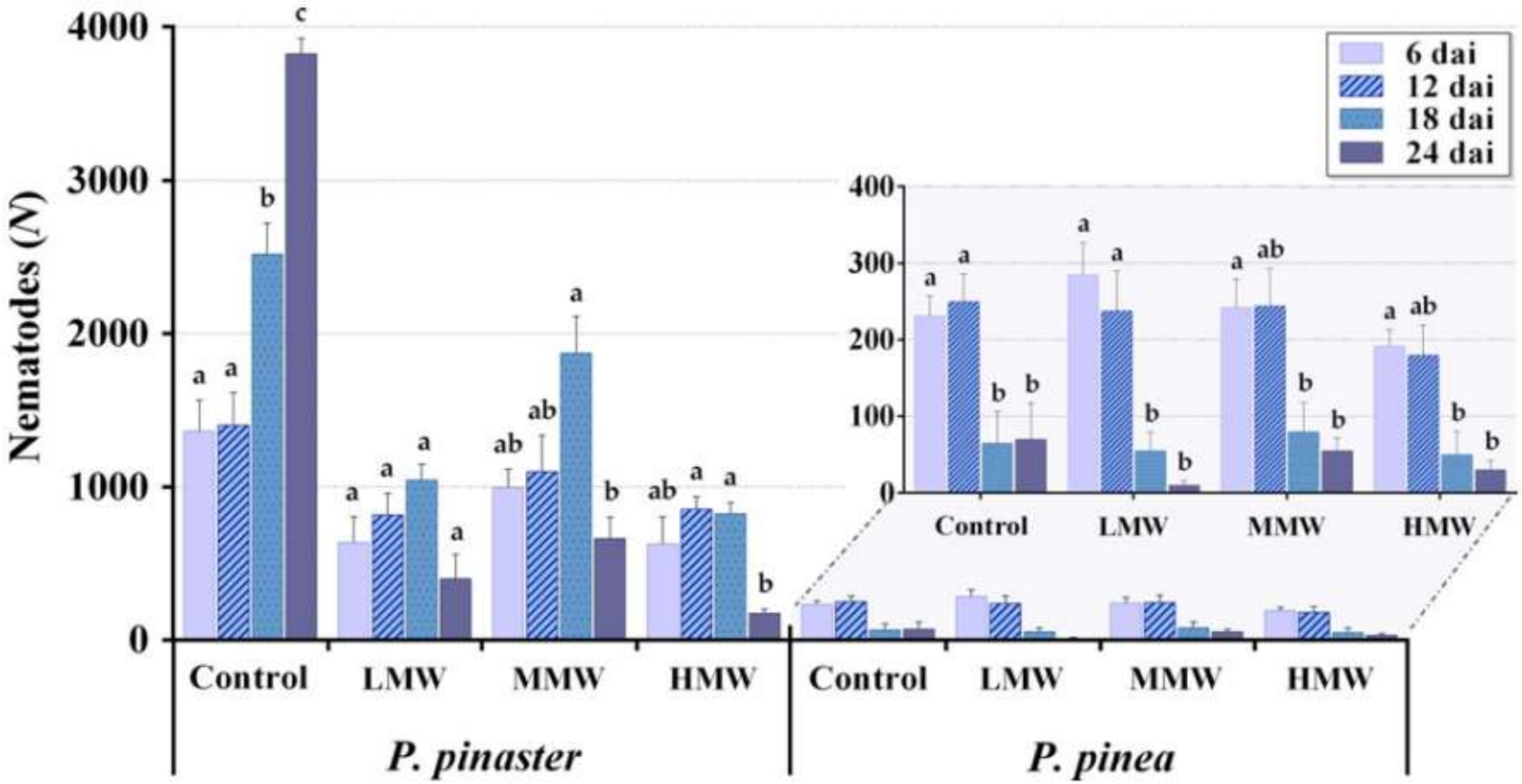
NEW BIOCONTROL TOOLS: CHITOSAN



Biofertilizer **prevented** water loss and chlorophyll degradation

Phenolics were induced at:
7 dpi in *P. pinea*
24 dpi in *P. pinaster*

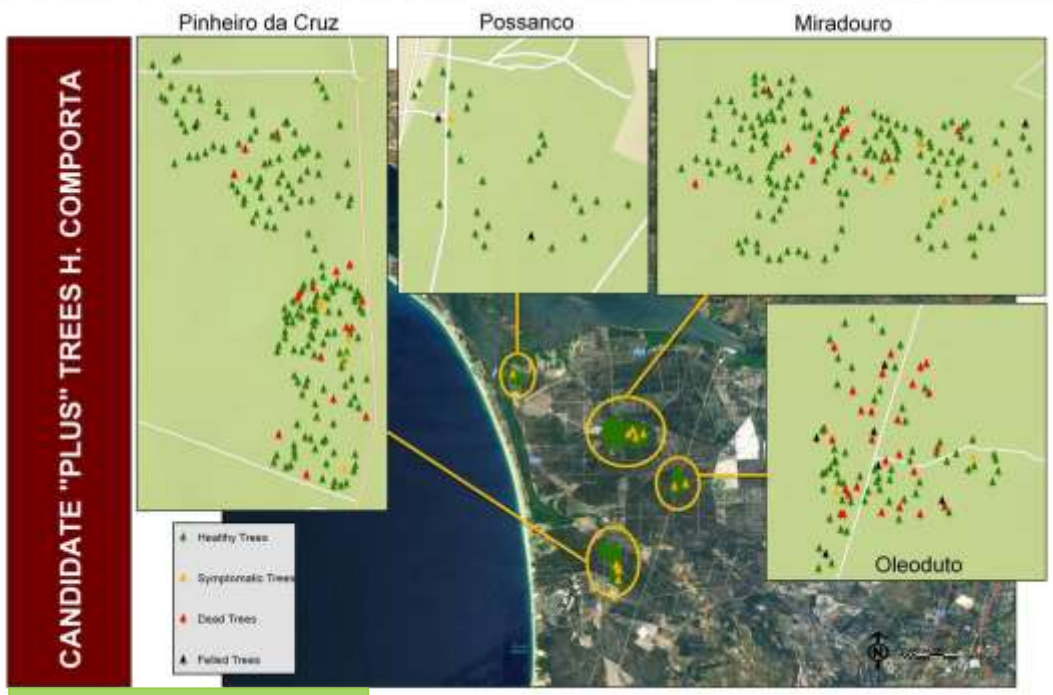
NEW BIOCONTROL TOOLS: CHITOSAN



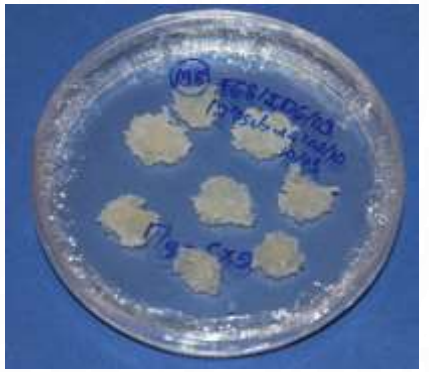
Nematode # is reduced

Antioxidant defenses enhanced

GENETIC TRANSFORMATION



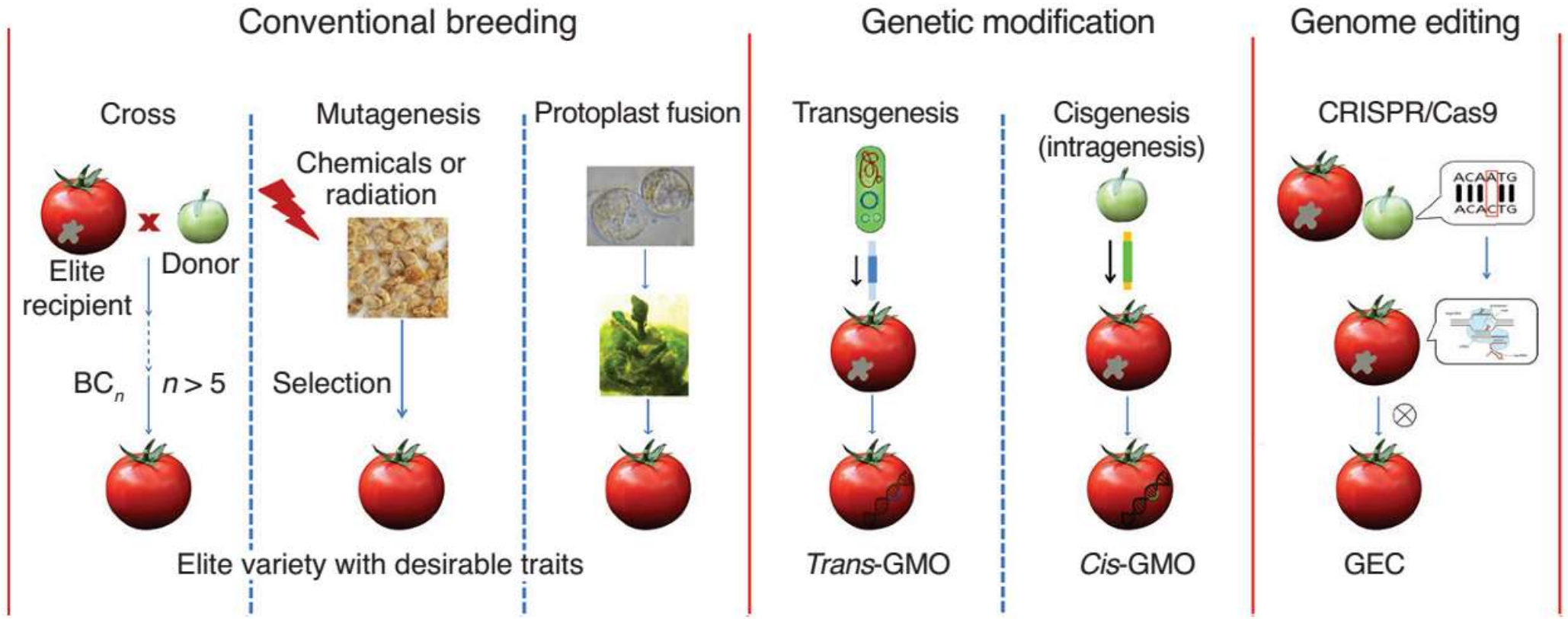
INIAV



IBET

Costa, et al (2011) **BMC Proceedings** 5, 80.

GENE EDITING?



- Targeted to the insect, bacteria, nematode, tree, fungi?

Huang (**2016**) Nature Genetics 48(2)

CONCLUSIONS (PART I)

- 16 years since the 1st plant genome was sequenced
- 5 years since the 1st draft PWN genome
- Genomics/transcriptomics/proteomics is now accessible to every lab
- Whole genome sequencing helped determine gene sets for host, insect, bacteria, fungi, nematode
- Our understanding of the PWD has made rapid headway

CONCLUSIONS (PART II)

- Transcriptomics studies provided hints on possible resistance proteins (Oxidative stress? Lignin? PR proteins?)
- Proteomics studies allowed determining *B. xylophilus* pathogenesis proteins (tolerance, migration and mimicry)
- Population based screening to look for genetic variants
- ´Genome surgery´: hexaploid wheat resistant to powdery mildew ➡ *P. pinaster* resistant to PWN?
- Novel biocontrol tools offer promise for the future of PWD management

Thank you very much for your attention

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