Using SYNCHRO.SAS, a program to facilitate phenological data processing, in a radiata pine seed orchard in northern Spain.

Codesido V[1]. and Fernández-López J.

[1] Verónica Codesido Sampedro

CINAM-Lourizán

Ap.127, 36080, Pontevedra, Spain

Tel.0034986805068 or mobil: 003462653172

Fax: 0034986856420

Email: vcodesido.cifal@siam-cma.org

ABSTRACT

Flowering process in a seed orchard is of great importance, since it affects the gene exchange between the clones and consequently the genetic composition of the seed produced. The knowledge of flowering phenology is of great importance and fundamental need for the successful operation of any seed orchard. Several different techniques have been developed for quantifying the degree of reproductive synchronization between all mating pairs of clones. A programme intended for SAS-pc (SYNCHRO.SAS) has recently been created to facilitate phenological data processing and to compute several phenological synchronization indices for each male-female combination and to enable construction of the male and female phenograms as well as other simple graphics that may help in the interpretation of phenological synchronization parameters. Reproductive phenology was studied in a radiata pine seed orchard, located in northern Spain. Timing of flowering was determined on the basis of data recorded by visual observations made three times a week in 2000, 2001 and 2002 flowering period. In general, the flowering periods of the different clones overlapped. The male flowering clones that best synchronised with the females appeared to be those that started flowering earlier. The phenological overlap index varied greatly among clones, whether male or female. SYNCHRO.SAS was a very useful tool for calculate the overlap indices and obtain phenological graphs.

INTRODUCTION

Radiata pine (*Pinus radiata* D.Don) is one of the most exotic planted forest in the world and was introduced in Galicia (northern Spain) at the middle of the 19th century.

Actually it is the third most important forestry specie in Galicia occupying the 12% of the Galician forest area (Anonymous, M.M.A., 2001), being one of the most important commercial specie for local wood industries that are an important economic sector in this area.

The main use of radiata pine in Galicia is for furniture, sawn-timber and poles. Therefore, a radiata pine tree improvement program was initiated in 1992 in Galicia by the CINAM (Centro de Investigación e Información Ambiental de Lourizán) with plus tree mass selection in Galician plantations and the use of this material for seed production in a clonal seed orchard (Sergude, A Coruña, Codesido and Merlo, 2001). The selection criteria for plus trees were superior height growth, an excellent straight stem, flat branch angle and they must to be freedom for disease and defect.

We recollected grafts to install a clonal seed orchard and seeds to install progeny tests from the plus trees selected. The seed orchard was installed far away from radiata pine plantations to avoid pollen contamination.

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Several different techniques have been developed for quantifying the degree of reproductive synchronization between all mating pairs of clones (Askew, 1988; Askew and Blush, 1990; Xie *et al*., 1994; Gömöry *et al*., 2000, 2003). A programme intended for SAS-Pc 6.12 (SYNCHRO.SAS) has recently been created to facilitate phenological data processing and to compute several phenological synchronization indices for each male-female combination and to enable construction of the male and female phenograms as well as other simple graphics that may help in the interpretation of phenological synchronization parameters (Zas *et al*., 2003)

The objectives of this study were:

- Determine the phenological variation in female and male flowering in a radiata pine seed orchard
- Describe the timing of female receptivity and pollen shedding
- Comprobe the utility of the SYNCHRO.SAS program in calculate phenological data

MATERIAL AND METHODS

The radiata pine seed orchard was established in Sergude between 1997-1998, and consists of 68 clones coming from selected plus trees in Galicia. The orchard is divided in 10 randomized complete blocks. Each block contains one ramet of each of 68 clones. The spacing is 1.5 x 3m.

In 2000, 2001 and 2002, flower bud development was monitored in 25 randomly selected clones in the orchard. Between January and late March, the reproductive phenology of three male and three female marked branches on each tree was observed three times a week to determine the phenological stage of each strobili in each branch at a given date, until all pollen was released and seed cones were no longer receptive (Codesido *et al*., 2005). The number of ramets per clone varied between 5 and 8. Observations of flowering were made on the same trees each year, but not always on the same branches. We distinguished four female stages and four male stages. The female stages were described by Matziris (1994) as follows: stage 1, the female bud is increasing in size, becomes cylindrical, but is still completely covered by the bud scales (0% female receptivity); stage 2, the apex of the enlarged cylindrical bud is opened and the first ovuliferous scales appear. At this stage the ovules are not receptive, but pollen grains may get inside the bud scales and if they survive they may to be able to take part in fertilization (20% female receptivity); stage 3, the scales of the female conelet are gradually separated and almost form right angles with the axis of the conelet. This is the stage of maximum receptivity (100%), and stage 4, the ovuliferous scales increase in size and thickness so that the strobili are no longer receptive (0% female receptivity).

