



Universidad de Valladolid



Aleppo pine provenances vary in susceptibility and secondary chemical response to *Gremmeniella abietina* infection

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*Gremmeniella abietina* (Lagerberg) Morelet



50ha area of affected *Pinus sylvestris* in Sweden  
(J. Witzell)



# *Gremmeniella abietina* (Lagerberg) Morelet

In Spain:

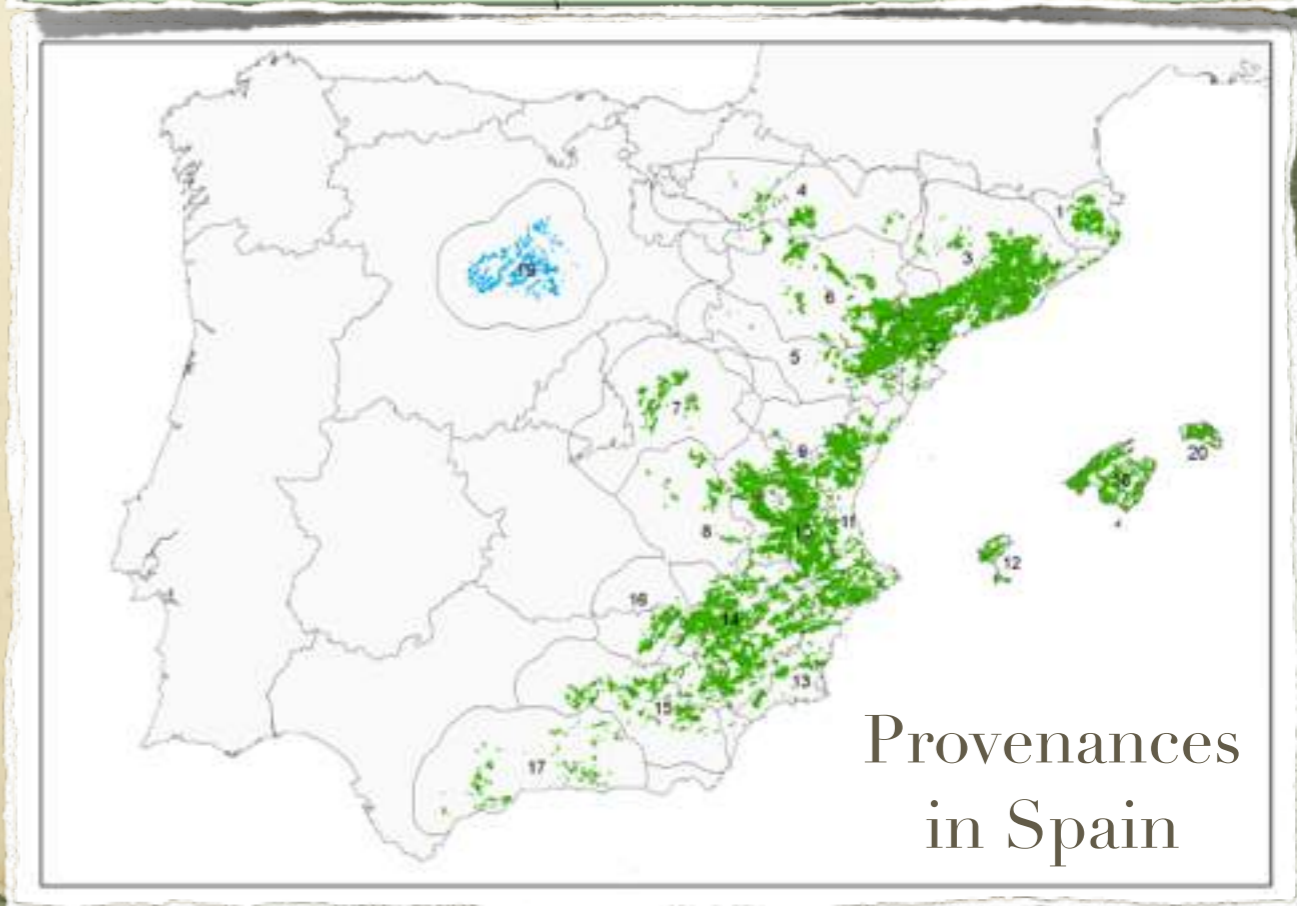
- Asexual stage
- No epidemic
- Defoliation
- Twig distortion
- Aleppo pine





# Aleppo pine (*Pinus halepensis* Mill.)

- **Natural distribution:** throughout the coast and in the central parts of the country.
- High heat and drought tolerance. Reforestation.
- Higher risk of infection when the tree species or provenances are growing outside its optimal habitat.

