Progress in INRA tree improvement program for maritime pine.

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Progress in INRA tree improvement program for maritime pine

Laurent Bouffier

INRA (French National Institute for Agricultural Research)
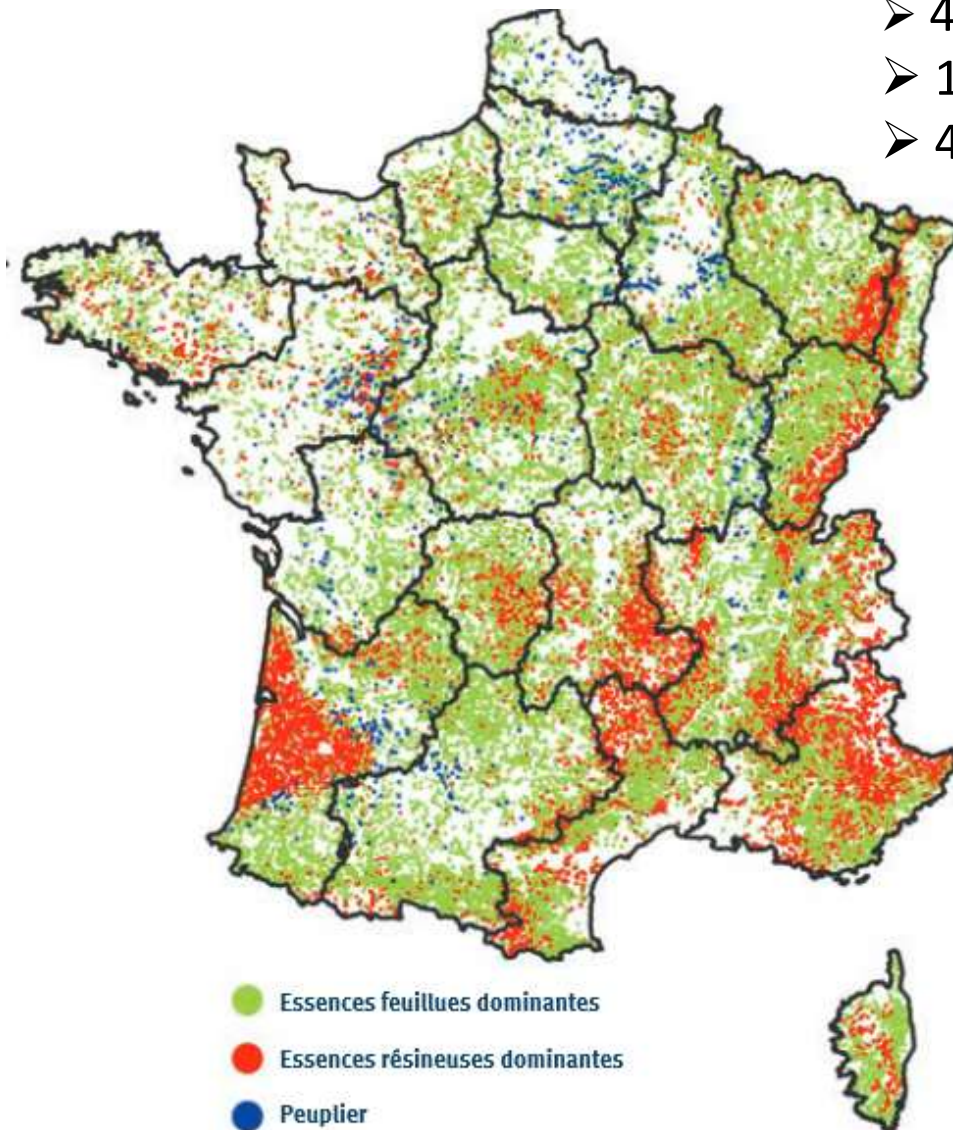
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STBA Technical Advisory Committee meeting
28-30 November 2017 - Melbourne

Outlines

➤ French forests and maritime pine plantations

➤ Breeding program: stakeholders, selection criteria, breeding population, seed orchards

➤ Dataplan / Treeplan since 2011

➤ Implementation of molecular markers (MM)

French forests

> 4th Forest in Europe (Sweden, Finland, Spain)
> 17 millions ha (30% surface area)
> 40% of growth not harvested

Essences feuillues dominantes
Essences résineuses dominantes
Peuplier

Source : e-IGN - 2014 ; campagne 2008 à 2012

	Area	Production (m3/ha/year)
Oak	24%	5.1
Beech	9%	5.6
Chesnut	5%	7.1
Other hardwoods	33%	3.9
HARDWOODS	**71%**	**4.7**
Spruce and fir	8%	11.8
Maritime pine	7%	6.6
Scots pine	6%	4.5
Douglas	3%	14.2
Other softwoods	6%	5.1
SOFTWOODS	**29%**	**7.9**

Forests in expansion in France

9 millions ha
(cadastre «napoléonien»)

16 millions ha
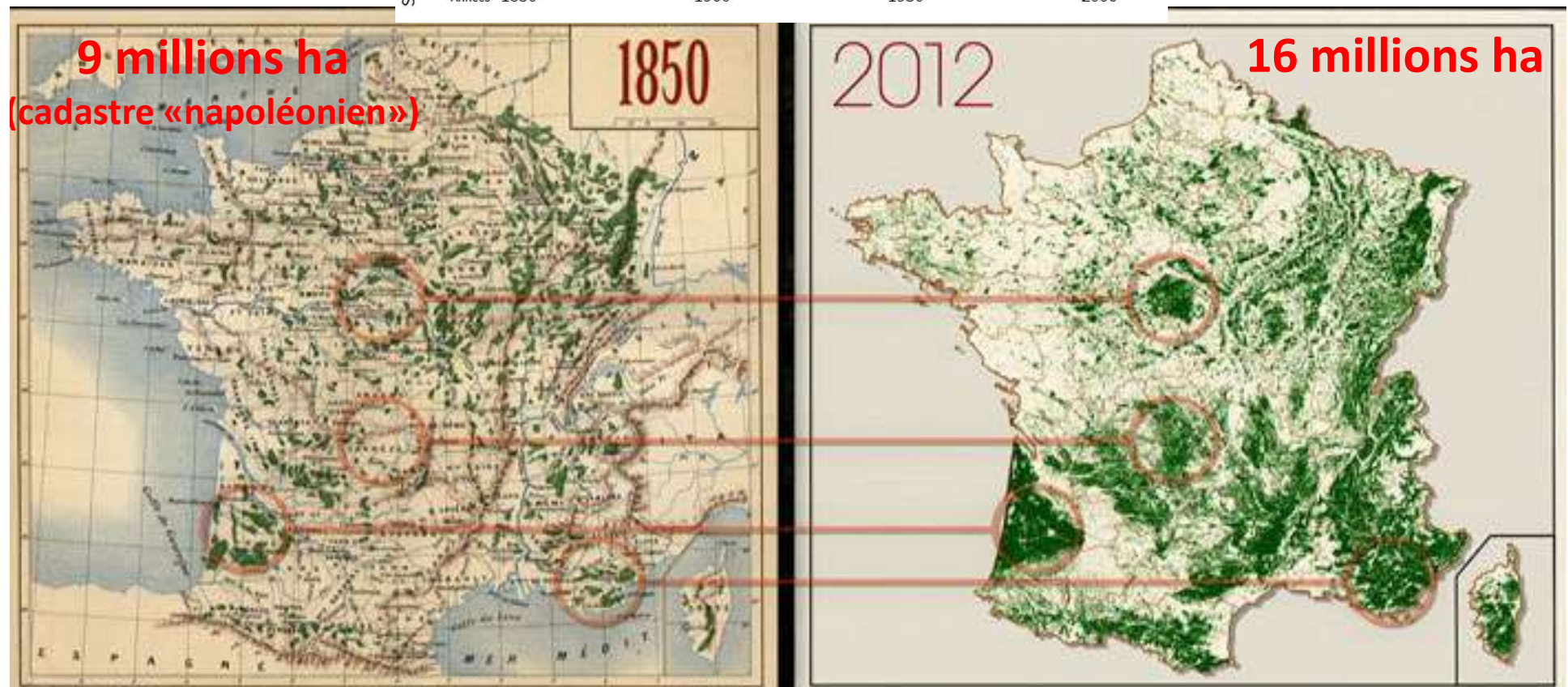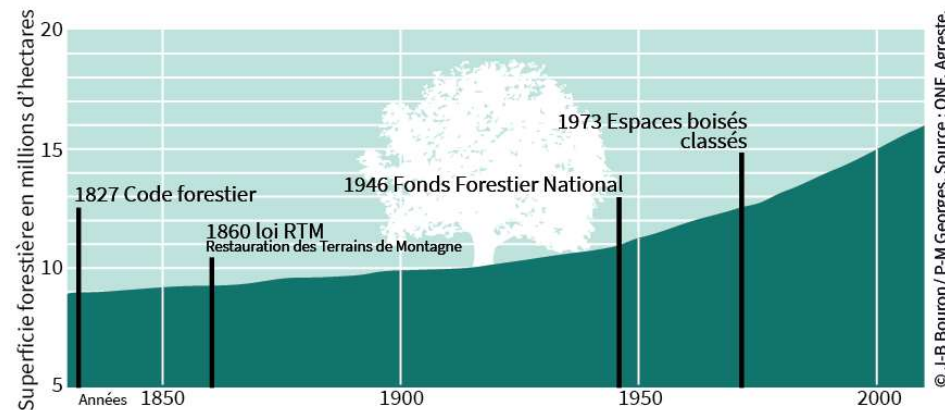
Landscapes in southwestern France

