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SYNCHRO: A SAS Program for Analysing the Floral Phenological Synchronisation in Seed Orchards

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Abstract

We provide a comprehensive SAS program to facilitate the analysis of the phenological synchronisation among all the genetic entries of a seed orchard. The program, intended for SAS-PC 6.12 under the Microsoft Windows, computes several phenological synchronisation indices for each male-female combination and performs the male and female phenograms as well as other simple graphics that may help in the interpretation of the phenological synchronisation parameters. An example of the phenological synchronisation study in a *Pinus pinaster* Ait. seed orchard in Northwest Spain is presented to demonstrate the use of the program and the features of the outputs.

Key words: Seed orchard, Genetic diversity, Floral phenology, Phenology synchronisation, *Pinus pinaster*, Computer program.

Introduction

The main objective of a seed orchard is to provide high genetic quality seed for reforestation. This genetic quality depends on both the genetic value and the genetic diversity of the seedlot (Kang *et al.,* 2001). The genetic diversity of the seed crop reaches its maximum when all clones in the orchard mate in equal proportions (called panmixis) and the resulting seedlot would contain equal contributions of all clones. However, panmixis is an ideal situation and clonal reproductive contributions vary with changes in many factors such as the strobili production, fecundity, distance among mates and flowering synchronisation (Xie *et al.,* 1994; Burczyk and Prat, 1997; Gömöry *et al.,* 2000; 2003).

Flowering phenology in an orchard is probably the single most important influence on outcrossing patterns (El Kassaby *et al.,* 1988; Erickson and Adams, 1989; Burczyk and Prat, 1997). Phenology affects the gene exchange among clones and the genetic compositions of the seeds derived from the seed orchard (Matziris, 1994; Burczyk and Chalupka, 1997). Differences in floral phenology lead to unbalanced contribution of clones, may preclude some combination crosses and, even, may remove the contribution of some clones in the seed crop. Thus, the lower the phenological synchronisation, the lower is the effective population size of the orchard seedlot. On the contrary, a high synchronisation can counteract the differences in male and female contributions among clones (Burczyk and Chalupka, 1997) as well as decrease the probability of background pollination (Webber and Painter, 1996). Furthermore, the quantification of the phenology synchronisation is fundamental in making decisions about orchard roguing, supplemental mass pollination or controlled pollinations within the orchard (El Kassaby and Ritland, 1986; Blush *et al.,* 1993).

The phenological data is often presented as time lines or phenograms in which the proportion of strobili in a given phenological stage is represented by bands on a line time. However, the degree of overlapping phenology is difficult to quantify by such empirical technique even though the differences are visually apparent (Askew and Blush, 1990). In order to overcome this problem, several authors have proposed different mathematical models for quantifying the reproductive synchronisation among all possible pair of clones in the orchard.

Askew and Blush (1990) propose the overlap index (PO_{ij}) which is a quantitative measure of the proportional symmetry of the female and male phenograms. The index PO_{ij} for the two comparison trees is the ratio of the common area to the maximum area between the female and male phenograms summed across all days. Askew (1988) and Xie *et al.* (1994) propose the RD_{ij} index, which is defined as the number of receptive days of clone *i* during which clone *j* is shedding pollen. Gömöry *et al.* (2000; 2003) use the Pearson correlation coefficients between the mean pollen shedding (or female receptivity) rate of each clone with the average female receptivity (or pollen shedding) rate of the complementary clones over all scoring dates. Coefficients near 1.0 indicate high synchronisation whereas low or even negative coefficients indicate poor overlapping phenologies.

Both, the elaboration of phenograms and the application of mathematical models to quantify the phenological synchronisation among clones, implicate a laborious and time-consuming management of field data. In this paper we present an elaborate SAS program that automatically generates, starting from field data, the female and male phenograms as well as the main reproductive synchronisation indices reported to date by various authors. We believe that this program, that does not required high SAS knowledge, will simplify the analysis of the reproductive phenology in seed orchards and other populations where phenology scores are available.

Features of the SAS program

The SYNCHRO program (available upon request from the authors) is intended for SAS-PC under the Microsoft Windows. It has been developed using SAS/Macros, simple data processing steps and several SAS procedures of the SAS/BASE 6.12 and SAS/GRAPH packages (SAS-Institute, 1988; 1990a; 1990b; 1990c).

