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CARACTERIZAÇÃO DA ESCOTOMORFOGÊNESE EM PLÂNTULAS DE *Araucaria angustifolia* (ARAUCARIACEAE)

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Un bambino al mare

"Conosco un bambino così povero
che non ha mai veduto il mare:
a Ferragosto lo vado a prendere,
in treno a Ostia lo voglio portare.
– Ecco, guarda – gli dirò –
questo è il mare, pigliane un po'! –
Col suo secchiello, fra tanta gente,
potrà rubarne poco o niente:
ma con gli occhi che sbarrerà
il mare intero si prenderà."

Gianni Rodari

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Characterization of skotomorphogenesis in seedlings of *Araucaria angustifolia* (Araucariaceae)

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Abstract:

Depending on light availability, plants may adopt two antagonistic developmental patterns: photomorphogenesis in the light and skotomorphogenesis in the darkness. Skotomorphogenesis is characterized by the use of the reserves to promote mostly stem elongation in order to seek light, while delaying leaf production and development of the photosynthetic apparatus. Despite being well described in angiosperms, it is poorly addressed in gymnosperms. This study aims to characterize this process in *Araucaria angustifolia*, an endangered conifer from South America, by imposing darkness to their aerial environments and forcing shoots to initially develop belowground. Seeds were either sown close to the soil surface or deep into the soil. Half of these seeds had access to light and half remained in the darkness. These plants were grown for 147 days, and then measured for several growth parameters. There was an increased investment on stem elongation at the expenses of leaf production when light was not available. Leaves that developed in the dark were smaller, lighter, and more widely spaced than those that developed in the light. Photomorphogenic shoots were greener and accumulated much more chlorophylls than the whitish skotomorphogenic ones. Darkness had no effect in the rate of consumption of seed reserves and on total dry mass accumulation. True leaves were not produced when shoots developed belowground, and these shoots were colorless, wider and invested more in dry mass in order to elongate when compared to shoots that extended aboveground. Skotomorphogenesis in *A. angustifolia* was characterized by a developmental pattern that increases the chances of plants reaching for light while saving as much carbon as possible. Darkness imposed by seed burial was also associated to the physical resistance offered by the soil, thus altering some aspects of the skotomorphogenesis.

Key words: Development, darkness, Brazilian pine.

Introduction:

In plants, light plays a fundamental role in multiple processes. It fuels photosynthesis, allowing the development of biomass, and it is involved in the detection of neighbour plants in systems with high density of individuals (Küpers et al. 2018; van Gelderen et al. 2018) and in the adoption of different patterns of development (Xu et al. 2015). However, during germination, most plants face the soil, a lightless environment, and must be able to survive as heterotrophic organisms for quite some time. This is possible because seed reserves are used as an energy source until the seedling is tall enough to reach for light and initiate its autotrophic growth based on photosynthesis (Seluzicki et al. 2017).

Depending on whether or not seedlings are exposed to light, two antagonistic developmental patterns arise. Skotomorphogenesis, which is the development in dark conditions, is characterized by the use of the reserves to promote stem elongation in order to seek for light. In this kind of development, a delay in the production of leaves and in the photosynthetic apparatus (Mathews 2005; Xu et al. 2015), as well as an elongated hypocotyl with apical hook, closed cotyledons that protects the shoot apical meristem and undifferentiated chloroplast precursors (Chory et al. 1989; Chory et al. 1996; Arsovski et al. 2012; Seluzicki et al. 2017) may be observed. Such elongation, however, makes the plants more susceptible to several pests due to the repression of induced defenses (Moreno et al. 2009; Cerrudo et al. 2012; de Wit et al. 2013), which emphasizes a trade-off, where the search for light is prioritized over protection (Gommers 2018). On the other hand, light induces photomorphogenesis, a process marked by the deceleration of hypocotyl elongation, the opening of the apical hook, the expansion of cotyledons and the maturation of chloroplasts, allowing the operation of the photosynthetic machinery (Xu et al. 2015; Seluzicki et al. 2017).

Many photoreceptors are involved in the perception of light, allowing the plants to properly respond to their surroundings. Cryptochromes, phototropines, UVB-RESISTANCE 8 (UVR8 for UV-B light), ZEITLUPE/FLAVIN-BINDING, KELCH REPEAT, F BOX 1/LOV KELCH PROTEIN 2 family of photoreceptors (ZTL/FKF1/LKP2) (for UV-A/blue light) and phytochromes (for red/far-red light) enable them to monitor a wide range of wavelengths of light at their establishment sites (Strasser et al. 2010; Xu et al. 2015). The participation of phytochromes in differentiated morphogenesis of seedlings under contrasting light conditions has been extensively studied both from physiological and evolutionary points of view (Mathews 2005; Castillon et al. 2007; Leivar et al. 2008; Strasser et al. 2010; Fortunato et al. 2016; Jung et al. 2016).

The shift from skotomorphogenesis to photomorphogenesis has been vastly addressed in eudicotyledons, especially in the model plant *Arabidopsis thaliana* (L.) Heynh, with focus on

the role of phytochromes (Yamaguchi et al. 1999; Strasser et al. 2010; Jung et al. 2016; Lee et al. 2016). Nevertheless, studies that characterize the skotomorphogenesis in gymnosperms, and address photoreceptor involvement in the transition to photomorphogenesis, are still very scarce when compared to those already performed with angiosperms. Even though research with angiosperms has an incomparable and undeniable repercussion for the understanding of molecular and evolutionary aspects of the processes involving light signals in plants, the results cannot be extrapolated to other groups and species with respect to morphological and ecophysiological patterns of response to light or lack thereof. Some patterns observed, such as the non-opening of the plumular hook, are exclusive of eudicotyledons, preventing the generalization to other groups (Leivar et al. 2008; Xu et al. 2015; Seluzicki et al. 2017). Thus, considering the genotypic and phenotypic differences among species, it is essential to characterize the skotomorphogenesis within different taxonomic and ecological groups, in order to deepen the knowledge about the diversity of growth patterns. Therefore, this study aims to characterize this process in the tree species *Araucaria angustifolia* (Bert) O. Ktze, a gymnosperm of the Araucariaceae family that grows in South America.