The male stages were described as follows (Codesido and Merlo, 2001): stage 1, the round brown strobili are covered by the bud scales (0% pollen shedding); stage 2, the male strobili burst through the bud scales and elongate (0% pollen shedding); stage 3, the yellow strobili start shedding their pollen (100% pollen shedding) and stage 4, end of pollen shedding. The male strobili wither and fall down (0% pollen shedding). When there was more than one female flower per branch, we considered the beginning of female receptivity in all of the branch when some of the flowers reached the receptive stage. In the same way, receptivity was not considered to be concluded until all the female strobili of the branch had reached the end of the receptive stage (Askew and Blush, 1990).

Phenological scores must be assessed on each branch in each ramet on each day of the study. The data were processed using the SYNCHRO.SAS programme, introducing the percentage of female receptivity or the percentage of pollen shedding associated with each phenological score and the date of the start and the end of assessment to obtain: the predicted beginning, end and duration of female receptivity and pollen shedding for each clone and for each ramet; the overall synchronicity graph, which represented the overall mean female receptivity and mean pollen shedding in all clones over time (e.g. Matziris, 1994); the male and female phenograms presented as bands over time, which represented the relative female receptivity (or pollen shedding) of each clone at a given date (see e.g. Askew and Blush, 1990).

RESULTS

The variation in the flowering patterns from year to year can be observed in the graph of overall phenology synchronicity (Figure 1). Maximum female receptivity was earlier than the peak of pollen shedding by 7 days in 2000 and by 2 days in 2001. Both peaks coincided in 2002. Note that in 2000, 15% of receptive female strobili remained in the seed orchard after pollen shedding stopped, so that 15% of the strobili were not pollinated in this year. This did not occur in the following two years. In 2000 the receptive period extended from February 3rd to March 18th and the pollen shedding period from February 11th to March 9th. The maximum number of receptive strobili (65%) occurred on February 20th, one week before the maximum pollen release (98%). In 2001 the receptive period extended from January 15th to March 1st and from January 25th to February 25th for pollen shedding. On February 5th, 90% of all of the flowers monitored were receptive and only two days later, 55% of male strobili were shedding pollen. In 2002 the pattern was very similar to that observed in 2000, with the receptive period occurring between February 2nd and March 17th, but on February 13th the percentage of receptive strobili was 90%. For male strobili, the period of pollen shedding occurred between February 11th to March 17th with a maximum of 70% on February 25th. Pollen shedding always began some days after the commencement of seed-cone receptivity (8 days in 2000, 11 days in 2001 and 11 days in 2002).

The large variation among clones in the initiation, cessation and duration of female receptivity and pollen shedding was evident in the phenograms (Figure 2). The differences between the clones that were earliest and latest in starting the receptive period were between 8 and 19 days for the beginning of the female flowering. The same clone (clone 41) was earliest in starting the receptive period in all three years, and clone 09 was always the latest. On the right of each phenogram appeared the overage clonal overlap index, POij index, which is a quantitative measure of the proportional symmetry of the female and male phenograms, is the ratio of the common area to the maximum area between the female and male phenograms summed across all registered days and for each pair of clones. The variation in the index calculated for female flowering ranged between 0.13 and 0.54 in 2000, and for male flowering, the index ranged between 0.17 and 0.47. In 2001 PO values varied between 0.19 and 0.37 for female and from 0.21 to 0.41 for male. In 2002, they varied between 0.23 and 0.51 for female and between 0.05 and 0.58 for male.

In general, the clone 42 was the best overlapping male flowering clone and the best female one was the 18. In 2000 and 2001 every female clone was able to be pollinated by every male clones and every male clone was able to pollinate every female clones. Nevertheless, in 2002 five pair of mating clones were not possible. There was no pollen available in clone 38 when clones 11, 12, 20, 39 and 41 were receptive.

The distributions of the phenological overlap index (Figure 3) varied greatly between years with averages of 0.34 in 2000, 0.29 in 2001 and 0.42 in 2002. The synchronization of male and female phenology for each pair of mating clones varied between 0.01 and 0.74 in 2000; between 0.07 and 0.52 in 2001 and between 0 and 0.82 in 2002. In 2000, 43% of the pairs of clones had POij values that were higher than the seed orchard mean, in 2001 it was 45% and in 2002 it was 55%. In general, most clones were synchronized, the overlap indices generally being higher than 0.40.

