Differences in cone production among stone pine clones

- an overview of the Spanish Breeding Program

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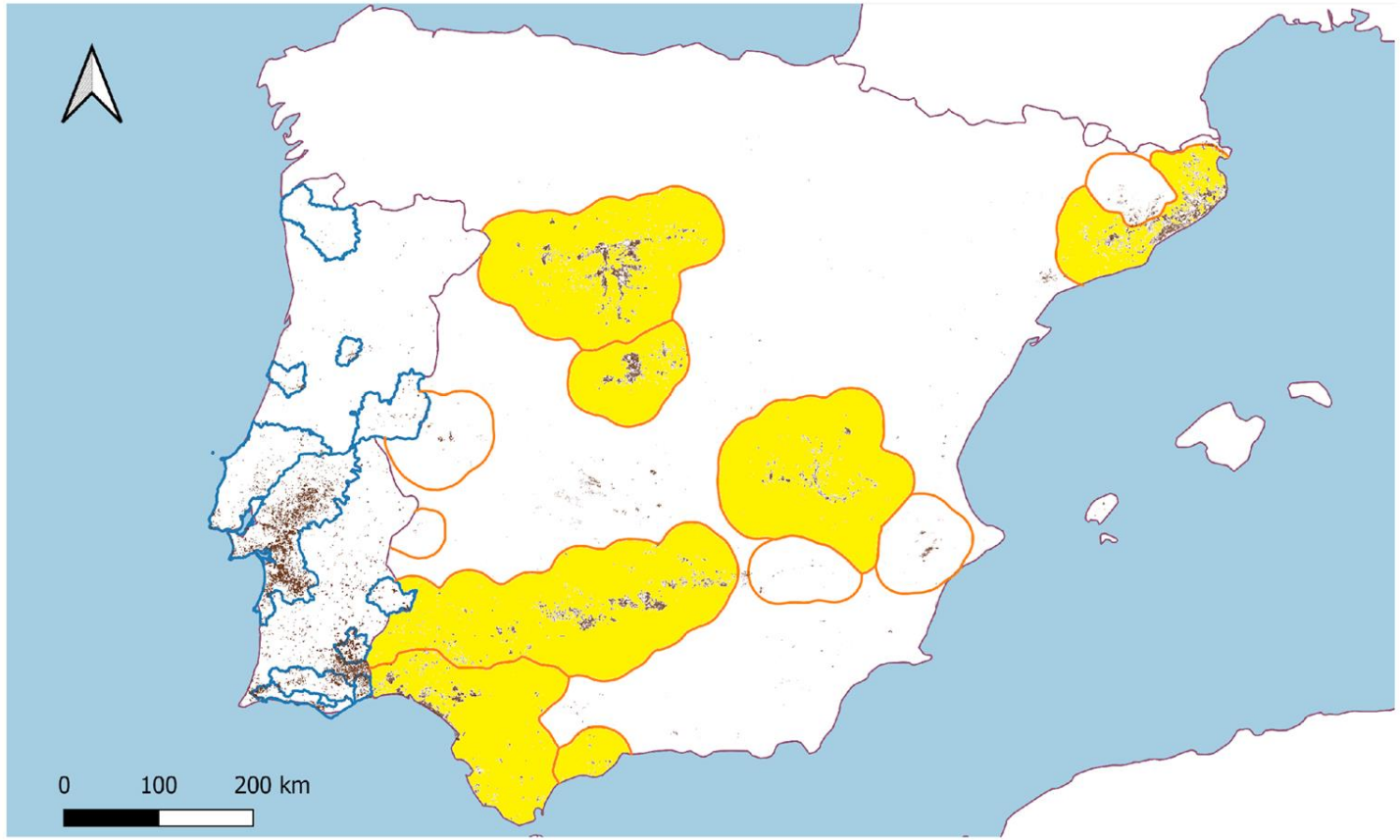
The beginnings of the breeding program

- **National and Regional programs (s. 1990s)**:
  - Ministry of Agriculture/Environment
  - Regional Forest Services
  - Universities
  - ICIFOR-INIA

- **Objective**:
  - Seed stands for Provenance Regions
  - Species domestication aiming at agroforestry plantations for pine nuts.

