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Reproductive phenology of *Pinus pinea*

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Abstract. Stone pine (*Pinus pinea*) is one important species for pine nut production worldwide, assuming an extreme economic, cultural and environmental importance in the Mediterranean Basin due to pine nut production. In Portugal, 70% of the national production comes from Alentejo region, where edafoclimatic conditions are propitious to high productivity and quality, rendering high economy revenue. Stone pine has a peculiar reproductive cycle, with approximately 3 years from bud differentiation to maturation. Recent reports point to a decrease in cone production and pine nut productivity, which could be associated with damages in specific phases of stone pine reproductive phenology. During two consecutive years, reproductive phenology was monitored in three plots, one located in Alcácer do Sal and two in Coruche. Observations and images were acquired over two growing periods, from beginning of March 2012 to end of August 2013. The periodicity of the observations ranged between one and three weeks depending on the time of the year. Male phenology was described in 3 main phases and female phenology was described in 7. The knowledge of reproductive phenology is important for relating production and productivity with climatic data or even in a major scale with climatic changes. Through plant phenological stages it is possible to assess damage that may be caused by insects that compromise the pine nut production.

Keywords. Stone pine – Phenological stages.

I – Introduction

Stone pine (*Pinus pinea*) is one important *Pinus* species worldwide, assuming an extreme economic, cultural and environmental importance in the Mediterranean Basin (Costa *et al.*, 2008). Pine nut production represents the most valuable and profitable activity for stone pine forests in Spain, Portugal, Italy and Turkey, where pine nuts have high commercial value (Fady *et al.*, 2004; Martinez *et al.*, 2004). Half of the Portuguese cone production comes from the Setúbal district, where edaphoclimatic conditions are propitious to high pine nut productivity and quality, rendering high economic income (Evaristo *et al.*, 2002; Costa *et al.*, 2008).

Recent reports from the main producers and industries (Pimpão, 2014) point to a decrease in cone production and pine nut productivity in the latest years, reflected by a huge number of aborted flowers and cones, leading to a production decrease, and an abnormal increase of pine cones with empty seeds, responsible for the productivity decrease. Reproductive development is one of the most important stages of a plant life cycle. Considering the kind of damages reported, it is evident that not only one reproductive stage is being affected. The absence of previous studies for stone pine in Portugal, though not in other countries (Italy-Francini, 1958; Spain-Abellanas and Pardos, 1989; Mutke *et al*., 2003), alerts to the importance of phenological and ontogenetic studies on this species, in order to associate the damages to biotic or abiotic factors. It is also very important to understand the type of damage infringed and the causal agent associated. Similar reports from Italy, Spain and Turkey refer that productivity decrease is essentially related following the introduction of an alien pest, the western conifer seed bug *Leptoglossus occidentali*s (Roversi *et al*., 2011; Bracalini *et al*., 2013).

Our objective is to contribute for a better understanding on the anatomy, morphology and timing of male/female reproductive phases which is intended to fill fundamental knowledge gaps about the impacts of biotic and abiotic factors and their interactions with the stone pine reproductive cycle.

II – Materials and methods

1. Phenology

This study was performed in three pure stone pine plots, one located in Alcácer do Sal (Portugal, 38º23'22.6'' N, 8º29'22.6''W; 64 m a.s.l.) and two located in Coruche (Plot 1 - 38°55'37.1''N, 8° 31' 25.4''W; 20 m a.s.l.; Plot 2 - 38°55'34.5''N, 8° 31' 25.6''W; 19 m a.s.l.). Phenological observations were conducted during 2 consecutive years, since March 2012 to end of August 2013, where a group of 70 trees were monitored with a frequency between 1 and 3 weeks, depending on the time of the year. The plot Alcácer do Sal had 30 trees and the 2 plots in Coruche had 40 trees (20 trees/plot). Trees were 12 years old in all plots, with a planting distance of 5 m × 5 m, where the selected trees are grafted. The tree diameters and heights were about 25 cm and 7 m, respectively. The phenological monitoring was performed in two layers: on the top of the crown, one branch was selected in 4 positions (N, S, W, E) for female structures observations and data collection; in the central zone other 4 branches for the same positions were selected for male structures. For each tree the most representative phenological state was considered in relation to the 4 positions. In field, images were acquired with a Canon® SX 30 IS digital camera. Some samples were brought to the laboratory and the detailed photos were taken with a Leica® DMS 1000 digital microscope with image acquisition software LAS V4.4.

