Germination after heat treatments of *Pinus tropicalis* Morelet and *Pinus caribaea* Morelet var. *caribaea* seeds of west Cuban forests

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Abstract – *Pinus tropicalis* Morelet and *P. caribaea* var. *caribaea* are two of the four endemic pine species in Cuba. They form mixed and pure stands in the western part of the island where fire is an important factor affecting pine distribution and forest structure. In this paper the effects of different heat treatments (90 °C, 110 °C, 150 °C and 200 °C) applied to seeds from both species for 30 s, 60 s and 300 s are studied to determine their tolerance to high temperature resulting from fires. Results show high resistance of seeds from both species to high temperatures and even a significant increase in germination percentage, especially in *P. tropicalis*. This could promote the increase of *P. tropicalis* distribution in comparison to *P. caribaea* var. *caribaea* in areas with high fire recurrence in W Cuba.

Pinus tropicalis / *P. caribaea* var. *caribaea* / seed / fire / tropical forest / Cuba

Résumé – Germination après des traitements thermiques sur des graines de *Pinus tropicalis* Morelet et *Pinus caribaea* Morelet var. *caribaea* de la forêt cubaine occidentale. *Pinus tropicalis* Morelet et *P. caribaea* var. *caribaea* sont deux des quatre espèces de pins endémiques de Cuba. Ils forment des peuplements purs et mixtes dans la zone occidentale de l'île, dans laquelle le feu est un facteur important qui affecte la distribution et la structure des forêts. Dans ce travail, on étudie les effets de différents traitements thermiques (90 °C, 110 °C, 150 °C et 200 °C) appliqués sur des graines des deux espèces pendant 30 s, 60 s et 300 s sur la tolérance des graines aux hautes températures résultant des incendies de forêt. Les résultats ont montré une haute résistance aux hautes températures des graines de ces deux espèces et un accroissement significatif du taux de germination, spécialement dans le cas de *P. tropicalis*. Ceci pourrait être une des raisons de l'expansion des populations de cette espèce par rapport au *P. caribaea* var. *caribaea* dans des zones occidentales de Cuba soumises à de fréquents incendies.

Pinus tropicalis / *P. caribaea* var. *caribaea* / graine / feu / forêt tropicale / Cuba

1. INTRODUCTION

Pine forests are located in a bipolar way in Cuba covering certain zones of the East and West part of the island. In W Cuba, pine forests are primarily abundant in the Pinar del Río province, from San Diego de los Baños to the W limit of Guanahacabibes peninsula and in the Isla de la Juventud, but they are also located in the more oriental zones such as Sierra Maestra and the Mayarí Region [2, 17].

There are four endemic pine species in Cuba [21]: *Pinus tropicalis* Morelet, *Pinus caribaea* Morelet var. *caribaea*, *P. cubensis* Griseb., and *P. maestrensis* Bisse. *P. cubensis* and *P. maestrensis* form open forests in areas at low altitude, whereas *P. tropicalis* and *Pinus caribaea* var. *caribaea* form extensive forests in mountain ranges of W Cuba [5].

Pinus tropicalis forests develop on sandy and ferrallitic-quartzitic soils occupying the deep low and dry soils of the San Cayetano formation, primarily on the top of the hills and mountains and covering the sunny exposures [9]. It is possible to find some *P. tropicalis* pure stands on sandy soils of the sa-

vannah [31]. *P. tropicalis* is well adapted to fire [13], although the post-fire dynamics of these pine forests are not well known. 10–20 years mature trees exhibit some needle fall [5] producing a significant amount of fuel on soils that promotes a rapid extension of fires. Seed germination is low ($14.1\% \pm 0.7$) [3]. Bonilla [7] increased the germination rate up to 70% using different pre-germination treatments.

P. caribaea natural distribution is formed by several populations from The Bahamas (27° N) to Nicaragua (12° N). Commercial plantations developed during the last 50 years due to this easy adaptation to disturbed soils. Nowadays, *P. caribaea* is a very used pine species for timber production in several tropical areas of Central and South-America [10] and it was even introduced to southern China in 1961 where 40 000 ha were planted and this area could be expected to reach 150 000 ha by the year 2010 [34]. In some of the mentioned areas, the introduction of this species appeared ecologically inappropriate (very poor soils and low altitudes), although it is used extensively on sandy soils after fires or where harvesting degraded the original vegetation [18]. Of the three recognized *P. caribaea* varieties (*P. caribaea* var. *caribaea*, *P. caribaea* var. *hondurensis* and *P. caribaea* var. *bahamensis*)

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only the first one formed natural stands in Cuba. *P. caribaea* var. *caribaea* is listed in The IUCN Red List of Threatened Species as “Vulnerable” [19].