The input data set must have one record per each assessed entry (ramet or branch) and sex and one phenological field for each assessment day. Up to 8 phenological phases per sex can be distinguished. User is asked at the beginning to enter the female receptivity rate and the pollen shedding rate for these 8 possible phases. Phenological phases can be assessed for identified branches on the trees or for the whole ramet, to which an average or modal value should be assigned. Details of the input data requisites will be given together with the program. Several checks of the input data are carried out before analysis and user is warned about the problems found. The program is self-adjusting and it is not necessary to change the code for different number of genetic entries, day measurements or phenological scores.

The program outputs are the following:

– Start, end and duration of the female receptivity and pollen shedding for each clone and for each ramet.

– An overall synchrony graph which represents the overall female receptivity mean and pollen shedding mean among all clones on a line time (e.g. Matziris, 1994).

– The male and female phenograms where floral phenology of each clone is presented as bands on a line time. The band width represents the relative female receptivity (or pollen shedding) at a given date (e.g. Askew and Blush, 1990).

– The phenological overlap indices (PO_{ij}) (Askew and Blush, 1990), the RD_{ij} indices (Askew, 1988; Xie *et al.,* 1994) and the r_{ph} correlation indices (Gömöry *et al.,* 2000; 2003) for each pair of clones. All these phenological indices are printed on the SAS-Output Window as cross-matrix, where column headings represent the genotypes serving as male parents and the row headings are the genotypes serving as female parents.

– Six frequency histograms, one for each phenological index and sex. The frequency histograms of the phenological synchronisation parameters may be useful to find genotypes that cannot find a mating partner within the seed orchard (e.g. Gömöry *et al.,* 2003).

The results are also stored in temporary data sets that can be subject to further analyses or can be used in the calculations of the relative gamete contribution. All the calculations performed by the SYNCHRO program are carried out both on a clone basis and on a ramet basis. The results on a ramet basis, *i.e.* for each male-female ramet combination, are not printed in the SAS-Output window but are stored in temporary data sets for further analyses.

The index proposed by Gömöry *et al.* (2003) gives a synchronisation index value for each clone serving as female or male parent. To quantify the individual synchronisation between female *i* and male *j*, the SYNCHRO program also calculates, for all possible pair of clones (or ramets), the correlation coefficient between the receptivity rate of clone *i* and the pollen shedding rate of clone j over all scoring dates.

Example of *Pinus pinaster* seed orchard

Floral phenological data from a clonal *Pinus pinaster* Ait. seed orchard is presented to demonstrate the use of the program and the features of the outputs. The seed orchard is located in Sergude (Santiago de Compostela, NW Spain (42°49' N; 8°27'W)) and was planted in 1991. The orchard follows a complete randomised design with 10 blocks and one ramet per block. Spacing is 5 x 5 m. A total of 116 clones, selected from the provenance 'Galicia Costera' (NW Spain) are present in the orchard. However, the data presented here consists of 10 clones assessed at 5 blocks each 3–4 days between February the 26th and April the 11th of 2001. Development of male and female

Table 1. – Description of the scored phenological phases in the *Pinus pinaster* seed orchard.

Male phenological phases			Female phenological phases		
Score	Description of the stage	Pollen-shedding rate	Score	Description of the stage	Receptivity rate
1	Developing, closed in integuments	0	1	Bud closed	0
2	Microsporangia visible but still tightly packed	0	2	Ovuliferous scales visible and gradually separated	20*
3	Yellow, fully shedding	100	3	Ovuliferous scales form right angle with the axis	100
4	Dry and brown. Not shedding	0	4	Ovuliferous scales thickened and closed	0

*: At this stage the ovules are not receptive, but pollen grains may get inside the bud scales and (if they survive) they may be able to take part in fertilisation (Matziris, 1994). The value of 20% was estimated based on own observations.

Table 2. – Indices of phenological overlap (Askew and Blush, 1990) for all possible pairs of clones serving as female and male parents.

	Male										
Female	1000	1020	1033	1046	1059	1062	2004	2057	2062	2083	Overall
1000		.65	.39	.37	.36	.37	.29	.58	.48	.10	.40
1020	.70		.45	.43	.42	.43	.32	.79	.56	.12	.47
1033	.49	.47		.53	.56	.52	.49	.55	.73	.29	.51
1046	.15	.08	.48		.55	.44	.72	.21	.41	.75	.42
1059	.14	.08	.47	.51		.43	.72	.19	.40	.73	.41
1062	.48	.47	.56	.55	.56		.48	.55	.72	.27	.52
2004	.46	.45	.61	.60	.65	.60		.54	.79	.37	.56
2057	.52	.55	.66	.65	.63	.65	.50		.83	.26	.58
2062	.60	.58	.46	.44	.43	.44	.35	.64		.16	.45
2083	.10	.04	.41	.44	.48	.37	.64	.16	.35		.33
Overall	.41	.38	.50	.50	.52	.47	.50	.47	.59	.34	.48

strobili was examined on all the resulting 50 ramets on three to five branches per ramet and sex. Four phenological phases were distinguished for male and female strobili (*Table 1*). The pollen-shedding and female receptivity rate values were specified at the start window dialog box as they appear in *Table 1*. The input data set had 463 records (50 ramets × 2 sex × 3–5 branches) and 19 variables (Clon, Ramet, Block, Branch, Sex, and 14 phenological fields, one for each assessment day).