2. Ontogeny

Samples of reproductive structures from the different phenological stages were fixed, during at least 48 hours, in FAA 1:1:18 - formalin: acetic acid: ethanol (70%) at 4°C (Johansen, 1940; Ruzin, 1999). Dehydration was achieved through progressive ethanol/water series. Samples were included in paraffin, 10µm thin sectioned using a rotary microtome (Leica RM2255) and were stained with Heidenheim Hematoxylin. Digital images of the histological sections were obtained with a ProgRes® CapturePro 2.8 - JENOPTIK Optical Systems coupled to an Olympus BX41 microscope, and were electronically processed using the software ProgRes® Systeme - JENOPTIK Optical Systems.

III – Results and discussion

1. Phenology

Male phenology is described by 3 main phases (Fig. 1): **M1** - Male strobili visible but without apparent pollen production; **M2** - Male strobili are yellow and pollen dehiscence occurs. The branches produce a notable pollen cloud when moved; **M3** - Most pollen has been released. Male strobili are dark brown and the fall is eminent.

Female phenology was described by 7 main phases (Fig. 2): **F1** - Female cones are evident but not fully developed; **F2** - Female cones are fully developed. Cones have a bright yellow/green colour. Receptivity; **F3** - Seed cones showing thickened scales sealing the cone; **F4** - Female strobili lignify and grows slightly, about 1 cm diameter the 1st, 2 cm the 2nd year (quiescent stage); **F5** - Growth restart. The scales junction area becomes greenish as a result of the activity resumption; **F6** - Fully developed, green cone, commercially mature; **F7** - Mature, brown cone opening in spring.

A chronogram for stone pine reproductive phenology was established after 2 years of observations (Fig. 3). The phenological behavior was homogeneous both within and between plots, as well between the two consecutive years. From mid-April to end of May, male conelets are dehiscent and female conelets are synchronously receptive in the first year of development, being the pollination the main event. In the second year, the female reproductive structure remains nearly quiescent, the conelets just increasing in size. Only in the third year the structure resume the development and the fecundation occurs. Cone maturation takes place during October and November (Fig. 3).

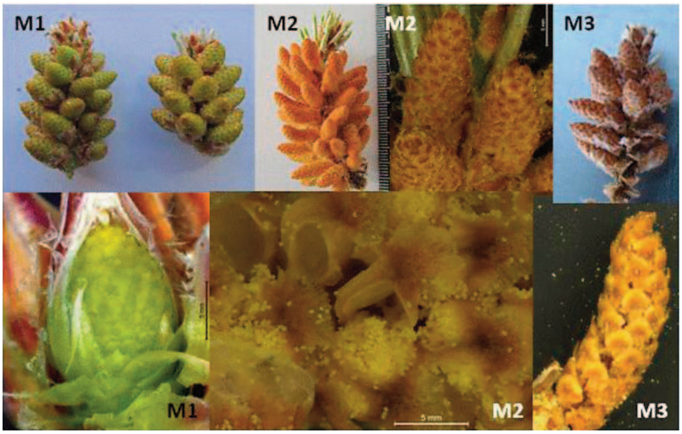

Fig. 1. Male phenological phases.

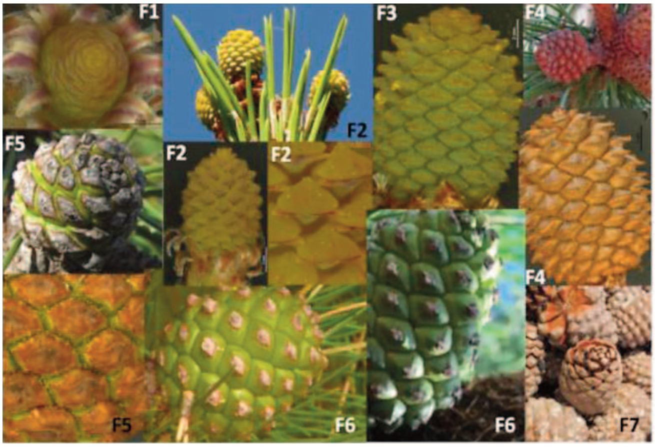

Fig. 2. Female phenological phases.