French vineyards

Forest area

0 - 15 %   15 - 45 %   45 - 100 %
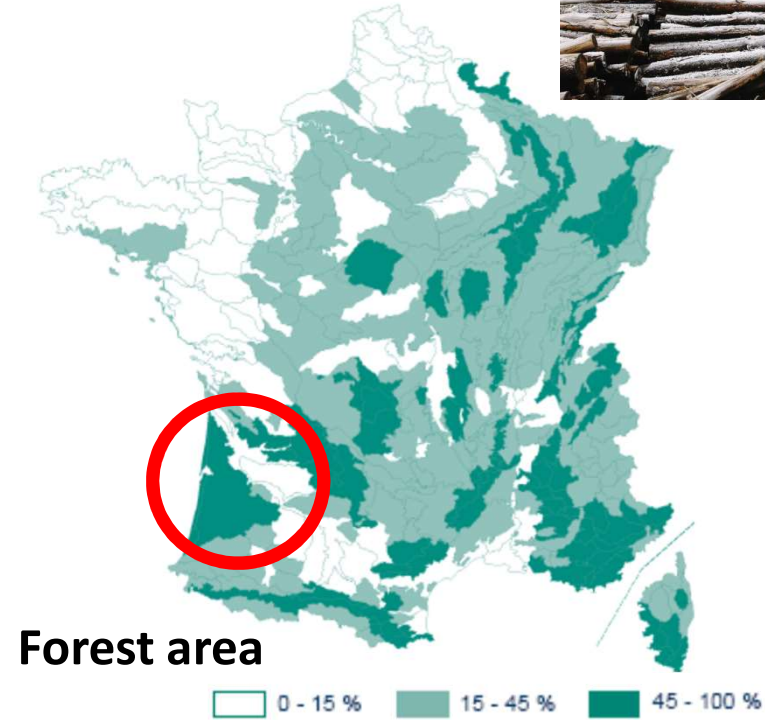
Maritime pine forest in southwestern France

Les Landes de Gascogne

- ☐ Massifs dunaires de la côte
- ☐ Forêt des Landes de Gascogne

➢ **Maritime pine forest established at the end of the 19th century**
  - ▪ Poor soils (sandy podzol)
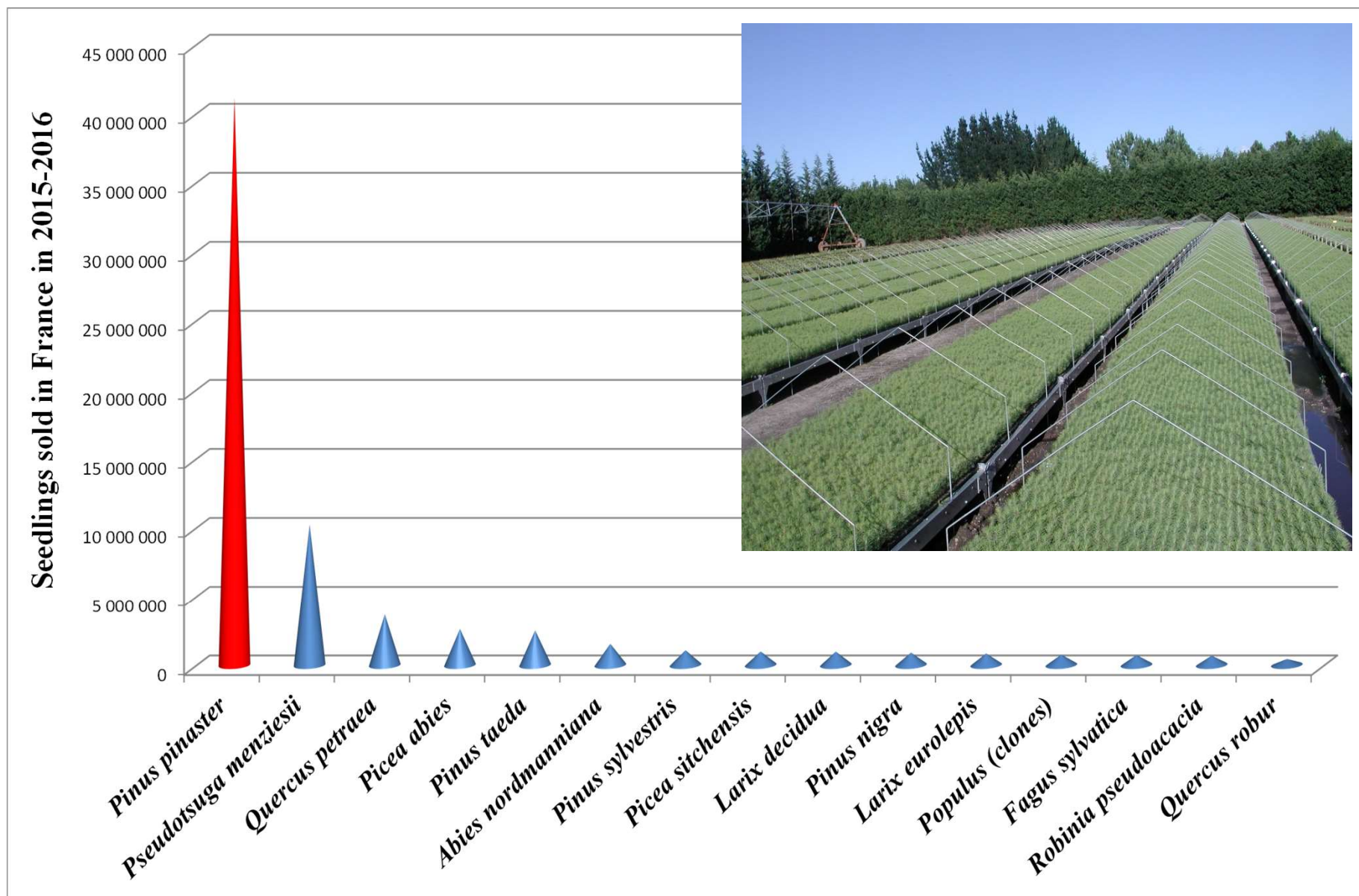 - ▪ Dry summers / wet winters (hydromorphic soils)

➢ **0.8 million hectares forest (7% of the French forest)**
  - ▪ Maritime pine plantations (>90% seedlings) except in sand dunes (natural regeneration)

➢ **24% of French wood harvest**
  - ▪ 11 m3/ha/year
  - ▪ 8.5 million m³ harvested /year
  - ▪ 60% saw timber / 40% industrial wood

Maritime pine = 1st plantation species in France

Breeding program managed by a consortium

➢ **Base population established in 1960's by INRA**

➢ **Two breeding programs (INRA, FCBA)**

➢ **Consortium since 1995 = Groupe « Pin Maritime du Futur »**

« Recurrent » fundings = Ministry of Agriculture + Région « Nouvelle Aquitaine »
Specific studies = European Union + Ministry of Education and Research (ANR projects)

Stakeholders for Maritime pine breeding

Maritime pine breeding program
Managed by GPMF (« Groupe Pin Maritime du Futur »)
IP = INRA + FCBA

Seed Orchards

Composition + Design
GPMF but SO must be validated by a national public authority (CTPS)

Ownership + Management
Public (ONF) and private companies

royalties

Nurseries
Private companies
(Forelite, Planfor, Naudet, Robin)

Current selection criteria

> ## Selection criteria:
> - Volume (girth, height)
> - Stem straightness
> - Rust resistance
> - Wood quality (branching quality, wood density, spiral grain)

	Height	Circumference	Sweep	Wood density	Spiral grain	Branch diameter	Branch angle	Fork nber
Circumference	0.69							
Sweep	0.17	0.31						
Wood density	0*	-0.11	0.13					
Spiral grain	0.03	-0.19	-0.09	0.18				
Branch diameter	0.53	0.70	0.28	0*	0*			
Branch angle	-0.16	-0.27	0,01	0*	0*	-0.14		
Fork nber	0.16	0.30	0.12	0*	0*	0.41	0.21	
Ramicorn nber	0.11	0.27	0.15	0*	0*	0.41	0.22	0.63