In Cuba, this species forms pure or mixed stands with *P. tropicalis* on acidic clayey soils, and soils with high iron content (lateritic soils of Cajalbana, W Cuba) quartzite and sandy soils in Pinar del Río and Isla de la Juventud. It grows best in frost-free areas up to 700 m in altitude on more fertile sites with good drainage and an annual rainfall of 1000–3000 mm [5]. The cones mature at the onset of the rainy season (May–October) but there is often variability among trees and stands. Cones tend to mature during the same period despite the variation in flowering times. Seed production in exotic plantations is often poor due to either cool temperatures that prevent flowers formation or humid conditions during flowering which do not allow pollination [4, 10]. When the tree is 3–4 years old, it begins to produce female cones but seed setting is low unless there are mature pollinating trees close by. Germination normally begins seven days after sowing and it reaches its maximum value after 12–15 days. It can last for several weeks [26]. High variations in germination capacity was recorded, depending on sites and station quality [6].

This is a heavily exploited species. Burning and logging of large areas of pine forest transformed the habitat into savannah. Frequent fires also prevented regeneration of the species in favour of *P. tropicalis*. Nowadays, about 70% of the original habitat in the Cuban Pine Forest ecoregion was lost, with only three remaining areas of intact habitat larger than 250 km² [14].

It is frequent to see mixed stands of *P. tropicalis* and *P. caribaea* var. *caribaea* in the Pinar del Río province. As fire acted regularly in W Cuba because of lightning or human action, landscape rapidly changed as *P. tropicalis* regeneration seemed to be better than that of *P. caribaea* var. *caribaea* [7]. Therefore, the aim of this paper was to study seed germination response of both species to different heat treatments trying to imitate fire conditions, once early responses of *P. tropicalis* forests after experimental fire was already studied [13].

2. MATERIAL AND METHODS

2.1. Seed collection site

It was broadly confirmed that pine germination capacity is influenced by the genetic characteristics of trees, weather conditions during flowering and fruiting, seed storage and seed age [8, 24, 29, 32]. These factors conditioned the choice of seed collection sites. Mature cones of *Pinus tropicalis* and *Pinus caribaea* var. *caribaea* were collected in several zones of Guaniguanico Mountain Range (22° 41' N, 83° 27' W), Pinar del Río (Cuba) in 2004. In previous experiments, these seed provenances showed similar germination percentages [7, 25, 28]. In the seed collection sites vegetation was made up of a tree canopy of *Pinus tropicalis* and *Pinus caribaea* var. *caribaea* with an average height of 12 m and 18 cm in diameter. Both species were strongly mixed and in some cases formed little mono-specific stands resulting in a mosaic landscape. Both the high pine density and the fuel amount produced by needle fall promote a rapid extension of fires in spite of the rich scrub layer formed by

Curatella americana L. (Dilleniaceae), *Amaioua corymbosa* H.B.K. (Rubiaceae), *Clusia rosea* Jacq. (Clusiaceae), a dense herbaceous layer: *Clidemia hirta* (L.) D. (Melastomataceae), *Xylopia aromatica* (Lam.) Mart. (Annonaceae), *Eragrostis pilosa* (L.) P. Beauv. (Poaceae), *Sorghastrum stipoides* (Kunth) Nash (Poaceae), *Odontosoria writghiana* Masón (Dennstaedtiaceae), liana species: *Cuscuta americana* L. (Convolvulaceae) and *Davilla rugosa* Poir. (Dilleniaceae). These pine stands present some endemic species such as: *Rondeletia correifolia* (Griseb.) Borhidi & Fernández (Rubiaceae), *Mitracarpus glabrescens* (Griseb.) Urb. (Rubiaceae) and *Tetrazigia coreacea* Urb. (Melastomataceae).

P. tropicalis and *P. caribaea* var. *caribaea* selected trees were 25–30 years old and all of them had a healthy appearance and abundant mature cones.

2.2. Experimental treatment

Once the cones were gathered, they were exposed to sun heat for 24 h and seeds were collected after natural opening [23]. Seeds of both species were stored in tight containers, in a cold room with controlled temperature (4 °C) and hygrometry (about 30%) during two weeks after collection and after that, they were submitted to four heat treatments in oven at 90 °C, 110 °C, 150 °C and 200 °C for 30 s, 60 s and 300 s respectively. Five 50-seed replications were used for each temperature and exposure time in both species. Five more 50-seed replications were not submitted to any heat treatment and were considered as control. Immediately after each treatment, seeds were sown in Petri dishes filled with sterilized humin-soil and placed in a greenhouse at 25 °C during the day and 16 °C at night. Petri dishes were moistened with de-ionized water every 2 days. Germination was checked daily, with germinated seeds being removed. Germination tests ended 10 days after the last new seedling was recorded. Ungerminated seeds were submitted to cutting and tetrazolium (TZ) tests in order to know the viability of those seeds [11]. In the case of TZ test, only those seeds that showed a significant respiratory activity (dark red) were considered as viable.

