

## Flowering acceleration in native Brazilian tree species for genetic conservation and breeding

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**Abstract.** Grafting and growth retardants are commonly used in breeding programs to stimulate flower production. However, little is known about their effects on Brazilian tree species. The aim of this study was to investigate the vegetative and reproductive development of grafted tree seedlings treated with paclobutrazol (PBZ) and grown under greenhouse or outdoor conditions. Potted seedlings of *Jacaranda mimosifolia*, *Handroanthus heptaphyllus*, *Swietenia macrophylla*, *Schinus terebinthifolius*, *Cariniana legalis*, and *Hymenaea courbaril* were evaluated. Shoot number, length, and circumference as well as flower and fruit numbers were determined at 50 and 90 days after PBZ application. Data were subjected to analysis of variance, and means were compared by Tukey's test ( $P \leq 0.05$ ). Growing conditions influenced the vegetative parameters of seedlings, especially after 90 days. *J. mimosifolia* and *S. terebinthifolius* responded positively to flowering induction, and their fruit and flower numbers differed between growing environments. Potted grafts of the six native tree species were successfully grown. Grafting and PBZ application induced early flowering in *J. mimosifolia* and *S. terebinthifolius*.

**Keywords:** clonal seed orchard, early flowering, greenhouse, plant breeding

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## Introduction

Plant breeding is an important tool that allows crossing diverse and superior genotypes to obtain heterotic offspring (Horsley et al. 2010). Seed orchards apply many breeding methods, but losses in productivity may arise from inbreeding, self-fertilization (Moran and Bell 1983, Potts et al. 1987), and external pollen contamination, especially when systems are established near other forests (Potts et al. 2008). Therefore, it is important to apply pollination control strategies in seedling seed orchards and clonal seed orchards.

Hybridization is one of the fastest and most efficient ways to enhance genetic gains in forest species (Dickinson et al. 2010, Randall et al. 2015). Many studies have been carried out with *Eucalyptus* (Assis et al. 2005, Horsley et al. 2010), *Corymbia* (Dickinson et al. 2010), and *Hevea brasiliensis* (Hamzah et al. 2002), resulting in increased productivity in forest-based industries (Assis & Mafia 2007). Genetic improvement programs for native forest species, however, are still incipient.

Growing seedlings in pots, in combination with adequate pollination techniques, eliminates the need to isolate individual flowers; instead, whole plants can be isolated (Assis et al. 2005). This strategy reduces the risk of pollen contamination, facilitates disease and pest management, shortens the time to flowering, and allows large-scale controlled crossings and propagation of high-quality full-sib families, a task previously considered technically and economically unfeasible (Silva et al. 2012). Some crucial aspects should be considered when planning indoor or outdoor seed orchards: parent selection, vegetative propagation techniques, flowering induction methods, pollen management (collection, processing, and storage), and pollination (Silva et al. 2012).

Grafting and growth regulators can be used to induce early flowering in plants (Martínez-Fuentes et al. 2013, Gardner et al. 2016,

Zhang et al. 2016, Soumya et al. 2017, Bindu et al. 2018). Paclobutrazol (PBZ) [(2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol] is a triazole fungicide with growth regulatory properties (Soumya et al. 2017). It has been shown to reduce growth (Hasan & Reid 1995), increase flowering (Gardner et al. 2016), and shorten generation cycles (Wei et al. 2018). PBZ acts by inhibiting the synthesis of gibberellins (GAs), a family of growth hormones (Kishore et al. 2015). *Mangifera indica* seedlings treated with PBZ produced higher amounts of phenolic and ascorbic acids (Srilatha et al. 2016, Bindu et al. 2018), important compounds for induction of flowering (Barth et al. 2006, Srilatha et al. 2016).

The target tree species of the study (*Jacaranda mimosifolia* D. Don, *Handroanthus heptaphyllus* (Vell.) Mattos, *Swietenia macrophylla* King, *Schinus terebinthifolius* Raddi, *Cariniana estrellensis* (Raddi) Kuntze, *Poincianellapluviosa* (DC.) LP Queiros and *Hymentaea courbaril* L.) are widely distributed in Brazil and have great relevance in several segments, such as: in the recovering of degraded ecosystems, in the enrichment of forests, in urban landscaping, in the biomonitoring and phytoremediation of areas with heavy metals and in the production of food, cosmetics and pharmaceutical products (Silva et al. 2010, Veggi et al. 2014, Ma et al. 2018, Oliveira et al. 2018, Balestrin et al. 2019, Costa et al. 2019, Domingos et al. 2019, Souza et al. 2019, Farias et al. 2020, Lisboa et al. 2020).

The aim of this study was to evaluate the vegetative and reproductive development of grafted Brazilian tree seedlings treated with PBZ and grown under greenhouse or outdoor conditions.

