

Pollen contamination and mating structure in maritime pine clonal seed orchards



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Maritime pine: first plantation species in France

0.8 million hectares (Landes forest, southwestern France)

- maritime pine (*Pinus pinaster* Ait.): fast growing species (rotation = 35 years) adapted to the Landes region (poor sandy soils, hydromorphic soils in winter, dry summers)
- 24% of French wood harvest (60% saw timber, 40% industrial wood)



A three-generation advanced breeding program

- developed from a base population selected in the Landes forest (~600 plus trees)
- recurrent breeding scheme, one breeding zone

Seed stocks: near 100% from seed orchards

- expected genetic gains: +30% for growth and +30% for stem straightness
- clonal and family open-pollinated seed orchards
- 41 millions seedlings produced in 2015-2016

Sampling procedure

Table 1: 2,552 seeds collected from 3 seed orchards over 3 years

Seed Orchard	Maternal genotype	Pollination year	Seed nber
CSO-1 [St Laurent2-VF3] surrounded by maritime pine plantations	center	2011	240 (60♀)
	border		240 (60♀)
	center	2013	116 (27-30♀)
	border		120 (30♀)
CSO-2 [Beyhac-VF3] some maritime pine plantations several km away	-	2014	147
	commercial seedlot		147
	♀A+♀B+♀C+♀D	2011	240 (60♀)
			2013
20♀	2013	590 (27-30♀)	
commercial seedlot		2014	142
CSO-3 [St Sardos-VF3] no maritime pine plantations within 20km	♀A+♀B+♀C+♀D	2011	240 (60♀)
			2013
	-	2014	149
			commercial seedlot

OBJECTIVES = Evaluate pollen contamination and paternal contributions according to (see Table 1):

Seed orchard location

3 clonal seed orchards (CSO) established with the same genotypes (CSO-1 in the Landes forest; CSO-2 in the outskirts of Landes forest; CSO-3 outside the Landes forest); 2 sampling zones in CSO-1 (center vs. border)

Pollination year

2011, 2013 and 2014 (i.e. seeds sampled in autumn 2012, 2014, 2015)

Maternal genotype

sampling either on 4 maternal genotypes with contrasted female phenology (♀A+♀B+♀C+♀D); or 20 random maternal genotypes (20♀); or with no consideration for maternal genotype (commercial seedlots)

Pedigree recovery

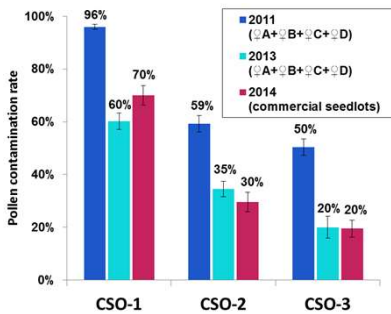
SNP genotyping

- DNA extracted from offspring (2,552 seedlings) and seed orchard parental genotypes (2 ramets per genotype)
- 2 plex (40 SNPs each) developed by Vidal et al. (2015): MAF > 0.45 and LD < 0.3
- genotyping using the Sequenom MassARRAY iPLEX Gold assays

Likelihood inference methodology (Cervus 3.0.7)

- paternity analyses when the mother is known
- parental analyses when both parents are unknown (commercial seedlots)
- assignment at 99% level of confidence (with maximum 1 mismatch)

Fig.1: Pollen contamination rate per orchard and per year



High pollen contamination

- Pollen contamination: CSO-1 >> CSO-2 > CSO-3 (Fig.1) can be explained by 3 parameters:
 - vicinity of maritime pine plantations
 - orchard age (CSO-1 established in 2006 vs. 2002-2003 for CSO-2 & 3)
 - CSO pedo-climatic conditions: CSO-2 and CSO-3 are early-flowering in comparison to CSO-1
- Pollen contamination: 2011 >> 2013 ≈ 2014 (Fig.1):
 - mainly due to climatic effects (precipitation, wind...) and partially to seed orchard age
- No significant variation in pollen contamination between CSO-1 center and border (data not shown)
- Pollen contamination varies significantly with maternal genotype (Fig.2)
- No correlation between female reproductive phenology and pollen contamination rate ($r = -0.15$, $p = 0.51$) (Fig.2)

Fig.2: Pollen contamination rate vs. phenology per maternal genotype (CSO-2, 2013)

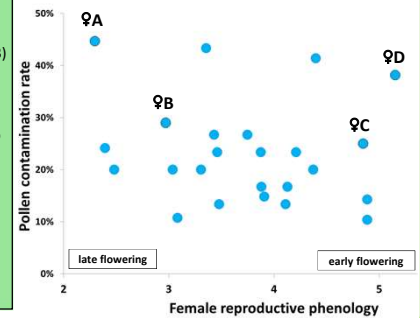


Table 2: Genetic diversity

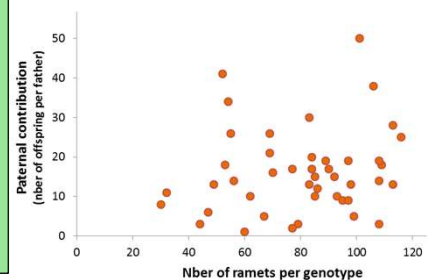
	CSO-1	CSO-2	CSO-3
N (census nber of genotypes)	46	47	48
N _{eff} (effective nber of genotypes)	39.1	43.1	38.6
N _{eff,father} (effective nber of fathers; 2011-2013)	14.6	31.2	13.1
N _{eff,father,cont} (effective nber of fathers including pollen contamination*; 2011-2013)	670.1	71.9	41.8

* each immigrant pollen is considered as a unique father

Heterogeneous parental contribution

- All genotypes contribute as father in the offspring
- Mean selfing rate in the three orchards = 5.4% (highly variable depending on genotype)
- Heterogeneous paternal contribution not related with the number of ramets per genotype (Fig.3)
- N_{eff} < N due to a variable number of ramets per genotype (Table 2)
- N_{eff,father} < N_{eff} due to heterogeneous paternal contribution (Table 2)
- Contamination increases genetic diversity in the CSO seedlots (N_{eff,father,cont}), specially in CSO-1 (Table 2)

Fig.3: Number of ramets per genotype vs. paternal contribution (CSO-2; 2011 & 2013)



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Conclusion and prospects

- 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...)