2.3. Statistical analysis

For all statistical tests, data were transformed using the log or $\sqrt{\text{variance}}$ transformation to meet the assumptions of normality and homoscedasticity. Tables and figures present untransformed data and standard error of the mean ($\pm$ S.E.). A One-Way ANOVA was used to test differences in total germination between species for each treatment and among treatments within species. Fisher's Least Significant Difference (LSD) procedure was used to compare mean values. All statistical analyses were conducted using a critical p -value ≤ 0.05 .

3. RESULTS

Table I shows that final germination percentages was significantly different for untreated seeds of both species, final *P. tropicalis* germination rate was $7.5\% \pm 5$ and *P. caribaea* var. *caribaea* one was $21.2\% \pm 2.58$. The lowest germination rates were obtained for the control. When heat treatments were applied, germination of *P. tropicalis* seeds was higher than that

Table I. Final average germination percentages ($\pm$ S.E.) for heated and control (C) seeds. First letter means significant differences at $p \leq 0.05$ between species for each treatment and second letter means significant differences at $p \leq 0.05$ among treatments for each species.

T (°C)	Duration heat treat.	TZ (<i>P. tropicalis</i>)	Cutting test (<i>P. tropicalis</i>)	TZ (<i>P. caribaea</i>)	Cutting test (<i>P. caribaea</i>)
90	30 s	19 $\pm$ 11.3 a	18 $\pm$ 9.2 a	40 $\pm$ 21.3 a	21 $\pm$ 9.8 a
	60 s	21 $\pm$ 10.3 a	24 $\pm$ 16.3 a	38 $\pm$ 19.4 a	22 $\pm$ 11.3 a
	300 s	15 $\pm$ 6.2 a	18 $\pm$ 12.6 a	37.2 $\pm$ 16.2 a	21 $\pm$ 16.2 a
110	30 s	28.5 $\pm$ 8.7 a	24 $\pm$ 12.8 a	35 $\pm$ 18 a	22 $\pm$ 14.6 a
	60 s	31 $\pm$ 10.4 a	25 $\pm$ 7.82 a	36.5 $\pm$ 14.4 a	22 $\pm$ 11.6 a
	300 s	20.5 $\pm$ 13.2 a	18 $\pm$ 12.2 a	45.5 $\pm$ 19.8 a	23 $\pm$ 10.3 a
150	30 s	20.5 $\pm$ 13.5 a	19 $\pm$ 18.8 a	32 $\pm$ 23.3 a	21 $\pm$ 12.4 a
	60 s	18.5 $\pm$ 12.5 a	20 $\pm$ 14.32 a	39.5 $\pm$ 22.7 a	20 $\pm$ 11.5 a
	300 s	38 $\pm$ 18.08 a	24 $\pm$ 16.8 a	44.7 $\pm$ 27.8 a	23 $\pm$ 13.6 a
200	30 s	16 $\pm$ 12.58 a	19 $\pm$ 9.8 a	36 $\pm$ 16.8 a	21 $\pm$ 11.5 a
	60 s	24 $\pm$ 15.02 a	16 $\pm$ 5.74 a	44.5 $\pm$ 17.2 a	21 $\pm$ 14.2 a
	300 s	41.2 $\pm$ 18.41 a	24 $\pm$ 6.78 a	45.2 $\pm$ 19.61 a	22 $\pm$ 15.6 a
C		64 $\pm$ 12.3 b	21 $\pm$ 19.6 a	60 $\pm$ 15.8 b	19 $\pm$ 13 a

Figure 1. Average cumulative rate of germination for replicates of 50 seeds of *Pinus tropicalis* and *P. caribaea* var. *caribaea* control seeds.

of *P. caribaea* var. *caribaea* in all tests. The highest differences between species were recorded for heat treatments applied for 30 s and 60 s. Differences among treatments in relation to total germination were also recorded primarily for 300 s of heat exposure. The most intense treatment (200 °C for 300 s) especially affected *P. caribaea* seeds germination (9.75 ± 4.78). The highest total average germination for both species was obtained at 90 °C for 300 s; $66.5\% \pm 9.28$ for *P. tropicalis*, and $37.82\% \pm 8.68$ for *P. caribaea* var. *caribaea* respectively.