As part of the SYNCHRO program output we present the cross matrix of the PO_{ij} indices (*Table 2*), the overall synchronisation graph (*Figure 1*) and the female and male phenograms (*Figure 2*).

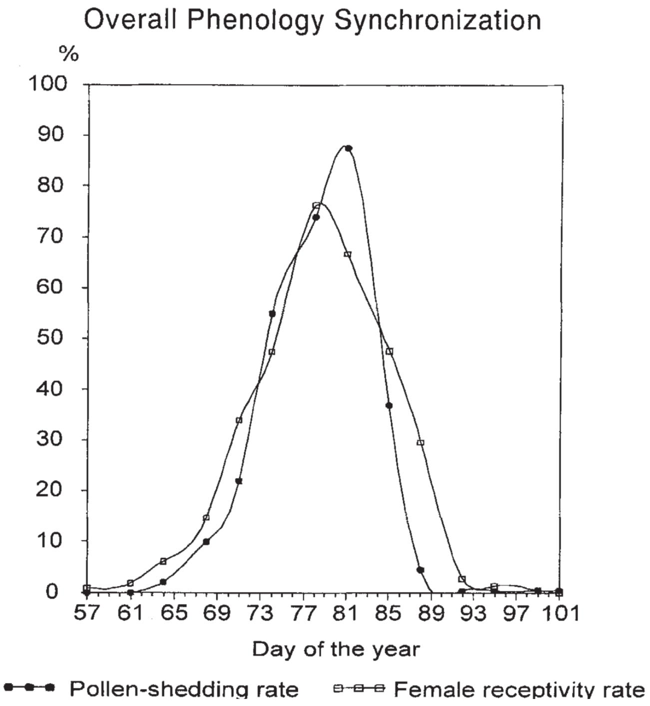

Figure 1. – Overall pollen-shedding and female receptivity rates for 10 *Pinus pinaster* clones at the Sergude seed orchard in 2001.

A high overall phenology synchronisation can be observed among all the 10 studied clones (*Figure 1*). The average index of phenological overlap was 0.48 (*Table 2*), slightly higher than that found by BURCZYK and CHALUPKA (1997) in a *Pinus sylvestris* seed orchard. As observed by MATZIRIS (1994), the pollen emission started a bit later than female receptivity and lasted for shorter period of time, and the date of maximum pollen shedding was slightly delayed in relation to the maximum female receptivity. Thus, late receptive clones may not be pollinated by early pollen shedding clones and vice versa.

The overall reproductive period was 43 days, slightly longer than that found in other conifer seed orchards (EL KASSABY *et al.,* 1984; BURCZYK and CHALUPKA, 1997). The length of the female receptivity period depends mainly on pollen availability (PULKKINEN, 1994). The high precipitation observed during the reproductive period may have impeded the permanence of pollen in the air and, thus, may be the cause of the relatively long female receptivity period.

Differences among clones in the commencement and duration of the male and female phenologies can be observed in the phenograms (*Figure 2*). The differences between the earliest and the latest clones were 12.5 and 15 days for the commencement of the female and male flowering respectively. These values were higher than those found by MATZIRIS (1994) for *Pinus nigra*, and indicated the existence of some weakly synchronised crosses.

The cross-matrix of the phenological synchronisation indices is useful to identify which pairs of clones have the lowest probability of mating. Considering male flowering, the earliest and latest clones (*i.e.* 2083 and 1020, respectively) showed the lowest overlap indices (*Table 2*). The clone 2083 serving as female parent had also poor overlapping. The r_{ph} and RPD_{ij} indices were also low for all these cases (data not shown).