➢ clone selection + grafting + low density plantation + tending

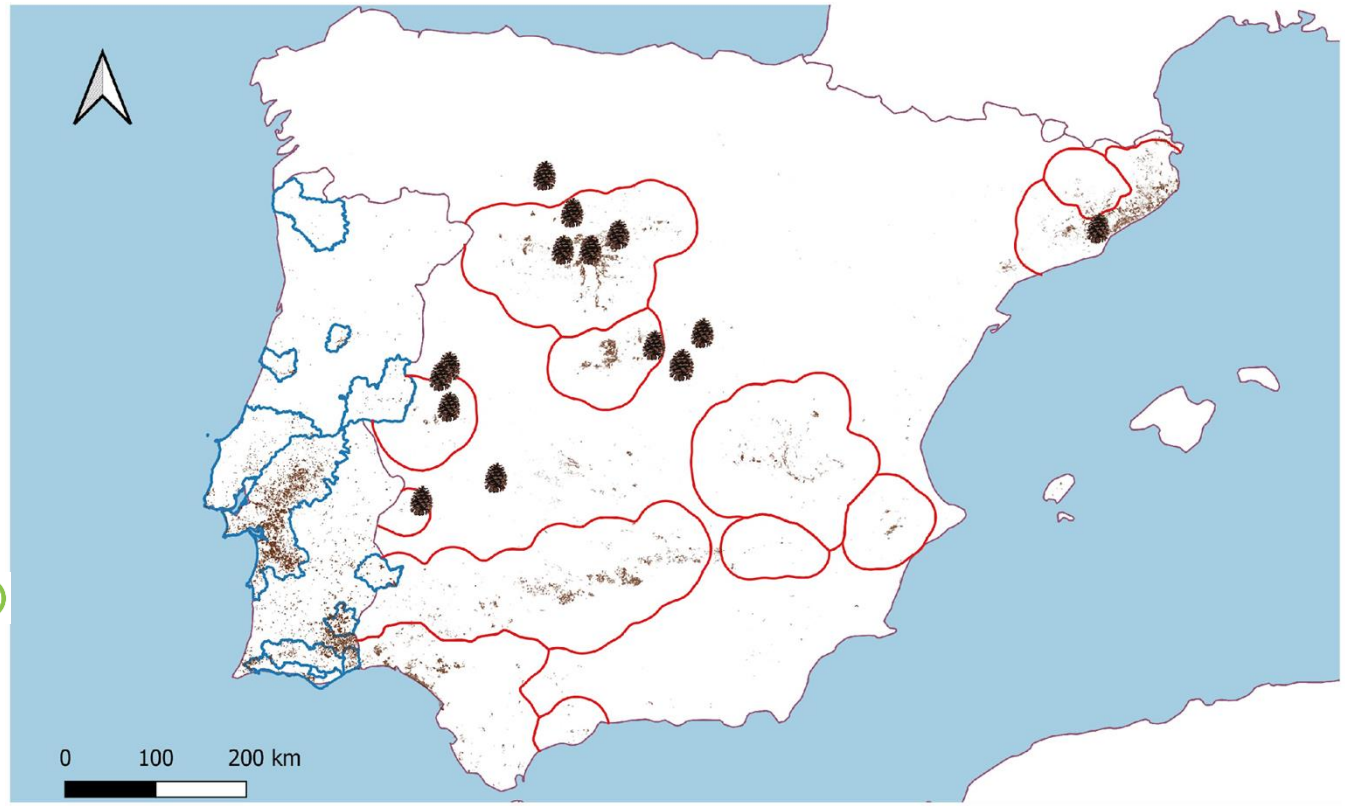
- ~ 360 genotypes (plus trees) from 4 provenances tested in clonal banks
- + 120 genotypes from Andalusian provenances