✓ Moderately unfavorable correlations growth – sweep

✓ No or slightly unfavorable correlation growth – wood quality

✓ Moderately unfavorable correlation growth - branching

✓ High heritability for height and wood quality

	Height	Circumference	Sweep	Wood density	Spiral grain	Branch diameter	Branch angle	Fork nber	Ramicorn nber
Heritability	0.34	0.18	0.24	0.42	0.46	0.15	0.17	0.06	0.08
Coefficient of additive variation	4.8%	7.0%	19.6%	7.4%	51.0%	10.3%	11.0%	61.7%	37.9%

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Selection criteria under study

➢ **Drought resistance**
- ▪ Multi-site evaluation (dry / semi-humid / humid sites)
- ▪ Inter-provenance hybridization

Use of inter-provenance hybridization to improve straigthness and drought tolerance
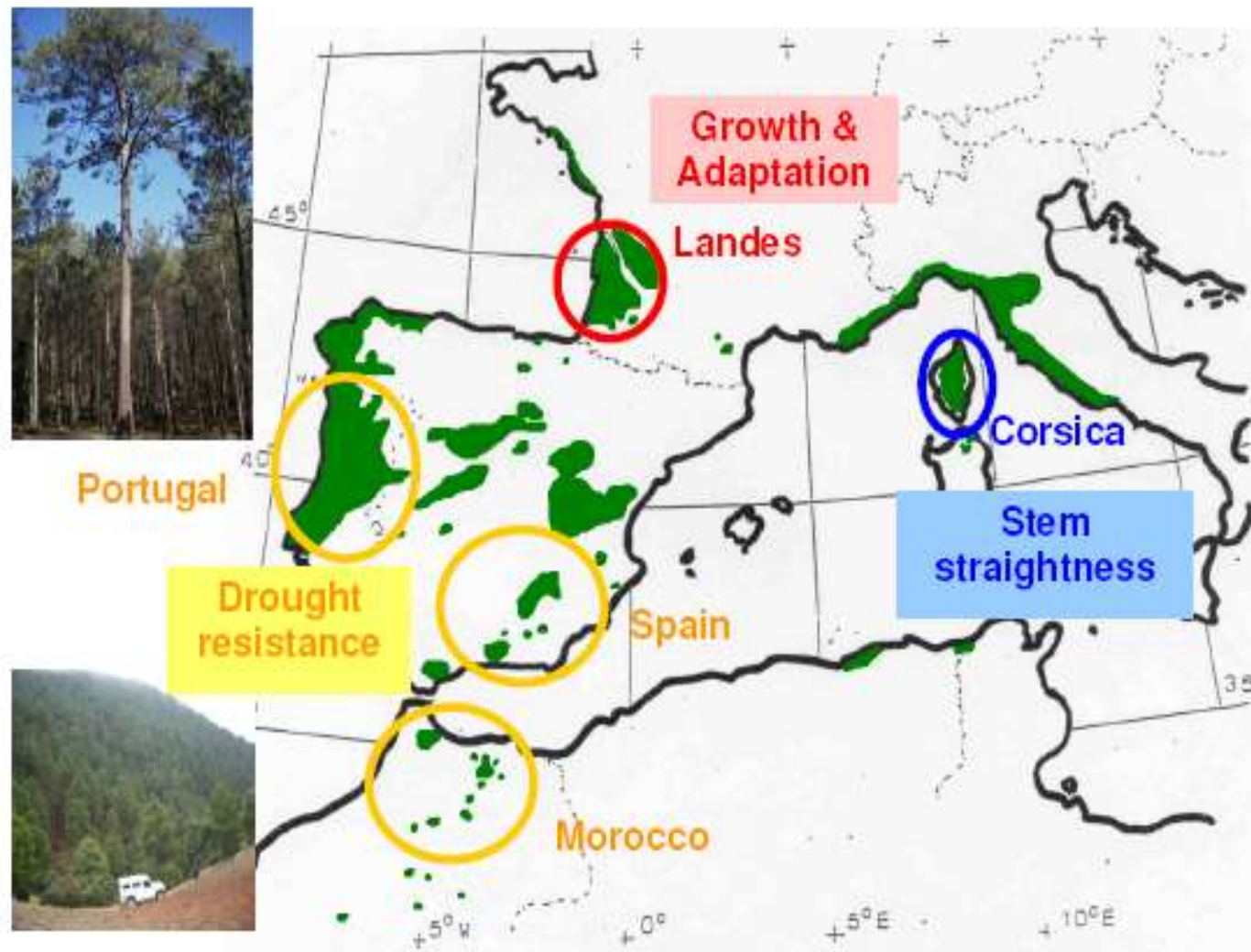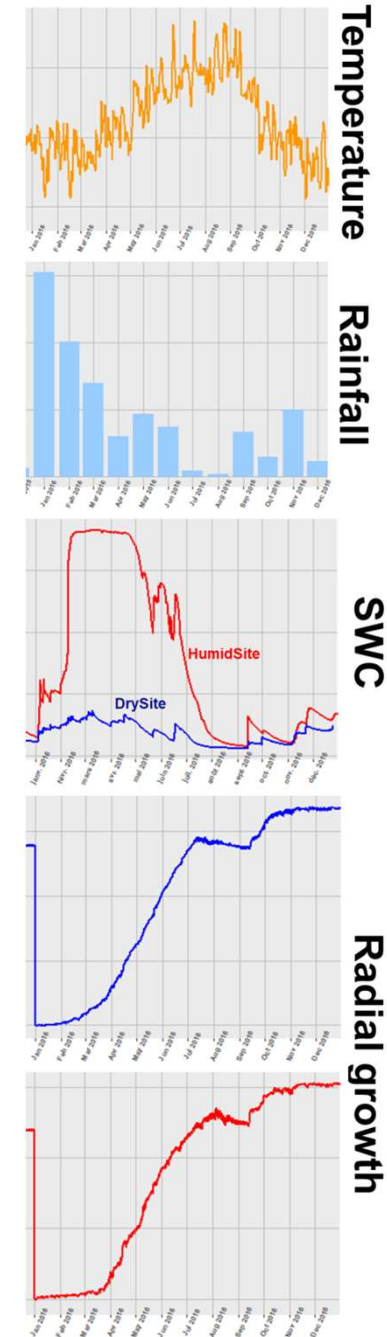
Selection criteria under study

➢ **Drought resistance**

- ▪ Multi-site evaluation (dry / semi-humid / humid sites)
- ▪ Inter-provenance hybridization
- ▪ Remote sensors (dendrometers and humidity sensors) to study variation of growth in relation to environment

Evaluate genetic variability of tree growth in water stress conditions

➤ Tree response to water stress measured through radial growth

➤ Definition of key parameters to characterize radial growth curves in water stress conditions and evaluate their genetic variability

➤ Identification of proxies (from wood density profiles) to simplify selection for drought adaptation
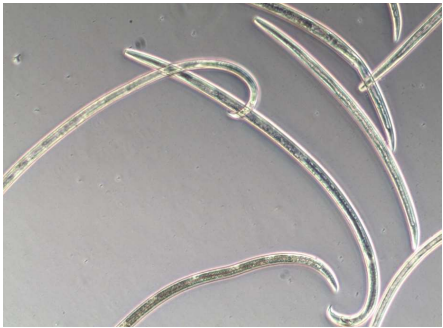
Selection criteria under study

➢ **Drought resistance**
- Multi-site evaluation (dry / semi-humid / humid sites)
- Inter-provenance hybridization
- Remote sensors (dendrometers and humidity sensors) to study variation of growth in relation to environment

