

# Clonal propagation of disease tolerant genotypes of highly relevant forest species in Spain (chestnut, cork oak and maritime pine)

Let's talk about clonal forestry  
September 16th, 2022



# 1 BRED MATERIAL PROPAGATION CONTEXT

**ENHANCED INTERNATIONAL WOOD DEMAND**      **TRANSITION TO BIOECONOMY + CLIMATIC CHANGE MITIGATION**

↓  
BIOMASS PRODUCTION INCREASE  
OPORTUNITY

↓  
**CARBON FIXATION**

↓  
BIOMASS PRODUCTION INCREASE  
NEEDED

GLOBALIZATION  
CLIMATE CHANGE

**BUT**

HIGH ECONOMICAL  
IMPACT PESTS &  
DISEASES RISK

**TOLERANCE BRED GENETIC MATERIAL + DELIVERY PROGRAM=...  
=.... COULD HELP INCREASING BIOMASS PRODUCTION**

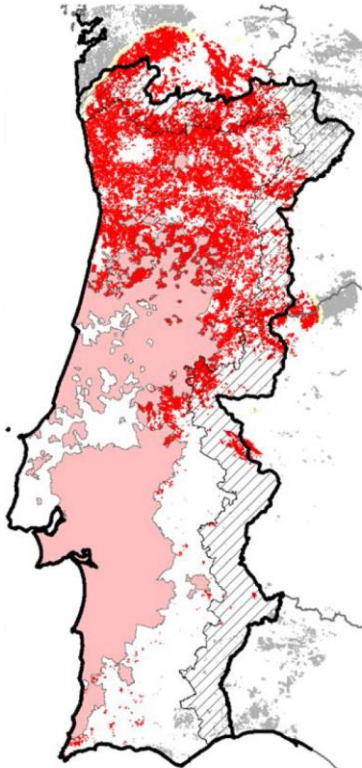
- EFFICIENT DELIVERY PROGRAM OF TOLERANCE BRED GENETIC MATERIAL MEANS:
  - **CLONAL** PROPAGATION OF ELITE GENOTYPES
  - **CLONAL / SEMINAL** PROPAGATION OF ELITE FAMILIES
  - **SEMINAL** PROPAGATION OF IMPROVED/SELECTED POPULATIONS



PINE NEMATODE (*Bursaphelenchus xylophilus*) DISEASE THREAT IN GALICIA

DE LA FUENTE ET AL.  
*J Appl Ecol.* 2018;55:2374–2385.

(d) 2028

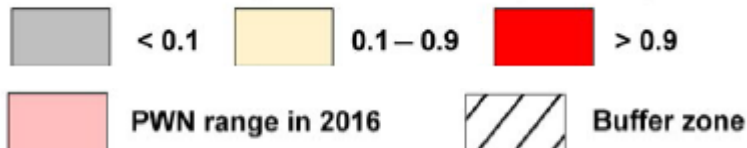


- In 1999, the pine wood nematode (PWN, *Bursaphelenchus xylophilus*) was first detected in Europe (south-western Portugal).
- Since then, it has spread producing large-scale damages in Portuguese forests and it is likely to broadly reach Spain conifers forest in 2020s decade



- *Pinus pinaster* Ait tolerance to PWN breeding program by CIFLOURIZÁN got 6 tolerance families (78% survival in inoculation trials, Díaz Vázquez et al 2020, Revista Foresta)
- Tolerance clones were registered in the Base Material National Catalogue, so to be legally commercialized as Family Progenitors numbered from PF-Q-26/15/001 to PF-Q-26/15/006)

Coniferous forest: predicted infection probability



# 3 PINUS PINASTER VEGETATIVE PROPAGATION

## OPERATIONAL MASS PROPAGATION OF PINUS PINASTER Ait. PILOT ACTIVITIES IN GALICIA



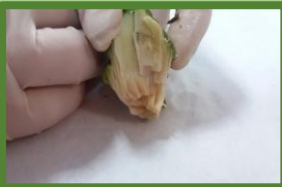
GERMINATED SOMATIC EMBRYOS

JUVENILE MOTHER PLANT FIELDS

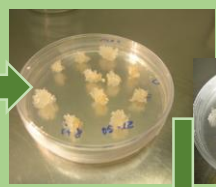
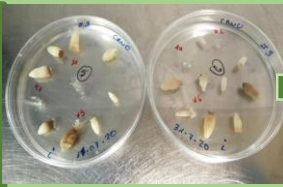
ROOTED CUTTINGS

