# Is Randomization Necessary in Seed Orchards?

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Abstract: Randomization of clones in seed orchards is commonly practiced to promote cross-fertilization and minimize selfing. While it is practiced for the "right" biological reason, randomization comes with added managerial burden during crop management and harvest. Evidence for extremely low selfing rates in most conifers' seed orchards and natural populations has lead to a re-evaluation of seed orchard designs. The clonal-row seed orchard design represents a viable option for reducing management burden, but it comes with increased estimates of correlated matings between adjacent clones (known as "neighbourhood effect"). Staggering of clonal rows was proposed to double the number of adjacent clones to reduce correlated matings; however, it limits every clone to only four neighbours. We propose a modification to the staggering rows with a "randomized, replicated, staggered clonal-row" design to allow the simultaneous realization of randomization and clonal-rows orchard designs benefits. An interactive computer program was designed for this purpose that allows controlling orchard size and layout, number of clones, number of rows and their length, selection of the physical distance between repeated rows of the same clone, level of "anti-randomization" tolerance imposed by the design parameters, and clonal deployment mode (equal clonal size vs. linear deployment).

Keywords: Seed orchards designs, randomized, clonal-row, selfing, correlated matings.

## INTRODUCTION

The genetic structures of seed orchards determine the type of mating events (El-Kassaby et al. 1986) while their layout (spatial arrangement of each clone's ramets relative to each other as well as to other clones' ramets) affects the magnitude and frequency of each mating type (El-Kassaby 1989; El-Kassaby and Askew 1998). In practice, randomization of parental ramets within a seed orchard is judicially practiced to minimize the impact of selfing/inbreeding and the permuted neighbourhood seed orchard design program was and still is the most commonly used for this purpose (COOL; Bell and Fletcher 1978).

The relative ease and access to genetic markers (El-Kassaby and Ritland 1998; Ritland and Ritland 2000), availability of powerful mathematical models for estimating mating system parameters (Ritland 2002), and the unique structure of conifers seed (haploid magagametophyte and diploid embryo) allowed the estimation of populations' and individuals' mating system parameters for many conifer species (see reviews by Adams and Birkes 1991; Mitton 1992).

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Results from studies conducted on natural and experimental populations (seed orchards) commonly highlighted, with very few exceptions (Perry and Knowles 1990; Xie et al. 1991; El-Kassaby et al. 1994; O'Connell et al. 2004), the fact that very little selfing is being observed and that the selfing rate of most natural populations is higher than that observed in seed orchards. These observations are not surprising considering conifers high inbreeding depression and the fact that, in most cases, selfing and most forms of mating among relatives do not develop to viable seed (Woods and Heaman 1989). In light of these biological facts and the added management burden caused by randomization, the development of new seed orchard designs is worth evaluation. The use of a clonal-row seed orchard design has been advocated and mating system parameters comparisons between clonal-row seed orchards and their randomized counterparts were conducted (El-Kassaby 2003; El-Kassaby et al. 2007).

Table 1. Outcrossing rate and correlated matings estimate comparisons between clonal-row and random seed orchard designs for seed samples collected from western hemlock and "interior spruce" seed orchards (95% confidence intervals).

| Species | Outcrossing rate | | Correlated matings | |
|---|---|---|---|---|
| | Random | Clonal-row | Random | Clonal-row |
| Western hemlock[a] | 0.970±0.021 | 0.899±0.037 | 0.077±0.032 | 0.349±0.166 |
| Interior spruce[b] | 0.989±0.030 | 0.948±0.027 | 0.043±0.043 | 0.093±0.037 |

[a]El-Kassaby 2003.
[b]El-Kassaby et al. 2007.

These comparisons revealed that clonal-row orchards consistently produced higher selfing (i.e., lower outcrossing) and correlated matings estimates than that of the random design (Table 1). The observed differences in outcrossing rates are manageable and even if they persist, their impact is expected to have a very small effect on seed yield (Woods and Heaman 1989); however, it should be noted that common seed orchard management practices such as supplemental-mass-pollination (El-Kassaby et al. 1993) and/or bloom delay (El-Kassaby and Davidson 1990, 1991) could drastically reduce these slight differences. On the other hand, the observed disparities in correlated matings estimates are real and were double or higher by an order of magnitude for the interior spruce and western hemlock orchards comparisons, respectively.