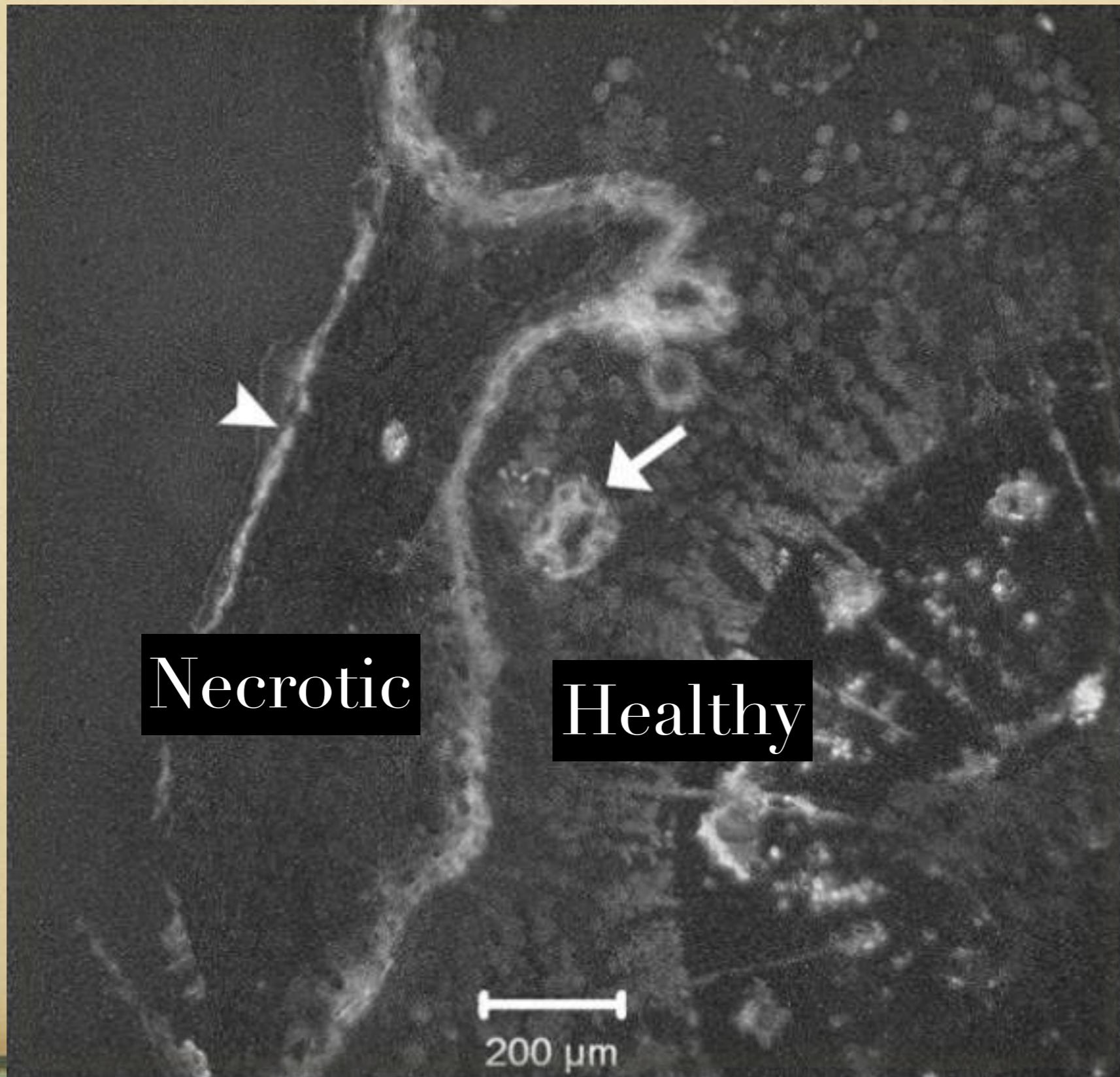




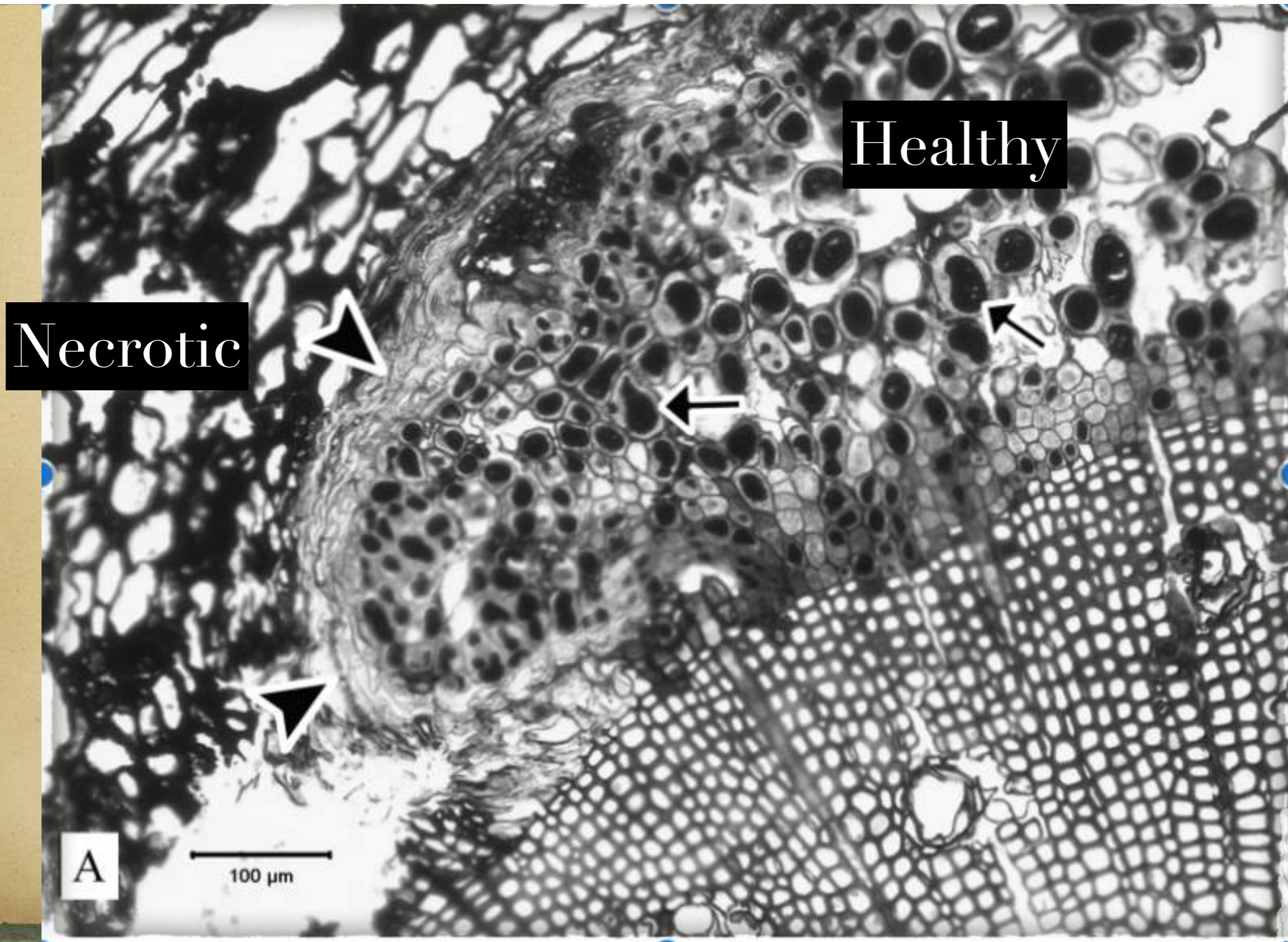
# Responses to *G. abietina* infections

- Both physical and chemical reactions in inoculated trees have been reported.
- Host's resistance to the *G. abietina* infection is likely to be related to:
  - (i) production of ligno-suberized tissues that help the tree compartmentalize the invaded tissues.
  - (ii) secretion of molecules such as phenolic compounds capable of degrading or altering the extracellular sheath of the pathogen.

Bernhold A, Hansson P, Rioux D, Simard M, Laflamme G, 2009. Resistance to *Gremmeniella abietina* (European race, large tree type) in introduced *Pinus contorta* and native *Pinus sylvestris* in Sweden. Canadian Journal of Forest Research 39, 89–96.



Bernhold A, Hansson P, Rioux D, Simard M, Laflamme G, 2009. Resistance to *Gremmeniella abietina* (European race, large tree type) in introduced *Pinus contorta* and native *Pinus sylvestris* in Sweden. Canadian Journal of Forest Research 39, 89–96.