Iberian *Pinus pinea* Provenance Regions

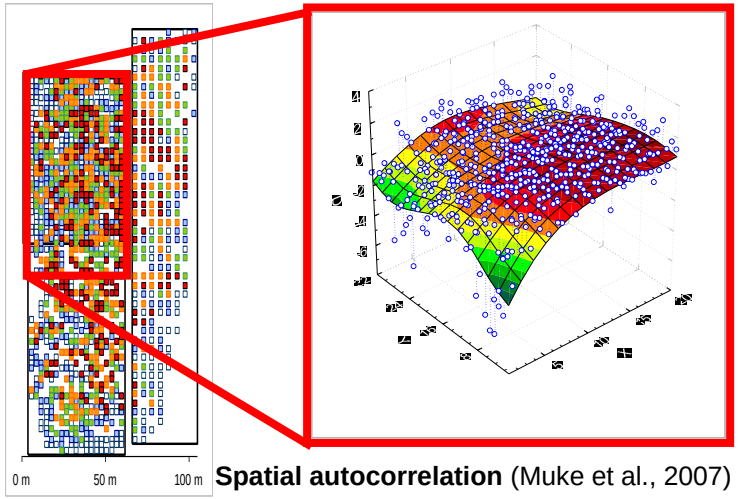
Current established grafted clone trials

Code	Institution	Center	Province	Area (ha)	Pl. Year
B23PH1	MITECO	CNRGF Puerta de Hierro	Madrid	0.6	1991
B23PH2	MITECO	CNRGF Puerta de Hierro	Madrid	0.7	1992
B23PH3	MITECO	CNRGF Puerta de Hierro	Madrid	0.6	1993
B23PH4	MITECO	CNRGF Puerta de Hierro	Madrid	0.8	1998
B23MN1	JCyL	Quintanilla de Onésimo	Valladolid	4.5	1991
B23MN2	JCyL	V. El Molinillo, Tordesillas	Valladolid	2.0	1998
B23MAY	JCyL	Mayorga	Valladolid	0.5	2008
B23MAT	Diput. VA	Matallana, Villalba de los Alcores	Valladolid	2	2008
B23PED	JCyL	La Pedraja de Portillo	Valladolid	1	2009
B23TOR	JCyL	V. El Molinillo, Tordesillas	Valladolid	1.5	2007
B23SER CAT	MITECO	CNRGF EL Serranillo	Guadalajara	0.7	2004
B23SER	MITECO	CNRGF EL Serranillo	Guadalajara	1.0	2007
B23ARG2007	IMIDRA	CTT La Isla, Arganda del Rey	Madrid	0.8	2007
B23IRTA	IRTA	Torre Marimón, Caldes de Montbui	Barcelona	1	2008-9-10
B23EXHP	Local council	Ayto. Hernán Pérez	Cáceres	3.5	2021
B23JEXVH	JEX	V. San Marcos, Holguera	Cáceres	0.86	2021
B23JEXVP	JEX	V. de Palazuelo, Villar de Rena	Badajoz	0.5	2021
B23JEXVLMG	JEX	V. La Moheda de Gata	Cáceres	0.89	2021
B23JEXVRCH	JEX	V. Rueda Chica, Puebl. del Guad.	Badajoz	0.78	2021
				24.2	



Spanish clone trials

1st Evaluation of clonal banks



Spatial autocorrelation (Muke et al., 2007)

- 6 common-garden comparative clone trials: 4 Ministry (MITECO) + 2 Regional Junta de Castilla y León

- Yearly cone harvest per tree (number, Kg, damages)
  - 10-20 yr individual tree production

- **Clone selection** (Mutke *et al.*, 2007):

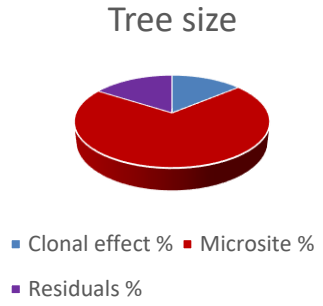
  Challenge: strong **spatial autocorrelation** that masks genetic gain

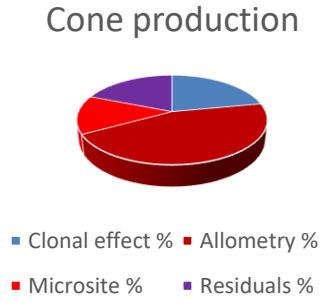
  Tree size = **14% clonal effect**, but 70% spatial autocorrelation (microsite)

  Cone production = **22% clonal effect**, but 45% due to tree size (allometry) + 14% microsite

  Solution: weight individual clone production with tree size and microsite effect as **covariates**

  Cone production (+ masting) + Allometry (Ø) + Microsite effect





- **Results**:
  - Clonal degree of genetic determination of cone production: ~ **20% (H^2)**
  - Gain: **+ 20-40%** with top 10% selected clones

