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Original Article

# Seed productions and reproductive indicators in *Pinus tropicalis* Morelet from seed orchard

# Producción de semillas e indicadores reproductivos de *Pinus tropicalis* Morelet en un huerto semillero

# Produção de sementes e indicadores reprodutivos de *Pinus tropicalis* Morelet em um jardim de sementes

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## ABSTRACT

In order to evaluate the viability and reproductive health of a seed mass of *Pinus tropicalis Morelet* as a management tool for a seed production center, seed production and the behavior of reproductive indicators were evaluated for three consecutive years, based on a sample of cones from 60 trees. The cones were dissected for their analysis. Potential and efficiency variables of seeds were estimated, as well as cone length, fertile scales, proportion of empty seeds, proportion of filled seeds and inbreeding estimator. The indicators were not similar for all three collection. The indicators of potential and seed efficiency were variable during the years evaluated. However, the percentage of empty seeds was higher than 15 % in all years, resulting in inbreeding estimators above the permissible for seed production areas.

**Keywords:** reproductive indicators; seed potential; inbreeding estimator; seed production.

## RESUMEN

Con el propósito de evaluar la viabilidad y salud reproductiva de una masa semillera de *Pinus tropicalis Morelet* como herramienta de manejo de una unidad productora de semillas, se evaluó la producción de semillas y el comportamiento de los indicadores reproductivos durante tres años consecutivos, a partir de una muestra de conos provenientes de 60 árboles. Los conos fueron diseccionados para su análisis.

Se estimaron las variables potencial y eficiencia de semillas, así como la longitud del cono, escamas fértiles, proporción de semillas vanas, proporción de semillas llenas y estimadora de endogamia. Los indicadores no fueron similares para las tres cosechas. Los indicadores de potencial y la eficiencia de semillas fueron variables durante los años evaluados. Sin embargo, el porcentaje de semillas vanas fue superior al 15 % en todos los años, resultando en estimadores de endogamia por encima de lo admisible para áreas de producción de semillas.

**Palabras clave:** indicadores reproductivos; potencial de semilla; estimador de endogamia; producción de semillas.

## SÍNTESE

A fim de avaliar a viabilidade e saúde reprodutiva de uma massa de sementes de *Pinus tropicalis Morelet* como ferramenta de manejo de uma unidade de produção de sementes, a produção de sementes e o comportamento dos indicadores reprodutivos foram avaliados durante três anos consecutivos, com base em uma amostra de cones de 60 árvores. Os cones foram dissecados para análise. As variáveis de potencial e eficiência das sementes foram estimadas, assim como o comprimento do cone, escalas férteis, proporção de sementes vazias, proporção de sementes cheias e o estimador de consanguinidade. Os indicadores não foram semelhantes para as três culturas. Os indicadores de potencial de sementes e eficiência de sementes foram variáveis durante os anos avaliados. No entanto, a porcentagem de sementes vazias foi acima de 15% em todos os anos, resultando em estimadores de endogamia acima do permitido para as áreas de produção de sementes.

**Palavras-chave:** indicadores reprodutivos; potencial de sementes; estimador de consanguinidade; produção de sementes.

## INTRODUCTION

The production of wood and other forest products in Cuba is supported by fast-growing species such as conifers. The country maintains pine trees as the priority species in its forestry development plan until 2030 for these purposes and supported mainly by the results of the genetic improvement programmes for each species and the selection of their seed sources.

*Pinus tropicalis* Morelet (tropical pine) is a species endemic to Cuba (Gernandt *et al.*, 2005) that is naturally distributed forming pure, extensive or sympatric stands with *Pinus caribaea*, in clayey soils of slate and quartzite sand in the province of Pinar del Río and sparsely in the Isla de la Juventud (Farjon and Styles, 1997) in an area of about 33 082.1 ha.

The species is considered as one of the main resources that the forest enterprises in the west have, especially the enterprise in the province of Pinar del Río. Being one of the priority species in reforestation plans, it is necessary to evaluate seed sources to guarantee the production of seeds associated to the genetic improvement program through mass or individual selection.

Cone analysis is considered a technique used in population monitoring (Santos-Sánchez *et al.,* 2018) and in turn facilitates the estimation of reproductive efficiency of populations, evaluating characteristics associated with seed production such as: cone size; number of filled and empty seeds per cone; proportion of aborted eggs; vigor and survival of seedlings and their germination (Quiroz-Vázquez *et al.,* 2017).