➢ **Pine wood nematode (or pine wilt nematode)**
- Nematode (*Bursaphelenchus xylophilus*) vectored by a sawyer beetle (*Monochamus galloprovincialis*)
- Pine nematodes move within infested host trees in tracheids and disrupti water uptake by the tree. Death of the tree is rapid, often within a few weeks or months.
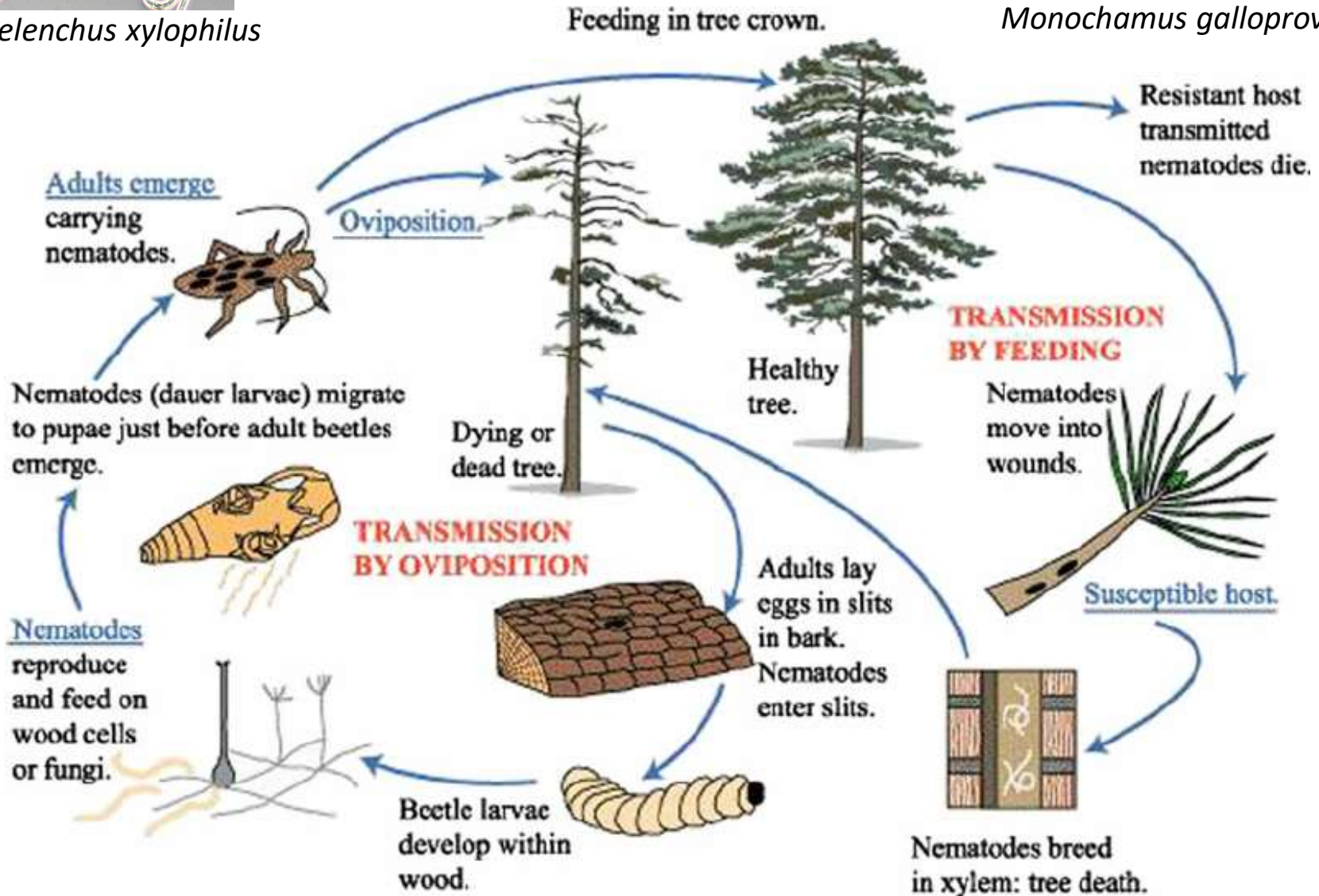
Pine wood nematode

Bursaphelenchus xylophilus

Monochamus galloprovincialis

Feeding in tree crown.

Resistant host transmitted nematodes die.

Adults emerge carrying nematodes.

Oviposition.

TRANSMISSION BY FEEDING

Nematodes (dauer larvae) migrate to pupae just before adult beetles emerge.

Nematodes move into wounds.

Healthy tree.

Dying or dead tree.

TRANSMISSION BY OVIPOSITION

Nematodes reproduce and feed on wood cells or fungi.

Adults lay eggs in slits in bark. Nematodes enter slits.

Susceptible host.

Beetle larvae develop within wood.

Nematodes breed in xylem: tree death.

Selection criteria under study

➢ **Drought resistance**
- Multi-site evaluation (dry / semi-humid / humid sites)
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- Pine nematodes move within infested host trees in tracheids and disrupti water uptake by the tree. Death of the tree is rapid, often within a few weeks or months.
- Detected in Portugal in 1999, in Spain in 2007
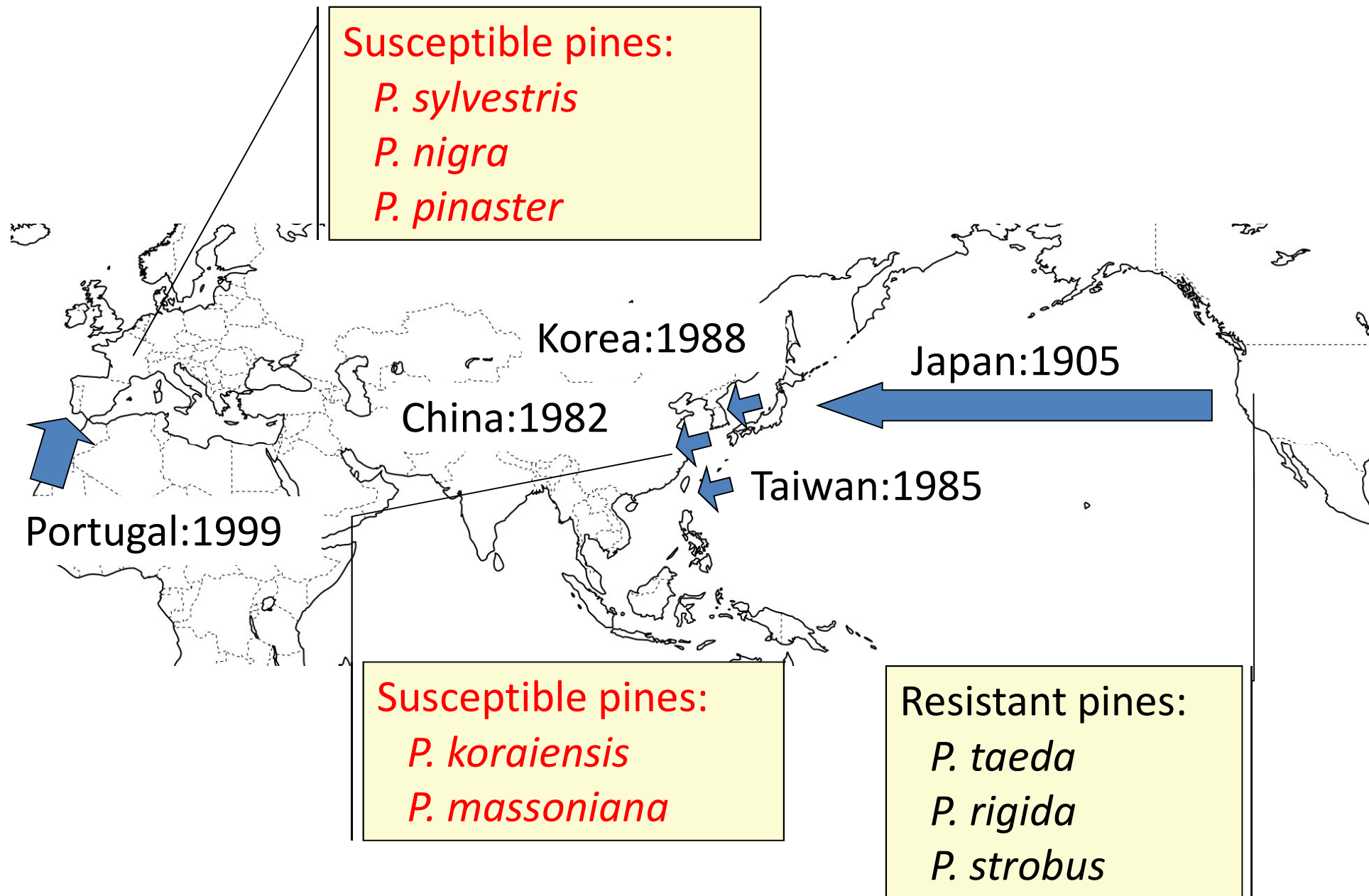
Breeding population
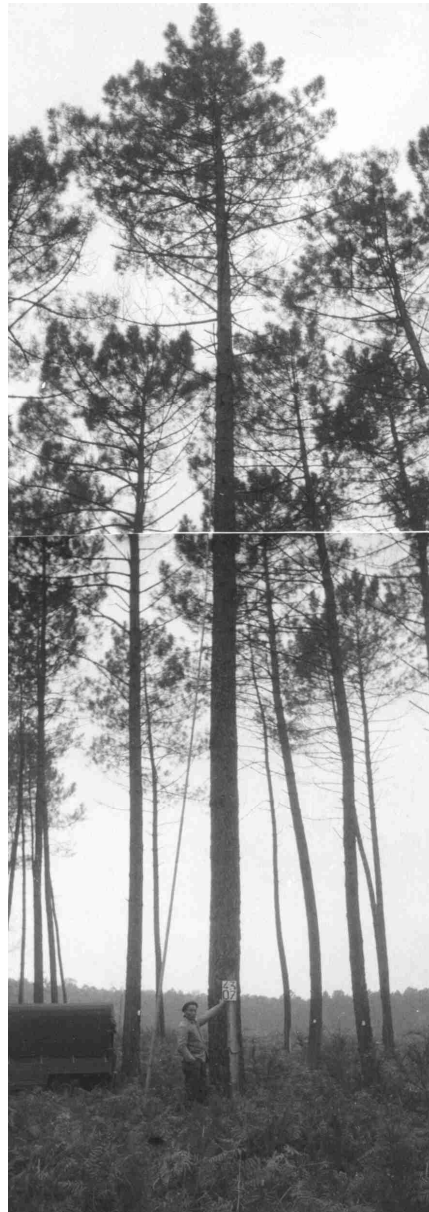
➢ Initiated in the 60's :