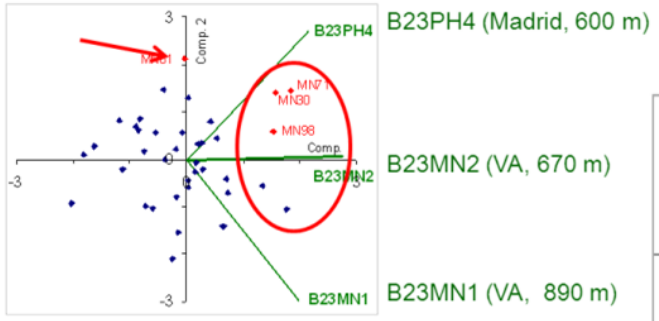
1st Registration of clones

- Clone identification: **microsatellite markers** (nuSSRs)
  - Adapted for *P. pinea* (Pinzauti *et al*., 2012, S. González Martínez, 2013)
  - 6 (+1) polymorphic nuSSRs
  - 1 very common molecular profile (20% of all samples)
  - Only a few unique profiles (allele combinations)
- Only 15 elite clones with distinct profile registered in National Register of Basic Materials (2015)
- Establishment of 1 stock nursery in NCFGR *Puerta de Hierro*, Madrid

RP	Clon	Name	Category	No. Trials	Mean gain (% cone production)
TESTED					
1	1011	Portillo-11		2	26%
1	1012	Portillo-12		3	20%
1	1073	La Vega		2	15%
1	1123	íscar		2	16%
1	1201	Valdegalindo		2	17%
QUALIFIED					
2	2004	Hoyo de Pinares		1	19%
2	2048	Almorox		1	19%
2	2068	San Martín de Valdeiglesias		1	24%
3	3029	El Provencio		1	31%
3	3048	Pozoamargo		1	21%
3	3057	Casas de Haro		1	23%
3	3063	El Picazo		1	22%
6	6010	Sta. Coloma de Farners		1	9%
6	6015	Llagostera		1	11%
6	6053	Dosrius		1	9%

Recommendations for use: provenance vs plasticity?

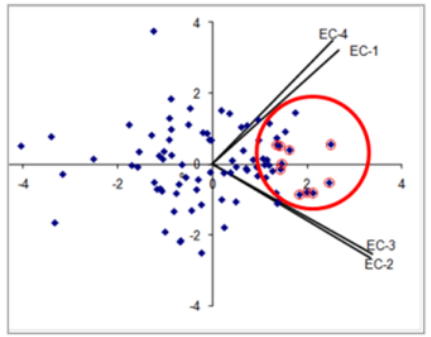
- Two studies on 3 and on 4 clone trials at different altitudes/climate zones showed differences in **phenotypic plasticity** along a continental ecological gradient:
  ~ some clones perform best in colder sites, but average in warmer, and vice versa.
  (Mutke, 2008)



B23PH4 (Madrid, 600 m)

B23MN2 (VA, 670 m)

B23MN1 (VA, 890 m)



EC-1, - 4
(Coastal plain)