Comparison among treatments revealed that only the highest temperatures applied for 300 s decreased average germination percentages for both species.

Germination curves proved to be quite different between species. *P. caribaea* var. *caribaea*, seed germination began 10–11 days after sowing in the control (Fig. 1) and the highest germination increase lasted from the 11th to the 50th day after sowing. Afterwards germination increased slowly up to the end of germination trial i.e. 80 days after sowing. Untreated *P. tropicalis* seed germination began 13 days after sowing and it rapidly increased during the following 12 days until stabilization (Fig. 1). When seeds were submitted to heat treatments, germination course changed. In all cases, *P. caribaea* var. *caribaea* seed germination began 6–7 days after sowing (Figs. 2a–2d). Furthermore the highest germination rate was reached earlier in comparison to the control. Although high temperature applied for 300 s determined a decrease in total germination capacity, the germination pattern was similar the other heat treatments (Fig. 2d).

P. tropicalis seed germination occurred 7–8 days after sowing for all treatments (Figs. 3a–3d), 6–7 days earlier than the beginning of germination in the control. As expected germination course was quite different in treated and untreated seeds. *P. tropicalis* treated seed germination occurred primarily during the first month after sowing but, afterwards seeds continued to germinate until the end of the study. Seeds heated at 90 °C and 110 °C for 300 s showed the highest germination percentage (Figs. 3a and 3b), whereas seeds heated at 150 °C and 200 °C for 300 s presented the lowest germination rate (Figs. 3c and 3d). *P. tropicalis* seeds heated at 200 °C for 30 s showed a low but continuous increase in germination throughout the experiment (Fig. 3d).

The results of cutting test on ungerminated seeds (Tab. II) showed that there were not significant differences on empty and damaged seeds among treatments and control for both species. Furthermore, results of TZ tests did not show significant differences among heated seeds for both species (Tab. II). In the case of control seeds, $64\% \pm 12.3$ of the total number of unheated seeds were viable for *P. tropicalis* and $60\% \pm 15.8$ were viable for *P. caribaea* var. *caribaea*.

4. DISCUSSION

Some species belonging to the genus *Pinus* are characterized by the presence of woody cones able to open even after a forest fire and which also protect seeds from heat damage

Figure 2. Average cumulate rate of the germination for replicates of 50 seeds of *Pinus caribaea* var. *caribaea* heated seeds. **a:** 90 °C for 30 s, 60 s and 300 s. **b:** 110 °C for 30 s, 60 s and 300 s. **c:** 150 °C for 30 s, 60 s and 300 s. **d:** 200 °C for 30 s, 60 s and 300 s.

Figure 3. Average cumulate rate of germination for replicates of 50 seeds of *Pinus tropicalis* heated seeds. **a:** 90 °C for 30 s, 60 s and 300 s. **b:** 110 °C for 30 s, 60 s and 300 s. **c:** 150 °C for 30 s, 60 s and 300 s. **d:** 200 °C for 30 s, 60 s and 300 s.

[30]. *Pinus tropicalis* and *P. caribaea* var. *caribaea* are obligate seeders that grow together in several zones in W Cuba. The germination capacity of their seeds is modified by the temperature reached during a fire and the time during which seeds are subjected to high temperatures [27]. Even seed production of pine regeneration can be modified by fire [16]).

Both species are endemic but when forming pure stands, they live in quite different ecosystems. *P. tropicalis* is well adapted to sunny exposures and siliceous soils with low nutri-

ent content, forming stands with a tree canopy composed only of this species. In contrast *P. caribaea* var. *caribaea* grows better in clayey soils with a higher nutrient content [1, 5]. Seed germination rate of *P. tropicalis* is low and presents significant variations depending on site quality [7]. These characteristics caused Cuban foresters not to choose this species for new plantations. Foresters usually prefer other species from nurseries with less germination problems and higher timber production [20, 22, 28].

Table II. Cutting and tetrazolium (TZ) tests for heated end control (C) ungerminated seeds at the end of the germination test. In the case of TZ test, only those seeds that showed a significant respiratory activity (dark red) were considered.