Araucaria angustifolia occurs in southeastern Brazil, primarily in the states of Paraná, Santa Catarina, Rio Grande do Sul, and locally in São Paulo, Minas Gerais and Rio de Janeiro, but also in some adjacent areas of Argentina (Misiones) and Paraguay (de Souza et al. 2009; Thomas 2013). It suffered intense logging in the last century, causing a severe reduction in its area of occurrence (Guerra et al. 2002), which originally covered about 200,000 km² (Reitz et al. 1988), but today is restricted to about 1 to 5% of the original extent, making it critically endangered (Thomas 2013). The collection of 3,400 tonnes per year of seeds for human consumption, habitat fragmentation and forest clearance are threats to this species (Thomas 2013). However, other authors suggest that the food usage of its seeds are contributing to preservation of this species (Barbosa et al. 2019), evidencing the need for more studies.

In the adult stage, *A. angustifolia*, a pioneer species in grasslands and an emergent tree in forests, is known for being a 'sun species'. Nevertheless, during its initial stages of development, it tolerates shade, being able to germinate and form seedling and sapling banks in the forest understory (Inoue et al. 1979; Duarte & Dillenburg 2000; Duarte et al. 2002). Also, many dispersers bury its seeds deep into the soil for future consumption (Iob & Vieira 2008; Ribeiro & Vieira 2014), which may result in full unavailability of light to the growing seedlings until they emerge from the soil surface. Considering the aforementioned and the scarcity of information regarding the development of gymnosperms in the absence of light, this study aims to characterize the skotomorphogenic development of seedlings of *A. angustifolia*, by imposing darkness to the plants both by burying the seeds deep into the soil and by suppressing light from their aerial environment.

Based on the current knowledge about plant skotomorphogenesis and taking into account the photomorphogenic development of *A. angustifolia* seedlings, we posed the following initial questions: (1) Are shoot and internode elongation increased under light suppression? (2) Does skotomorphogenesis compromise stem support? (3) Is leaf development delayed under darkness? (4) Is shoot greening (chlorophyll accumulation) suppressed in the absence of light exposure? (5) Does darkness result in a greater consumption of seed reserves? (6) Do dark-grown plants differ from light-grown ones on their overall mass accumulation and on the relative investments of such mass? (7) Is skotomorphogenesis development different, depending on whether light was suppressed by seed burial or by shoot exposure to a light-deprived aerial environment?

Material and methods:

Seed gathering and planting:

Pine seeds were obtained from three different municipalities in the state of Rio Grande do Sul, Brazil: Progresso (29° 14 '38 "S 52° 18' 43" W), Barros Cassal (29 ° 05 '34 "S 52° 34' 58" W) and Cacique Doble (27° 46'12 "S 51° 39'36" W), and stored inside plastic bags under 4 °C for 15 days. Such seeds from different locations were then mixed. They were disinfected by a 20-minute immersion in a 1:1 (v:v) solution of distilled water and sodium hypochlorite (with 2-2.5% of active chlorine). Subsequently, they were washed in running water for 5 min and rinsed with distilled water. Each seed was weighed in an analytical balance (Analytical Balance FA2104N, Bioprecisa, Curitiba, Brazil) to obtain its fresh mass (FM). On June 4, 2018, they were planted in PET bottles, filled with a 1:2.5 mixture of medium-sized pre-washed sand and a commercial organic substrate (Substrato orgânico pronto para vasos HUMOSOLO, Vida, Porto Alegre, Brazil).

To estimate the dry mass (DM) of the planted seeds, an extra lot, composed of 30 seeds, had their FM and DM measured, in order to estimate their water content (on a fresh weight basis). Seed drying took place on a forced-air drying oven (Forced-air drying oven DL-AF, DeLeo, Porto Alegre, Brazil) at 70 °C until mass became constant. Their average water content (0.43 g) was then used to estimate the DM of each planted seed.

Growth conditions and light treatments:

The experiment was conducted in the Plant Ecophysiology Laboratory at Federal University of Rio Grande do Sul, located in Porto Alegre, RS, Brazil (30° 04'33.0"S 51° 07'29.1"W) for a period of 147 days (from June 4, 2018 to October 29, 2018). Four different treatments were

assigned to 10 plants each: **(1) Light-surface (LS):** Seeds were sown close to the soil surface (2 cm deep) and received light; **(2) Light-buried (LB):** Seeds were sown deep in the substrate (10 cm deep) and received light; **(3) Darkness-surface (DS)**: Seeds were sown close to the soil surface (2 cm deep) and did not have access to light; **(4) Darkness-buried (DB):** Seeds were sown deep in the substrate (10 cm deep) and did not have access to light. The purpose behind burying the seeds was to create similar conditions to those imposed by some dispersers.

Darkness was imposed by the use of a black canvas in the growing environment, while the control groups were kept near windows and had access to natural light. Plants were kept well-watered throughout the experiment. When watering the seedlings that remained in the dark, the laboratory had its windows previously darkened with black canvas, and a green LED lamp was used to illuminate the room, since plants do not absorb much of the green light wavelengths.

Growth parameters:

Height, total shoot length, diameter and biomass: Plant height was measured throughout the experiment. Upon harvesting, all individuals had their total shoot length measured and were separated into lateral roots, main root (which included the underground hypocotyl), shoot, and attached seed. Concerning LB and DB plants, the portion of the shoot that grew below the soil surface (belowground shoot) was separated from the segment that emerged from the soil (aboveground shoot). Stem diameter at mid-height was measured with a caliper, and in the case of LB and DB plants, it was measured both in the below- and aboveground shoot sections. The plant material was oven-dried at 70 °C until constant mass was reached. Th weighed in an analytical balance (Analytical Balance FA2104N, Bioprecisa, Curitiba, Brazil). These individual masses were used to compute some biomass ratios: shoot/root, shoot/lateral roots, and lateral roots/main root.

Specific stem length (SSL): This parameter was calculated by dividing the shoot length by its dry mass. In the case of LB and DB plants, the SSL was calculated both for the below- and aboveground shoot sections.

Internode length (IL): It was estimated by dividing the number of leaves in 1/5 of the overall shoot length, by the respective shoot length. A large number of leaves per unit of shoot length was then interpreted as a short internode length.

