



# Scientific novel: *Araucaria angustifolia* branch may be converted into orthotropic trunk by grafting<sup>1</sup>

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10.1590/0034-737X202067010012

## ABSTRACT

Grafting technique has been presented as an alternative for *Araucaria angustifolia* propagation. However, vegetative rescuing mature trees and obtaining large numbers of propagules is difficult, because the use of branch grafts has not been indicated due to its plagiotropic and morphogenic characteristic, generally resulting in undesired growth of the graft. So we aimed to describe the unpublished fact occurred in four plants of *A. angustifolia* grafted with grafts from primary branches that resulted in conversion to trunk. Plant growth and morphology were evaluated ten years after grafting. We verified vertical growth and organization of trunk and persistent branches with defined whorls, same to plants grafted with trunk grafts. Field tests with grafted plants using grafts obtained from the four plants resulted in same development pattern, proving the permanent morphogenic change and opening new perspectives for araucaria vegetative rescue and grafting. We concluded that trunciform shoots can be obtained by *A. angustifolia* grafting with plagiotropic primary branches. Despite the morphological clarity of trunciform shoots on grafted plagiotropic primary branches, it has not yet been possible to identify the causes of this reversion.

Keywords: conifer; tropism; vegetative propagation; brazilian pine.

## INTRODUCTION

*Araucaria angustifolia* is a native species of South America. With its characteristic canopy architecture, this species can reach an average of 25 meters in height and 1.5 meters in diameter. Its trunk is monopodial, cylindrical and presents rhythmic growth. Adult plants usually have regular rows of primary branches arranged in whorls (between 6 and 10 branches in each whorl) and 8 to 15 whorls. It is the dominant tree of the Mixed Ombrophilous Forest and can live 300 years or more (Zanette *et al.*, 2017).

The economic, social and environmental importance of *A. angustifolia* is significant in its naturally occurring states. In the second half of the twentieth century, its timber was heavily targeted by industry leading the species to risk of extinction (Carlucci *et al.* 2013). Currently araucaria cutting is prohibited by law, except for plantations. The extraction of its seeds is still common,

requiring investments in the establishment of orchards for the production of pine nuts. Otherwise, this activity may interfere with the natural regeneration of the species and, consequently, its maintenance.

Although still not significant, some initiatives have been observed for araucaria plantations to produce pine nuts, which may contribute to reduce the species risks of extinction. To meet this demand, research has sought highly productive genotypes, differentiated by production time, pine nut size (Zanette *et al.*, 2017) and precocity. In parallel, alternative methods of propagation by cuttings (Wendling *et al.*, 2017a) and by grafting (Wendling *et al.* 2017b), which maintain the desired characteristics of the plants, were also improved. Propagation by seeds (most common) does not allow the identification of sex before the plant enters the reproductive phase, nor does it guarantee the maintenance of the genetic quality of the selected plant.

Submitted on August 16<sup>th</sup>, 2019 and accepted on November 10<sup>th</sup>, 2019.

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Despite the reduction of areas with *A. angustifolia*, it is relatively easy to identify plants with productive characteristics of interest and multiply them by established methods of vegetative propagation. However, the dimorphism of its branches and trunk, historically reported in the first grafting studies with the species (Gurgel & Gurgel Filho, 1967; Kageyama & Ferreira, 1975; Zobel & Talbert, 1984), is still considered the main barrier for its propagation on a large scale.

Morphoanatomic studies have shown that terminal buds and dormant buds along the araucaria tree trunk are the only meristems normally capable of producing orthotropic (verticalized) shoot growth (Nikles, 1964). Although recent findings (identification of strigolactones, elucidation of their biosynthesis and signaling) provide relatively clear information on the molecular regulation of axillary shoot growth (Domagalska & Leyser, 2011; Smith & Li, 2014; Flematti *et al.*, 2016), our understanding on axillary meristem initiation at the molecular level is still incomplete (Wang & Jiao, 2018). In the case of araucaria, it is suggested that there are different mechanisms of internal regulation in the axilla of trunk and branch needles, as they originate plants with different crown architecture.

This paper reports araucaria grafted from plagiotropic branch propagules that resulted in plants with vertical growth and stem with morphology and trunk function. There is no mention in the literature for *A. angustifolia* or other Araucariaceae trees regarding this, a fact that makes this discovery unprecedented and allows to make some hypotheses about such behavior.