– Local provenance for growth and frost resistance

– Base population ~450 plus trees selected in local stands

– Recurrent selection scheme
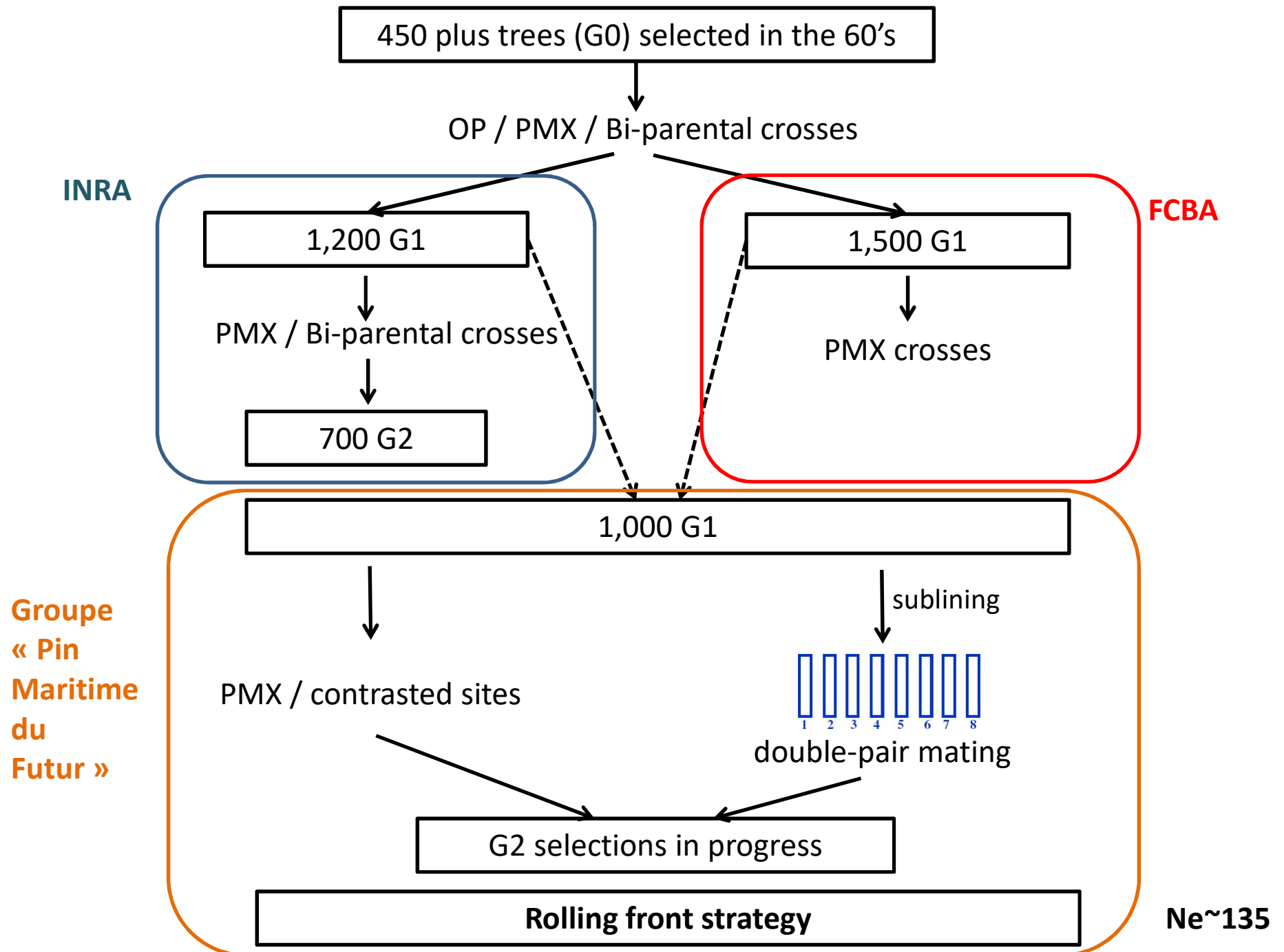
19
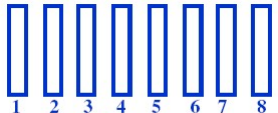
Seed orchards

- clonal and family open-pollinated seed orchards

- Density ~300 trees/ha
- SO area ~10-20 ha

- No flowering induction, no fertilisation, no pest control…

- ~40 genotypes selected to established several SO (one for each company)

- about one SO composition every 15 years
~1980: VF1
~1995: VF2
~2010: VF3

- Perspectives: to accelerate deployment (consortium to manage SO) and management intensification

Dataplan - BLUP calculation with Treeplan®

➢ 2011: pilot project
  2017: 78 trials / 7,489 families / 590,396 trees

➢ A single database for INRA and FCBA

➢ Promotion of historical trials (first trial established in 1962): re-analysed with spatial analyses

➢ More powerful genetic evaluation (BLUP comparable across trials, generations)

➢ Treeplan BLUP used for VF4 (backward and forward selections)

➢ Used of STBA tools to design future progeny trials

Development of a 9k SNP array

High-density SNP assay development for genetic analysis in maritime pine (*Pinus pinaster*)

C. PLOMION,*† J. BARTHOLOMÉ,*† I. LESUR,*‡ C. BOURY,*† I. RODRÍGUEZ-QUILÓN,§
H. LAGRAULET,*† F. EHRENMANN,*† L. BOUFFIER,*† J. M. GION,*¶ D. GRIVET,§ M. DE MIGUEL,*†
N. DE MARÍA,§ M. T. CERVERA,§ F. BAGNOLI,** F. ISIK,†† G. G. VENDRAMIN** and
S. C. GONZÁLEZ-MARTÍNEZ§

➔ 9k Illumina Infinium SNP array developped in 2016 by Plomion *et al.* (previously available SNPs + EST data + candidate genes)

Next stage: Genotyping by Sequencing (GBS)?