# Aims of the study

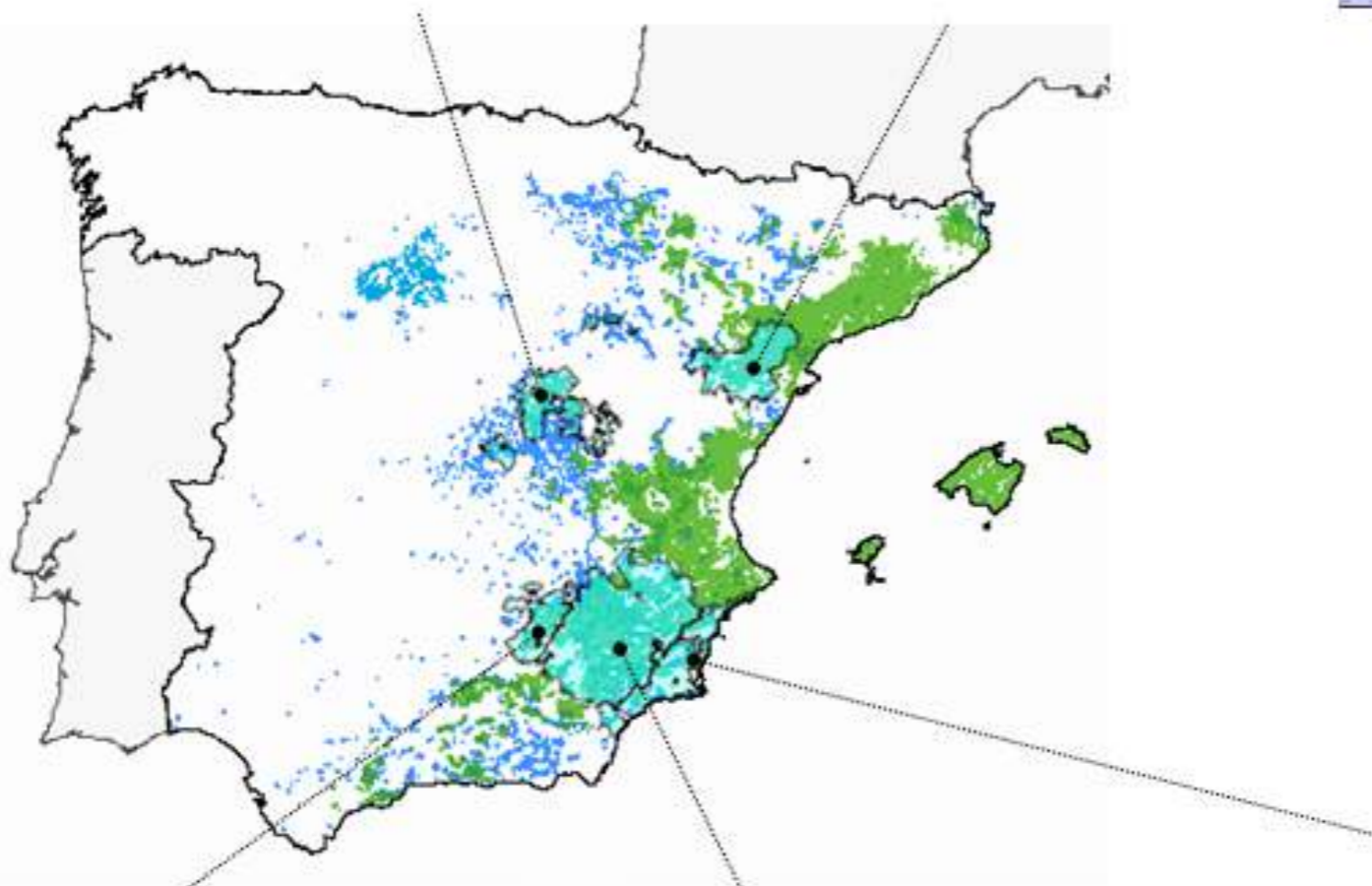
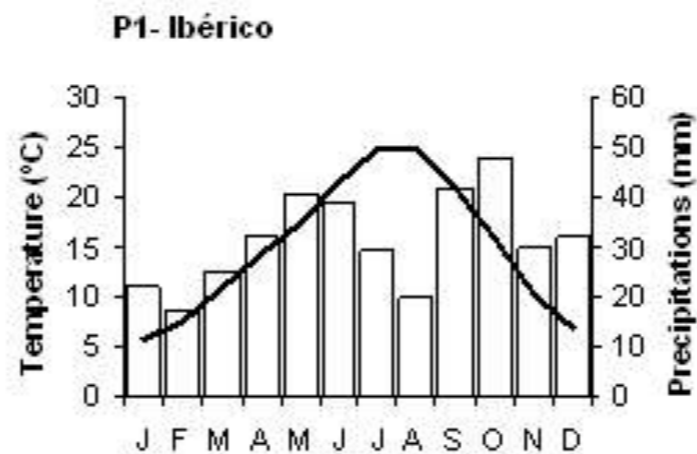
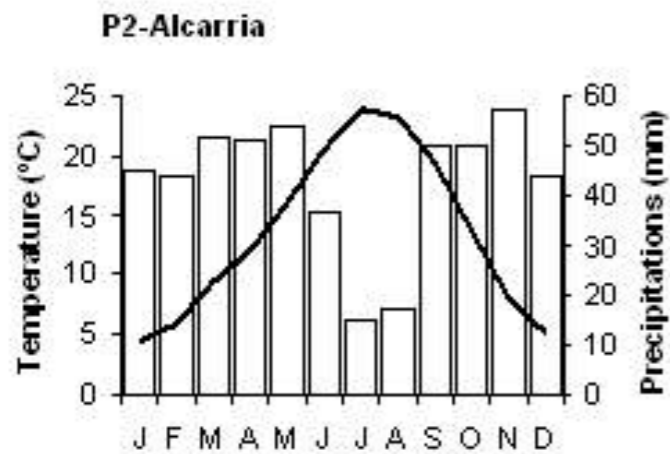
- To explore the response of the provenances to the disease.
- Susceptibility of the provenances was quantified by measuring:
  - (i) The **necrosis** produced by the progression of the pathogen.
  - (ii) The **concentration of some UV-absorbing compounds** in the seedlings produced in response to the infection.





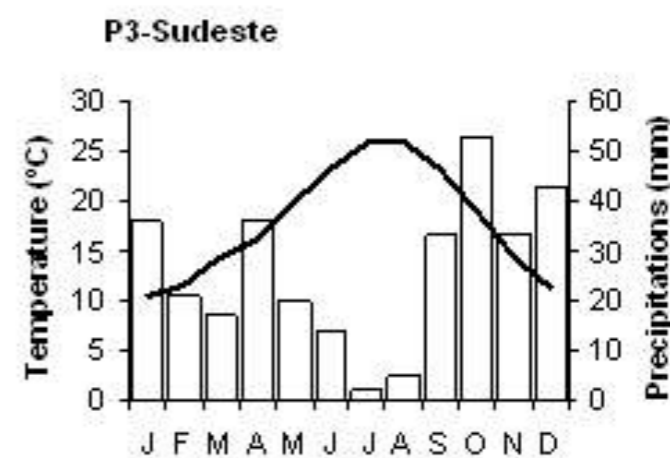
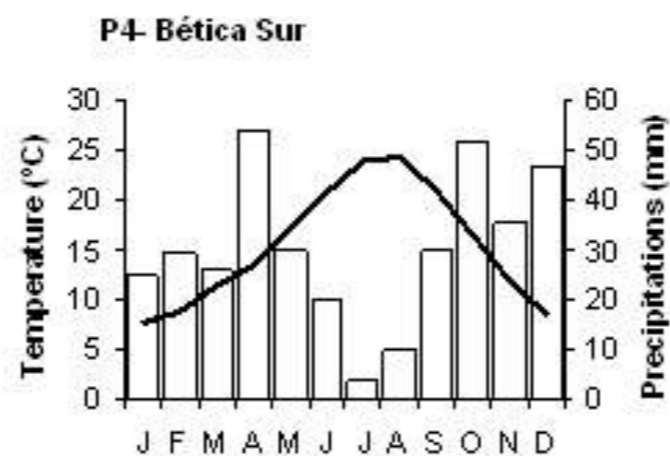
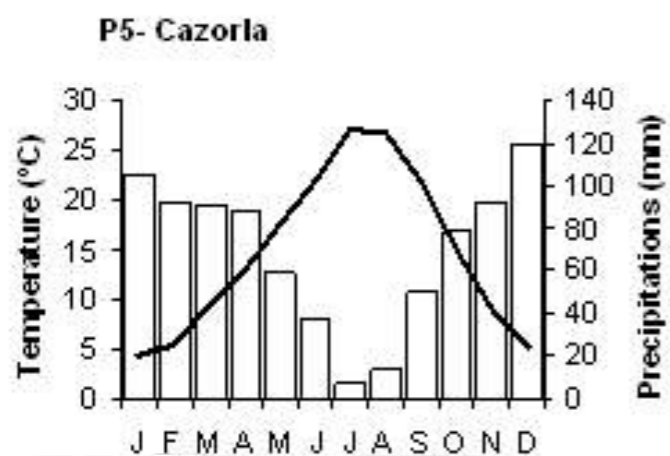
# Materials & Methods