## MATERIAL AND METHODS

### *Experimental condition*

We evaluated grafted plants established in the field in an experimental area of Embrapa Forests, located in Colombo-PR (Lat. 25° 19,206 'S, Long. 49° 9,514' O, Alt. 928 m). The region's climate is classified as temperate (Cfb according to Köppen-Geiger climate classification), with the coldest month temperature between -3 and 18 ° C, humid, well-distributed rainfall throughout the year. Average annual rainfall around 1,500 mm without the occurrence of a dry season.

The grafts used in the grafting were obtained from shoots naturally emitted on canopy branches of 30-year-old adult trees, as can be seen in Wendling *et al.* (2016 - Figure 1A). The grafting technique employed was by cleft graft in rootstocks of approximately 1.5 years and 80 cm in height produced by seeds, kept in a pot.

The grafts were finally transplanted at the field on September 2007. The plants were kept clean by periodic crowning during the first year and later by mowing. Graft growth habits, morphology and sexual reproduction initiation were evaluated.

### *Field test to confirm grafts reversion*

The field test was composed of four plants that stood out for the change of their growth habit and morphology not previously verified in the araucaria. The main objective was to confirm the repetition of plant growth behavior after grafting, as well as the morphogenic change of the grafted branches. Propagules were removed from the apical region of the grafts sprouts, as well as from buds that appeared on the plagiotropic grafts for new grafting using the chip budding (Wendling *et al.*, 2017b) in rootstocks produced from seeds and kept in plastic bags of 3.6 L. Similar to what happened in the previous stage, after grafting success, the plants were definitely transplanted to the field and monitored.

This research has a descriptive character, since it presents unpublished facts and phenomena resulting from grafting with branch propagules. Understanding how these phenomena occur will be an important step to enable the vegetative rescue of mature trees from branches, as well as for the production of large-scale grafted plants.

## RESULTS AND DISCUSSION

Two distinct morphogenic behaviors were observed that resulted in four plants with vertical growth and trunk morphology, in none of them was verified flower initiation. In the first one, the grafts presented clearly plagiotropic initial growth and branch morphology (Figure 1A). Over time, they began to have an orthotropic growth and trunk morphology, indicating morphogenic changes in the plant (Figure 1D).

