Floresta e Ambiente 2020; 27(1): e20170386
https://doi.org/10.1590/2179-8087.038617
ISSN 2179-8087 (online)

ORIGINAL ARTICLE – Silviculture

The Comparative Photosynthetic Responses of *Pinus caribaea* var. *caribaea* and *Pinus tropicalis*, from Western Cuba

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Abstract

This study aimed to evaluate the photosynthetic responses of *Pinus caribaea* Morelet var. *caribaea* Barret & Golfari and *Pinus tropicalis* Morelet in the ecological conditions of Pinar del Río, Cuba. Data were collected in March 2015 using an open system IRGA-porometer LI-6400. The response of both species was measured by increasing concentrations of carbon dioxide and photosynthetically active radiation. Results indicated that under the same environmental conditions, *P. caribaea* var. *caribaea* is more productive than *P. tropicalis*, since *P. caribaea* var. *caribaea,* showed higher values of net CO_2 assimilation and reaching the CO_2 compensation point at 77 µmol mol^{-1}, against the 113 µmol mol^{-1} registered by *P. tropicalis*. The species *P. caribaea* var. *caribaea* reached light saturation at lower values than *P. tropicalis*, and showed greater efficiency for the carboxylation of Rubisco. The results indicated that both species perform C_3 photosynthetic mechanism.

Keywords: CO_2 compensation point, photosynthesis, Pinus, net assimilation rate, photosynthetic active radiation.

1. INTRODUCTION AND OBJECTIVES

Primary processes such as gas exchange and water relations primarily determine plant development (Taiz & Zeiger, 2006), and therefore, studying the influence of the environment is important for understanding plant habitats and niches (Bertrand et al., 2017).

The evolution of ecology and eco-physiology as integrative sciences of the biology of organisms, and relationships with both its physical habitat and their co-inhabitants are experimental sciences that generate basic knowledge; therefore, they participate in the process of planning and forecasting production (Kimball et al., 2016). Ecological principles can generate new paradigms and deliver management recommendations for production systems, which are more suited to each particular habitat; besides, they are more environmentally friendly (Diaz, 2001).

These perspectives allow us to determine which attributes are large-scale eco-physiological parameters in order to ensure pertinent management and better planned use of forest resources, on the basis of scientifically sound and well-understood mechanisms of acclimation and adaptation of the plant development.

Physiological techniques have enabled incorporating information related to the functional features of the state of the plants (Pelegrín et al., 2005; Singh & Nangoy, 2016). Gas exchange is one of the most frequently used eco-physiological variables (Koussoroplis et al., 2017; Vallejo et al., 2003). Thus, the assessment of this variable has become more complex and opened up a wide spectrum for the interpretation of the plants' response, ranging from purely

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morphological traits to addressing the function of a species (Vilagrosa et al., 2005).

Pine forests are relevant given their position, due to their physiognomic range, the diversity of their uses and a high economic and ecological value (Torngern et al., 2017). In Western Cuba, forests are composed only by two endemic pine species, of which little is known about their ecophysiology. Also, there is a lack of specific studies on the photosynthetic response to allow the understanding of the levels of productivity. Therefore, the study of the environment-species relationships in the ecological conditions of Pinar del Río deserves special attention. This research aimed to evaluate and compare the photosynthetic response of *P. caribaea* and *P. tropicalis* in the same ecological conditions.

2. MATERIALS AND METHODS

2.1. Geographical location and sample selection

The trial was conducted in an experimental area of the Pinar del Río Botanical Garden, located at 22°20'45" N, 84°00'40" W, at an altitude of 196 masl. The site has clayey soil, with little organic matter content, gravel, and has low natural fertility; annual rainfall of 1,600 mm and an average temperature of 24.2 °C (Frías, 2013; García et al., 2013). We selected five *P. caribaea* var. *caribaea* and *P. tropicalis*, considering their health status, phenotype, development and exposure to light.