Specific leaf area (SLA): It was computed as the ratio between the area of six leaves taken from the shoot mid-section and their respective dry mass. The area was calculated using leaf photographs, with the software SketchAndCalc (Dobbs 2011; available at www.sketchandcalc.com).

Chlorophyll content: Chlorophylls were extracted from a sample of six fresh leaves (the same used for SLA), using absolute ethanol. The absorbance readings were performed in a

spectrophotometer (Spectrophotometer PM 2K, Zeiss, Co., Oberkochen, Germany) at wavelengths of 649 and 665 nm. The chlorophyll concentration was calculated according to Wintermans & De Mots (1965), and their content was expressed on leaf area and leaf dry mass basis.

Seed reserves consumption: It was estimated using the final dry mass (FDM) and the initial dry mass (IDM) of the seeds, and then calculating seed consumption with the equation (IDM - FDM)/IDM.

Data analysis

To make comparisons between all treatments, parametric data was submitted to a one-way analysis of variance (one-way ANOVA), while one-way ANOVA on ranks (Kruskal-Wallis test) was used when data did not show homogeneity of variances and normality of distribution. In both cases, ANOVA was followed by Tukey's test. To compare parameters between two groups, a t test was executed and, in case of non-parametric data, a Mann-Whitney *U* test.

In the case of SLA, ANOVA was followed by a Dunn's test due to different sample size, since three individuals (that belonged to LS, DS and DB groups) were excluded from this analysis.

All tests were performed with $P \leq 0.05$, using Sigmaplot 11 (Systat Software Inc., San Jose, California, USA).

Results:

Plant height among treatments did not differ until the last measurement, when DS and DB plants had a significantly higher height than LS and LB (Figures 1 and 3). The total shoot length was higher in DS than in LS plants, while LB and DB did not differ. Seed burial did not affect this parameter, even when considering the presence of absence of light (Figure 2). The internode length was also longer in dark-grown plants, as revealed by the smaller number of leaves per unit of shoot length in comparison with light-grown plants (Table 1).

Biomass and its ratios were not significantly different among the groups (Table 1 and Figure 4). LB seedlings consumed more seed reserves than LS, but this difference was not observed when DB and DS treatments were compared to each other. There was also no difference in seed consumption between light-grown (LS and LB) and dark-grown (DS and DB) plants (Table 1).
The SSL of the aboveground shoots was higher in DB than in all other plant groups, which did not differ from one another, while SSL of the belowground shoots was the same (Table 1). For plants that had their seeds deeply buried (LB and DB), SSL did not differ between their

belowground shoot sections. On the other hand, their belowground shoots had a much lower SSL than their respective aboveground shoot sections (Table 1).

The section of shoots that developed belowground (present in LB and DB plants) did not produce leaves (Figure 5). The area and dry mass of individual leaves of the aerial shoots were much greater in light-grown (LS and LB) than in dark-grown plants (DS and DB). These leaf parameters were not affected by seed burial. SLA was greater in darkness (Figure 6), but only in plants that had their seeds buried into the soil (DB *vs*. LB). Leaf chlorophyll *a* and *b*, as well as total chlorophyll, had a much higher content on light-grown plants than in their dark counterparts, both when expressed on a leaf area and on a leaf dry mass basis (Figures 7 and 8). In fact, leaves from dark-grown plants were completely pale.

Discussion:

DS and DB plants had a very distinct phenotype, characterized, from the physiognomical point of view, by the longer (but self-supporting) stems, the tinier leaves and the overall lack of the characteristic green color of the shoots, when compared to LS and LB. Some interesting results also arose when looking at the plant responses to the deep seed burial that was imposed. We will now specifically address the initial questions posed in this study, and try to get an overall picture of the skotomorphogenic development of seedlings of *A. angustifolia* and the possible physiological and ecological significance of such development.

Are shoot and internode elongation increased under light suppression?

An increased elongation of the internodes and/or the hypocotyls is a typical skotomorphogenic response reported for angiosperms (Josse & Halliday 2008; Seluzicki et al. 2017). The hypocotyl of *A. angustifolia* has an underground development, and we would not expect it to elongate in response to either shade or darkness. Instead, the main shoot (epicotyl) of the young plants were significant longer (resulting in taller plants) when plants had the light suppressed from the aerial environment (DS and DB) than when light was made available to the plants (LS and LB). This increase in stem elongation was associated to longer internodes, as revealed by the smaller number of leaves per unit of stem length of the dark-grown plants. A greater investment in height is also the most common response to shaded environments (e.g., Cancian & Cordeiro 1998; Poorter 1999; Ferrer & Dillenburg 2000; Jurado et al. 2006), particularly in the so called ‘sun plants’, and this response also involves the balance between active and inactive forms of the phytochromes (Morgan & Smith 1979). This elongation response in response to shading has also been reported for young plants of *A. angustifolia* (Inoue & Torres

1980; Franco & Dillenburg 2007), though it seems to be a transitory, initial growth response (Franco & Dillenburg 2007).

A possible ecological significance of the greater initial investment in height when seedlings are cultivated under shade or, in this case, in total darkness, consist in overcoming whatever is reducing or suppressing light incidence in the growing plant: the competing vegetation in dense stands (Schmitt et al. 1995; Donohue et al. 2000; Pierik et al. 2003), or a thick layer of litter or soil. By speeding up height growth, seedlings will have an increased chance of more rapidly improving their light environment compared to seedlings that do not exhibit this response. This 'seek-for-light' strategy may ensure the survival of the photoautotroph organisms, but will most likely come at the expenses of other growth investments. It is well known that some angiosperm skotomorphogenic seedlings will allocate their resources toward hypocotyl elongation, at the expense of cotyledon and root development (Josse & Halliday 2008). In the case of *A. angustifolia*, dark-grown seedlings had less leaves per unit of stem length than light-grown ones, reflecting the greater internode elongation of the former, and the increased investment on stem elongation at the expenses of leaf production when light was not available. Such type of response has also been observed in shading experiments (Egara & Jones 1977; Li et al. 2010). Unlike the strategy of just growing taller, which requires a greater investment of dry matter, the internode elongation is a resource saving strategy, since fewer leaves are produced. Internode extension plays a crucial role in forest gaps, allowing seedlings to grow away from dark or shaded areas and neighboring plants in the competition for light resources, with little investment of dry matter, since fewer leaves will be produced (Schmitt & Wulff 1993; Peer et al. 1999). In other words, it is a cheap strategy of growing tall and seeking for light.