CONCLUSIONS

1. Flowering phenology was characterized by a steady increase in the number of receptive trees and the absence of pollen donors at the beginning of the receptive period. After a few days of female receptive period in the seed orchard, there was a steady increase in pollen production with a peak in pollen shedding.
2. The percentage of strobili shedding pollen was greater than the percentage of female receptive strobili in 2000 but not in 2001 and in 2002, possibly because of the rainfall patterns. Conditions were drier in 2000 than in 2001 and 2002.
3. During the three years under study, we observed a gradual shift in flowering phenology, probably due to the juvenility of the seed orchard, with synchronization between female receptivity and pollen shedding periods improving with time.
4. Stabilization between all pair of clones appeared to be reached, each clone could pollinate each other at some moment of the pollen shedding process and could also be pollinated by any clone in some moment of its receptivity phase.

5. The indices varied greatly among any one male or female parent and also among years. The overall seed orchard PO index value was 0.34 in 2000, 0.29 in 2001 and 0.42 in 2002.
6. In 2002 the orchard reached desirable PO values, except for clone ID38 which was severally affected by *Rhyacyonya buoliana* the summer before and all its ramets appeared to be unhealthy. This was the only clone in 2002 that could not pollinate some of the other clones. If we eliminate this clone from the orchard we could obtain good flowering synchronization, with an orchard mean PO value about 0.6.
7. The best overlapping male flowering clones appeared to be those clones that started flowering earlier. Nevertheless, the highest overlap indices for female flowering were observed for intermediate flowering clones
8. The reproductive synchronization of the seed orchard was not perfect, but improved each year.
9. The PO values were useful for following the development of young seed orchards until reaching reproductive stability.
10. SYNCHRO.SAS was very useful to help us to calculate the overlap indices and to draw phenological graphs

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FIURES

Figure 1. Graphs of overall phenological overlap. The percentage of receptive strobili and shedding pollen strobili are shown.

a)2000 b)2001 c)2002

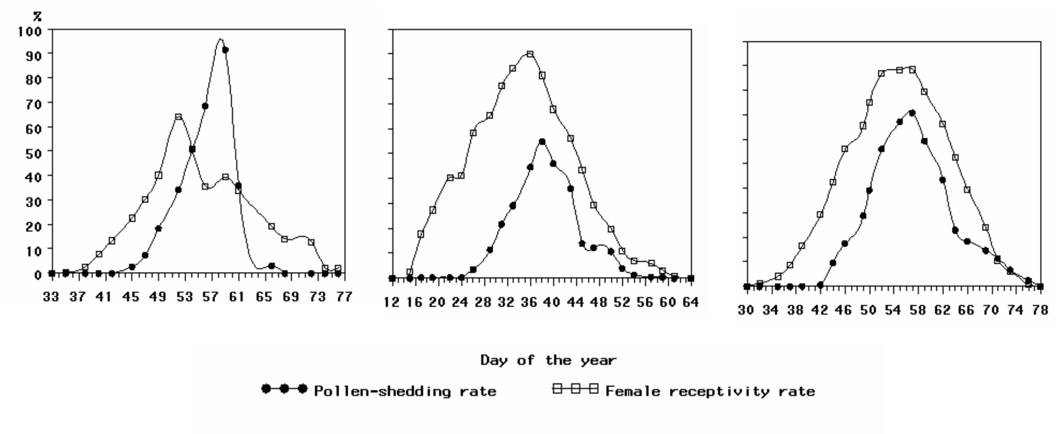


Figure 2. Flowering phenograms. PO_{ij} mean clonal index values are shown in boxes on the right hand side.