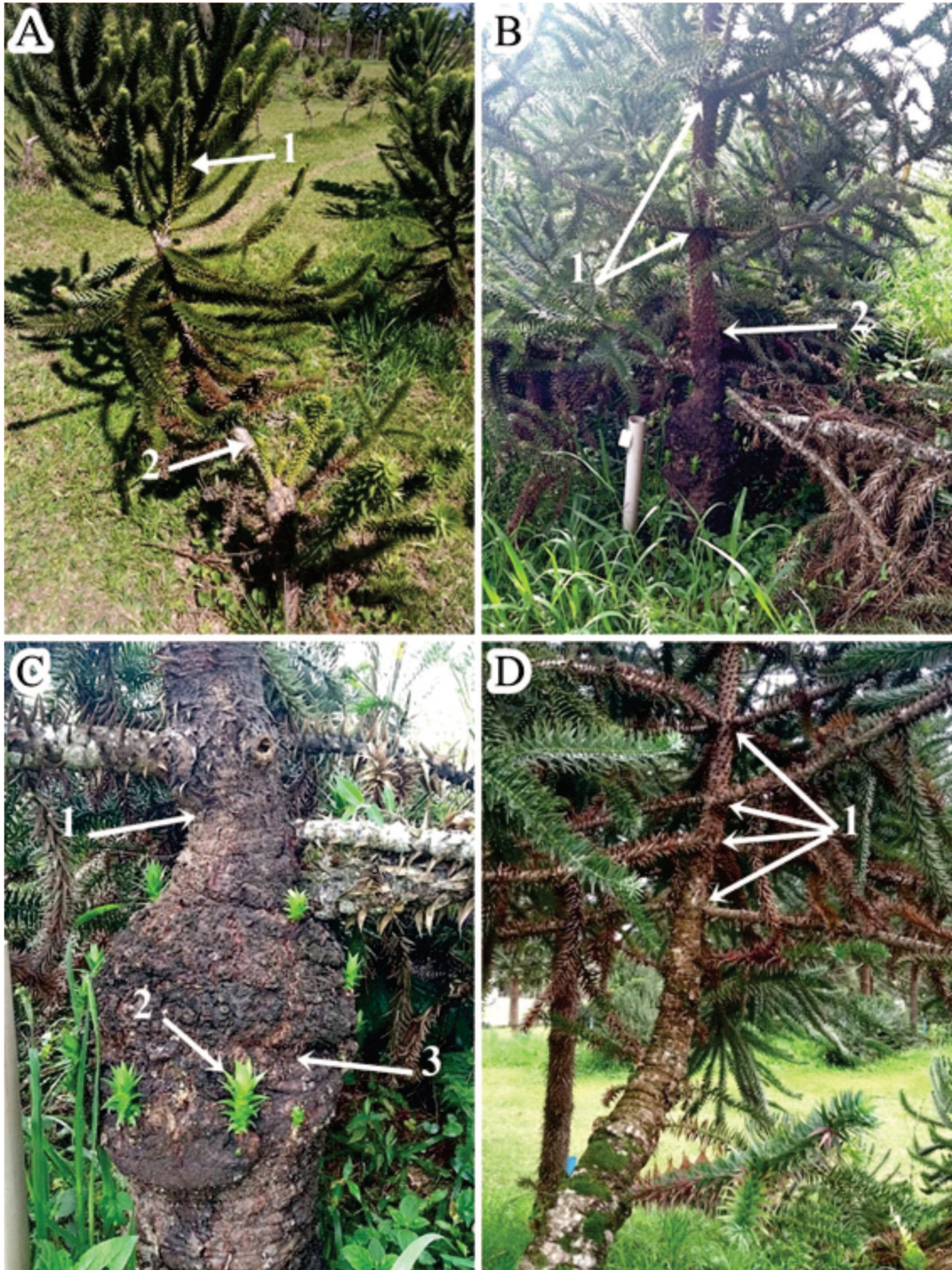
In the second behavior, the development (induction) of vertical sprouting on the upper surface of plagiotropic graft was verified, also accompanied by morphogenic changes (Figures 2B and C).

In general, grafts from primary branches presented buds with initial inclined growth (plagiotropic) and disorganized secondary branching, identical to their place of origin (Figure 1A). At ten years of age, however, four plants were found with some growth characteristics considered unusual in araucaria grafted with this type of material, mainly because they occurred simultaneously, constituting important markers related to the change of tropism and morphogeny.

### *Vertical growth and organization in whorls*

In Figure 1B it is possible to clearly observe the primary branch vertical growth. Although uncommon, this growth characteristic contradicts the reports of Nikles (1964) who stated in his work that the terminal bud and the dormant ones along the Araucariaceae trunk were the only meristems capable of producing buds with orthotropic growth.





**Figure 1:** Different growth behaviors of *A. angustifolia* plants grafted from primary branches: A) Plant with disorganized secondary branching (1) and plagiotropic growth (2); B) Plant with secondary branching arranged in whorls (1) and vertical growth (2); C) Differentiation in growth rate: primary branch - determined growth (1), trunk - undetermined growth (3) and induction of orthotropic shoots in the rootstock (2); D) Plant with reversal of plagiotropism and branch-to-trunk morphology (approximately 10 years) with secondary branch organized in whorls (1).



In Figure 1D the oldest branch (secondary branches) of the stem near the grafting region is disorganized. Plagiotropic graft tend to form a canopy architecture similar to that from which the material was obtained. In the intermediate region, however, branching began to line up in definite whorls, where it is possible to observe a fourth order of branches (Figure 2A), common in *araucaria*, but uncommon in plants grafted with plagiotropic branches, leading to believe in a possible morphogenic change (reversal) to the trunk.

Vertical growth of grafts from primary branches was observed in a previous survey (unpublished data) in at least 10% of the three-year-old grafted plants. However, the verticalization process was not accompanied by the organization of the secondary branches into well-defined whorls, which would characterize trunk formation, as occurred in this research. The authors attributed the type, age and position of the propagule in the canopy of the mother plant as probable determining factors for such growth behavior.