Does skotomorphogenesis compromise stem support?

All plants were able to fully support their shoots in a vertical position after about five months of growth. For dark-grown plants, this means that the skotomorphogenic development of their shoots, which includes a great investment on elongation, did not compromise the support of such shoots. Remaining erect is crucial for seeking for light, and this was at least partly accomplished by not compromising stem radial growth. Regardless of the presence or absence of light, plant shoots developing aboveground all had a similar stem diameter, which means that stem elongation of dark-grown plants did not come at the expenses of stem radial growth. On the other hand, the taller shoots presented by the dark-grown plants may require an even larger diameter or an extra-mechanical support (compared to light grown plants), in order to ensure that their longer shoots can keep themselves erect. The SSL is a proxy of biomechanical stability (Kleyer et al. 2019), and the higher SSL of the aboveground shoots of the DB plants, when compared to LB, means that the former are producing the same length of stem with less

material, which could compromise stem stability. Taking into account that both plants had similar stem diameters, we can suggest that stem tissues are less dense in DB than in LB plants, causing them to have a larger SSL. It is possible that light-grown plants were able to provide stems with tissues that allow for mechanical stability, meaning greater investment in cell wall material and lower SSL. Dark-grown plants, on the other hand, may have accomplished their stability through a greater investment in turgor of the stem cells. The stems of the dark-grown plants did indeed look more succulent than those of light-grown plants, but anatomical investigations will be needed to help us elucidate these questions.

An interesting result was that DS plants did not differ in either SSL or stem diameter when compared to LS plants. Not having to face a deep layer of soil may have allowed these plants to invest more carbon material in the construction of their shoots, which led them to have a much smaller SSL than those shoots that were also in the dark, but that also had part of their extension growing belowground (DB plants). Our results show that DS shoots were more capable than DB shoots to sustain themselves erects as they grew taller, but this possible difference in stem stiffness was not evaluated in our study.

Is leaf development delayed under darkness?

A delay in leaf development is a key characteristic of the skotomorphogenic development of plants (Mathews 2005; Xu et al. 2015). Leaves are of no use if light is not available, and expanding fewer leaves and reducing their expansion (responses observed in the dark-grown plants of *A. angustifolia*) will result in the economy of carbon, which can then be used for shoot extension in the search for light.

On average, leaves that developed in a dark aerial environment had about 25% of the area of the ones that developed in the light. In terms of leaf mass, the reduction was in the same extent for plants whose seeds were planted close to the surface, but for those whose seeds were deeply buried, leaf size was only 18% of the size of light-grown leaves. As a result, SLA of DB plants was larger than all other plants, meaning that they invest less mass in the construction of a given areas than all others.

Specific leaf area (SLA) is the most important factor responsible for the variation in a plant's relative growth rate, recognized for its association with different traits, such as morphology, physiology and biochemistry (Lambers et al. 1998). Environmental conditions, such as temperature and irradiance, are capable of modulating SLA. In coniferous trees, shading is responsible for the development of wider and thinner leaves, which increases SLA when compared to high-irradiance conditions (Kimmins 1987). Leaves with high SLA are considered 'cheap' leaves, and shading commonly result in the increase of this parameter, revealing a greater relative investment in area at the expenses of mass. This response may reflect a survival

mechanism based on resource savings, since it means a lower investment of dry mass on a given light harvesting surface (Boardman 1977; Givnish 1988; Barrett & Fox 1994). However, maximizing leaf area by an increment in SLA is linked to additional exposure to drought, herbivory and frost (Valladares & Niinemets 2008), becoming limiting factors for such an increase.

Previous studies have shown SLA of young plants of *A. angustifolia* to increase in response to shading (Duarte & Dillenburg 2000), although such increase seems not to persist over time (Franco & Dillenburg 2007), which probably resulted from the fact that various factors modulate this parameter. Our present results also show inconsistent response of SLA to light availability, since darkness did not result in increased SLA when seeds had not been deeply buried. In summary, plants deprived of light saved carbon for shoot elongation by expanding fewer, smaller and lighter leaves, and, when plant shoots also had to face the soil environment, the leaves that later emerged also had a greater SLA, probably as a result of the additional carbon investment that had to be made to overcome the physical resistance offered by the soil.

Is shoot greening (chlorophyll accumulation) suppressed in the absence of light exposure?

LS and LB plants produced much more chlorophyll than DS and DB. It is interesting to note that, despite being in the dark, the etiolated seedlings did produce a little amount of chlorophyll as well, even though they appeared either whitish or yellowish.

During chlorophyll (Chl) biosynthesis, the reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) is an essential step, being responsible for the plant greening phenotype. Two different Pchlide enzymes are able to catalyse this reaction, the light-dependent NADPH-Pchlide oxidoreductase (LPOR) and the the light-independent Pchlide reductase (DPOR) (Armstrong 1998; Gabruk & Mysliwa-Kurdziel 2015; Stolárik et al. 2018).

Gymnosperms that produce chlorophylls in the dark have both Pchlide reductases, while angiosperms usually lack the DPOR enzyme, which prevents them from producing chlorophyll without light. However, among conifers and within the gymnosperm group, there is a significant variability in the chlorophyll biosynthesis as well as in the formation of the photosynthetic apparatus (Armstrong 1998). When grown in darkness, *Picea abies* (L.) Karst accumulated the highest amount of Chl of all Pinaceae (Mariani et al. 1990; Fujita & Bauer 2003; Kusumi et al. 2006; Demko et al. 2009). On the other hand, *Ginkgo biloba* L. is unable to synthesize the pigment in the absence of light, despite the presence of the corresponding genes, which may not be expressed in a significant quantity (Burke et al. 1993; Chinn & Silverthorne 1993; Richard et al. 1994; Pavlovič et al. 2009).