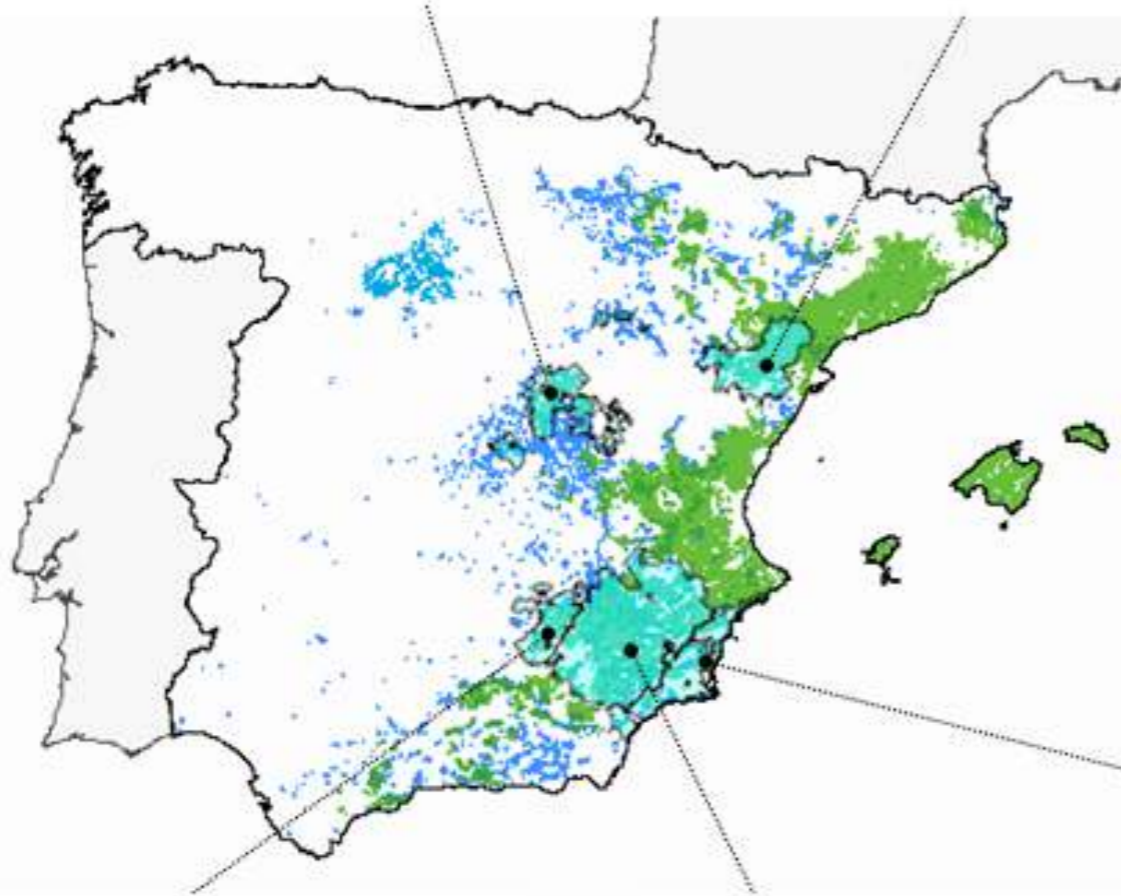
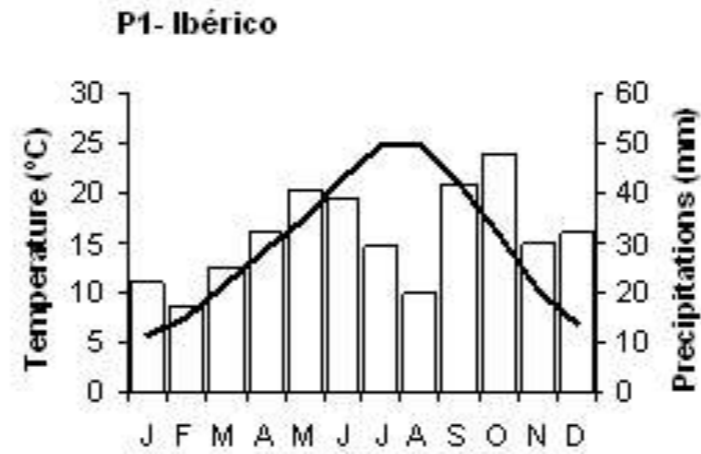
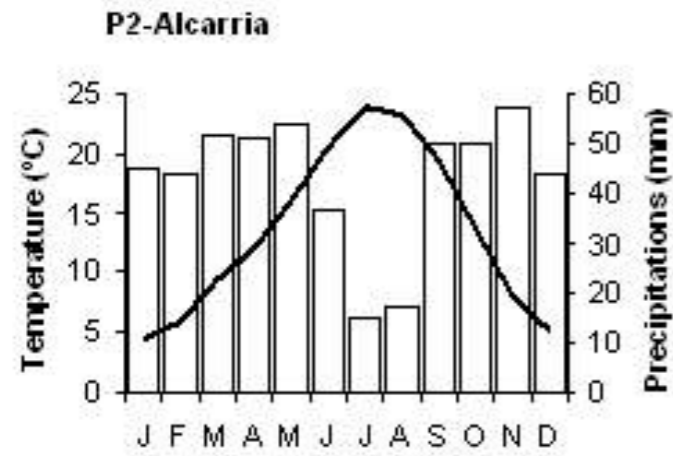




### Legend

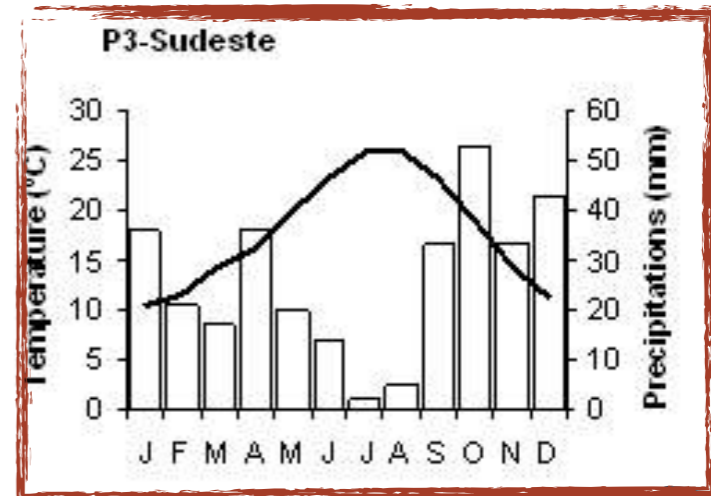
- Natural stands of *P. halepensis*
- Selected provenance regions
- Artificial reforestations of *P. halepensis*