## PROPOSED DESIGN

El-Kassaby (2003) and El-Kassaby et al. (2007) advocated the use of clonal-row design and proposed two modifications that could substantially reduce the "neighbourhood effect" that is responsible for the production of higher selfing and correlated matings. In the classical clonal-row seed orchard design (Figure 1, left); each clone is spatially contiguous to two other clones, producing a complete side-by-side clonal flanking, thus promoting both within-row selfing and between-rows correlated matings. The first modification (Figure 1, center); involves staggering the adjacent rows, so that each individual clone will be flanked by four unrelated clones, thus promoting outcrossing and effectively breaking down the "neighbourhood effect" resulting in reduced correlated matings. The second modification (Figure 1, right), involves replicating and randomizing the staggered rows of a specific clone throughout the seed orchard, thus at every

replication, each clone is flanked by four different clones and these clones are different from those selected at the other replications.
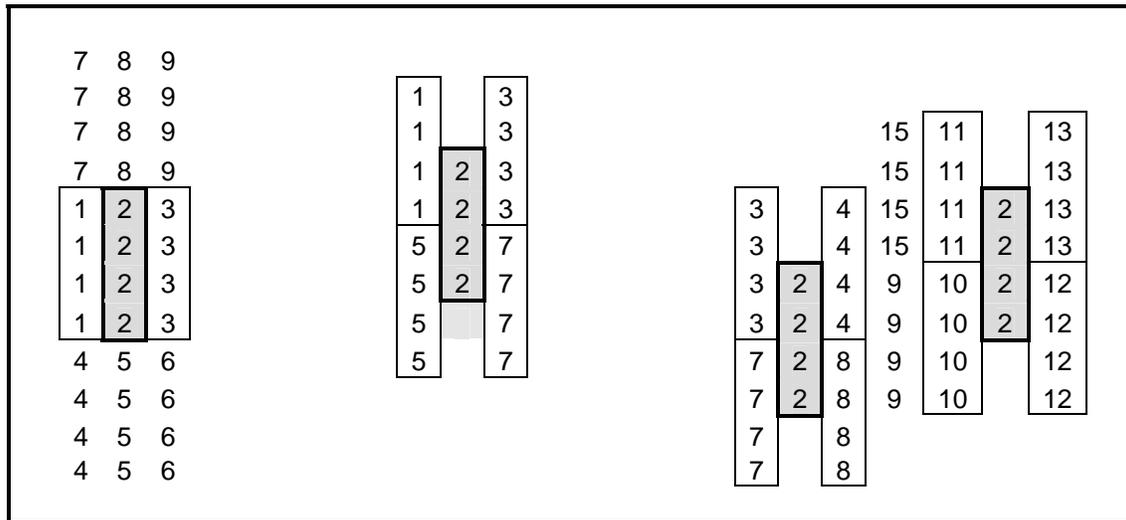


Figure 1. Illustration of three clonal-row seed orchards designs. The 1st (left) shows classical clonal-row with 50% flanking of a specific clone, the 2nd (center) shows the effect of row staggering where every clone is surrounded be four other clones (i.e., 25% flanking), and the 3rd (right) shows the effect of staggering and randomization of clonal-rows where each clone is surrounded by four different clones at each replication.

These proposed changes are expected to reduce both selfing and correlated matings observed in the classical clonal-row design (observations from the western hemlock and interior spruce studies) and the staggered clonal-rows orchards, creating a "compromise" between the systematic and randomized designs and the benefits of both clonal-rows and randomization are simultaneously realized. We propose designating this seed orchard design as "randomized, replicated, staggered clonal-row" ($R^2$SCR).

The generation of the proposed $R^2$SCR design layout is challenging since restrictions such as staggering of rows, row length, and the selection of the physical distance (i.e., number of rows) separating repeated rows of the same clone were imposed on the randomization process. An interactive computer program was developed and after each run, multiple orchard layouts are generated. The orchard's design parameters include: the number of clones, seed orchard size, length of the rows (single ramet which is equivalent to the COOL program or multiple-ramets row that must be in an even number to allow staggering), the physical distance separating rows of the same clone, seed orchard configuration (real estate barriers or lack of), level of "anti-randomization" tolerance (estimated using least square technique), and deployment mode (equal clonal size vs. linear deployment (Lindgren and Matheson 1986)).

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