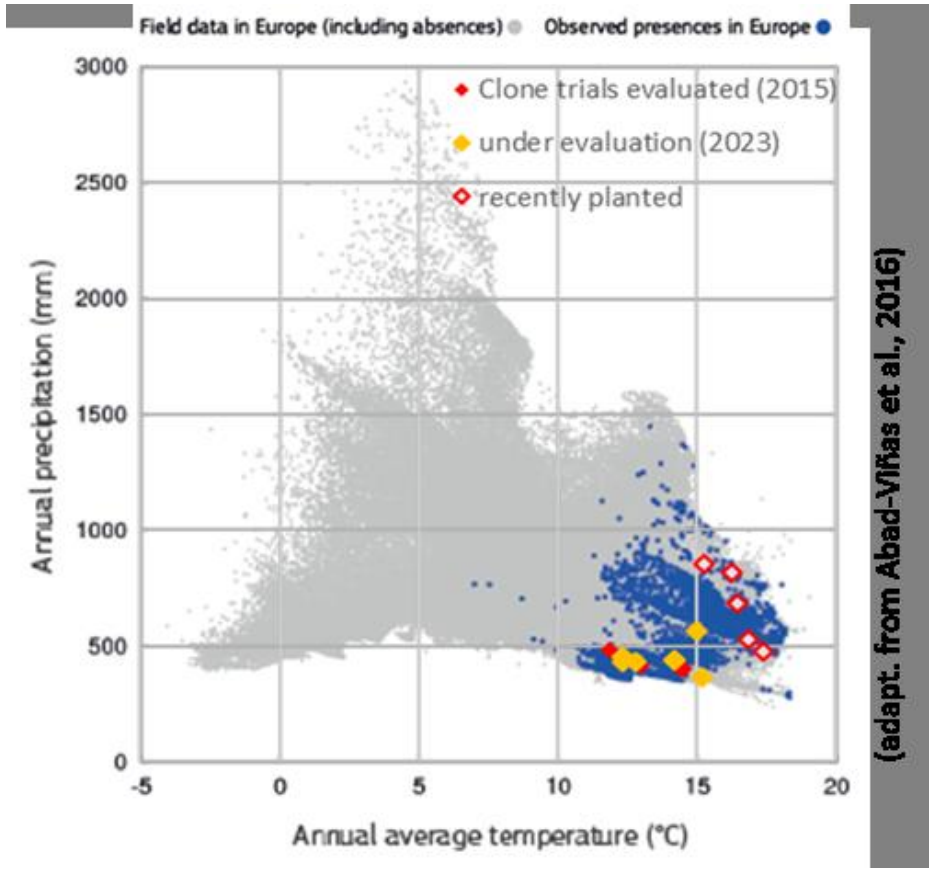
EC-2 ,- 3
(Sierra Morena)

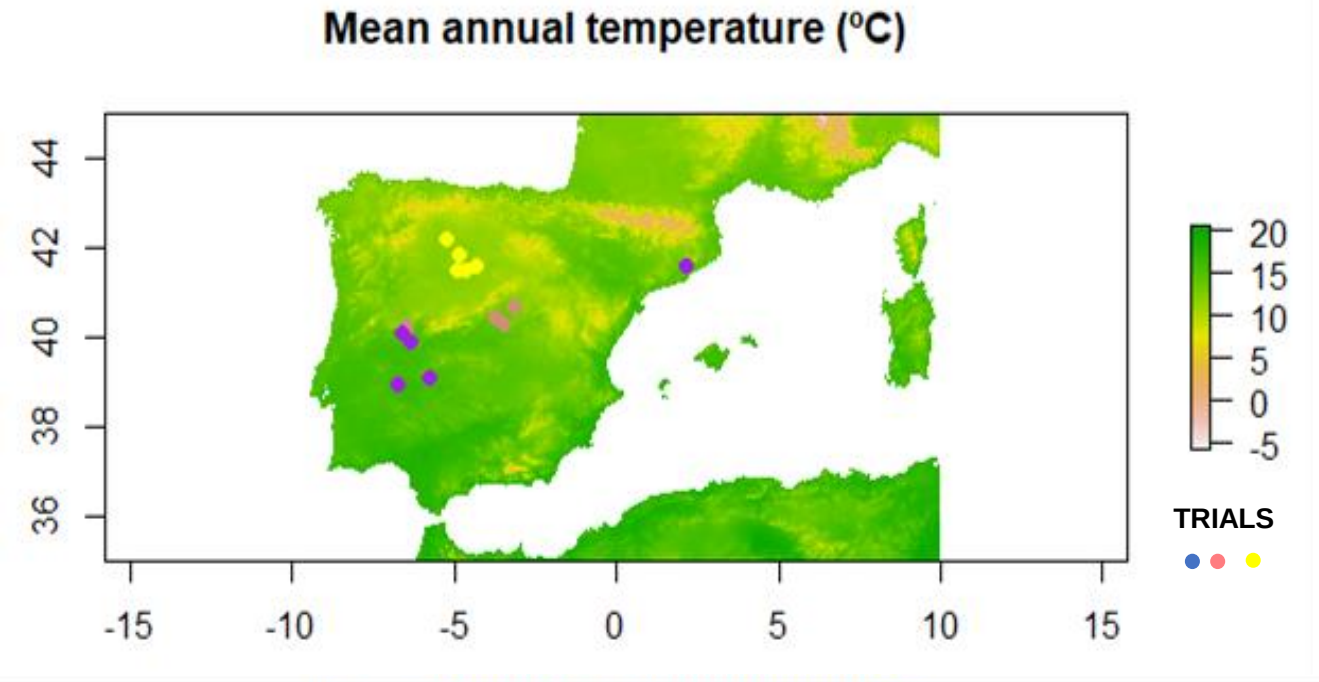
- Some previous studies in common gardens established some provenance differentiation, but significant only after adjusting for microsite effect (spatial autocorrelation)