PLANTATIONS

X12-20 v/a



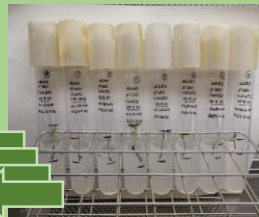
INDUCTION



PROLIFERATION



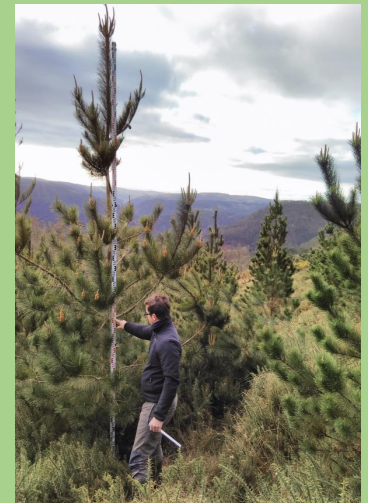
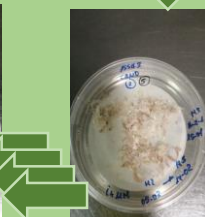
ACCLIMATION



GERMINATION



MATURATION



**MASS PROPAGATION SCALING OF TOLERANCE TO PINE NEMATODE OPERATIONAL TEST IN PROGRESS (initiated in 2022) :**

- 1 lab
- 6 nurseries

# 4

## PINUS PINASTER VEGETATIVE PROPAGATION

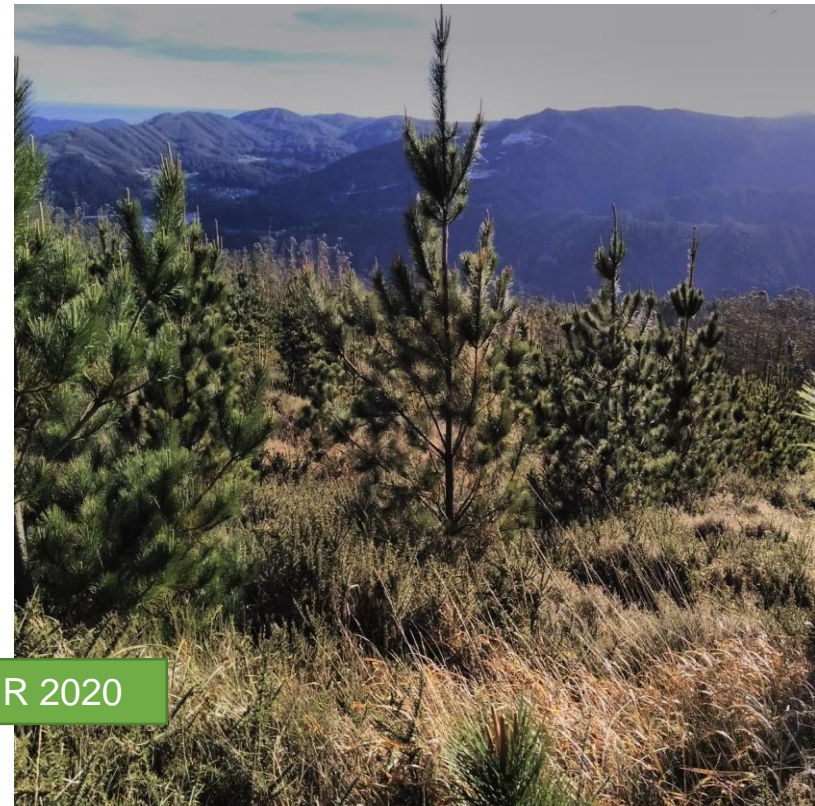


SPRING 2014



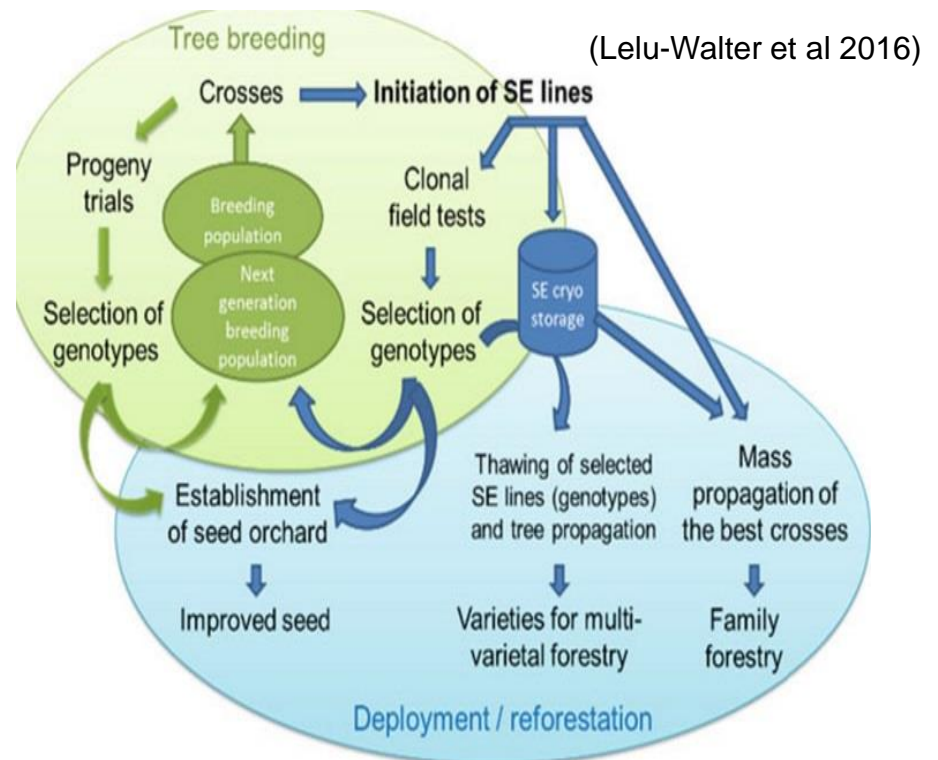
WINTER 2020

6 YEARS OLD *Pinus pinaster* BRED FOR GROWING ACITUDES ROOTED CUTTINGS .  