The species has reported a low percentage of germination in the last 10 years, which could be related to problems in the technological discipline of seed harvesting or by an unequal frequency of crosses determined by the effective size in its seed source.

This phenomenon is reported for species where their populations have been fragmented due to forest or habitat exploitation, which has brought about both a decrease in germination and germination capacity, and the appearance of a large number of abnormal plants due to increased inbreeding (inbreeding). This remains a question mark for foresters and propagators (Ramírez-Mandujano *et al.,* 2017; Rajora and Mosseler, 2001) in the process of selection and creation of seed orchards.

For this purpose, it is necessary to correctly evaluate the potential of a seed source, based on indicators that express its genetic health, and not only the phenotypic health of the individuals that integrate it. The objective of this study is the evaluation of reproductive indicators and seed production in a sapling seed nursery during three consecutive years.

## MATERIALS AND METHODS

Cone samples of 60 trees from the "Ceja del Negro" seed garden of *Pinus tropicalis* were taken in June in the years 2014, 2015 and 2016. This seed production center belongs to the UEB of the municipality of Consolación del Sur, lot 9: stand 5, with production category: seedling orchard and associated with the program of genetic improvement of *Pinus tropicalis*. The area is characterized by a lixiviated yellow quartzite ferrallitic soil and occupies a total area of 25.8 ha of pine forest on flat land (Consolación del Sur Silvicultural Center Management Project). The orchard is 31 years old and has an average height of 15 m.

Fifty cones were taken at random from the pool, available at the Pinar del Rio Seed Processing Enterprise in 2014, 2015 and 2016, respectively. The cones were measured in their length and then dried at room temperature for one month until the beginning of their opening and seed extraction. Each cone was dissected to count the Total Number of Scales (NoE), including Fertile (EF) and Infertile (Einf) Scales, Full Seeds (SLL) and Vanes (SV). A seed certification test was carried out under Cuban Standard NC: 71-04/87.

The indicators were calculated using Bramlett's formulas (Quiroz-Vázquez *et al.,* 2017): Seed Potential (PS)=Fertile Scales x 2; Developed Seeds (SD)=Sain Seeds (SV) + Full Seeds (SLL); Seed Efficiency (ES)= (SLL/PS) x 100; Proportion of Full Seeds (SLLP)= SLL/PS; Proportion of Vain Seeds (SVP)= SV/PS; Index of Endogamy (IEND)=SV/SD.

To establish the relationship with some inbreeding induced process and seed viability, a germination test was performed. The germination count was performed up to 45 days after the establishment of the trial. Germinated seed was considered to be that whose radicle length exceeded the length of the seed. For this purpose, the percentage of germination and the percentage of abnormal seedlings (PAn) were determined.

Biometric Analysis: an analysis of variance (ANOVA) of one factor and a Duncan mean comparison test were used to detect differences between collection years.

## RESULTS AND DISCUSSION

Cone and seed variables: As shown in Table 1, the dimensions of the seeds were similar in all years, but not for the length of the cone. These values are generally associated with the amount of seed and its germination capacity. Domínguez-Callero *et al.,* (2016) suggest that there is a close relationship between cone size and seed weight. While Flores-López *et al.,* (2005) express that this relationship also exists between cone size and the size of the largest seeds. In this sense, for many conifers, larger seeds produce more robust seedlings at least during the first year of life, which is a competitive advantage during the plantation establishment phase (Flores-López *et al.,* 2005). However, it has been found that cone and seed variables can vary between populations, between individuals and between regions for species with a wider distribution (Boratyñska *et al.,* 2005).

**Table 1**. - Cone and seed variables

| Variables | 2014 | 2015 | 2016 |
|---|---|---|---|
| **Cone length (cm)** | 7,14[a] | 6,99[b] | 6,55[c] |
| **Seed length (mm)** | 14 | 14 | 13 |
| **Wing length (mm)** | 5,5 | 5,3 | 5,2 |

***Note**: means with different letters have significant differences at P< 0.05*

### Variables corresponding to seed production

The potential of Average Seed (PS) and Fertile Scales (EF) was similar in all years (Table 2), although the striplings collected in 2015 and 2016 were smaller than those reported for 2014, the number of fertile scales was very similar.

One of the elements related to the genetic structure of each population or area of seed production is the seed potential, because it represents the effectiveness of the process of pollination, fertilization and maturation of the seed (Fernando, 2013); at the same time, the effective population size determines the proportion of pollen needed to make the allogamy effective (Fernando, 2013; Flores-López, 2014).