80 SNPs for applied researches

9,000 SNPs available

5,652 polymorphic SNPs

169 SNPs with MAF>0.45 in the breeding population

121 SNPs with low LD (<0.3)
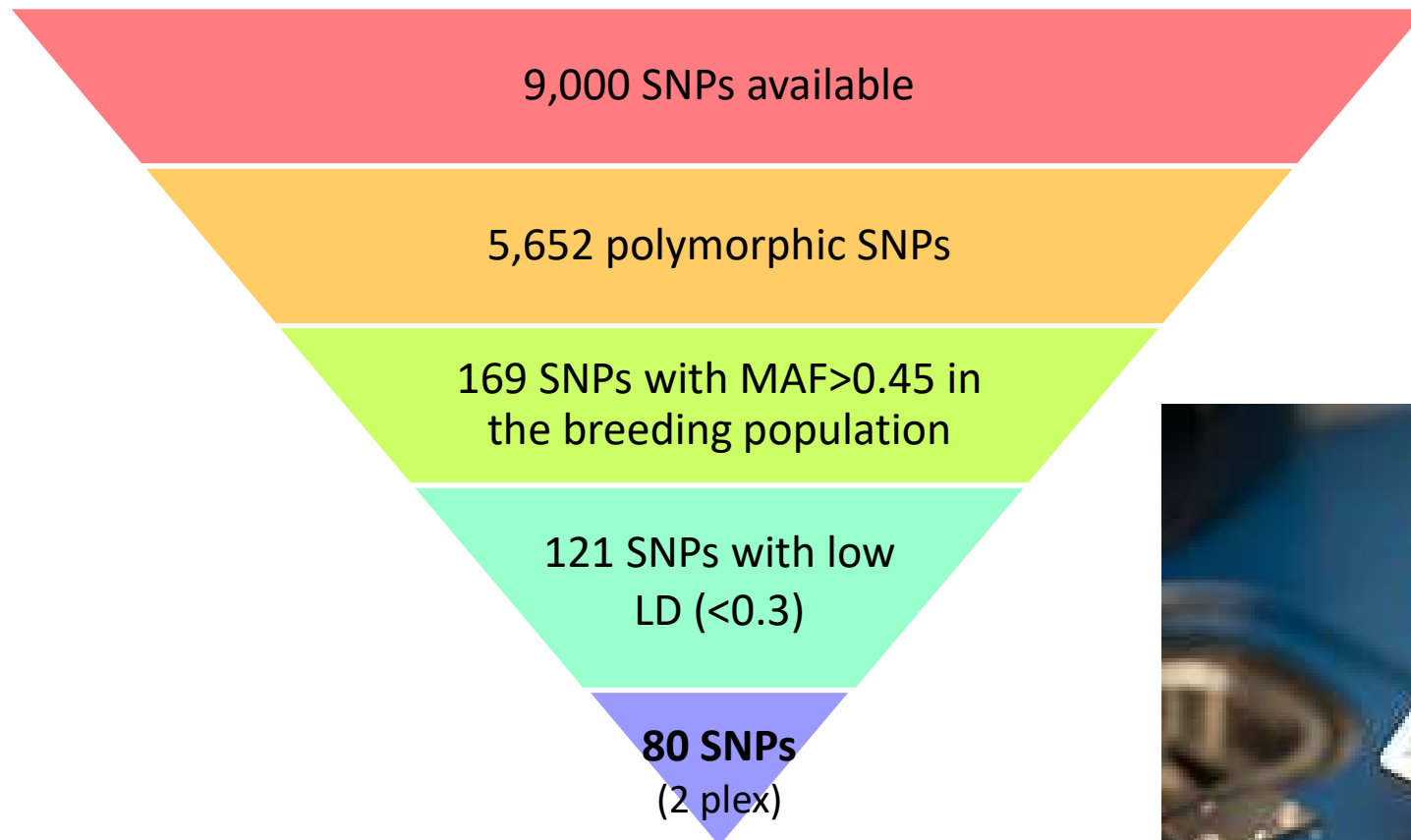
80 SNPs
(2 plex)

Genotyping using Sequenom's Mass Array technology (mass spectroscopy)

(cost ~8.5€ per sample)
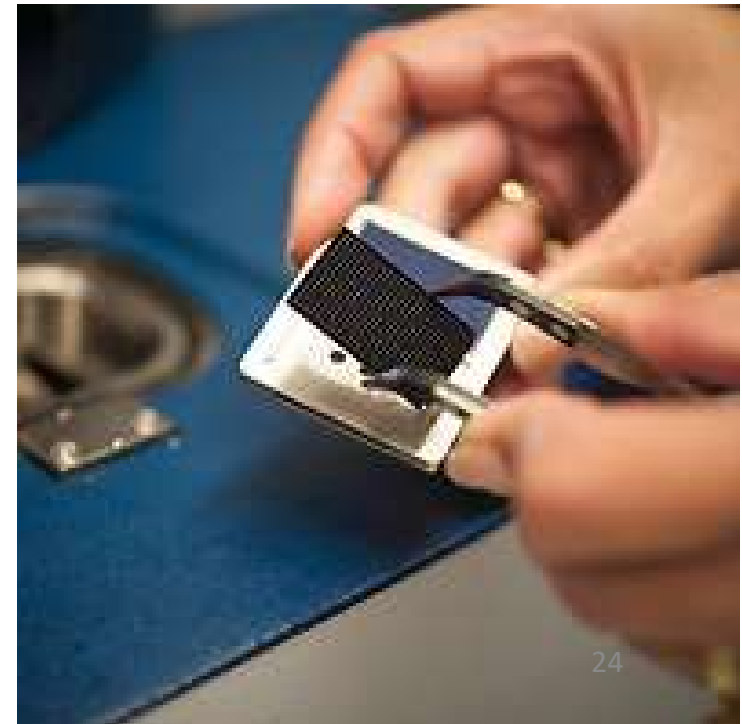
80 SNPs to check id / pedigree

Parental recovery carried out on 3,009 G2 trees (from several polycross trials)

mother id confirmed + father recovered + grandparents' pedigree validated	2174	72%
mother id confirmed + father recovered + grandparents' pedigree not validated	242	8%
mother id confirmed + father not recovered	348	12%
mother id not confirmed	245	8%

Mistakes are cumulative since the beginning of the breeding programme (~ 50 years ago) and can have various origins.

Objectives :
- « id card » for each genotype in clonal archives
  - ➔ **3-year-project (2018-2020) to genotype 7,000 trees**
- **to correct (when possible) the pedigree mistakes in Dataplan**
- **Field labelling with bar codes**

➔ **Higher accuracy for the breeding values**
➔ **1st mandatory step to implement MM in breeding programs**

Collecting grafts on selected trees — E₁

Grafting in nursery — E₂

Planting in clonal archives (several ramets per genotype) — E₃

Crosses
Pollen X Female flowers
Collecting, drying, storing
Isolation — E₄

Collecting cones, drying, seeds extraction, storing — E₅

Seedling in nursery — E₆

Establishment of progeny trial — E₇

(from Marjorie Vidal PhD, 2016)
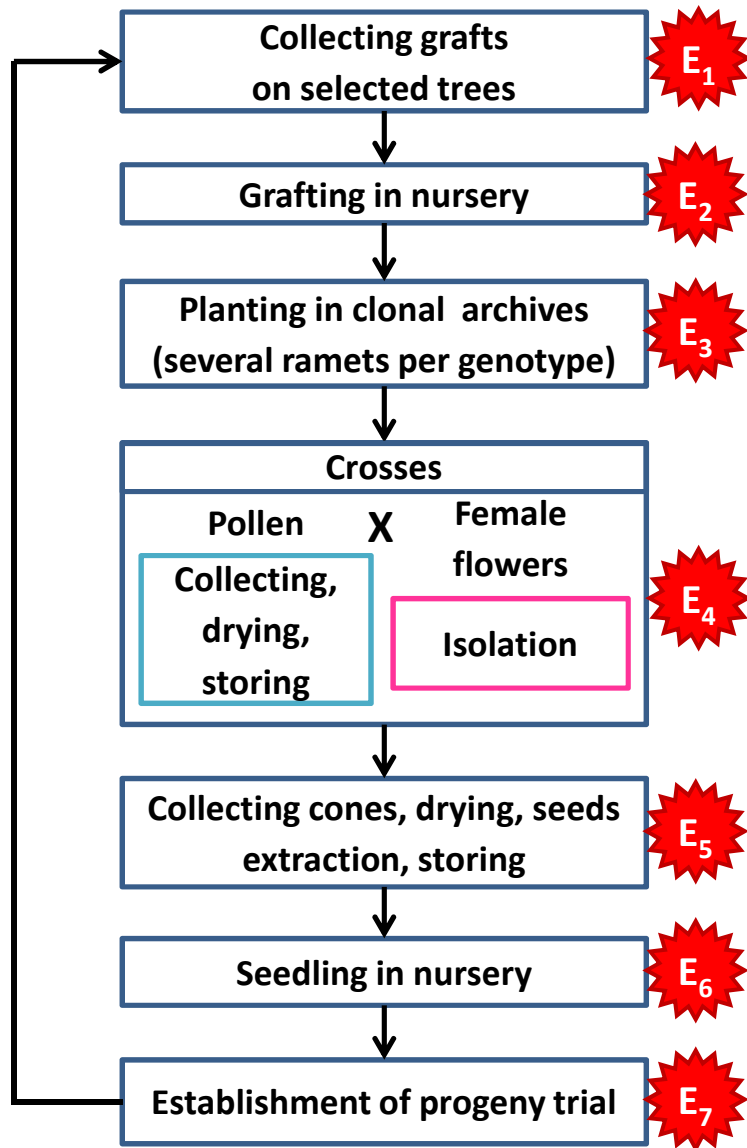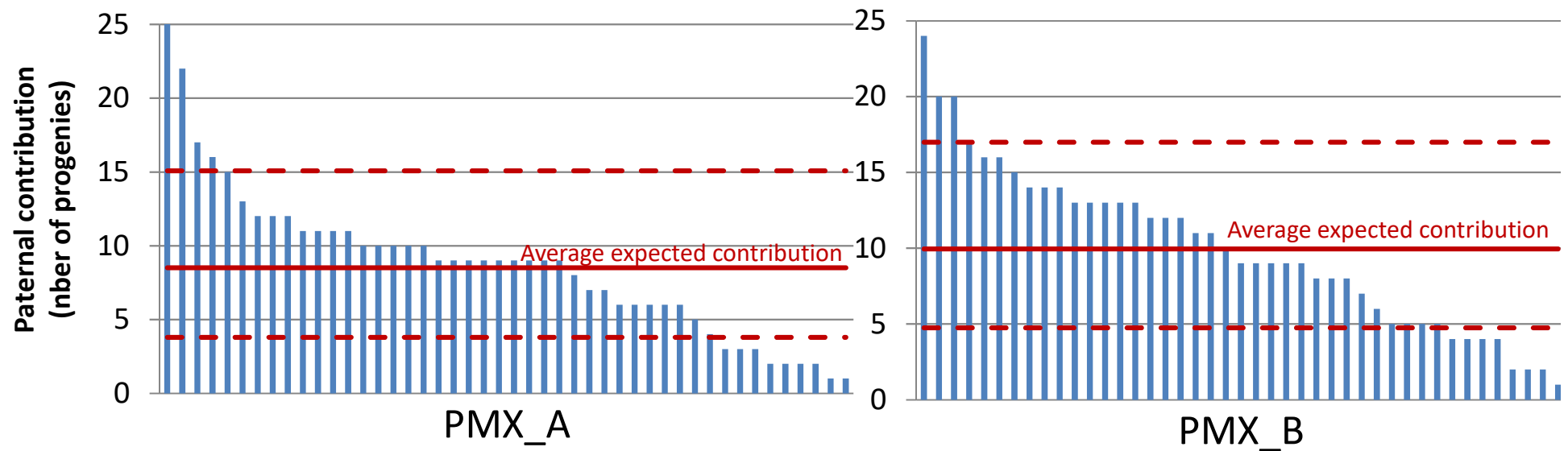
➢ Various sources of identity errors