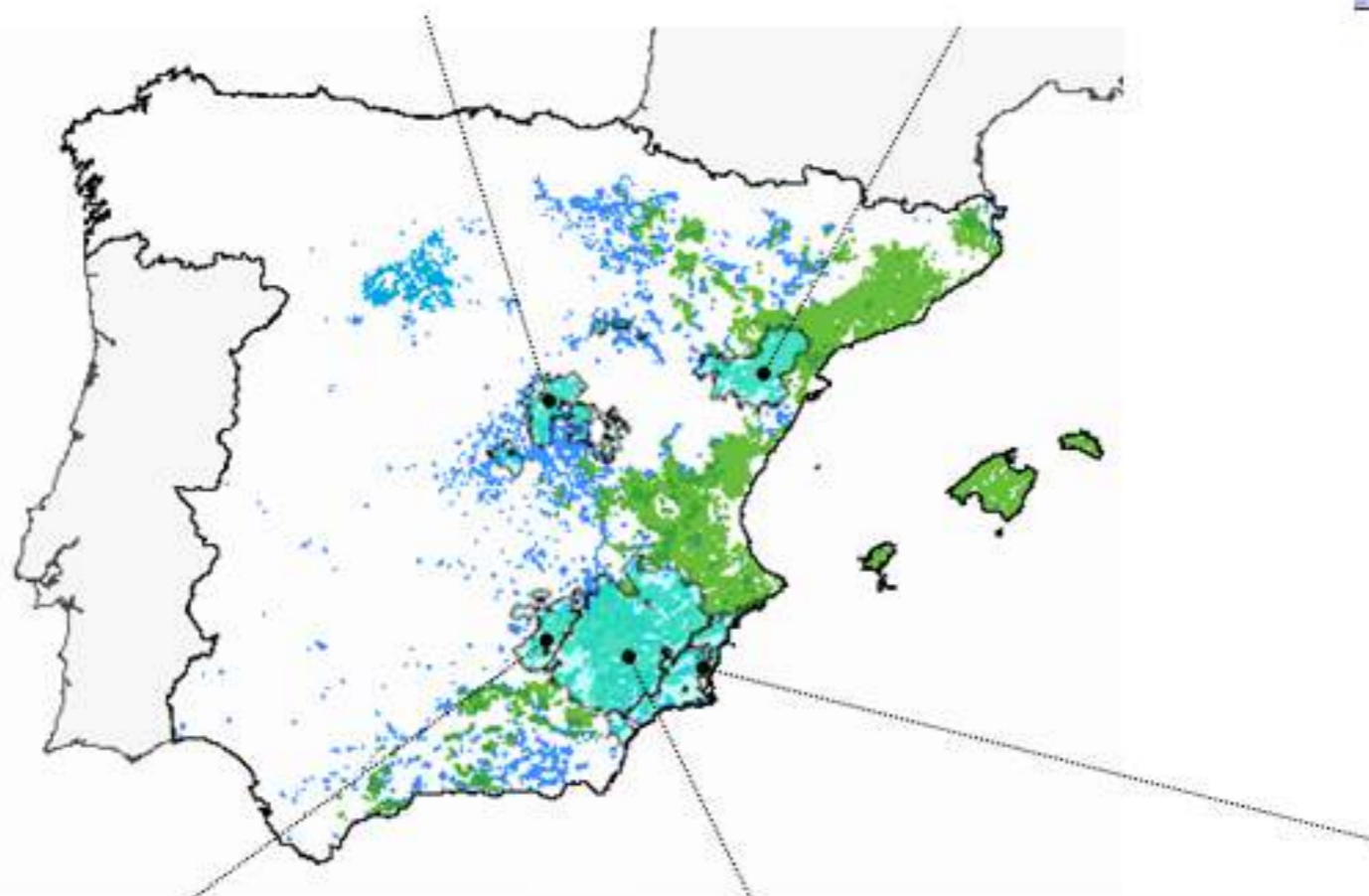
**Legend**

- Natural stands of *P. halepensis*
- Selected provenance regions
- Artificial reforestations of *P. halepensis*



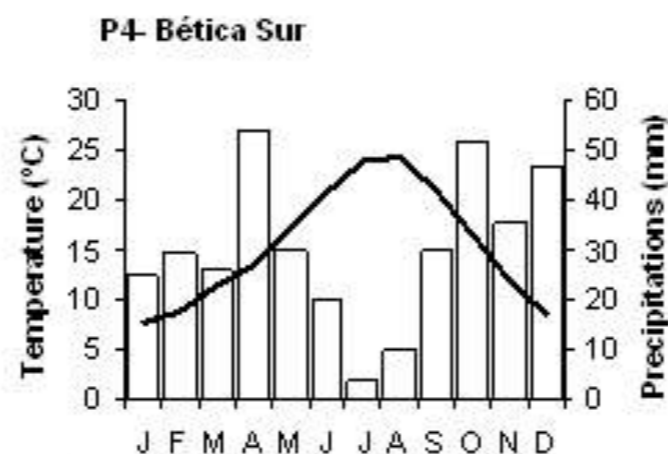
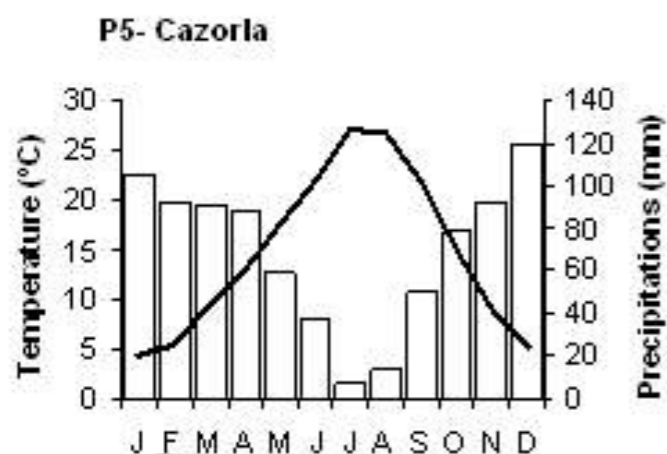
**Continental climate**

# Extreme continental climate



## Legend

- Natural stands of *P. halepensis*
- Selected provenance regions
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# Provenances

18 months



*Pinus halepensis*, Aleppo Pine seeds

Photo © Cándido Gálvez Ramírez & Rafael Mª Navarro Corral



*Pinus halepensis*, Aleppo Pine seedlings

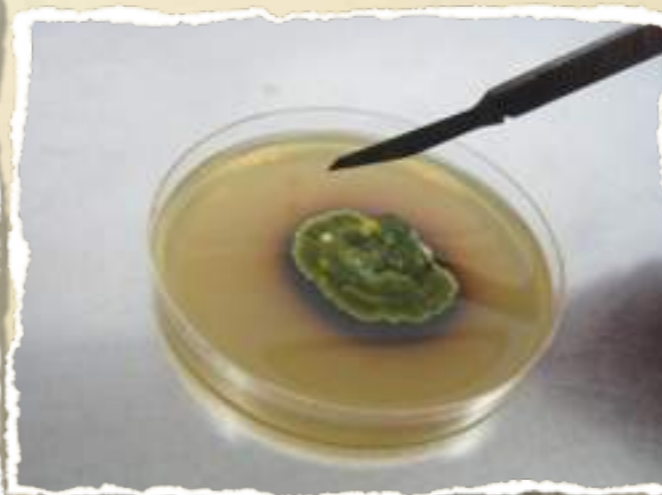
Photo © Cándido Gálvez Ramírez & Rafael Mª Navarro Corral





# Inoculations

- Made in **January** to mimic natural infection.
- **Eight *G. abietina* isolates** were used. Control seedlings: agar.
- **Mycelia** was used: no success with conidial suspensions.
- **3 weeks later**: the experiment was repeated. Total: **630 seedlings**.