MAÑÓN-A CORUÑA-NORTHWESTERN SPAIN



## WHAT NEXT?

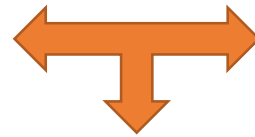
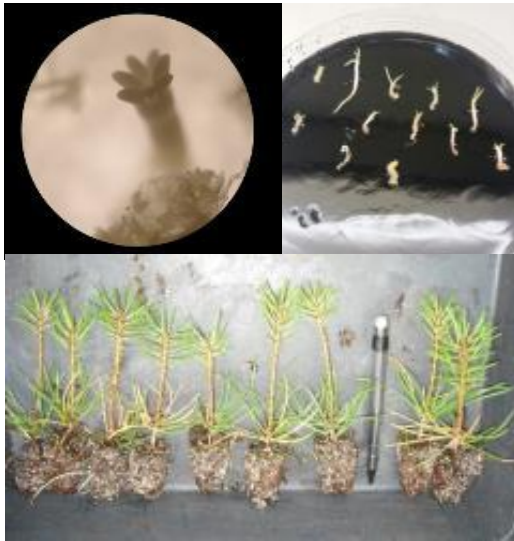
- WAIT FOR RESULTS FROM THE MASS PROPAGATION SCALING OF *Pinus pinaster* TOLERANCE TO PWN
- START A NEW SCALING MASS PROPAGATION PROCESS OF *Pinus radiata* DISEASE TOLERANCE (FINISHING OBTENTION OF NEW TOLERANCE GENETIC MATERIAL INCLUDED)
- INTEGRATED CRYOPRESERVATION ALONG WITH SOMATIC EMBRYOGENESIS IN TO THE BREEDING PROGRAMS TO GET ADVANTAGE OF THE CLONAL BREEDING



In R&D projects to:

- Clone outstanding selected genotypes
- Deploy clonal trials
- Cryopreserve selected genotypes