2.2. Gas exchange measurements

The gas exchange measurements were performed using the portable photosynthesis device called the IRGA-porometer LI-6400 (Licor, Inc.; Lincoln, NE, USA). Specific measurements of the photosynthetically active radiation (PAR) were conducted in March 2015.

2.3. Photosynthetic kinetics

Photosynthetic kinetics was recorded with five replications in response to different PAR intensities and several levels of intracellular CO_2 concentration (Ci) (Tezara et al., 2003; Tezara et al., 2014; Warren et al., 2011).

The kinetics in response to Ci was recorded between 11 a.m. and 1 p.m., maintaining the value of the incident radiation on the pine needles (800 μmol $m^{-2}s^{-1}$). The fit of the curves (Equation 1) was performed using temperature (32 °C) of the chamber assimilation constant, and varying the levels of CO_2 Ci from 0 to 600 μmol mol^{-1}, with relative humidity of 23 ± 2% (Tezara et al., 1998).

$$A = b + d \times e^{(K \times C_i)} \cdot (1)$$

where b: CO_2-saturated photosynthetic capacity; (A_{sat}) and (b + d) = y ± intercept (Tezara et al., 1998). Carboxylation efficiency (CE) was calculated from the initial slope of the curve.

The kinetics in response to the PAR was carried out from 1 p.m. to 3 p.m., maintaining the concentration of CO_2 (400 μmol mol^{-1}) and the temperature of the assimilation chamber (32 °C) constant, varying the humidity of 19 ± 2%, at PAR levels ranging from 0 to 2,000 μmol m^{-2} s^{-1}, every 2.5 minutes for each PAR level.

Photosynthetic kinetics was recorded with five replications in response to different PAR intensities, and various levels of intracellular Ci, using ideas from Prado & Moraes (1997) for fitting the curves.

For kinetics measures, the portable photosynthesis device LI-6400 (Licor, Inc.; Lincoln, NE, USA) was used, because it allows one to control the temperature of the assimilation chamber, the incident radiation on the pine needles using a programmable lamp (6400-02B LED Light source), and the CO_2 concentration with a dosing attachment of this gas (6400-01 CO_2 Mixer). It also allows one to fix the maximum acceptable variation coefficient for each recorded value; in this case such maximum was set at 2%.

The averages and standard deviation were plotted using the data obtained on the kinetics of assimilation (A) in response to Ci and PAR from the five repetitions. Ci kinetics were determined in three physiological parameters: compensation point, CO_2 saturation point, and the efficiency of the Rubisco enzyme (CE) were estimated based on the initial slope of the kinetics obtained (Farquhar & Sharkey, 1982). On the PAR kinetics, the compensation points and light saturation were determined.

Stomatal conductance and perspiration were monitored for five days, throughout the day in both species, recording values at 8 a.m., 10 a.m., 12 p.m., 2 p.m. and 4 p.m., with IRGA-porometer.

Statistical significance of photosynthetic parameter was assessed through one-way of variance (ANOVA) at $p < 0.05$, using the SPSS software ver. 22.0.

3. RESULTS AND DISCUSSION

3.1. Kinetics and photosynthetic parameters

The kinetics of assimilation (A) in *P. caribaea* and *P. tropicalis* were plotted against intercellular C_i (Figure 1). Such kinetics revealed that with a constant 800 μmol m^{-2} s^{-1} PAR, the saturation point (Γ) of photosynthesis occurred at 473 μmol mol^{-1} C$_i$ for *P. caribaea* var. *caribaea,* and at 417 μmol mol^{-1} CO$_2$ for *P. tropicalis*. Assimilation was higher in *P. caribaea* var. *caribaea* with 11.39 μmol mol^{-1} CO$_2$ m^{-2} s^{-1}, while in *P. tropicalis* it was 7.36 μmol mol^{-1}.