This is the first study addressing the development of seedlings of *A. angustifolia* in darkness, since previous works only focused on the role of shading. Franco & Dillenburg (2007) reported

a higher chlorophyll content per unit of leaf dry mass in plants receiving 10% of full irradiance than in those which were fully irradiated. This contrasted to the results presented by Duarte & Dillenburg (2000), who did not observe an increase in chlorophyll concentration in response to a similar level of shading. An increase in the amount of chlorophyll per unit of leaf mass in plants exposed to low irradiances is a quite common response (Boardman 1977; Barrett & Fox 1994; Henry & Aarssen 1997; Nicotra et al. 1997), and has been interpreted as an adjustment of the photosynthetic apparatus aiming to absorb a greater amount of photons that travels through the leaf tissue. However, some studies have proposed that a reduction in total chlorophyll content may also be a proper physiological response (Boardman 1977; Kozlowski et al. 1991; Barrett & Fox 1994; Stenberg et al. 1995). Strauss-Debenedetti & Bazzaz (1991) have proposed that the response of chlorophyll concentration to light availability depends on plant life habits, but, taking into account the N costs involved in synthesizing chlorophylls, one would expect it to also be responsive to soil chemical conditions. When it comes to darkness, chlorophylls are of no use, and not synthesizing them until light is available may save carbon and nutrients to the growing plant. Our results do indicate that seedlings of *A. angustifolia* do not respond to darkness in the same way it usually respond to shade. As previously stated, this response could represent an economy of resources in such a hostile environment, where photosynthesis is not possible and the seed's finite resources have to be parsimoniously used. We hypothesize that, alike other gymnosperms that do not synthetize chlorophyll in the dark, the DPOR gene may be absent or non-functional in this species, and we suggest this possibility should be investigated in future studies.

Does darkness result in a greater consumption of seed reserves?

One would expect that, due to the deprivation of light, seedlings of *A. angustifolia* would more readily consume the abundant seed reserves than those under light. Darkness, however, had no apparent effect in the rate of consumption of seed reserves. Dillenburg et al. (2010), when investigating the role of the underground hypocotyl during seedling growth, reported that, regardless of whether seedlings were developing under light or darkness, seed consumption followed the same pace, and its reserves were mostly restored in the underground hypocotyl for later use in sustaining shoot growth. It does seem that, by having the possibility of restoring the seed reserves, the species drains its supporting seeds in a manner that is not responsive to how much resources are available for growth.

If light per se did not affect the rate of seed consumption, seed burial did, but only when the aerial environmental was not shaded. These results will be later discussed.

Do dark-grown plants differ from light-grown ones on their overall mass accumulation and on the relative investments of such mass?

Despite the contrasting phenotypes between light- and dark-grown plants, the total amount of dry mass they accumulated did not differ. On a first look, this seems quite surprising considering that plants in the dark were unable to photosynthesize. Dillenburg et al. (2010) reported differences in mass accumulation between light- and dark-grown plants, but only after 100 days of growth. This major delay in detecting the negative effects of light absence on mass accumulation probably results from the plentiful reserves provided by the seed, combined with a slow pace of growth. The dark-grown plants in our study would most likely accumulate less mass than those exposed to light, had we allowed them to grow a little longer.

Another important aspect relates to the distribution of the total mass among the different plant parts. Darkness did not result in any significant changes in the shoot : root and shoot : lateral roots biomass ratios. Increasing the allocation to shoot growth in response to shading is regarded as a universal response (Pearcy & Sims 1994), but it may reduce plants' ability to compete for belowground resources (Walters et al. 1993). Duarte & Dillenburg (2000) and Franco & Dillenburg failed to detect such differences in their 5-month experiments, while Inoue and Torres (1980) did report an increase in the shoot : root mass ratio of *A. angustifolia* in response to shading in a 9-month experiment. In contrast to these previous studies, we imposed darkness and not shade to the growing seedlings. Our experiment also lasted almost five months, and we also did not observe any changes in either the shoot : root or shoot : lateral roots mass ratios in response to darkness. These lacks of responses displayed by seedlings may reveal the importance of the root system for the very initial stages of plant development and indicates that the degree of plasticity of these ratios probably depends on the stage of plant development.

The experimental seedlings were also unresponsive to the presence or absence of light regarding the relative investment in lateral roots vs. main root. This unresponsiveness may be again related to a lack of plasticity at the very early stages of plant development, but we can also speculate that, for as long as the young seedlings survive under darkness, changing this mass allocation may have no impact in its success in the search for light.

Is skotomorphogenesis development different, depending on whether light was suppressed by seed burial or by shoot exposure to a light-deprived aerial environment?

Two major considerations should be made regarding plants that originated from seeds that were buried deep into the soil. One concerns and intra-plant and intra-organ response of below- *vs* aboveground sections of the same shoot. The other relates to the possible interactive effects of seeding depth and light presence in the aerial environment.

When the two different sections of the same shoot were compared in LB and DB, important differences did appear. When belowground, true leaves were not produced in the shoots, and what we found were scale-like structures. These underground shoot sections were also green-less, but we did not make any attempt to quantify chlorophylls or SLA in those scaly leaves. We can then say that, regarding leaf development, that being buried into the soil resulted in more profound reductions in leaf development than only being in the dark, and made the tiny leaves grow attached to the stem surface, which certainly facilitated shoot extension through the soil. As for chlorophyll accumulation, no additional effect of the soil around the shoots would be expected other than suppressing the light and then preventing them from producing these photosynthetic pigments, so they were as colorless as the aboveground sections that were in the dark (DB plants) and deeply contrasted to the green aboveground sections when these were irradiated (LB plants). Stem diameter was also dramatically different between the two sections, both in LB and DB plants, being much larger in the below- than in the aboveground section. This increased diameter most certainly allowed the stems to more successfully 'dig' through the soil without breaking and is analogous to the already reported increased diameter of roots in response to increased soil compaction (Mósena & Dillenburg 2004). Consistent with the diameter response, the sections of the shoot that were growing below the soil surface had a much lower SSL than the sections that were growing above the soil surface, indicating a much greater investment of mass in order to achieve a given extension of the shoot. As previously discussed, this smaller SSL may also have resulted from an increased density of the root tissues, an aspect which has not been evaluated in this study. It is known that switching from a thin-walled stem tissue to a dense and lignified one increase stem vigor and prevent plants from collapsing under their own weight as they grow taller (Steingraeber 1982; Niklas 1994). A similar switch might be needed when a stem elongates in a high resistance environment such as the soil, instead of elongating facing the much lower resistance offered by the air. To our knowledge, this is the first study to address the impact of seed burial in the shoot growth and development of *A. angustifolia.*

