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Comparison of seedling growth among three co-occurring varieties of *Araucaria angustifolia* (Bertol.) Kuntze under greenhouse conditions[1]

André Luiz Coutinho[2] and Lúcia Rebello Dillenburg[3,4]

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RESUMO – (Comparações do crescimento de plântulas entre três variedades co-ocorrentes de *Araucaria angustifolia* (Bertol.) Kuntze em condições de casa de vegetação). *Araucaria angustifolia* (Bertol.) Kuntze é uma espécie de porte alto e vida longa, que cresce como árvore emergente em florestas de araucária no sul do Brasil. Quatro variedades foram descritas para esta espécie, e três delas foram selecionadas para este estudo: "angustifolia" (variedade típica), "caiova" e "indehiscens". Estas variedades co-ocorrentes diferem umas das outras na coloração externa de suas sementes e em relação ao tempo de maturação das mesmas. O objetivo deste estudo foi comparar o crescimento inicial de plantas oriundas de sementes destas três variedades, a fim de testar a hipótese de que estas variedades também diferem no crescimento inicial das plântulas. As sementes foram coletadas em um mesmo local da floresta, e 60 plantas de cada variedade foram cultivadas em vasos por um período de 244 dias. A variedade "angustifolia" acumulou significativamente mais massa (tanto na parte aérea quanto na raiz) do que as outras duas, mas as três variedades não diferiram na partição de massa entre parte aérea e raiz e entre raízes laterais e a raiz principal. O maior crescimento da variedade típica pode ajudar a explicar a sua maior abundância natural, uma vez que um maior tamanho inicial teria efeitos positivos sobre o recrutamento e sobrevivência da mesma.
Palavras-chave: Araucariaceae, pinheiro brasileiro, pinheiro macaco, pinheiro caiova, variação fenotípica

ABSTRACT – (Comparison of seedling growth among three co-occurring varieties of *Araucaria angustifolia* (Bertol.) Kuntze under greenhouse conditions). *Araucaria angustifolia* (Bertol.) Kuntze is a tall, long-lived tree species, which grows as an emergent tree in the araucaria forests of southern Brazil. Four varieties have been described for this species, and three of them were selected for this study: "angustifolia" (the type variety), "caiova" and "indehiscens". These are co-occurring varieties that differ in external seed color and timing of seed maturation. The purpose of this study was to compare the initial growth of plants originated from seeds of these three varieties, in order to test the hypothesis that these varieties also differ in the initial growth of their seedlings. Seeds were collected from a single forest location, and 60 plants per variety were grown in pots for a period of 244 days. The "angustifolia" variety accumulated significantly more mass (both in shoot and root) than the other two, but the three varieties did not differ in mass allocation between shoot and root and between lateral roots and the main root. The greater growth of the type variety may help explain its greater natural abundance, since attaining a larger size will have positive effects on seedling recruitment and survival.
Key words: Araucariaceae, Brazilian pine, monkey pine, caiova pine, phenotypic variation

Introduction

Araucaria angustifolia (Bertol.) Kuntze (Brazilian pine or, simply, araucaria) is a conifer, member of the Araucariaceae family, that has a striking impact on the physiognomy of its forest habitats, because of its large size and peculiar architecture (Jarenkow & Baptista 1987). The species inhabits the so-called araucaria forests on the high plateaus of southern Brazil, which appear either as continuous formations or as forest islands in a grassland-forest mosaic (Rambo 1994). In the past, these forests comprised about 35% of the vegetation cover of the three southernmost states of Brazil. This contribution changed dramatically to only 2 to 4% because of massive logging of *A. angustifolia* (Guerra *et al.* 2002). Despite being legally protected today, the species is now critically endangered (IUCN 2008).

