



New insights in seed orchards pollen contamination, study case in an advanced breeding program

Ricardo Alia based on peer reviews by **Eduardo Notivol** and 1 anonymous reviewer

Laurent Bouffier, Sandrine Debille, Pierre Alazard, Annie Raffin, Patrick Pastuszka, Jean-François Trontin (2023) Pollen contamination and mating structure in maritime pine (*Pinus pinaster* Ait.) clonal seed orchards revealed by SNP markers. bioRxiv, ver. 2, peer-reviewed and recommended by Peer Community in Forest and Wood Sciences.

<https://doi.org/10.1101/2022.09.27.509769>

Submitted: 29 September 2022, Recommended: 08 August 2023

Cite this recommendation as:

Alia, R. (2023) New insights in seed orchards pollen contamination, study case in an advanced breeding program. *Peer Community in Forest and Wood Sciences*, 100109. [10.24072/pci.forestwoodsci.100109](https://doi.org/10.24072/pci.forestwoodsci.100109)

Published: 08 August 2023

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This preprint (Bouffier et al, 2023) analyses different biological (tree genotype, age, flowering phenology) and environmental factors (vicinity with external pollen sources, orchard structure, soil type, climatic conditions) with influence on the of seed lots in seed orchards of an important forest tree species (*Pinus pinaster* Ait.). The analysis is based on an optimized set of 60 SNP markers that constitute a new tool for characterizing improved material in the breeding program of the species.

One of the main questions when managing seed orchard is to obtain a precise estimation of pollen contamination, as it causes major losses to genetic improvement from selection and breeding (Di Giovanni and Kevan, 1991) but also will determine the adaptive potential of the species (Kremer et al. 2012). The results indicate that contamination rates were highly variable between seed lots (from 20 to 96%), with a mean value of 50%. The main factors determining these rates include the distance between the seed orchard and external pollen sources, rain during the pollination period, seed orchard age, soil conditions and seed parent identity.

A second point of interest in this paper is the determination of the overall self-fertilization rate. This factor also determines the quality of the seed-lots and was estimated as 5.4%, with high variability between genotypes (from 0% to 26%). The overall value is of the same order of magnitude than in other species.

These results are used to define some recommendations for managing seed orchards in the French breeding program, but that can be generalized to other species (eg. Mullin and Lee, 2013). As an example, they recommend that sampling 100 seeds annually should be sufficient to estimate pollen contamination (with a

standard error of 5%). Also, they suggest that one of the main measures to reduce pollen contamination is carefully selecting the location of the orchard, in terms of its distance from external pollen sources and soil conditions, and not collecting seeds from young trees (below 8 years old).

The present preprint revisits an important topic of research with interest for the biology of tree species, but also with great implications in applied breeding activities. The main conclusions are essential to understand the importance of different factors in managing seed orchards and in the future performance of the reproductive material.

In conclusion, this paper stresses the need for more studies, taking advantage of new genomic tools, to advance the knowledge of factors influencing the success of breeding programs.

References:

Bouffier L, Debille S, Alazard P, Raffin A, Pastuszka P, Trontin JF (2023). Pollen contamination and mating structure in maritime pine (*Pinus pinaster* Ait.) clonal seed orchards revealed by SNP markers. bioRxiv, 2022.09.27.509769, ver. 2 peer-reviewed and recommended by Peer Community in Forest and Wood Science. <https://doi.org/10.1101/2022.09.27.509769>

Di-Giovanni F, Kevan PG (1991) Factors affecting pollen dynamics and its importance to pollen contamination: a review. *Can J For Res* 21(8):1155-1170.
<https://doi.org/10.1139/x91-163>

Kremer A, Ronce O, Robledo-Arnuncio JJ, Guillaume F, Bohrer G, Nathan R, Bridle JR, Gomulkiewicz R, Klein EK, Ritland K, Kuparinen A, Gerber S, Schueler S (2012) Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecol Lett* 15(4):378-92.
<https://doi.org/10.1111/j.1461-0248.2012.01746.x>

Mullin TJ, Lee SJ (2013) Best practice for tree breeding in Europe. Skogforsk, Uppsala, Sweden. ISBN: 530 978-91-977649-6-4. <https://www.skogforsk.se/contentassets/42acda01f83843bf925f690bd0a6ed37/best-practice-hela-low.pdf>

Reviews

Evaluation round #1

DOI or URL of the preprint: <https://doi.org/10.1101/2022.09.27.509769>

Version of the preprint: 1

Authors' reply, 21 June 2023

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Decision by [Ricardo Alia](#), posted 17 February 2023, validated 17 February 2023

The paper could be accepted with a minor revision

The paper covers a very interesting topic for managing seed orchards, and the extensive study is highly relevant for the area of research. As one of the reviewers points out, there are minor suggestions to improve the manuscript, dealing with a more detailed description of objectives and reducing the discussion. But the quality of the research and writing allows the acceptance of this manuscript.