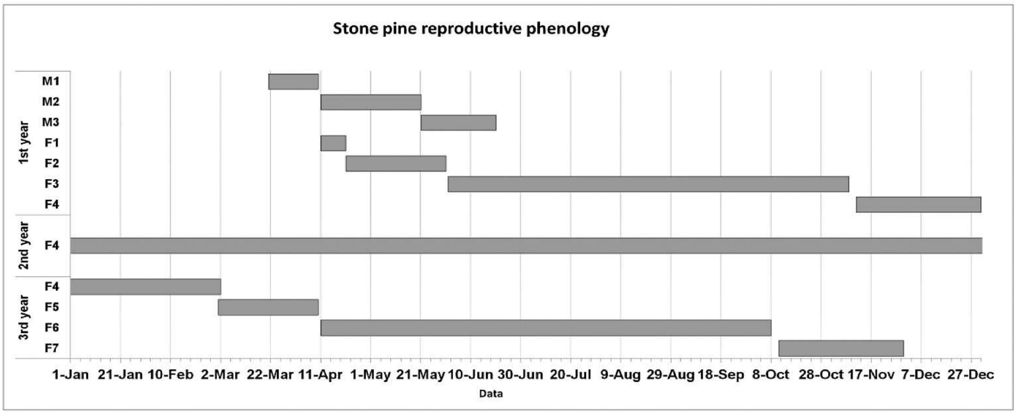

Fig. 3. Chronology of male and female reproductive structures evolution for the 3 years of development. M1 to M3 male structures. F1 to F7 female structures. Adjusted values for 2012-2013.

2. Ontogeny

Histological studies reveal the main internal sequential events of female reproductive structures development (Fig.4). **F1** - Beginning of scales formation; **F2** - Cone scales are separated, primordia of ovule differentiation are visible and structure is ready for pollination; **F3** - Cone scales become imbricated and ovule development continue. **F4** - Quiescence, the longest stage, when few morphological or anatomical changes in the ovules are observed; **F5** - Female gametophyte develops and the entire reproductive structure grows; **F6** - Ovule is ready for fecundation. After this stage it was not possible to process with histological observations due to the hardness of pine nut shell.

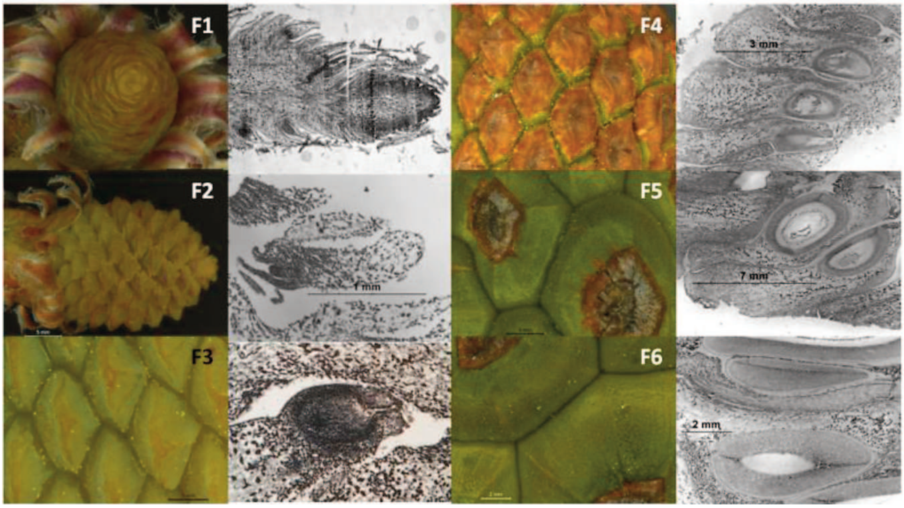

Fig. 4. Female phenological stages and correspondent longitudinal histological sections.