Reitz & Klein (1966) were the first to suggest the existence of varieties within the species, and they characterized 9 different ones, based mostly on the coloration and phenological characteristics of the seeds (the so-called "pinhões"). Later, Mattos (1972) reduced the number of varieties to four: *A. angustifolia* var. dependens Mattos; *Araucaria angustifolia* var. angustifolia (Bertol.) Kuntze; *A. angustifolia* caiova Reitz and Klein; and *A. angustifolia* var. indehiscens Mattos. This study focused on these last three varieties, which co-occur in the state of Rio Grande do Sul, inhabiting the same forest stands. *Araucaria angustifolia* var. angustifolia is the type variety, whose seeds mature from April to May; *A. angustifolia* var. indehiscens, also known as monkey pine, is mainly characterized by the fact that the seed-bearing structure (gymnostrobilous) remains attached to the tree, even after seed maturation (October to January); *A. angustifolia* var. caiova matures its seeds between June and July. In addition to the delayed maturation of seeds of these last two varieties, they are also much less abundant than the type one in co-occurring areas (personal observation). At present, growth performance of these varieties has not been compared at any plant stage.

Taking into account (1) the ecological importance of *A. angustifolia*, a key-species in the forest and in the grass-forest ecotone; (2) its great significance for agroforestry development in southern Brazil; (3) its great commercial value (food, resin, wood); and (4) the complete absence in the scientific literature of information on its botanical varieties, this study aims to investigate the possible expression of variation in the initial growth of three different varieties: "angustifolia", "caiova" and "indehiscens". The hypothesis to be tested is that these varieties also differ in

[1] Part of the Master's dissertation of the first Author
[2] Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Ecologia, Porto Alegre, RS, Brasil
[3] Universidade Federal do Rio Grande do Sul, Departamento de Botânica, Porto Alegre, RS, Brazil
[4] Corresponding author: lucia.dillenburg@ufrgs.br

terms of initial seedling growth. Such investigations will be of special relevance in characterizing a yet uninvestigated aspect of the phenotypic variability of Brazilian pine and in orienting strategies for the conservation and management of the species.

Material and methods

Plant material and germination - Pine seeds were collected from the São Francisco de Paula National Forest in southern Brazil (29° 24'S and 50° 22'W, altitude 912 m a.s.l), directly from cone-bearing trees of each of the three varieties, on May 17 ("angustifolia") and on June 22 ("caiova" and "indehiscens"), 2005. Six trees from each variety were used as seed donors. Seeds were stored in polyethylene bags under refrigeration (Fowler *et al*. 1998) for either 97 ("angustifolia") or 64 ("caiova" and "indehiscens") days. Despite different maturation dates, seeds from these last two varieties were harvested at the same time, because seed maturation occurred earlier than usual, which caused an overlap of the maturation period between them. According to local seed gatherers, this anticipation was probably related to the major summer drought that took place in 2005.

Prior to germination, seeds were disinfested with a 2% sodium hypochlorite solution for 20 min and then rinsed thoroughly with distilled water. Seeds were then scarified by removing the hard coats from the proximal third of the seed. This procedure allows for faster and more uniform germination (Ferreira & Handro 1979). Finally, seeds were sowed in trays containing wet vermiculite, and placed on lab benches.

Plant growth - On August 31, 2005, germinated seeds, with well-formed radicles, were selected for planting. PVC tubes with 10 cm diameter and ~45 cm height were filled with 3 L of a homogeneous mixture of medium-sized, washed sand and a commercial organic substrate (Plantimax HA/Eucatex) (2:1; *v:v*). Plants were grown for 244 days (until April 24, 2006) in a glasshouse at the Federal University of Rio Grande do Sul (30º01' S, 51º13' W, 4 m a.s.l.), under naturally fluctuating conditions. Mean minimum and maximum temperatures from April to December in the region vary from 9 to 18 ºC and from 19 to 29 ºC, respectively. Pots were watered to saturation every ten to fifteen days. Irradiance on bench tops was measured with a quantum (LI-190S-1, Licor Inc.), and averaged 937 μmol m^{-2} s^{-1} at noon time of a clear day in late August.

Experimental design - Potted plants were arranged in the greenhouse following a randomized complete block design, each block consisting of 18 experimental units (six plants from each of the three varieties, each seed coming from a different tree donor), placed in close proximity on the greenhouse bench, in order to keep irradiance within blocks as homogeneous as possible. There were a total of 10 blocks and 180 experimental units.

