

Pollen contamination and mating structure in maritime pine clonal seed orchards



BOUFFIER Laurent^{a,b}, DEBILLE Sandrine^c, ALAZARD Pierre^c, PASTUSZKA Patrick^d, RAFFIN Annie^{a,b}, HARVENGT Luc^c, LELU-WALTER Marie-Anne^e, MUSCH Brigitte^f, TRONTIN Jean-François^c



^a INRA, BIOGECO, UMR 1202, 33610 Cestas, France
^b University Bordeaux, BIOGECO, UMR 1202, 33400 Talence, France
^c FCBA, Biotech & Advanced Forestry Dpt., 33610 Cestas, France
^d INRA, Experimental Forest Unit Pierroton, UE 570, 33610 Cestas, France
^e INRA, UAGPF, UR 588, 45075 Orléans, France
^f CGAF, USC ONF-INRA, 45075 Orléans, France

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	-	commercial seedlot	147
	♀A+♀B+♀C+♀D	2011	240 (60/♀)
	20 ♀	2013	238 (58-60/♀)
	commercial seedlot	2014	142
CSO-3 [St Sardos-VF3] no maritime pine plantations within 20km	♀A+♀B+♀C+♀D	2011	240 (60/♀)
	20 ♀	2013	90 (17-29/♀)
	commercial seedlot	2014	149

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

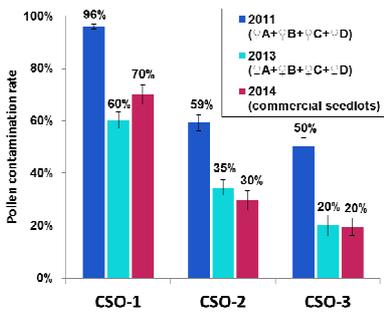
SNP genotyping

- 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)

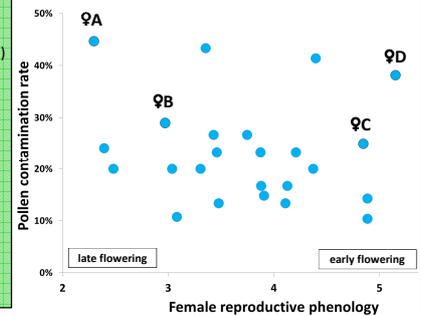


Table 2: Genetic diversity

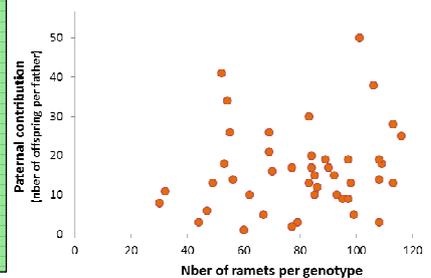
	CSO-1	CSO-2	CSO-3
N (census nber of genotypes)	46	47	48
N _{eff} (effective nber of genotypes)	39.1	43.1	38.6
N _{eff, father} (effective nber of fathers; 2011-2013)	14.6	31.2	13.1
N _{eff, father, cont} (effective nber of fathers including pollen contamination*; 2011-2013)	670.1	71.9	41.8

* 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)



Acknowledgements: This project was supported by the French Ministry of Agriculture (QUASEGRAINE/2014-352/coord. ONF) and funds from Region Nouvelle Aquitaine (IMAF/12009468-052/coord. FCBA) & Region Centre-Val de Loire (IMTEMPERIES/2014-00094511/coord. INRA). We thank the Maritime Pine Breeding Cooperative (Groupe Pin Maritime du Futur) for its support through the FORTIUS project (coord. INRA) granted by Region Nouvelle Aquitaine and the French Ministry of Agriculture. SNP genotyping was performed at the Bordeaux Genomic Facility granted by Region Nouvelle Aquitaine (20030304002FA, 20040305003FA). FEDER funds from European Union (2003227) and ANR (XYLOFOREST/ANR-10-EQPX-16).

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...)