No major differences were observed between the phenology in these two years in the main area of pine nut production in Portugal and the data obtained by Abellanas and Pardos (1989) and Montero *et al.* (2004) in Spain.

Considering phenological and ontogenetic studies in other pine species, such as *Pinus contorta* Dougl. (Owens, 2006) and *Pinus monticola* Dougl. (Owens, 2004), *Pinus pinea* needs one year more than most pine species, implying longer vulnerability of reproductive structures to biotic and abiotic factors.

Adverse weather conditions during pollen release (M2) can compromise pollination, causing a production decrease. However, considering the recent producer reports on the production and productivity decrease, female reproductive structures are the most sensitive. In the initial female flowering phases (F1 to F3), the effect of biotic factors may void the structure development, leading to early death and directly affecting the production. If this damage is caused in a more advanced phenological stage (F4 to F6), where pollination has already occurred and megagametophyte is developing, ovules abortion may occur, causing a productivity decrease (high number of empty nuts).

IV – Conclusions

With these results it was possible to adapt the timing of stone pine reproductive phenology to Portuguese climate conditions (Fig. 5). We have represented five years instead of three, where year 0 corresponds to reproductive structures differentiation; year 1 to reproductive organ formation and pollination; year 2 represents the quiescent stage; in year 3 occurs the fecundation and cone maturation; the year 4 corresponds to the natural dehiscence. Despite this last year being part of the natural cycle, it is eliminated by commercial cones collection at maturity in winter, before dehiscence.

The establishment of a reproductive phenological model is an important tool for: (i) breeding as a base for performing controlled crosses; (ii) climate changes due to phenology being a fingerprint of climate; (iii) pollination efficiency between clones through flowering synchronization; (iv) modeling and prediction of production to improve the producers ability to plan management practices; and (v) relation with pests and diseases assessing damages in reproductive structures and contributing to identify the causes of pine nut production and productivity losses.

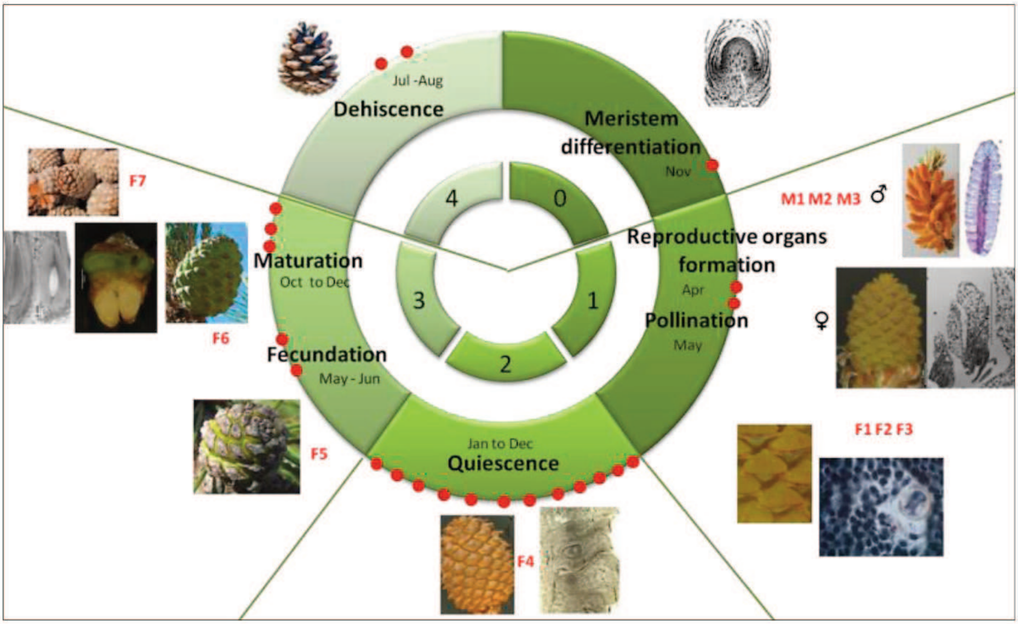

Fig. 5. Stone pine reproductive phenological model.

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