### *Pinus pinaster* and *P. radiata*



### *Castanea spp.*



### *Quercus suber*

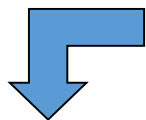


# 7 SELECTION OF CHESTNUT FOR INK DISEASE TOLERANCE

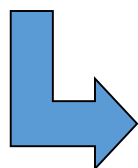
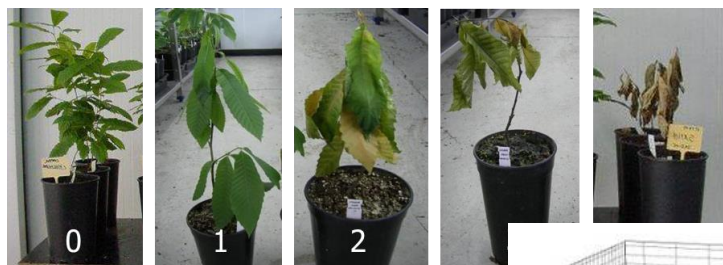


**ASYMTOMATIC ADULT TREES SELECTED IN NATURAL STANDS WITH HIGH AFFECTION OF *Phytophthora cinnamomi*.**

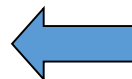
Multiple copies by micropropagation



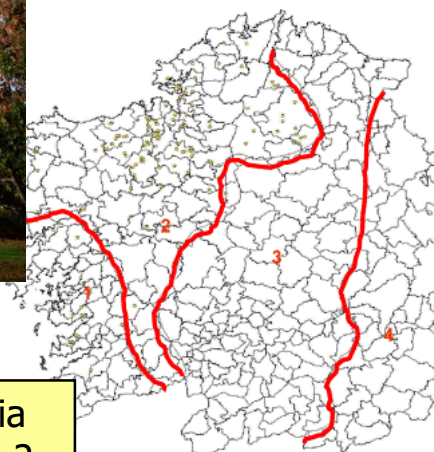
Resistance tests



Molecular characterization



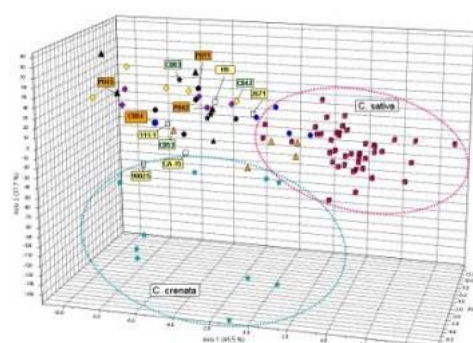
✓ prospection of 18,809 ha in Galicia  
✓ 206 trees selected in areas 1 and 2



Field trials deployment



Registration of 8 resistant hybrid clones as rootstocks

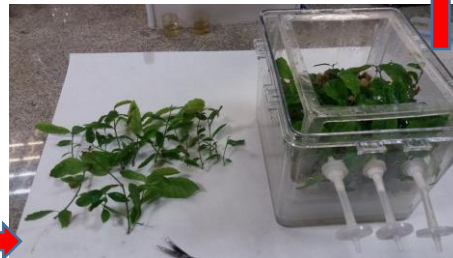




## CUTTINGS



## MICROPROPAGATION



CORK PRODUCTION



Phytophthora TOLERANCE

Field selection



Acorn collection from asymptomatic trees within damaged stands



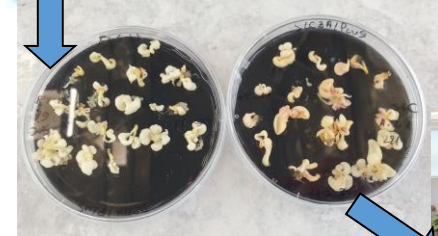
Somatic embryogenesis induction



Acorns sown in a split-plot design



Pc inoculation and water stress treatment



Plant production by SE

Deployment of field trials (2007-2010)



28 highly cork producing genotypes about to enter into FRM Register



Selection of surviving seedling and mother plants with highly surviving progenies, to be cloned