# Visual severity: scale



0-Symptomless



1-Chlorosis



2-Advanced chlorosis



3-Dieback



4-Necrotic



5-Dead



# Relative necrosis length

Total length of the plant



Cut lengthwise



Necrosis

$$\text{RNL} = \frac{\text{Necrosis}}{\text{Total length of the plant}}$$







# High-performance liquid chromatography (HPLC) analysis



(1) 10cm-piece



(2) Freeze-dry 24h



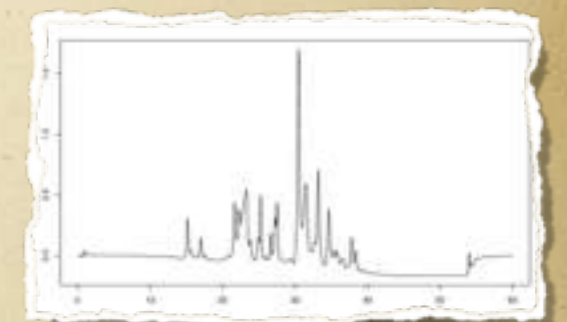
(3) Grind until powder



(4) Extract with methanol



(5) HPLC



Two peaks: eriodictyol  
and naringenin  
dominated the HPLC  
profile at 280 nm.



# Statistics

- Relative necrosis length: Mixed Model.



*(high heterogeneity of the variance of the data)*

- Content of phenols: erioctydiol and naringenin:  
non parametric Kruskal-Wallis test.

- Relationships among variables: non parametric  
Spearman correlation matrix.