In addition to affecting shoot developmental patterns within the same plant, seed burial also modulated some of the plant responses to the presence of light and *vice versa*. One would expect that plants originating from deeply-buried seeds would attain a smaller height than those coming from seeds located close to the soil surface, because, in the first scenario, part of the shoot vertical extension took place belowground. Interestingly, this effect was only perceived in dark-grown plants. We suggest that the great carbon investment that had to be made by the underground stems (which had a smaller SSL), did not prevent the LB plants from attaining the same height as LS plants. The seed consumption data show that the former had used more of the seed reserves by the end of the experiment than the latter, and, taking into account that this difference in seed consumption was not noticed when comparing DS and DB plants, it is

possible that the LB plants speeded up seed consumption only when the shoots emerged from the soil and acquired a photomorphogenic development. This final 'boom' in seed consumption allowed their shoots to get as tall as those that were never belowground. Seed consumption did not differ between DS and DB plants, which resulted in those whose shoots came from deep in the soil to attain a smaller height than those that started from the surface. It was also noticeable that a deep seed burial in dark-grown plants (DB) not only resulted in shorter plants but also in aboveground shoots with much greater SSL (more slender shoots), when compared to plants that were also in a dark environment but did not have to face a deep seed burial. A similar response also occurred at the leaf level: the same DB plants also had the greatest SLA among all plant groups, indicating that they invested much less carbon to build a given leaf area than did the dark-grown plants that did not face deep seed burial.

It was already mentioned earlier in the discussion, that an increase in the shoot : root ratio in response to darkness was observed only when plants originated from deeply-buried seeds. This resulted from the unexpected and yet unexplained fact that deep-seed burial led to an increase of root mass and a consequent decrease in shoot : root ratio in plants exposed to light. This resulted in LB plants having a shoot : root ratio that was smaller than all other groups.

What next?

Futures investigations of the skotomorphogenic development of *A. angustifolia* should look deep into anatomical aspects of shoots and leaves to better understand the mechanisms behind the observed responses. The temporal dynamics of seed consumption would also help understand the distinct responses to light availability between plants that arose from deeply sown seeds and those that emerge from seeds located close to the soil surface. It could also be quite interesting to investigate the biochemical and molecular limitations of the skotomorphogenic seedlings regarding chlorophyll accumulation.

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Tables and Figures

Table 1. Growth and physiological parameters measured in *Araucaria angustifolia* seedlings grown under four different treatments. All means are followed by standard errors. Treatments means with different letters within rows and with asterisks (*) between rows are significantly different at $P \leq 0.05$.

Parameter	Treatment			
	LS	DS	LB	DB
Shoot : Root (g g^{-1})	1.82 ± 0.24a	1.87 ± 0.24a	1.03 ± 0.09b	1.36 ± 0.16ab
Shoot : Lateral roots (g g^{-1})	5.11 ± 1.00ab	6.43 ± 1.03a	2.42 ± 0.22b	4.11 ± 0.57ab
Lateral roots : Main root (g g^{-1})	1.64 ± 0.35ab	1.15 ± 0.20b	2.01 ± 0.17a	1.57 ± 0.21ab
Seed reserves consumption (g g^{-1})	0.69 ± 0.02bc	0.74 ± 0.02bc	0.80 ± 0.01a	0.77 ± 0.02ac
Stem diameter - Aboveground shoot (cm)	0.27 ± 0.01ab	0.33 ± 0.02a	0.26 ± 0.02b*	0.29 ± 0.01ab*
Stem diameter - Belowground shoot (cm)	-	-	0.51 ± 0.03a*	0.41 ± 0.02b*
Specific stem length - Aboveground shoot (cm g^{-1})	34.58 ± 3.10b	43.99 ± 2.45b	40.46 ± 1.52b*	73.19 ± 9.43a*
Specific stem length - Belowground shoot (cm g^{-1})	-	-	24.44 ± 1.94a*	29.81 ± 3.35a*
Internode length (leaves cm^{-1})	4.97 ± 0.45ac	2.43 ± 0.14b	5.16 ± 0.56ac	2.87 ± 0.48b

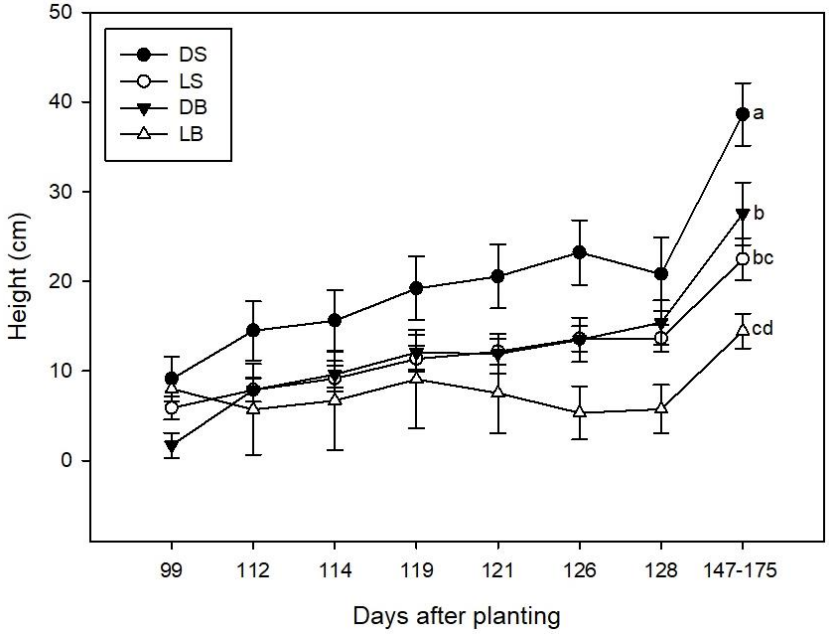

Figure 1. Seedling height growth. Vertical bars indicate the mean standard error. Significant differences between treatments ($P \leq 0.05$) were detected only in the last measurement.