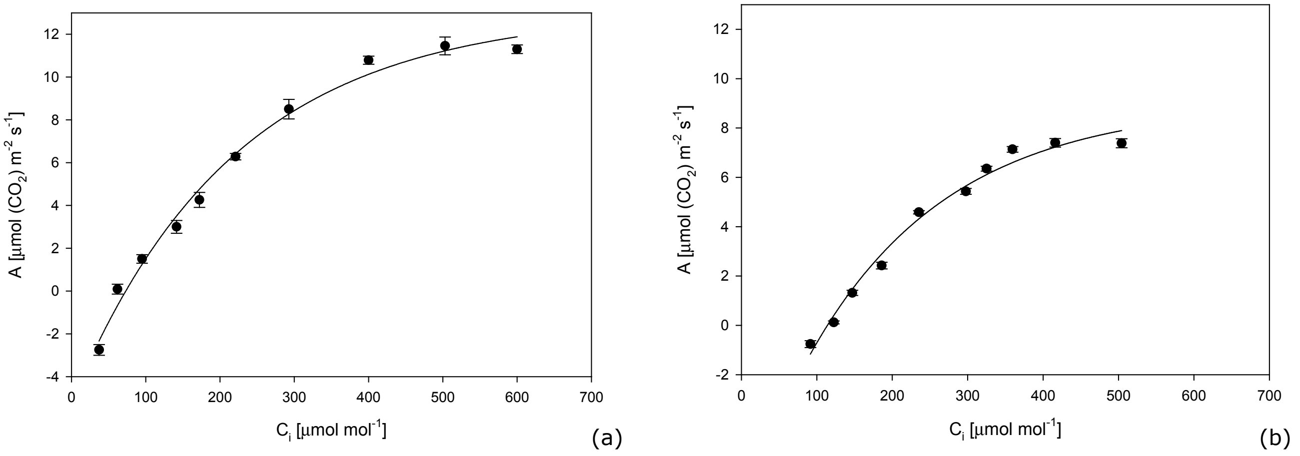

Figure 1. Curves of net photosynthetic rate (A) to intercellular CO$_2$ concentration (Ci) of (a) *P. caribaea* var. *caribaea* and (b) *P. tropicalis.*

The PAR compensation point (Γ), which represents the CO$_2$ concentration, and photosynthesis equals respiration, was 77 μmol mol^{-1} for *P. caribaea* var. *caribaea*; and even higher in *P. tropicalis,* with values close to 113 μmol mol^{-1} (Figure 2). These values correspond to plants with C$_3$ metabolism according Begon et al. (1988), and indicate that *P. caribaea* var. *caribaea* showed higher photosynthetic efficiency under the same ecological conditions for both species, such as soil nutrient content, light and water availability, and topography.

Carboxylation efficiency for both species (equivalent to the slope of the line drawn with the points recorded from 0 to 200 μmol of CO$_2$ per mole) was 0.046 μmol per mole of CO$_2$ air for *P. caribaea* var. *caribaea*, indicating that Rubisco is more efficient in the capture of CO$_2$ in comparison to *P. tropicalis* which showed an efficiency of 0.038 μmol mol^{-1}.