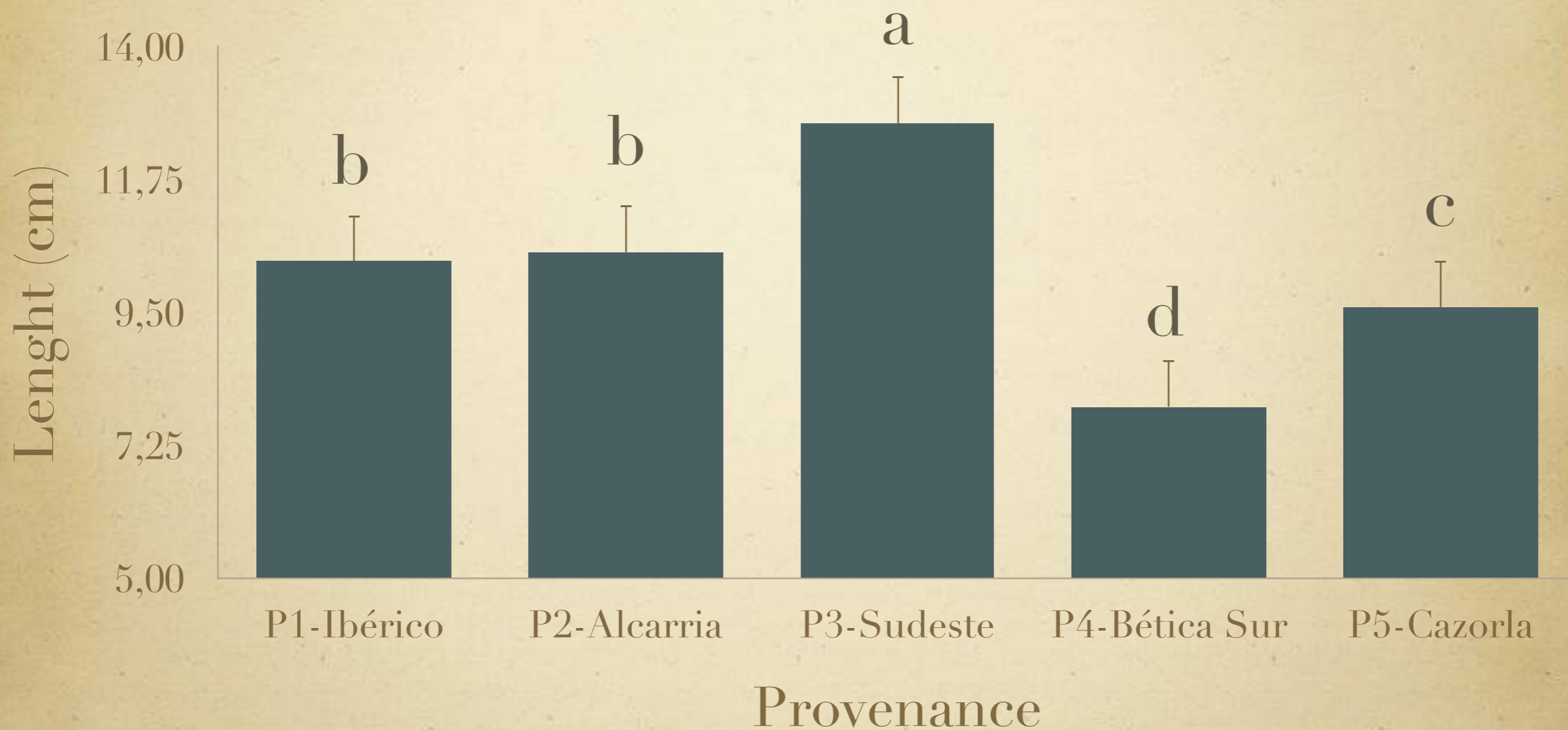


# Results





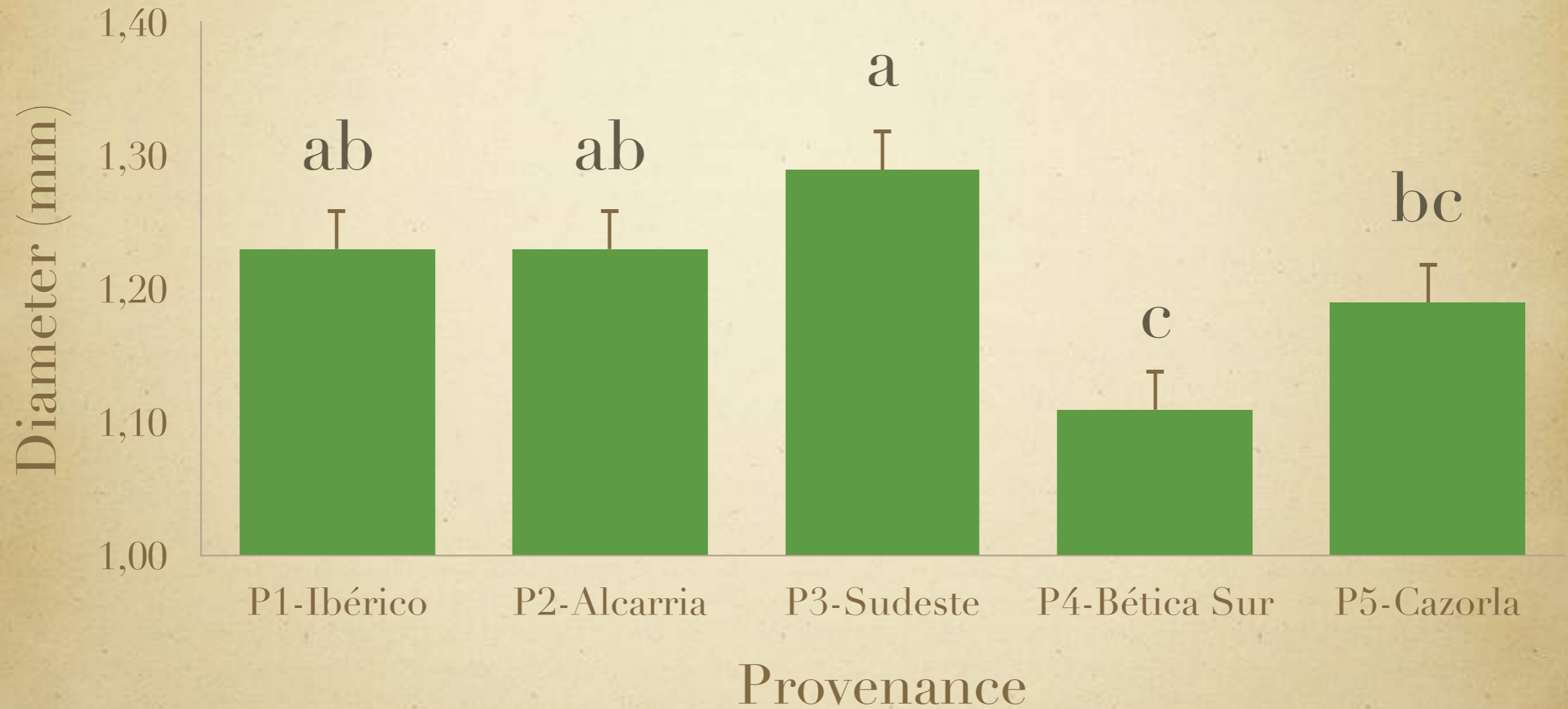
# Length of the seedlings



\* Means without a common letter in the same column show values significantly different from  $p < 0.05$  (ANOVA Tukey's HSD Test).