#### ***Longevity and thickening of branches***

Besides the change of tropism, the plants also presented secondary branches with different growth in thickness and longevity. In Figure 2A it is possible to observe that the secondary branches are thicker and of longer life when compared to branches of the plants that kept the tropism similar to the place of origin (Figure 1A), indicating physiological alterations with reflex in the plant morphogenesis. In general, the *araucaria* secondary branches are flexible, have anisotropic growth (without preferential direction) and a limited life cycle between 5 and 7 years (Zanette *et al.*, 2017). For a better understanding of the branches of *A. angustifolia*, Hackbarth *et al.* (2017) studied the different stems physiology of the species and found differences in the amino acids of trunk, branches and vines (“grimpas”). The result confirms the particularities of each stem and, therefore, for morphogenic change to occur (a primary branch is generated in place of a secondary branch), there must be some mechanism responsible for reprogramming gene expression and acquiring cellular competence. These issues, however, are little clarified so far (Rocha *et al.*, 2016).

The interaction between graft and rootstock may explain, in some cases, the morphology, anatomy and physiology of grafted plants (Hartmann *et al.*, 2011; Santarosa & Souza, 2016). However, there are no studies on this topic in *araucaria* grafting with a view to morphogenic change, and it is more common to relate the interaction to the compatibility between the parts (Wendling *et al.*, 2017b) and the crown morphology or tropism (Constantino & Zanette, 2018).

A possible explanation for the transformations that occurred is the existence of silencing signals from genes transmissible to the graft. This concept may be the key to a new approach to the graft transformation puzzle (Mudge *et al.*, 2009). Recent research further broadens this view in conjunction with the likely involvement of epigenetic inheritance mechanisms.

The environmental condition can induce epigenetic changes and thus reveal a hidden genetic variability. Although information about this mechanism has been growing rapidly in recent years, helping to understand various biological processes, its understanding is still a great challenge (Vieira, 2017).

Another concept also poorly understood is the “hybrid graft” in which the transfer of genetic material from the rootstock can promote graft transformation, leading to hereditary changes (Goldschmidt, 2014).

On-site observations, however, suggest a process of stress on grafts due to plant age. The *araucaria* trunk presents indeterminate radial growth (Figure 1C), unlike the branch that is determined (Zanette *et al.*, 2017). Therefore, the growth rate of the rootstock can lead to incompatibility (Wendling *et al.*, 2017b), and thus a growth stress capable of generating stress and to influence plant development.

Regarding the growth behavior of the plant in Figure 2B and C, it is possible that there has been a stress process due to local conditions (long and low growth under vegetation, competition, suffering with frequent mowing in the area in which it is found), since environmental disturbances and stress at ground level are some of the most important factors influencing the development of epicormic sprouts (Burrows, 2008; Colin *et al.*, 2010).

Different from other species in which this type of sprouting may be the product of morphogenesis when a cambium cell transformation occurs to give rise to the new sprout (Rast *et al.*, 1988), in the case of *araucaria*, sprouts originate from a set of meristematic cells in the axilla of the needles (Burrows *et al.*, 2003). These meristems usually do not differentiate into other tissues without some stimulation (Burrows, 1990; Meier *et al.*, 2012). Therefore, the changes observed in this work may be related to the existence of a physiological process capable of triggering a signal that reflects the morphogenic competence of cells leading to a particular and permanent development of the plant.

#### ***Field test***

The growth behavior of the grafted and field-maintained plants was similar to that of the origin plants (plants that showed vertical growth and morphogenic change). However, the growth was vertical from the beginning of the graft sprouting and the whorls were