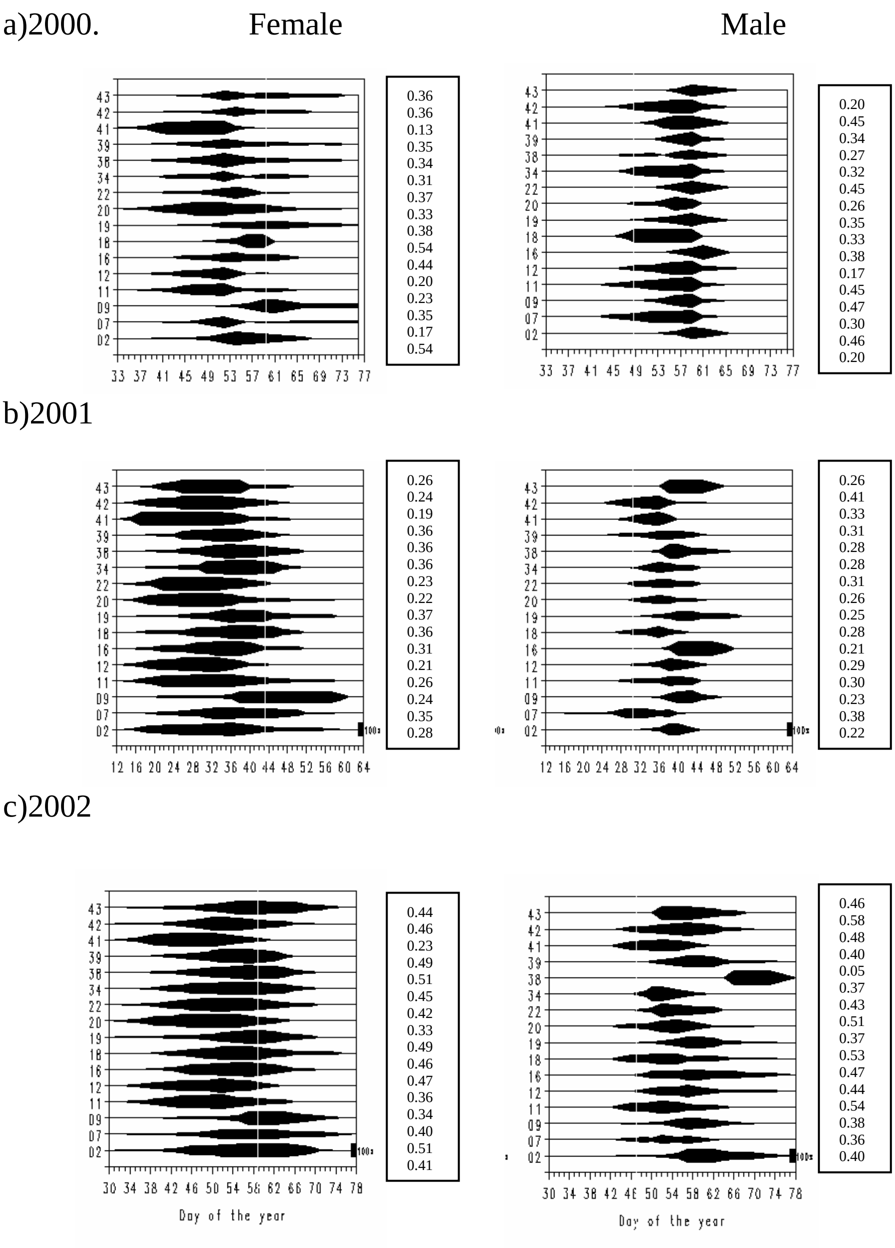
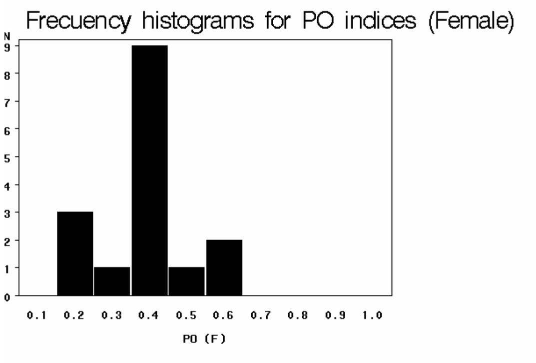
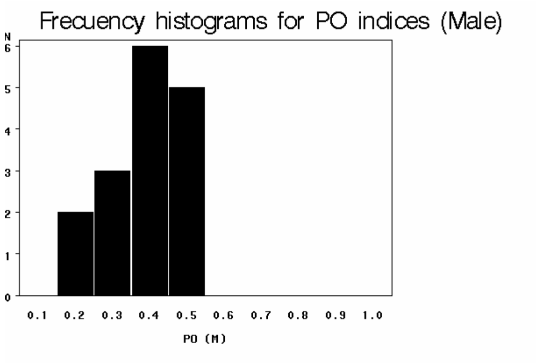


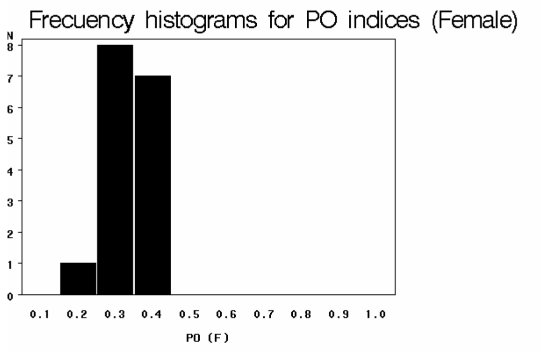
Figure 3. Frequency histograms for PO indices.

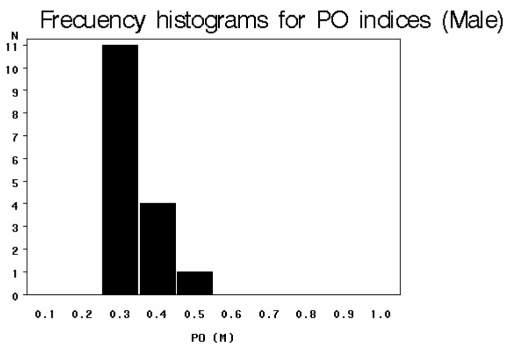
a)2000.





b)2001





c)2002