**Table 2**. - Behavior of reproductive indicators during three harvests

| Variables | 2014 | 2015 | 2016 |
|---|---|---|---|
| PS | 93,02 | 97,04 | 90,1 |
| ES | 43,01[a] | 40,79[b] | 42,92[a] |
| SD | 46,5 | 48,2 | 45,0 |
| SLLP | 0,43[a] | 0,41[b] | 0,43[a] |
| SVP | 0,07[b] | 0,09[a] | 0,07[b] |
| IEND | 0,13[b] | 0,18[a] | 0,14[b] |
| SV (%) | 15,5 | 18,7 | 23 |
| SLL (%) | 80 | 81,3 | 77 |
| EF | 46,54 | 48,52 | 45,05 |
| Einf | 40,95[c] | 31,73[b] | 47,78[a] |
| G (%) | 26,5[b] | 12,8[c] | 60,6[a] |
| PAn (%) | 12 | 10 | 30 |

**Note:** means with different letters have significant differences at *P< 0.05*

Pines, as cross-pollinated species, have developed different morphological and genetic mechanisms to ensure cross-pollination; however, these are not sufficiently effective (Bower and Aitken, 2007), so a decrease in seed potential has been detected, generally associated with inbreeding (Owens *et al.,* 2005; Flores-López *et al.,* 2005). On the other hand, there is a particularity for conifers in arid zones, where the seed potential is lower compared to conifers that are found in better conditions (Quiroz-Vázquez *et al.*, 2017).

The most representative variable of seed production is the seed efficiency defined as the amount of filled seed in relation to the seed potential expressed as a percentage. This indicator varied substantially between the 2014 and 2016 harvests with respect to the 2015 harvest. Fernando, (2013) reviews the reproductive cycle of pines in the tropics, in which most of them undergo a three-calendar-year reproductive cycle, from pollen release to cone maturation and seed output. Low seed efficiency is generally attributed to four causes: low pollination, presence of lethal genes, insect damage (Bustamante-García *et al.,* 2012) and low availability of pollen with asynchrony in pollen release (Fernando, 2013); however, studies in tropical pines are scarce (Fernando, 2013) and do not allow to reach a regularity in the causes. According to the development of the cone from flowering to formation and maturity, there are also losses of strobbles, which were not considered in the study. Owens and Fernando (2007) and Fernando, (2013) report that this loss can reach up to 50 % or more of the seed harvest and the total seed production can be evaluated by combining the life tables of the cone harvest.

Likewise, the indicators associated with full and empty seeds differed between 2014 and 2016 harvests with respect to 2015, with the lowest value in 2015 being observed in the SLLP variable. A possible cause of this behavior is associated with the absence of cross-pollination and cross-fertilization that affects the efficiency of the seed and that is to be expected in small populations or in single trees. This could help to detect inbreeding rates of more than 12 %.

The results show that there is a high percentage of empty seeds. This value is usually recommended to be below 20 % (Sorencen, 2001; Ledig *et al.,* 2002) and for conifers in seed source areas, it is accepted that it is around 12 % as an indicator of unaffected inbreeding depression (Sorencen, 2001; Ledig *et al.,* 2002).

An extremely important indicator for silviculturists is the Endogamy Index (EII), as this reflects not only stand health, pollen availability and effective cross-pollination, but also the amount of viable seed to be produced (White *et al.,* 2007). This parameter was relatively high in relation to what should be expected for a seed-producing area. The effects of inbreeding depression generate not only low percentages of seedling production in nurseries and an increase in abnormal seedlings, but also reduce the number of filled seeds and increase the number of empty seeds, production of weak seedlings, albinos with lower survival capacity (Ramírez-Mandujano *et al.,* 2017).

The percentage of germination varied considerably with the years of harvest, but did not reach 70 %. In 2015 the worst reproductive indicators of the species were reported, which also corresponds to the lowest germination rates. However, the number of abnormal plants was higher in 2016, reaching values of almost half of the germination itself. All this pointed to the fact that only a third of the seed production in 2016 will actually be viable.

The seedling orchard "Ceja del Negro" presented irregularities in the reproductive indicators during the three years.

The inbreeding estimator is higher than 12 %, which could determine the low percentages of germination and amount of abnormal plants.

The reproductive variables are evidence of the fluctuations and reduction in the effective size of the orchard.

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The authors have participated in the writing of the work and analysis of the documents