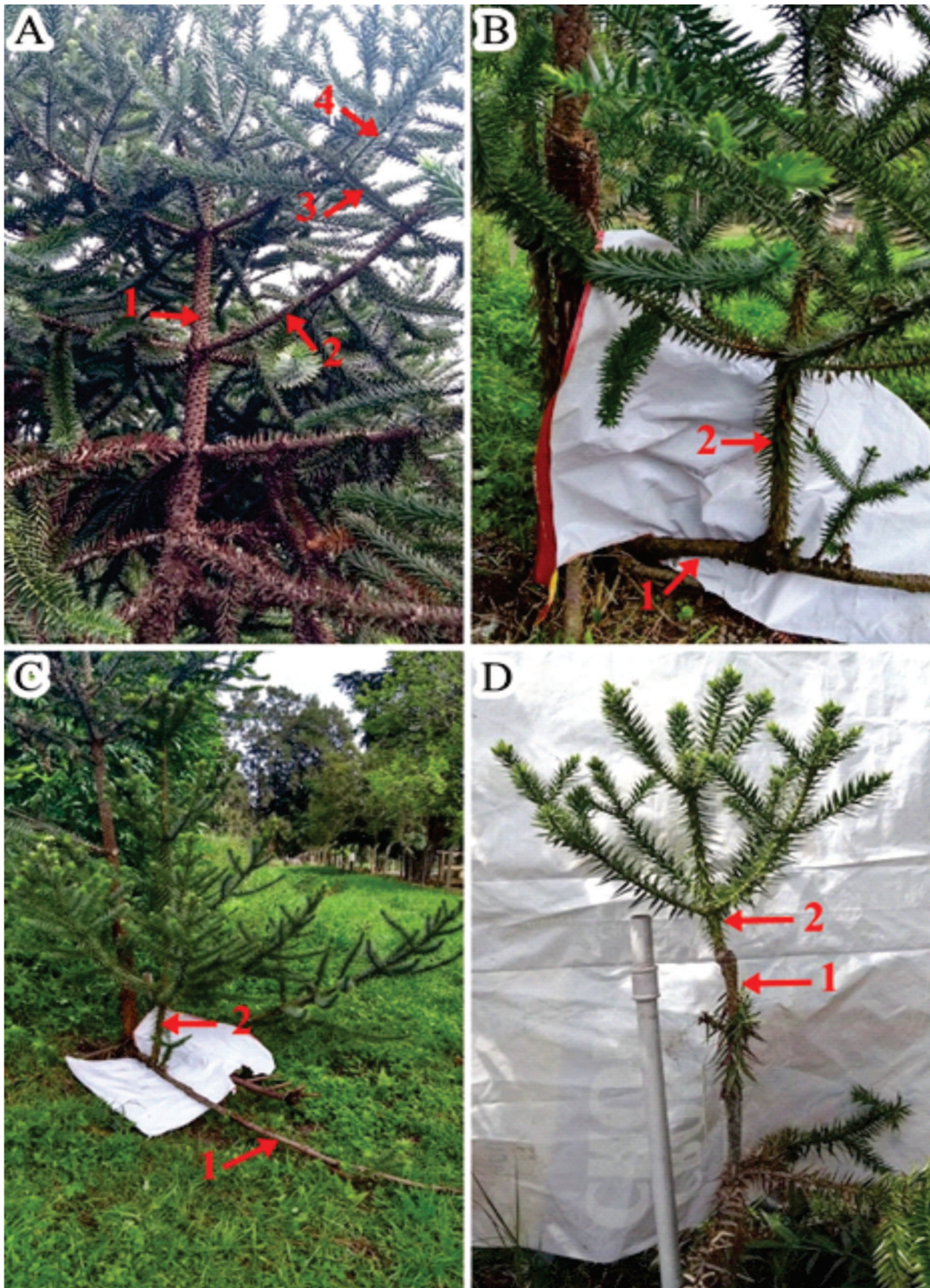


Figure 2: Thick and lignified branches arranged in definite whorls, highlighting the primary branch that was converted into main trunk (1), secondary (2), tertiary (3) and fourth order branches (4) (A); Induced orthotropic budding (2) on plagiotropic branch (1) (B and C); Field test: grafting with material obtained from plant that showed morphogenic and tropism changes (2), rootstock (1) (D).



organized with well distributed branching (Figure 2D), reflecting an order identical to that of a plant grafted with trunk propagules of the same age. This allows us to conclude that the morphogenic changes that occurred in araucaria plants were definitive. Thus, understanding what factors are involved in this growth habit and morphology reversion from branch to trunk may open new perspectives for the vegetative rescue and grafting of araucaria branches.

## CONCLUSIONS

Trunciform shoots can be obtained by *A. angustifolia* grafting with plagiotropic primary branches.

Despite the morphological clarity of trunciform shoots on grafted plagiotropic primary branches, it has not yet been possible to identify the causes of this reversion.

There is no conflict of interest in conducting this research and publishing the manuscript.

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