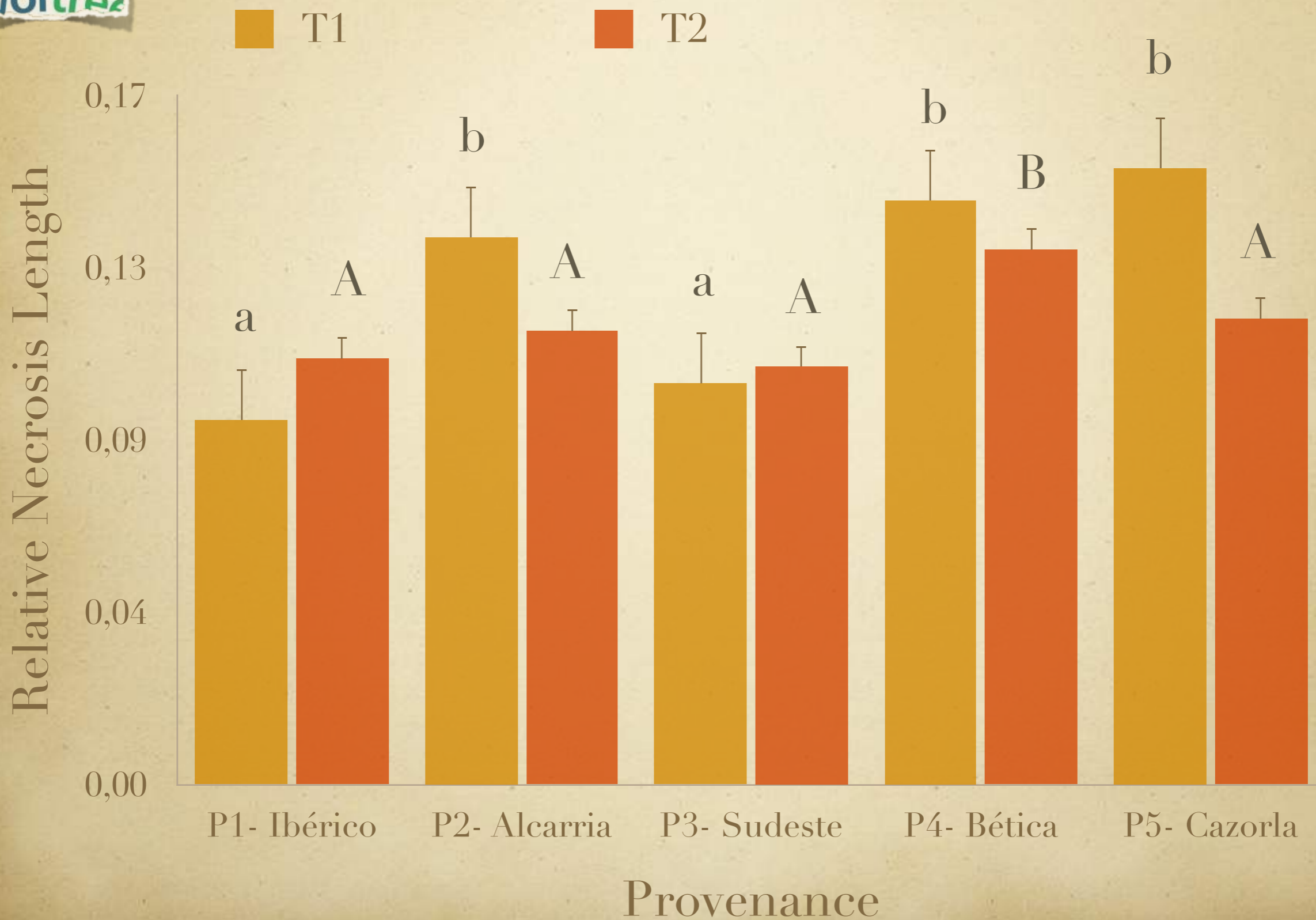
# Diameter of the seedlings



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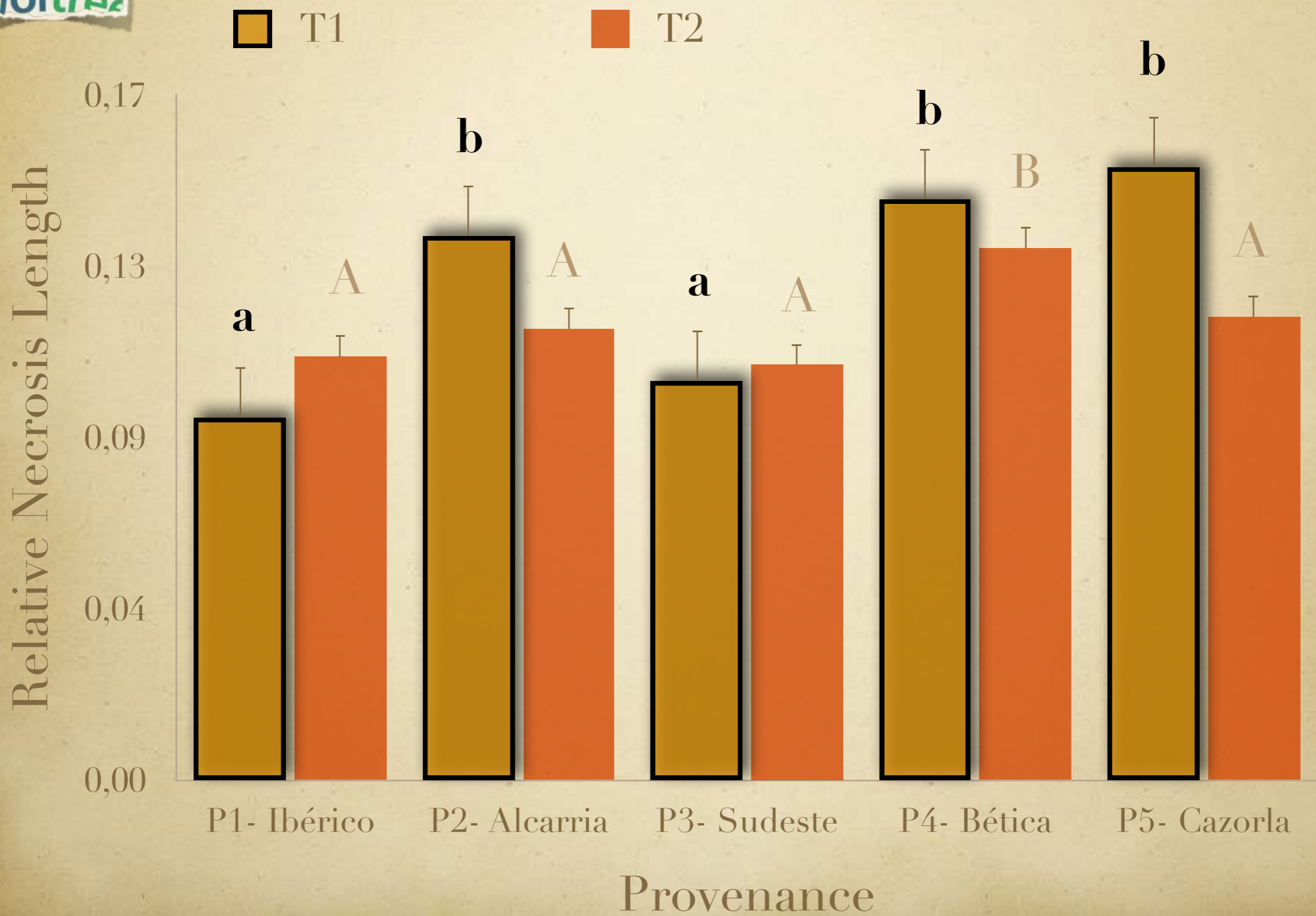


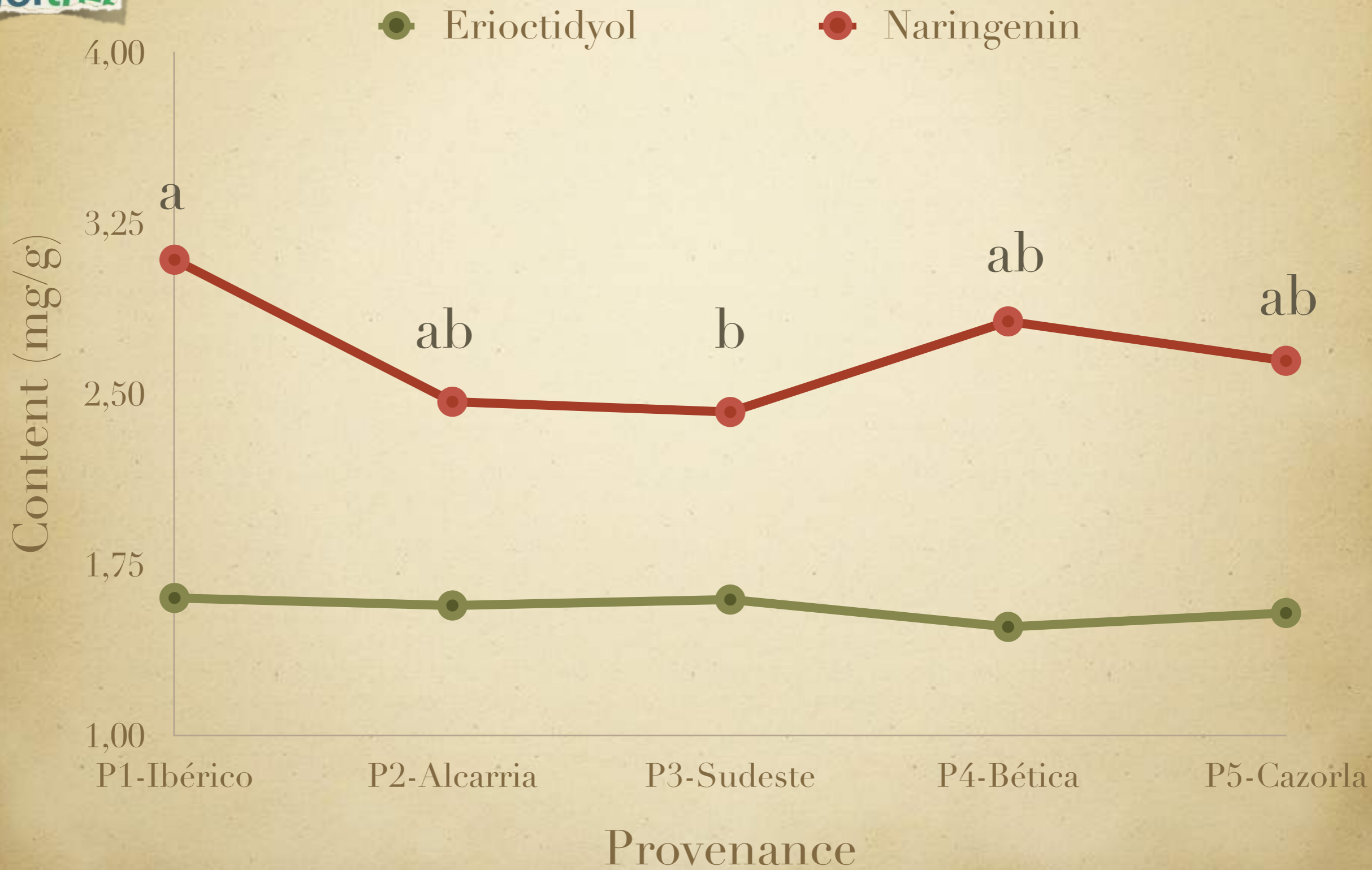
# Relative Necrosis Length





# Relative Necrosis Length















# Conclusions

1. The provenances of Aleppo pine showed a different response to the *G. abietina* infection in terms of relative necrosis length.
2. Higher elevation and precipitation, lower resistance to disease.
3. Increasing damage as the latitude of origin decreases.



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4. The observed difference in resistance may be explained by the fact that these high- and low-altitude provenances presented significant differentiation in adaptive traits mediated by maternal effects.
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*Thanks for your attention*

*Merci pour votre attention!*