Plant measurements - Plant height (H) and total branch length (TBL, sum of lengths of all lateral branches) were recorded every other week with the aid of a ruler. Total shoot length (TSL) was computed by adding H and TBL. At the end of the experiment, plants were harvested for mass determination and measurement of tap root length (TRL). Plants were fractioned into main tap root, lateral roots and shoot, roots were washed to remove soil debris, and all plant material was oven-dried at 60 °C until constant weight and weighed.

Data analysis - Data were submitted to two-way analysis of variance (block and variety), with randomization test, using the statistical program MULTIV v.2.4 (Pillar 2006). Euclidean distance was used as a dissimilarity measure for all data. Means were compared through orthogonal contrasts ($P \leq 0.05$).

Results

After 90 days of growth, TSL of the "angustifolia" variety was significantly greater than the TSL of the other two (Fig. 1) because of a significantly greater TBL of the former (plant height did not differ between the three groups of plants; Tab. 1). This difference persisted until the end of the experimental period (244 days), when TSL of "angustifolia" plants was 10 and 15% greater than that of "caiova" and "indehiscens", respectively.

Total mass accumulation by plants at the end of the experiment also revealed a 19.8 and 29.2% greater mass in "angustifolia" plants than in "caiova" and "indehiscens", respectively (Tab. 1). Differences in plant mass were due to proportional differences in both root and shoot mass, such that the root:shoot mass ratios were the same for all three varieties (Tab. 1). At root level, reduction in the mass of the tap root in "caiova" and "indehiscens", compared to "angustifolia", occurred at the same proportion as reduction in the mass of lateral roots, such that the mass ratio between these two root fractions did not differ between the varieties either (Tab. 1).

Discussion

The results of this study led to the confirmation of our initial hypothesis, which stated that the varieties should

Table 1. Growth parameters of the three varieties *of Araucaria angustifolia* measured at the end of the experimental period (244 days). Means followed by different letters are significantly different from each other ($P \leq 0.05$).

Growth parameter	Variety			
	"angustifolia"	"caiova"	"indehiscens"	*P* value
Height (cm)	37.66a	35.09a	34.84a	0.0633
Total shoot length (cm)	106.83a	96.79ab	93.32b	0.0004
Tap root length (cm)	47.92a	46.14a	48.06a	0.1418
Shoot (S) mass (g)	8.04a	6.62ab	6.13b	0.0001
Tap root (TR) mass (g)	2.14a	1.82b	1.65b	0.0144
Lateral root (LR) mass (g)	0.99a	0.61b	0.61b	0.0001
Root (R) mass	3.13a	2.43b	2.16b	0.0003
Plant mass (g)	10.84a	9.05b	8.39b	0.0001
LR : TR	0.393a	0.343a	0.380a	0.1628
R : S	0.367a	0.364a	0.368a	0.4459
LR : S	0.103a	0.091a	0.100a	0.5954

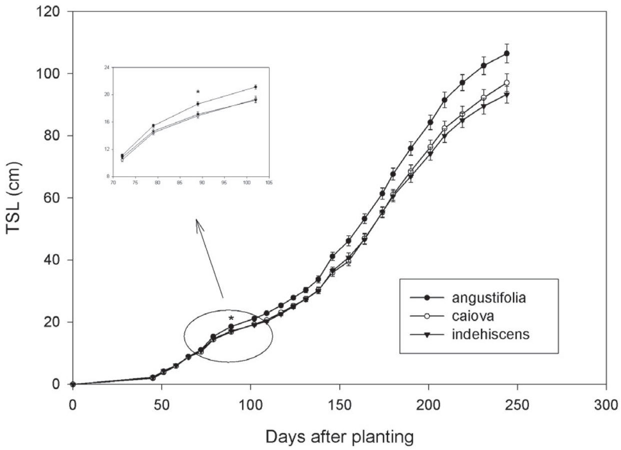

Figure 1. Increases in total shoot length (TSL) along the experimental period (244 days) for the three varieties of *Araucaria angustifolia*. Vertical lines indicate the standard errors of the means. Inset graph shows the growth responses at the time interval when differences were found to be significant. The asterisk indicates the time when differences between the "angustifolia" variety and the other two started to be significantly different ($P \leq 0.05$).

also differ in their initial seedling growth. Indeed, the type variety ("angustifolia") grew more than the other two, and the "caiova" and "indehiscens" varieties did not differ from each other for all the growth parameters we measured. The fact that tree populations of these three varieties do co-occur (Reitz & Klein 1966) is already a strong indicative that the different phenotypic expressions of adult individuals of *A. angustifolia* do not result from phenotypic plasticity, but are very likely an expression of genetic variation. The expression of differences among varieties during initial plant growth further supports the genetic basis of such variation.