Despite the poor phenological synchronisation in some cases, all possible crosses are able to mate. However, it is possible that, under optimal environmental conditions for pollination, the receptivity periods may shorten and the probability of mating for some pairs of clones become much lower. However, all clones should be included in the further analyses and the inter-year variability should be assessed before considering comple-

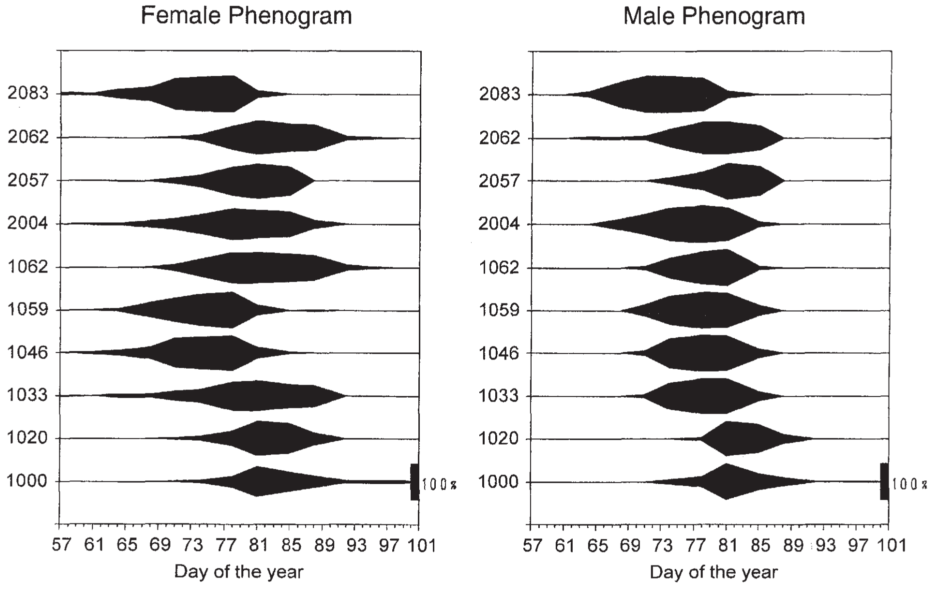

Figure 2. – Female and male phenograms for 10 *Pinus pinaster* clones in 2001. The width of the horizontal bands represents the percentage of total receptivity (or pollen-shedding) for all the assessed branches of the given clone at a census date.

mentary treatments, such as supplemental mass pollination or artificial controlled pollination.

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Genetic Parameters for Spiral Grain in Scots Pine and Norway Spruce

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Abstract

Genetic parameters were estimated for grain angle, growth and exterior quality traits in two 18-year-old Scots pine (*Pinus sylvestris* L.) progeny trials and for grain angle and growth traits in two 12-year-old clonal trials of Norway spruce (*Picea abies* L. Karst.). Mean grain angles under bark ranged from 1.4 to 2.0 degrees and from 2.1 to 2.6 degrees in the Scots pine and Norway spruce trials, respectively. Heritabilities for grain angle were high in Scots pine ($h^2 > 0.40$) and moderate in Norway spruce ($H^2 > 0.30$). The genetic standard deviations were approximately, or slightly less than one degree. In general, grain angle was genetically and phenotypically uncorrelated with the growth and exterior quality traits. All traits showed low levels of genotype by environment interaction, and there was no tendency for grain angle to be more stable than the other traits studied.

A recently developed device for measuring grain angle was tested and found to be suitable for measurements in genetic tests. The device pushes a wedge through the bark and into the wood, following (and thus revealing) the inclination of the tracheids.

Key words: Pinus sylvestris L., *Picea abies* L. Karst., grain angle, heritability, genetic correlations.

Introduction

Spiral grain, i.e. the helical orientation of the tracheids in a tree stem, is a wood property that has attracted much research interest in recent years, because it has proven to be closely associated with the shape stability (Northcott, 1965; Balodis, 1972) and strength of lumber (Dinwoodie, 2000), which are key properties for the quality of sawn products (Perstorper et al., 1995). Forestry-oriented research has focused on studies of the silvicultural effects on grain angle (Pape, 1999), and the patterns of variation within and between trees (Cown et al., 1991; Säll, 2002) as well as between stands (Danborg, 1994). Technically-oriented research has focused on studies of the relationship between grain angle and end-use properties, particularly twist (see for instance, Ormarsson, 1999; Forsberg and Warensjö, 2001), as well as measurement techniques. Recently, Nyström (2003) presented a method for automatically measuring grain angle on logs that will enable logs to be sorted with respect to grain angle, and thus the propensity of the lumber to twist.

In breeding-related research various genetic parameters have been estimated, particularly in radiata pine (*Pinus radiata* D. Don) and Sitka spruce (*Picea sitchensis* Bong. Carr.) In

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