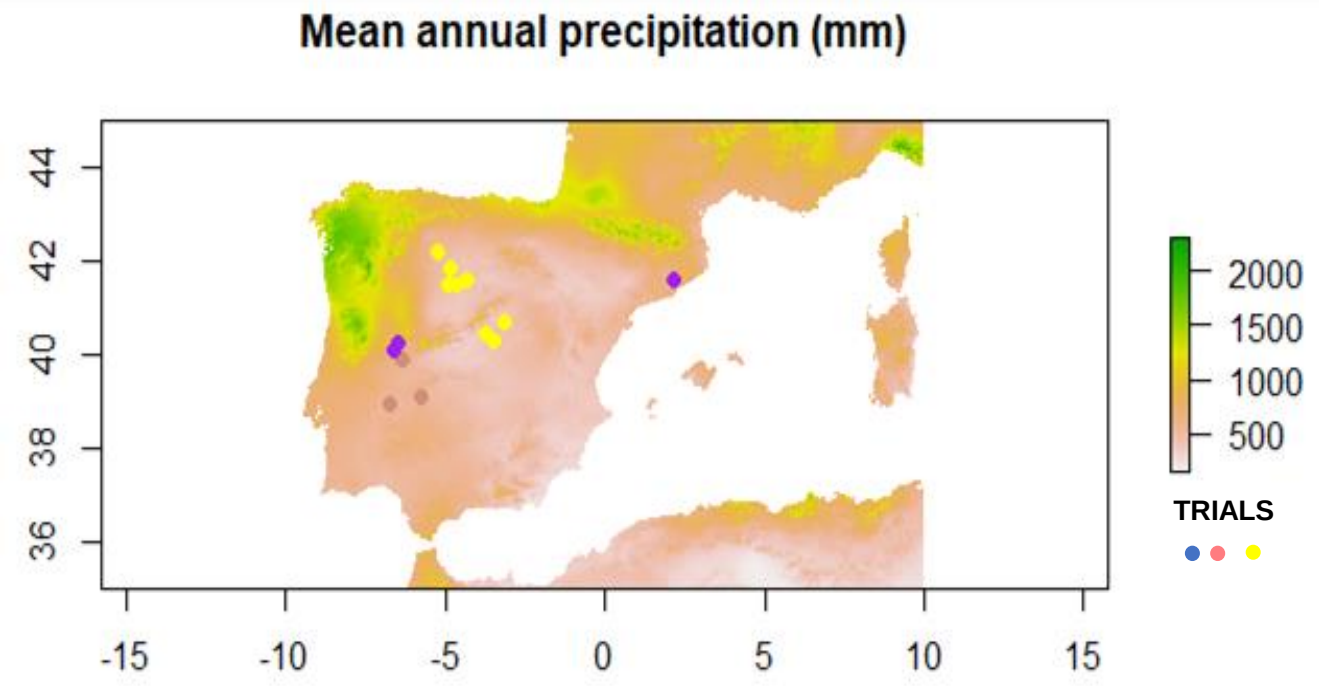
PENDING: TEST CORRELATION BETWEEN ELITE CLONE PRODUCTION AND ENVIRONMENTAL CONDITIONS

Climate envelope

Have we covered the species climate range?







Next actions

- Formalisation of SNPs as a tool for clone identification method
- Evaluation of new clone banks (2 MITECO, 1 IRTA, 1 JCyL)
- Re-examination of “old” banks to expand number of elite clones (using SNPs identification)
- Registration of the new resulting clones and revision previous registered clones.
- Establishment of demonstration plots (Extremadura)
- Test planting layouts and pruning intensity effect
- Test clone effect on production damage (*Leptoglossus occidentalis* H.)

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LISBOA 2023

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THANKS FOR YOUR ATTENTION!

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