Of particular concern when interpreting the results was the fact that the growth differences we measured in this study could have resulted from contrasting size and viability of seeds. With this respect, we unfortunately did not measure mass or size of the collected seeds in order to test if the varieties did differ in this respect. These three varieties have been distinguished from each other in terms of color of seed coverings and maturation times (Mattos 1972), and not in terms of size. Because seeds from the three varieties mature at different times of the year, it was inevitable to store them for different time periods in order to run the experiments with all three at the same time. Thus, seeds from the "angustifolia" variety were collected first and stored longer, and seeds from the "caiova" and "indehiscens" varieties were collected last and stored for a shorter period of time. Considering the recalcitrant nature of the seeds (Panza *et al.* 2002), reduction of seed viability upon storage could be expected. However, we believe that any unaccounted difference in seed size and/or viability did not affect the significance of our results: first, because seedlings that grew more were the ones that came from seeds that were stored longer; and second, because if seed vigor and/or size were to play a role in seedling growth, differences in plant size would have manifested themselves early in the growing period, since the most obvious impact of seed mass is on initial seedling size (Westoby *et al.* 2002; Kennedy *et al.* 2004). This was not the case in our experiment, because significant differences in total shoot length between varieties only appeared at about 90 days after seed germination. Based on our own observations (unpublished results) and on Ferreira & Handro (1979), most of the seed reserves were probably gone by this time.

Despite significant differences in mass accumulation, there were no contrasting patterns in terms of mass allocation. The three varieties exhibited remarkably similar root to shoot mass ratios, indicating equal functional balance between root and shoot activities. Also, the relative investment between the lateral roots and the main tap root did not differ significantly among the varieties. A growth allocation pattern which promotes a greater mass investment in lateral roots at the expense of the tap root results in greater efficiency of absorption (Boot 1989). This leads us to suggest that, at this young plant stage, these three varieties show the same functional balance between the two types of roots.

Considering that seeds from the different varieties mature at different times of the year, the potential advantages of shedding seeds at one time or another deserves future investigation. Would one of these periods be more advantageous for seed germination and seedling establishment? In southern Brazil, particularly in the state of Rio Grande do Sul, precipitation events are more frequent, intense and long-lasting in the fall-winter period than in the spring-summer one (Moreno 1961). The "angustifolia" and "caiova" varieties drop their seed cones in the wettest part of the year, while the "indehiscens" variety displays seed maturation in the drier period. However, since the cones do remain attached to the tree in this variety, seed dispersal is already seriously compromised. According to Mattos (1972), this variety is doomed to extinction.

The superior growth of the type variety might be one of the causes for the larger proportion of its individuals within populations that mix all three varieties. Attaining a greater size at the establishment phase will increase the chances of survival because of a greater ability to compete for resources and to cope with different environmental stresses (Walters & Reich 2000; Myers & Kitajima 2007). *Araucaria angustifolia* shows significant year-to-year variation in seed production within populations, characterizing a mast seeding dispersal strategy (Monks & Kelly 2006). Would this strategy apply only to the most abundant and typical variety? Based on the study by Iob (2007), who showed that sites with greater availability of seeds of *A. angustifolia* showed less seed removal by predators, we suggest that less seed predation will take place at the time the "angustifolia" variety is maturing its seeds when compared to the "caiova". This would represent an additional advantage for the type variety over the others, by increasing recruitment and establishment of the seedlings.

Although focusing on the very young growth stage of the species, the results of this study also indicate that projects aiming to protect the Brazilian pine and its ecosystem should take into consideration the different growth potentials of these varieties, and ensure the use of the type variety in restoration programs.

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