➢ It can be difficult to detect the error origin

➢ Cumulative process over generations

80 SNPs to recover pedigree in PMX

> *Significant departure from equal contribution only due to few fathers*

> **Pedigree recovery allows forward selection in PMX trials**

80 SNPs to study pollen contamination in SO

- Pollen contamination and parental contribution analysed in open-pollinated SO

- 3 parameters analysed:
  - SO location
  - Pollination year
  - Maternal genotype
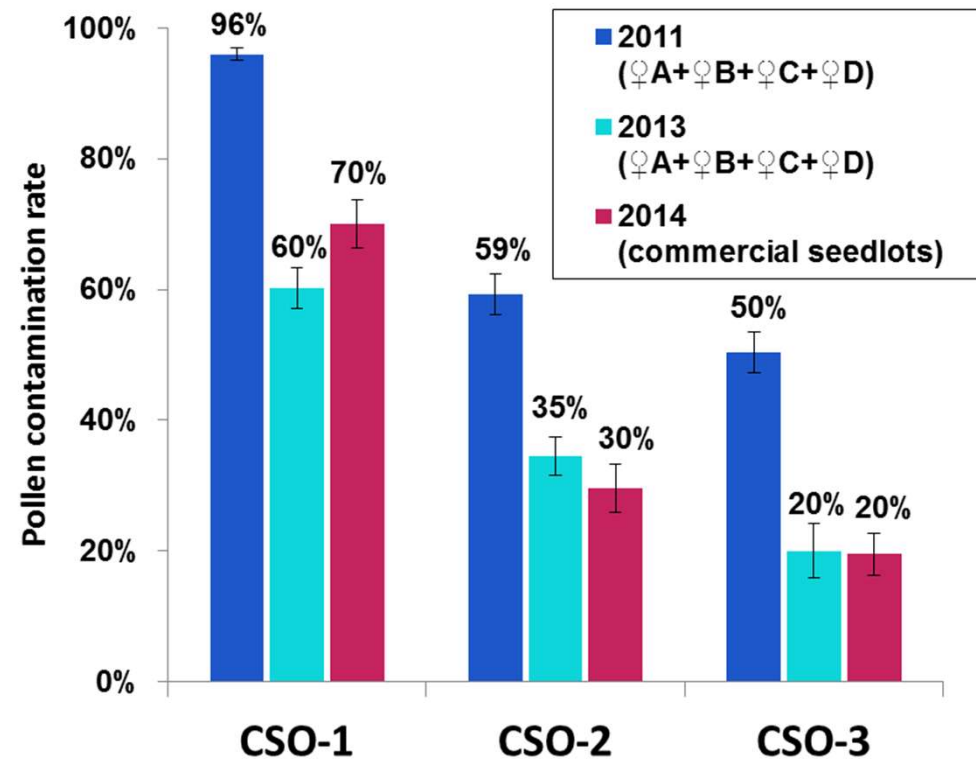
> **High pollen contamination rate (mean for the 3 orchards over three years = 49%) which decreases genetic gains (expected genetic gains drop from 30% to 24%)**

> **For the future:**
  - explore reproductive success to understand heterogeneous parental contribution
  - explore annual variation in pollen contamination (climatic data)
  - develop methods to decrease pollen contamination (orchard location, tent isolation, supplemental mass pollination…)

Low density genotyping (80 SNPs) now used for:

➢ **« Id card » for each genotype in clonal archives**
  - ✓ Check and correct id errors in clonal archives and in Dataplan
  - ✓ Genotyping of each new selection
  - ✓ Less pedigree errors in the futur crosses

➢ **Use pedigree recovery for forward selection in PMX trials**
  - ✓ Promote existing trials
  - ✓ New breeding strategies can be designed using this new tool

➢ **Enhance studies in SO to better understand pollen contamination and parental contribution**
  - ✓ Deployment stage poorly studied until now
  - ✓ More collaboration needed with SO managers to collect data on phenology and seed productivity

High density genotyping for Genomic Selection

DEVELOPMENT OF PREDICTIVE MODEL

Cross validation

GENOTYPES • SNP data

PHENOTYPES • Trait data

Predictive models

$$Y = Xb + Zh + e$$

Predictive model updating

Field trial and phenotype

"TRAINING" POPULATION
(e.g. progeny trial N ≥ 1,000 of a breeding population with $N_e = 60$)

BREEDING

GENOMIC SELECTION CYCLE

Flower induction of top ranked GEBV seedlings

GENOTYPES • SNP data

Selected seedlings

Clonal trial of top ranked GEGV seedlings

Elite clones

SELECTION CANDIDATES
(e.g. 100 full or half-sib families of 100 offspring each = 10,000 seedlings)

Genomic selection

BMC Genomics

RESEARCH ARTICLE **Open Access**

CrossMark
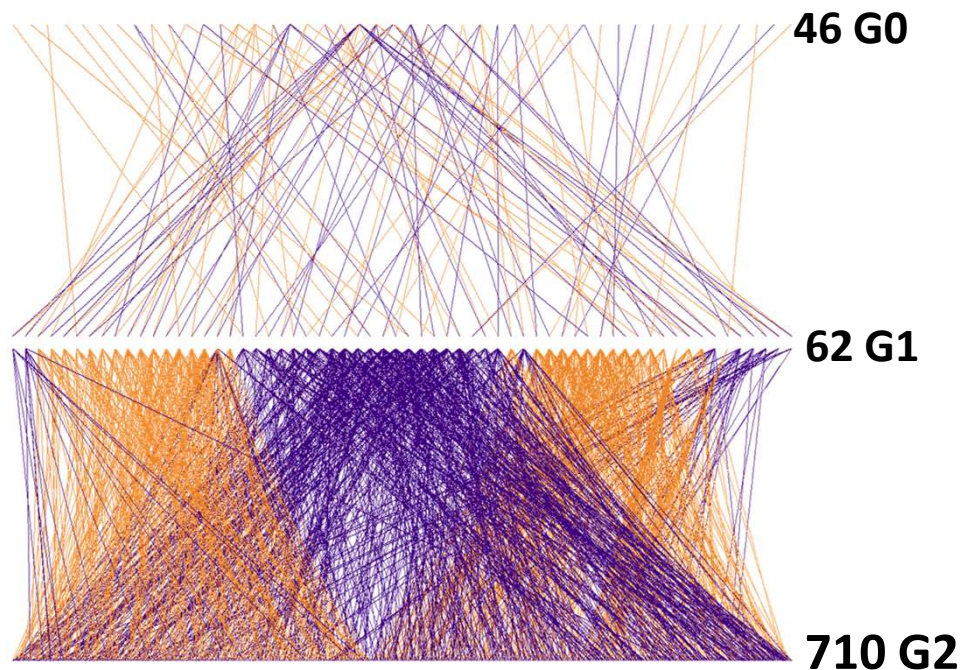
Performance of genomic prediction within and across generations in maritime pine

Jérôme Bartholomé[1] iD, Joost Van Heerwaarden[2], Fikret Isik[3], Christophe Boury[1], Marjorie Vidal[1,4],
Christophe Plomion[1] and Laurent Bouffier[1*]