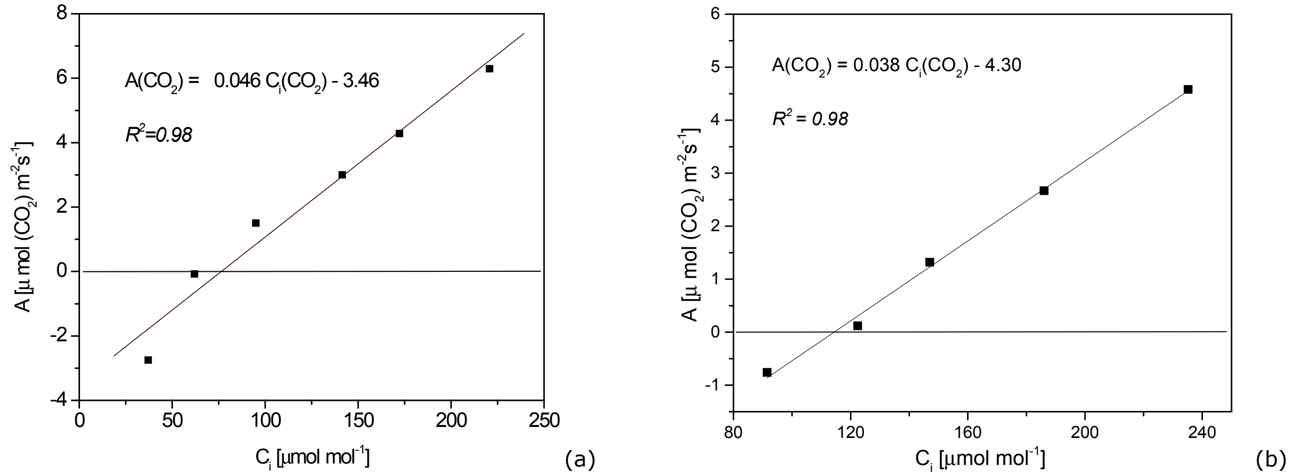

Figure 2. CO$_2$ compensation point for (a) *P. caribaea* var. *caribaea* and (b) *P. tropicalis.*

In response to PAR, the kinetics of A depending on the light (PAR) is shown in Figure 3. In this case, a constant concentration of CO$_2$ of 400 μmol mol^{-1}, *P. caribaea* var. *caribaea* reached photo-saturation with 1,509 μmol m^{-2}s^{-1}, with a rate of A of CO$_2$ close to 6 μmol m^{-2}s^{-1}. The light compensation point occurred at 37 μmol m^{-2}s^{-1} with an A zero, since this is when photosynthesis matches respiration. Similar results were obtained by Ingwers et al. (2016) in a study of physiological attributes of three and four needle fascicles of *Pinus taeda* (loblolly pine), where they obtained an assimilation of 4 μmol m^{-2}s^{-1}.

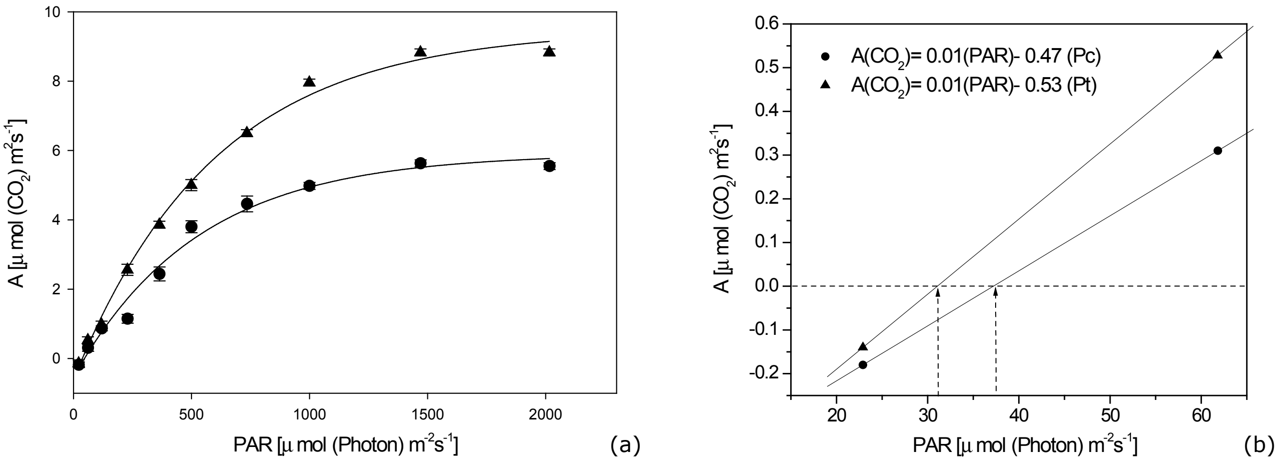

Figure 3. Curves of assimilation of CO_2 (A) to photosynthetically active radiation (PAR) of *P. caribaea* var. *caribaea* (Pc) and *P. tropicalis* (Pt) (a), and Light compensation point for *P. caribaea* var. *caribaea* and *P. tropicalis* (b).