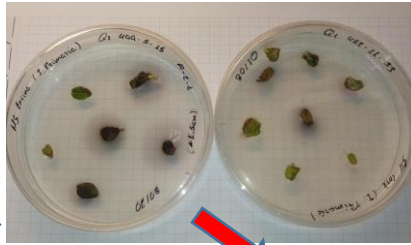


Mortality data recording

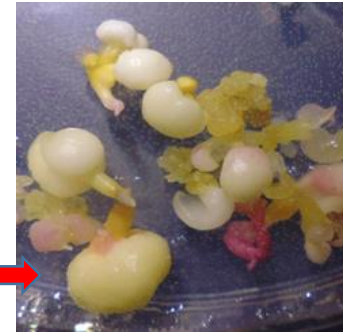
# 10 MICROPROPAGATION OF CORK OAK BY SOMATIC EMBRYOGENESIS



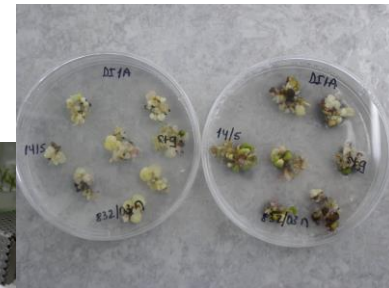
Induction of SE on somatic tissues (leaves)



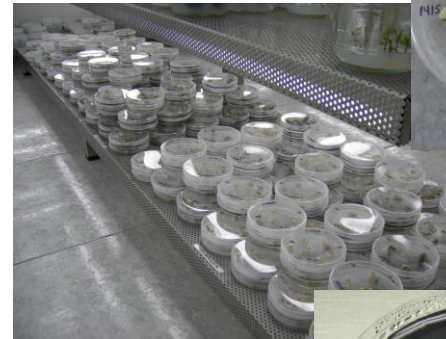
Newly formed SE



Recurrent or secondary SE



Proliferation phase



Maturation phase



Acclimatization phase



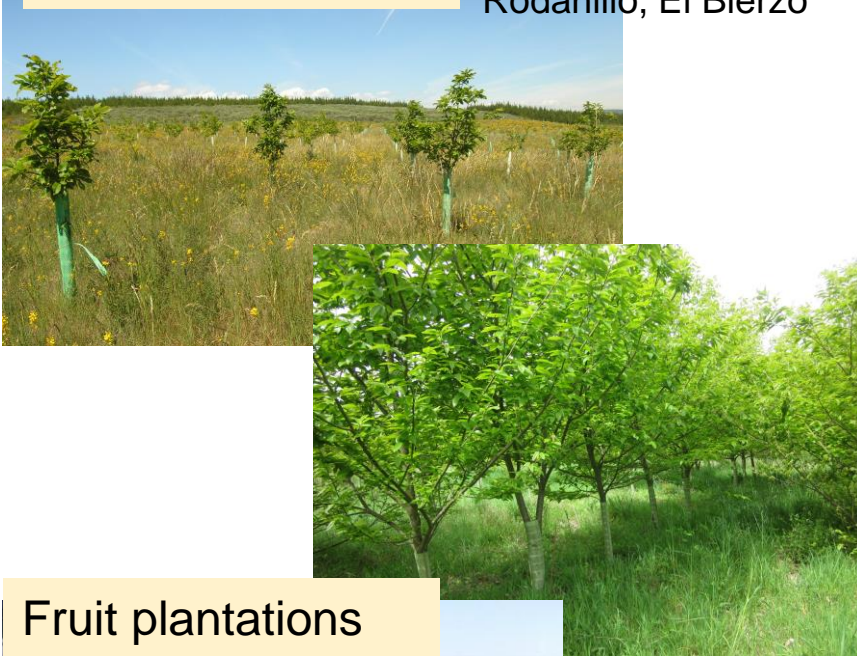
Germinated SE

Emblings growth

# 11 CLONAL PLANTATIONS OF CHESTNUT AND CORK OAK

## Timber plantations

Rodanillo, El Bierzo



## Experimental plots



2012  
Rueda Chica, Badajoz  
2021

## Fruit plantations



Villuercas, Cáceres



## Dehesa redensification



Ballesteros, Cáceres



## NEXT STEPS:

- Increase of chestnut production capacity by the building of a new lab
- Immediate registration of cork producing genotypes as RFM
- Deployment of experimental plots for tolerance of cork oak
- Development of cork oak SE protocol to industrial scale



Monte Medo, Maceda



# Thank you!

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