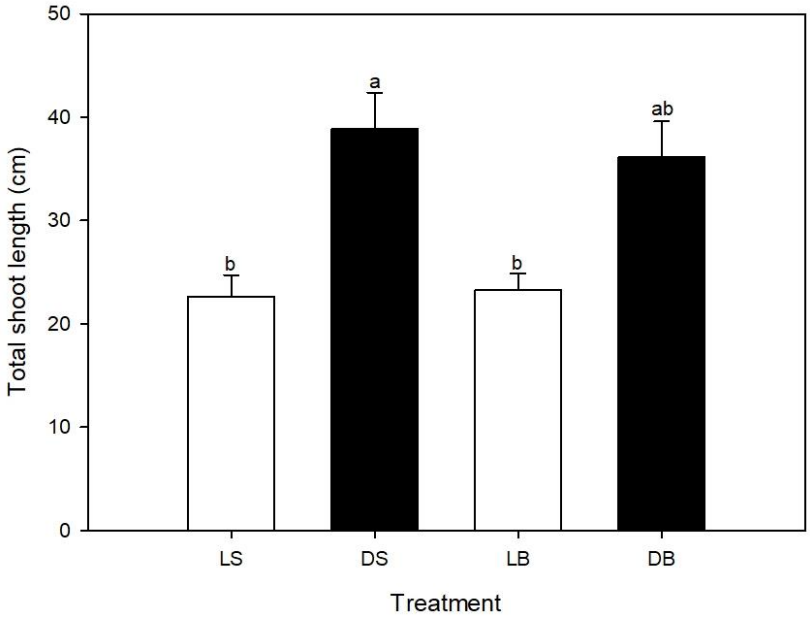

Figure 2. Total shoot length of the seedlings. Vertical bars indicate the mean standard error. Different letters above bars indicate significant differences between treatments ($P \leq 0.05$).

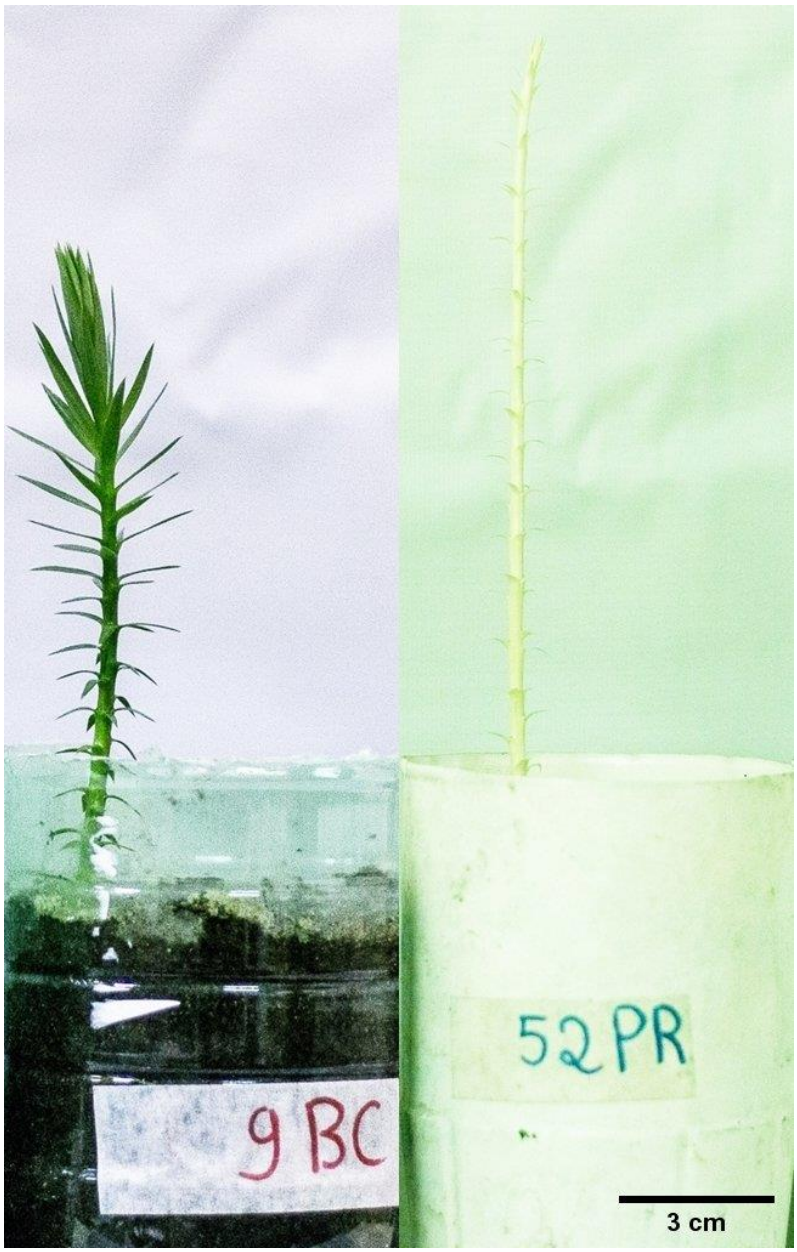

Figure 3. A photomorphogenic (left) and a skotomorphogenic (right) *Araucaria angustifolia* seedling. Note the paler and longer stem, as well as the tinier leaves of the skotomorphogenic plant, when compared to the photomorphogenic one. The skotomorphogenic plants did not lose the capacity for self-support.

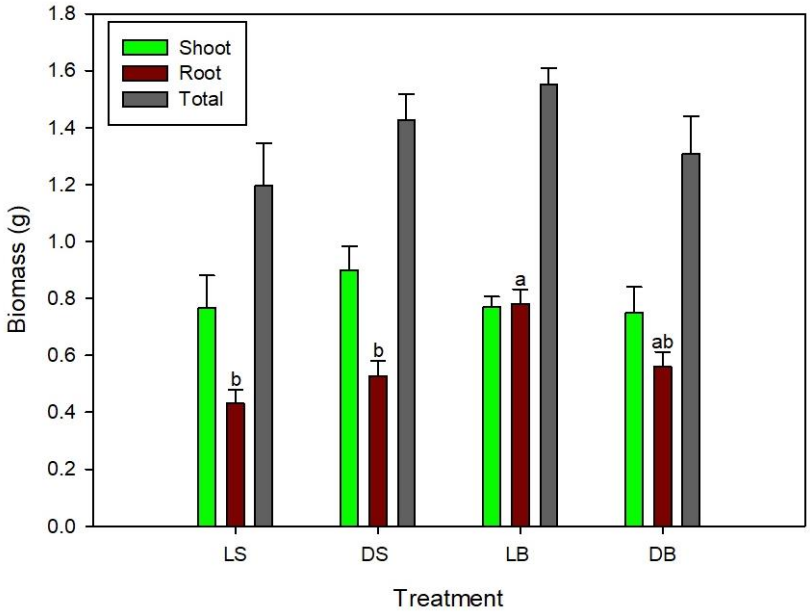

Figure 4. Plant biomass at the end of the experiment. Vertical bars indicate the mean standard error. Different letters above bars indicate significant differences between treatments ($P \leq 0.05$). There were differences only in the root biomass.