In *P. tropicalis*, the light saturation reached 1,605 $m^{-2}s^{-1}$, with a rate of an A of CO_2 close to 9 µmol $m^{-2}s^{-1}$, and the light compensation point occurred at 31 µmol $m^{-2}s^{-1}$. These results also indicate that *P. caribaea var. caribaea* under these ecological conditions photo-saturates at slightly lower values than *P. tropicalis*. García et al. (2013) gave requirements concerning light, in which *P. tropicalis* presents a more heliophilous character than *P. caribaea var. caribaea*. In the case of *Pinus strobus*, Fréchette et al. (2016) observed that, under different light conditions, it reached values of assimilation close to 9 CO_2 µmol $m^{-2}s^{-1}$ and compensation point values between 20 and 45 µmol $m^{-2}s^{-1}$. On the other hand, Busch et al. (2007) obtained values of assimilation lower than 3 CO_2 µmol $m^{-2}s^{-1}$ for *Pinus banksiana* (Jack pine).

Table 1 shows the ANOVA analysis of the two variables of pine species: assimilation (A), saturation point (Γ) and carboxylation efficiency (CE). The results indicated that they have significant (sig.) difference in the photosynthetic parameters ($p < 0.005$).

Table 1. ANOVA for photosynthetic parameters.

		Sum of Squares	df	Mean Square	F-Value	Sig
A (µmol $m^{-2}s^{-1}$)	Between groups	19.279	1	19.279	804.862	0.000
	Within groups	0.096	4	0.024		
	Total	19.375	5			
Γ (µmol mol^{-1})	Between groups	2560.343	1	2560.343	2680.001	0.000
	Within groups	3.821	4	0.955		
	Total	2564.165	5			
CE (µmol mol^{-1})	Between groups	0.000	1	0.000	96.000	0.001
	Within groups	0.000	4	0.000		
	Total	0.000	5			

A: assimilation; Γ: saturation point; CE: carboxylation efficiency; df: degrees of freedom; Sig: statistical significance.

The results above are in agreement with the behavior of the transpiration rates and stomatal conductance reached by both pine species (Figure 4). As observed, *P. caribaea* recorded values are higher than *P. tropicalis*. It is known that both stomatal conductance and transpiration are indicators of stomatal opening, which favors the entry of CO_2 into the cellular interior and consequently manifests itself in greater photosynthetic activity (Anev et al., 2016; Azcón-Bieto & Talón, 2008; García et al., 2014; Hogan et al., 1995). This indicates that *P. caribaea* reaches higher rates of CO_2 fixation and is more productive.