46 G0

62 G1

710 G2

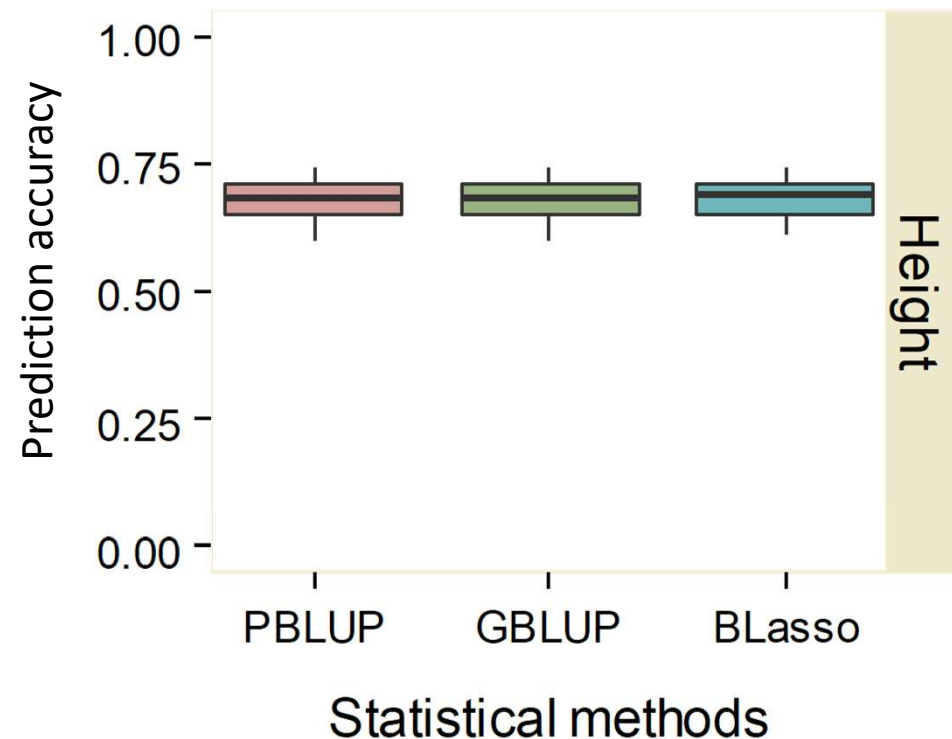➢ 818 individuals (Ne=24)
➢ G2 + all their ancestors (G0 + G1)
➢ G2: 32 HS families (≈ 22 individuals)
      355 FS families
➢ 4,300 SNPs
➢ Growth, sweep

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Genomic selection

Training population:
G0 + G1 + 80% G2

Validation population:
20% G2

> Similar accuracy for the pedigree-based method (PBLUP) and marker-based methods (GBLUP and Blasso)
> Weaknesses: low nber of trees per FS families; no clonal replication for phenotyping

Use of MM in the maritime pine breeding program

Operational

Short term
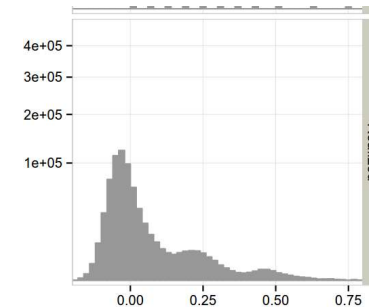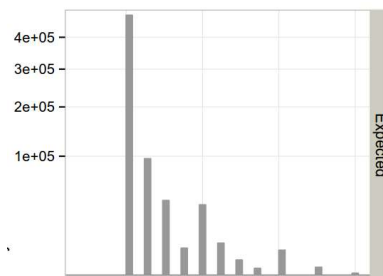
Mid-term

1/ Identities / pedigree
- Higher accuracy for BLUP evaluation
- First mandatory step to implement MM in the breeding program

2/ Polymix breeding with paternity recovery
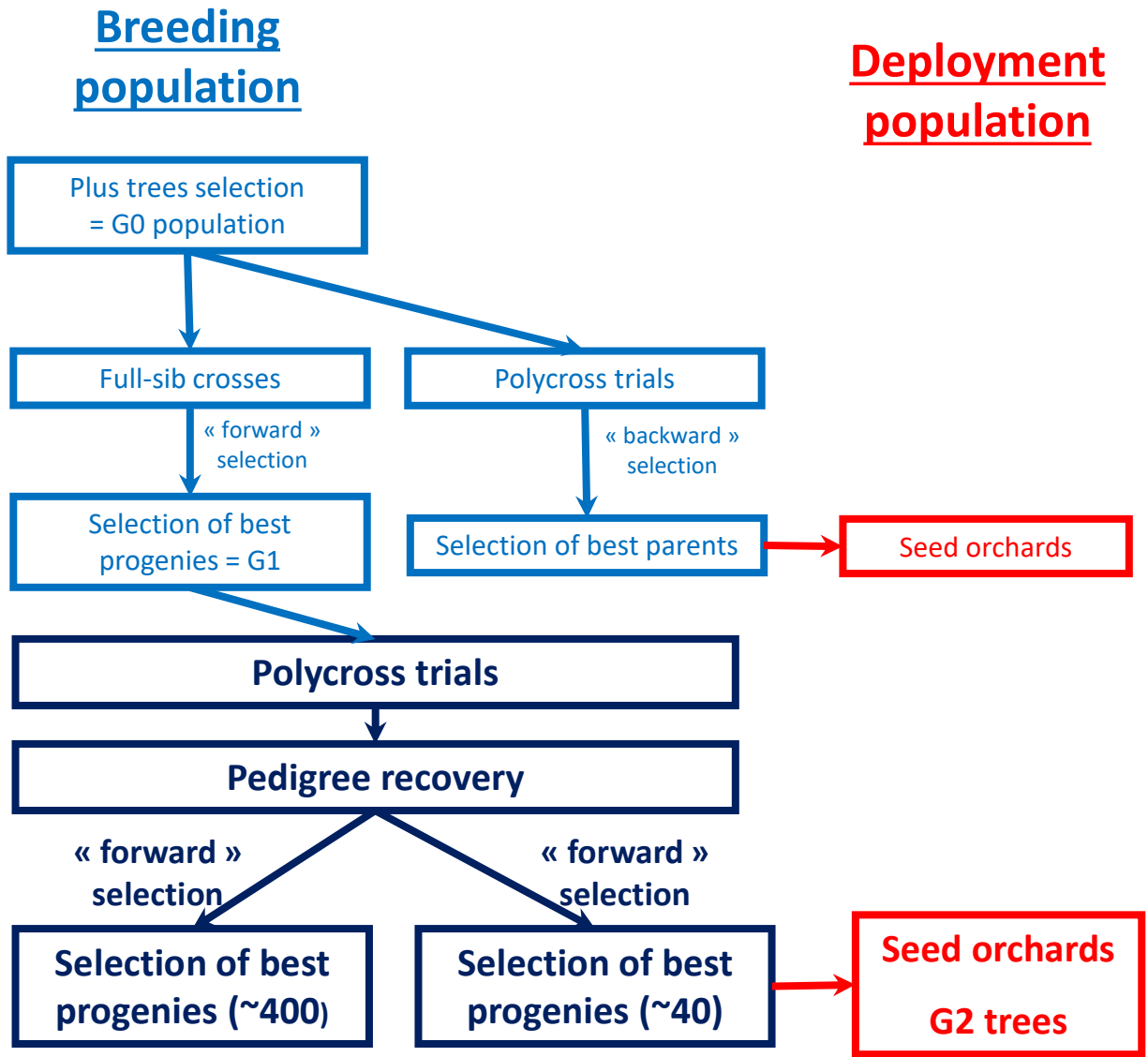- To simplify the mating process and maximize number of families evaluated
- Optimization needed with simulations

3/ Genomic selection
- Accuracy mainly due to pedigree recovery
  - → Increase number of trees per FS families and number of markers to be able to predict mendelian sampling
- Implementation of realized genetic relationship (single-step method) to combine pedigree and marker information

Breeding strategies simulations to implement MM

Breeding population

Deployment population

```
Plus trees selection
= G0 population
```

```
Full-sib crosses                    Polycross trials
```

« forward » selection   « backward » selection

```
Selection of best                   Selection of best parents  ───►  Seed orchards
progenies = G1
```

```
Polycross trials
```

```
Pedigree recovery
```

« forward » selection   « forward » selection

```
Selection of best          Selection of best          Seed orchards
progenies (~400)           progenies (~40)   ───►
                                                      G2 trees
```

Polymix breeding with paternity analysis

(Lambeth *et al.*, 2001)

Breeding strategies simulations to implement MM

- **Advantages of polymix breeding with paternity recovery:**
  - Simplification of the mating design
  - Maximization of crosses
  - Avoid pedigree mistakes
  - Accelerate deployment of genetic gains
- **Simulations (Popsim, stochastic model) / various breeding contexts**
  - Number of polymix / number of trees per family
  - Polymix composition (number of fathers, relatedness, genetic merit)
  - Cost / benefit analysis to assess economical efficiency of this strategy
- **Simulations for genomic selection implementation**
  - Determinist models
  - Nber of markers, training population design

Conclusion

➢ **Rolling front strategy with more emphasis on gain (vs. diversity)**

➢ **Deployment more dynamic (SO renewed more often + management intensification)**

➢ **New selection criteria under study (climate change and disease resistance)**

➢ **Low genotyping data implemented in the program**

➢ **High genotyping under study: data set available for H-matrix**

➢ **Simulations to anticipate the evolution from "traditional" breeding towards the use of MM**