Temperature (°C)	Duration of heat treatment	<i>P. tropicalis</i>	<i>P. caribaea</i> var. <i>caribaea</i>
90	30 s	62 ± 10.06 aa	31 ± 5.28 ba
	60 s	54 ± 9.08 aa	35 ± 6 ba
	300 s	66.5 ± 9.28 ab	37.82 ± 8.68 bb
110	30 s	45.5 ± 7.72 aa	36 ± 2.82 aa
	60 s	44 ± 12.6 aa	33.5 ± 7.72 aa
	300 s	62.5 ± 3.4 ab	31.5 ± 4.42 bb
150	30 s	60.5 ± 2.5 aa	37 ± 8.08 ba
	60 s	60.5 ± 2.5 aa	31.5 ± 4.42 ba
	300 s	33 ± 8.08 ab	27.32 ± 7.38 ab
200	30 s	65 ± 2.58 aa	36 ± 8.48 ba
	60 s	59 ± 5.02 ab	29.5 ± 5.74 ba
	300 s	27.8 ± 5.41 ac	18.35 ± 6.78 bb
C		7.5 ± 4 a	21.2 ± 2.58 b

Fire is a very important factor that occurs in tropical pine forests. Site quality, vegetation structure and composition before fire should also be considered to determine the early stages of secondary succession [12, 15, 35]. As pointed out by De Las Heras et al. [13], fire-stimulated germination of seeds stored in soil seed banks could contribute to the regeneration of many species in tropical pine forests. In the case of *P. tropicalis*, a significant decrease in its frequency was noted after experimental fire.

It seemed that the major part of mature seeds in the cones and those in the soil bank die during fire, so regeneration comes primarily from seeds dispersed by trees located in the surrounding unburnt areas. However, this study proved that *P. tropicalis* seeds are stimulated to germinate after heat treatments. As the viability of *P. tropicalis* and *P. caribaea* var. *caribaea* seeds lasts approximately for 3 years [28], the role of the soil seed bank is expected to be less important than that of the aerial seed bank. Furthermore, both species have no serotinous cones as it is usual in other pine species well adapted to fire [23]. Typically, in Mediterranean pines such as *P. halepensis* and *P. pinaster*, seed germination is not stimulated by heat [23] although these species are considered as active pyrophytes [33] because fire could favour their colonization ability by means of a better opening of their cones and thus a better seed dispersion. Their regeneration after fire was not always assured and it was linked to their heliophilous characteristic [23].

In *Pinus caribaea* var. *caribaea*, heat stimulated germination, but not as strongly as in *P. tropicalis*. De las Heras et al. [13] studied pure *P. tropicalis* stands, and they noted that many seeds came from surrounding areas unaffected by fire where *P. caribaea* var. *caribaea* could be found. As *P. caribaea* var. *caribaea* was not recorded after the experimental fire despite the proximity of mature trees, the lower adaptation to high temperatures of *P. caribaea* seeds in comparison to *P. tropicalis* could explain the natural expansion of *P. tropicalis* in areas regularly affected by fire in W Cuba. Only human action is responsible for the formation of pine mixed forests.

On the other hand, several endemic plant species are strongly linked to mature *P. tropicalis* forests and their presence and abundance is regulated by fire, De Las Heras et al. [13]. Some of these species such as *Byrsonima spicata* and *Sterculia* sp. have timber value and the degradation of their ecosystems could be problematic for foresters. This was studied in a *P. caribaea* plantation after fire in Trinidad and Tobago [19]. The abundance of commercially-important timber species in the most fire-damaged area with *P. caribaea* stands was 93% lower than for least fire damaged sites of mature mora (*Mora excelsa* Benth.) forest.

Fire acts as an important modelling factor in W Cuba as reported in De Las Heras et al. [13]. In this paper, the floristic composition of *P. tropicalis* forest one year after fire is related to other tropical pine forests such as *P. elliotii* var. *densa* Little & KW Dorman and *P. palustris* Miller in central Florida, with a known fire regime [25], although fire response of Cuban pine forests presented significant differences. The low *P. tropicalis* regeneration, the null presence of *P. caribaea* var. *caribaea*, and the differences in germination percentages after heat treatments, seemed to point out differences in the post-fire regeneration of both species. Although the germination seed rate of both species was favoured after high temperatures, the increase in germination rate of *P. tropicalis* seeds was significantly higher.

As a conclusion, *P. tropicalis* and *P. caribaea* var. *caribaea* seeds increased their germination rate after treatments at high temperatures. However, the response of *P. tropicalis* seeds was significantly better. The pattern of mixed pine forests in W Cuba depended on fire regime and they were well adapted to this disturbance. Nevertheless the differences in germination rates after fire of the two main endemic pine species could modify tree canopy structure. Finally, heat shocks may be considered as an efficient and inexpensive treatment to increase germination of *P. tropicalis* and *P. caribaea* var. *caribaea* seeds in Cuban nurseries.

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