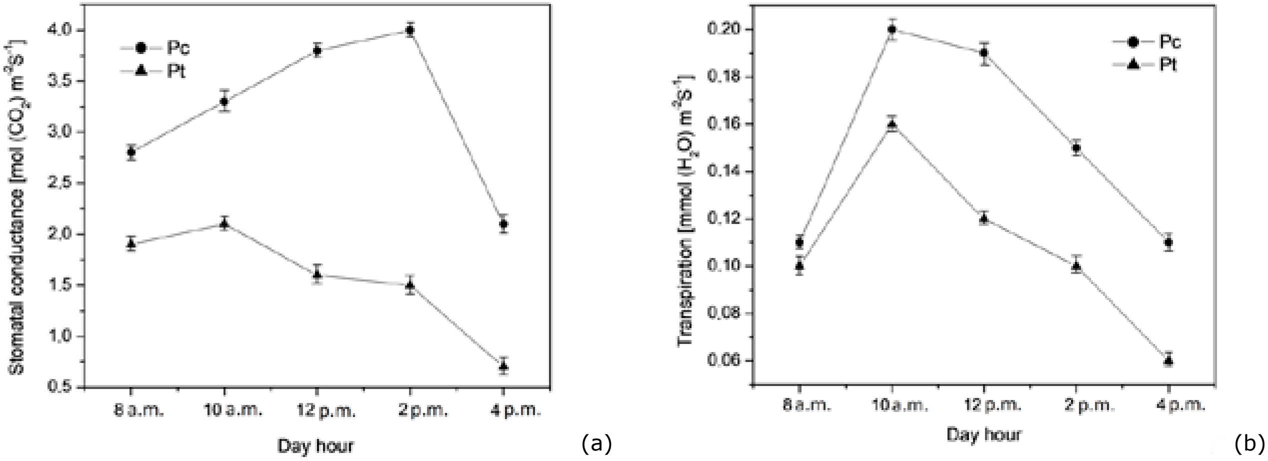

Figure 4. Measures of stomatal conductance for *P. caribaea* var. *caribaea* (Pc) and *P. tropicalis* (Pt) (a). Transpiration for *P. caribaea* var. *caribaea* (Pc) and *P. tropicalis* (Pt) (b).

Many studies based on experiments under greenhouse conditions reveal that changes in stomatal conductance were the main cause of decreased photosynthesis. Different studies (Flexas & Medrano, 2002; Galmés et al., 2011; Saibo et al., 2009) led to the conclusion that a reduced rate of photosynthesis had a strong correlation with conductance as shown in potted grape wine plants. This strong relation led to the assumption that the down regulation of photosynthesis depends more on the availability of CO_2 in the chloroplast than on leaf water content or water potential.

Riikonen et al. (2016) found that, for *Picea abies* and *Pinus sylvestris,* the assimilation values were lower than 4 µmol $m^{-2}s^{-1}$ and stomatal conductance less than 0.10 mol $m^{-2}s^{-1}$.

Conifers of western Cuba, although sharing the same habitat, respond in different ways to the ecological conditions of Pinar del Río. *P. caribaea* var. *caribaea* is more productive with higher values of CO_2 assimilation, reaching its CO_2 compensation point and light saturation at lower values than *P. tropicalis* and has greater efficiency at the carboxylation of Rubisco. These results can be attributed to the anatomical features of their needles and fundamentally to the difference in the number of stomata per unit area, where a greater amount has been reported for *P. caribaea* var. *caribaea* (García et al., 2013; Pérez del Valle et al., 2016). The species *P. caribaea* var. *caribaea* presents more needles per fascicle than *P. tropicalis*, which could explain its higher productivity. Ingwers et al. (2016) reported higher assimilation of CO_2 when *Pinus taeda* presented four needles per fascicle.

This study shows that both species perform a C_3 photosynthetic mechanism. The obtained results contribute to the scientific basis for the prudent and planned management of pine species, which will contribute to optimize forest production. The gas exchange processes under the controlled environmental conditions of Pinar del Río allow a better understanding of the differences between the species, which provides key information for the foresters who are dedicated to the establishment of mixed masses of *P. caribaea* species and *P. tropicalis*. This will allow decisions to independently establish these species in a way that optimizes their growth, survival, development and production.

4. CONCLUSIONS

The species *P. caribaea* var. *caribaea* presented greater photosynthetic efficiency than *P. tropicalis* in the same ecological conditions, expressed through measures of assimilation of CO_2, light compensation point, stomatal conductance and transpiration. The differences found can be attributed to the anatomical characteristics of their needles and fundamentally to the difference in the number of stomata per unit area, which infers a more productive character. These results provide valuable information for the management of the two pine species that are established as a mixture in the western zone of Cuba.

ACKNOWLEDGEMENTS

The authors thank the Universidad Estatal Amazónica for the financial support.

SUBMISSION STATUS

Received: 30 Mar. 2017
Accepted: 31 July 2018
Associate editor: João Latorraca

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