Figure 5. A photomorphogenic seedling (from the LB group) whose seed was buried deep into the soil. Note the absence of leaves in the belowground shoot.

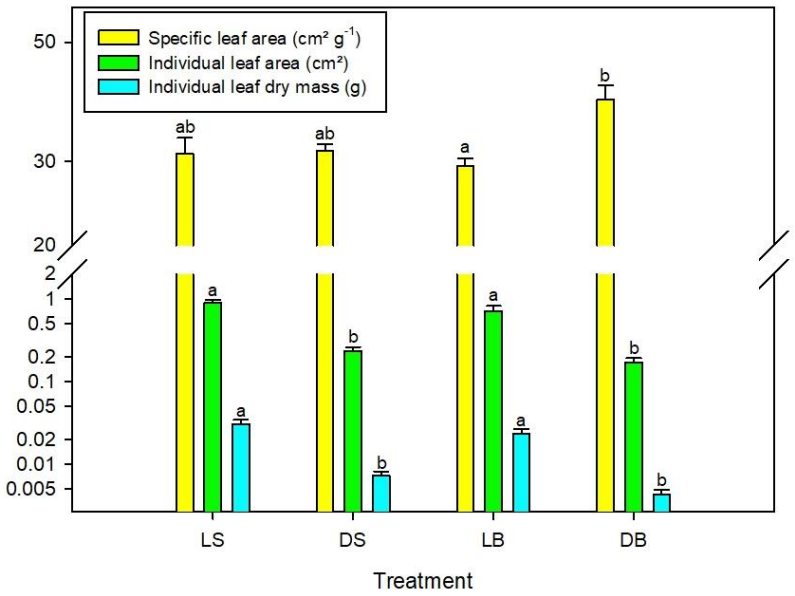

Figure 6. Specific leaf area, individual leaf area and individual leaf dry mass at the end of the experiment. Vertical bars indicate the mean standard error. Different letters above bars indicate significant differences between treatments ($P \leq 0.05$).

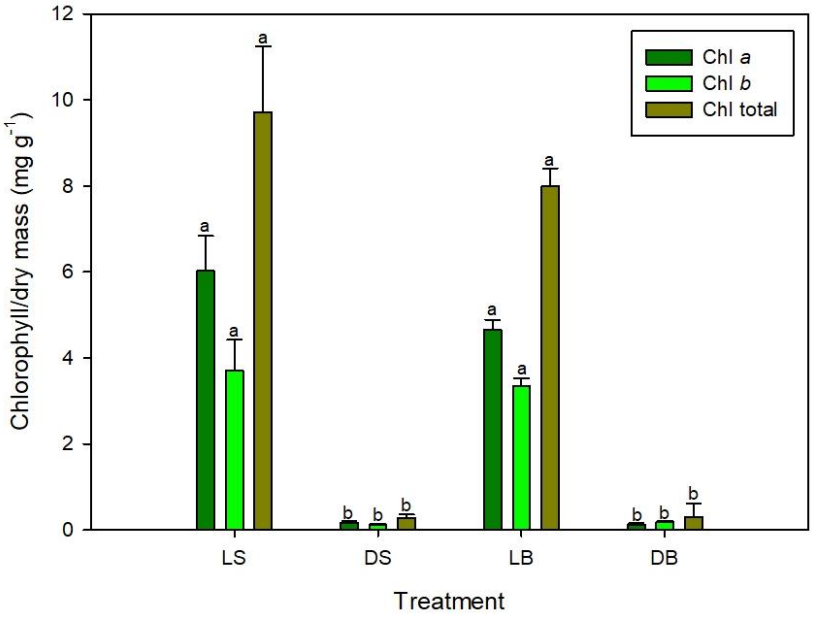

Figure 7. Chlorophyll content on a dry mass basis. Vertical bars indicate the mean standard error. Different letters above bars indicate significant differences between treatments ($P \leq 0.05$).

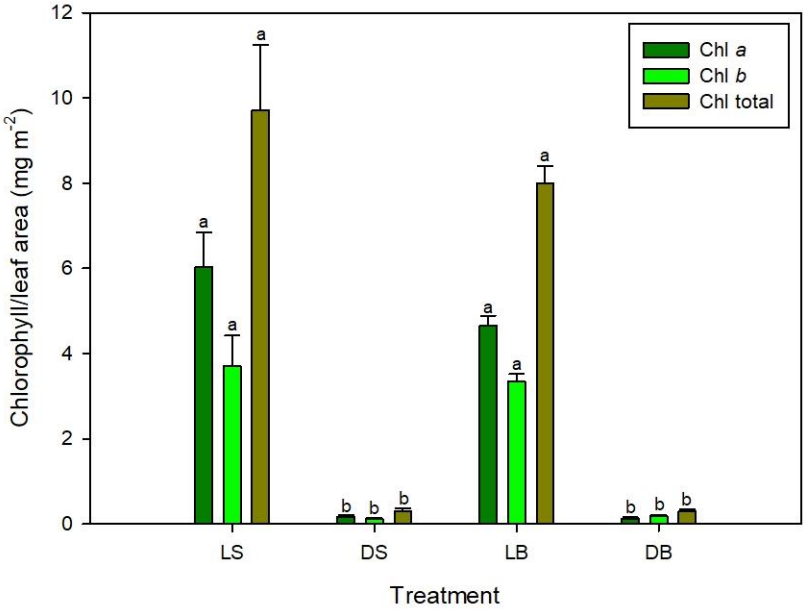

Figure 8. Chlorophyll content on a leaf area basis. Vertical bars indicate the mean standard error. Different letters above bars indicate significant differences between treatments ($P \leq 0.05$).