

Dear Editor,

Thank you for your positive feedback and for the very pertinent reviewers' comments. You will find below our answers to each comment. They helped us to improve the manuscript and hopefully to make it clearer.

The new manuscript is attached with and without tracked changes.

Sincerely,

Laurent Bouffier

1- Comments by Ricardo Alia

“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 point out, there are minor suggestions to improve the manuscript, dealing with a more detailed description of objectives and a reduction in the discussion. But the quality of the research and writing allow the acceptance of this manuscript.”

We have modified the initial manuscript following the reviewers' recommendations. We have re-organized the introduction and the discussion parts. More specifically, the objectives are now better detailed in the introduction and the discussion has been shortened (the conclusion part was removed). We now give more information about tree age in Table 2 and we present in Figure 1b the results on pollen contamination rates within seed orchard.

2- Comments by Eduardo Notivol

“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.”

Yes, the intensity of flowering in maritime pine increases with tree age but becomes optimal for commercial harvesting as early as from 8-10 years. For pollination year 2011, CSO-1 (only 5 years old) showed very high pollen contamination rates compared to CSO-2/CSO-3 (8-9 years old) and pollen contamination observed 2 and 3 years later in CSO-1 was significantly reduced. Therefore, our data suggest that that pollen contamination rates decrease with tree maturity. It would be necessary to study older orchards to deepen the effect of seed orchard age on pollen contamination but we currently speculate that a lower rate of fertile male and female strobilus is likely in CSO-1 compared to CSO-2 and CSO-3. Internal pollen flows at CSO-1 at age 5 years (pollination year 2011) could be insufficient to compete both quantitatively and qualitatively with external sources and result in very high rates of pollen contamination.

Therefore, to address your comment we added in Table 2 the age of seed orchard for each pollination year. And we mentioned (now in the last part of the discussion, line 405) that a way to reduce pollen contamination rates would be to avoid collecting seeds from young trees although it would be necessary to complete our data with studies on orchards of contrasting ages (especially older orchards) to define more precisely the effect of seed orchard age on contamination.

“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?”

No, 30% is the expected genetic gain considering no pollen contamination in the orchards and also that the contributions of the parental genotypes are random/balanced (the two major assumptions to achieve the expected genetic gain, see lines 289-291).

To make this sentence more meaningful, we have detailed the argumentation lines 291-299. Furthermore, we have corrected a mistake: assuming pollen contamination rate of 50%, the genetic gain drops to 22.5% and not 24% as written in the initial manuscript.

3- Comments by anonymous reviewer

“The manuscript offers a 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 detailed 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. Firstly, the key effect of the surrounding 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 beginning?"

We rewrote the objectives section at the end of the introduction (lines 86 to 96) and the abstract accordingly (lines 24-26).

"These drawbacks are indeed related with a too long discussion, that could be shortened with a closer match between the results and hypotheses / expectations."

The discussion section has been re-organized to better fit with the objectives expressed at the end of the introduction and subtitles were added.

Finally, the conclusions MUST be reduced considerably, such that only real conclusions are left, without repeating the argumentation of the discussion and keeping a lower level of speculation."

We finally considered the current conclusion to be entirely a discussion of the prospects for optimizing the deployment of future seed orchards. We have moved it to the end of the discussion.

Specific comments:

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

We rewrote this sentence in a more direct style. "Interpretative factors included ..." (line 30)

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

We rewrote this sentence (line 34) to specifically include the observed "considerable variability" (from 0% to 26%).

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

We added in text (lines 34-38) the main issues for both seed orchard management (seed orchard location, soil and climate optimal conditions, minimum age for commercial seed lots harvesting) and for identifying new research perspectives (exploring links between pollen contamination and climatic data, genetic control of flowering traits).

"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."

We re-organized the introduction to mention the core question earlier (see lines 69-71).

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

Yes, we used the iPLEX® Gold assay available from Sequenom, Inc. (San Diego, CA). This multiplex SNP genotyping platform allows for assaying up about 40 markers in 384 individuals at the same time.

“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.”

We re-wrote the end of the introduction (see lines 86-96).

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

We added the column “Nearest maritime pine stands” in Table 1 to complete the qualitative information given in the text (lines 108-114).

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

The CSO acronym (for Clonal Seed Orchard) has been introduced at the end of the introduction (line 93) and used throughout the manuscript. We have added an acronym list (including CSO).after the abstract

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

We think we use a sufficiently neutral style here. We are just stating the result (contamination rate significantly higher in CSO-1) and there is no indication that this is unexpected result. However, we have adjusted the discussion (l.333) to mention that this result was expected.

“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.”

We now mention in the introduction (line 91) ‘localization within the orchard’ as one of the factors that has been addressed in our study. We have introduced a new figure (Figure 1b) to illustrate this result (no significant difference was found in contamination rates between the two sampling zones, central vs. border) and this result is discussed lines 342-345.

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

The number of ramets (genotype representativeness) has been added in the introduction (objective section) as a factor considered for interpretation of results.

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

The discussion has been re-organized in-depth. We now start the discussion with the main results of the study and their consequences on the genetic gain and diversity in seed orchards.

“Line 288: As expected ...” and “Line 298-300: Again, highly expectable”

We rewrote the sentence (line 333) to anticipate that the location of the CSO is a key factor to explain pollen contamination.

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

We have modified the sentence line 345. However, the soil condition and the orchard location are two confounded effect in our study which justifies to keep these factors altogether. This is now explained lines 350-352.

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

Done in the introduction and throughout manuscript (for example a new column with tree age was added in Table 2) as tree age is indeed a main factor investigated in our study.

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

Done in the introduction and throughout manuscript. Orchard structure (center/border location of trees) is indeed a factor investigated in our study (SS1 sampling in CSO-1). We have also added a Figure (Figure 1b) to detail the results.

“Lines 328-332: Idem about yearly rainfall”

We introduced in the objective section of introduction rainfalls during the pollination period (meteorological conditions) as an interpretative factor of results.

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

Self-fertilization (part of parental contribution) has been introduced as an objective of our work, similarly to pollen contamination and genetic diversity (see the end of the introduction).

“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.”

You are totally right! The conclusion section has been removed as the discussion has be re-organized.