Reviewed by Eduardo Notivol, 14 October 2022

The paper deals with a very interesting topic and it provides important information and methodology to tackle with pollen contamination in pine seed orchards.

The introduction and the plot are well presented, trying to shed some light with updated and improved methods to quantify the amount of external pollen in the improved seed production. Selfing, as a different issue for inbreeding and genetic diversity reduction is considered as well.

The three sampling strategies used provide sound results on the problem studied, in particular the number of genotypes included in the study and the use of SNPs for it.

The variability of the results in the three orchards monitored is huge and it is properly discussed on the basis of distance of surrounding stands of the same species, pedological characteristics, age (intensity of flowering) of the ramets and meteorological factors (dryness).

In general, after reading the manuscript, the main idea is that pollen contamination is high, mean rate of 50% with peaks until 96%, and this mean value is reflecting the early? age of the three seed orchards (10-11 years old in the best case), so is it expected, perhaps, a higher contamination in the near future when the orchards reach the maximum size, flowering intensity and productivity?. I suggest including some comment about it in conclusions.

The sentence "*Assuming that the foreign pollen comes from unimproved stands, we calculated that the expected genetic gain would fall from 30% to 24%...*" needs to be explained in deep. Is because the expected gg of 30 % took into account a similar rate of contamination that the obtained in the manuscript?

All in all IMHO, if the las two raised questions could be elucidated, the draft is worth for publication in a high impact journal.

Reviewed by anonymous reviewer 1, 15 February 2023

The manuscript offers an thorough experimental assessment on pollen contamination in Pinus pinaster seed orchards in France. From my limited expertise in this particular field, I believe the experimental design and data analysis are both flawless. The paper is well written in a correct, neutral style, so it generally reads well with the exceptions that will be deailed below.

My main concern about the manuscript is how the main questions, results and discussion is presented and organized, and also a (perhaps) excessive will to explain everything beyond the possibilities of the experiment.

The reader finds too often relevant (or highly expectable) results that were not properly detailed in the objectives section at the end of the introduction. Fistly, the key effect of the sorrounding forest populations among the three seed orchards cannot be presented as a "finding" but rather as a confirmation of the expected result. Second, the effects of plant age and the location within the orchard are not even mentioned in the objectives, but they are mentioned both in the results and discussion. Similarly, environmental factors related to the yearly differences only pop up at the end of the discussion. Why not mentioning them from the very beggining?.

This drawbacks are indeed related with a too long discussion, that could be shortened with a closer match between the results and hypotheses / expectations. Finally, the conclusions MUST be reduced considerably, such that only real conclusions are left, without repeating the argumentation of the discusion and keeping a lower level of speculation.

Specific comments:

Line 28: avoid too vague wording like "Several interpretative factors were highlighted". Declare exacly which ones instead.

Line 33: Similarly: "considerable variability" is not specific enough

Line 35: Similarly "identifying new research perspectives" is too vague. Please, be more specific.

Line 62: This is the first mention of the core question of the paper. I find this is too demanding for the reader. An earlier mention would help

Line 74: "easy to multiplex", is this right??

Lines 79-84. This part needs a complete rewriting in the line stated above: declare hypotheses / expectations related to the effect of the surrounding stands of the species, the environmental effects, the position within each orchard and the age of the orchard plants.

Line 122 (Table). I think including here the distance to the nearest large maritime pine stands would improve the reading.

Line 192. Please avoid acronyms whenever not necessary. Here use "seed orchard" instead.

Line 197-198: This cannot be shown as something "unexpected", but rather the confirmation of a reasonable expectation.

Line 200: I think this result highly relevant, and actually it is mentioned later on in the discussion. Therefore, I think enough importance should be given in the objectives, and showing the figures.

Line 230. Was this mentioned before as an objective??

Line 264-267: I recommend something more "catchy" to start the discussion, giving a clearer idea of your contribution beyond the previous knowledge.

Line 288: As expected....

Line 298-300: Again, highly expectable

Line 301. I suggest starting by declaring the evident importance of reproductive phenology, and then how the soil and climate conditions affects it.

Lines 308-309. As already stated, give plant age the necessary relevance throughout the work, or remove it!

Lines 318-319: The same thing about position within the orchard

Lines 328-332: Idem about yearly rainfall

Lines 351-352. Again, a totally new idea that had not been mentioned elsewhere (unless I'm wrong!)

Lines 357-390. Forgive me, but this is fine example of what IS NOT a Conclusions section. Extremely long and little conclusive. Please, reduce it to one fourth, and let it be real conclusions.