## Materials and methods

### Study site

The experiment was conducted in Viçosa,

Minas Gerais, Brazil, from June to September 2018. Viçosa is located in the Zona da Mata mesoregion of Minas Gerais (20°45'S 42°51'W, 652 m above sea level) and has a moderate humid subtropical climate (Cwb), according to the Köppen classification, with an average annual rainfall of 1,341 mm and relative humidity of 80%. The annual average, maximum, and minimum temperatures are 22.31, 28.2, and 16.4 °C, respectively.

### Plants and growing conditions

The rootstocks of *J. mimosifolia*, *H. heptaphyllus*, *S. macrophylla*, *S. terebinthifolius*, *C. estrellensis*, *P. pluviosa* and *H. courbaril* were produced from seeds in 5 L bags, which were watered daily. Fertilization was performed using 10 g of 21-00-21 (NPK) slow release fertilizer (Polyblen®, Compass Minerals, São Paulo, Brazil) every 90 days. At the time of grafting, the seedlings were 1 year old, about 45 cm high and with 4 cm in circumference of the basis of the plant.

The grafts used were collected from adult branches with a tendency to orthotropic growth, containing 20 to 30 cm in length of mother trees in the field, placed in a thermal box containing gel ice, to avoid dehydration.

The rootstocks were prepared by cutting the tip of the yolk above the first node, making a longitudinal V-shaped cut of about 3 cm and preserving the lateral branches for a photosynthetic activity. The grafts were excised in the form of a wedge (Figure 1a) and inserted into the rootstock. (Figure 1b). A junction was selected with selection and wrapping with parafilm to avoid water loss and keep the exchange tissues in close contact (Figure 1c). The grafts were performed by a single experienced graft and, weekly, new shoots were removed from the stock to avoid competition with the young ones.

Up to 90 days after grafting, the seedlings were treated with 2.0 ml of paclobutrazol (PBZ) diluted in 250 ml of water (0.5 g of active ingredient). Five days after applying the

PBZ, the seedlings were transferred to 20 L pots, completing the volume of the container with substrate without product, so that part of the roots is in contact with the PBZ and part without contact with the product to avoid toxicity. Fertilization was carried out with 40 g of Polyblen® 21-00-21 (NPK). As grafted seedlings, they were grown in a greenhouse or outdoors.

### Climate

Greenhouse temperatures ranged from 16.31 °C to 43.77 °C, with an average humidity of 50%. Outdoor temperatures ranged from 15.19 °C to 35.68 °C, with an average humidity of 65%. Total precipitation in outdoor environment during the study months was 158.4 mm.

### Data analysis

The experiment was conducted in a randomized block design and factorial arrangement, containing two factors called orchard type (indoor and outdoor) and species (with six species). Totaled 12 treatments with 15 repetition. Vegetative parameters (shoot number, length, and circumference) and reproductive parameters (flower and fruit number) were evaluated at 50 and 90 days after PBZ application.

Vegetative data were subjected to analysis of variance (ANOVA). When significant differences were found, means were compared by Tukey's test ( $P \leq 0.05$ ) using Genes (Cruz 2013). Flowering and fruiting data were not normally distributed and were therefore analyzed by descriptive statistics. Statistical analyzes were performed using RBio software (Bhering 2017).

## Results

### Vegetative parameters

The interaction effects of tree species and growing environment were significant ( $P \leq$

0.05) for shoot number at 50 days after PBZ application. At 90 days after PBZ application, the interaction had a significant effect ( $P \leq 0.01$ ) on shoot number and circumference. Shoot length varied significantly between environments and tree species. These data are presented separately in tables and figures.

At 50 days after PBZ application, the shoot number, length, and circumference of greenhouse plants were significantly higher than those of outdoor plants (Table 1). At 50 days after PBZ application, *C. legalis* had the largest shoot length (55.78 cm) and *S. terebinthifolius* the second largest (Fig. 2A). *J. mimosifolia* (0.95 cm) and *S. macrophylla* (0.94 cm) had the largest shoot circumference (Fig. 2B).

Plant species had a significant effect on shoot number, regardless of the growing environment (Table 1). Under greenhouse conditions, *C. legalis* had the largest number of shoots (Fig. 3A), and, under outdoor conditions, *S. terebinthifolius* had the largest number (Fig. 3B).

At 90 days after PBZ application, the mean shoot length (Fig. 4A) and sprouts number (Fig. 4B), differed significantly among plants grown in the greenhouse and plants grown out-

doors.

*J. mimosifolia* had the largest shoot circumference at 90 days compared with other plants grown in the greenhouse (Fig. 5A). A small variance in shoot circumference was observed among plants grown outdoors. *J. mimosifolia*, *H. heptaphyllus*, and *S. macrophylla* showed the largest shoot circumference (Fig. 5B).

Shoot circumference at 90 days after PBZ application was significantly influenced by the growing environment, as also observed at 50 days post-treatment. This parameter was higher ( $P \leq 0.05$ ) in all plants grown in the greenhouse than in those grown outdoors, except for *C. legalis* and *H. courbaril*. The shoot circumference of these species did not differ between environments (Fig. 6).

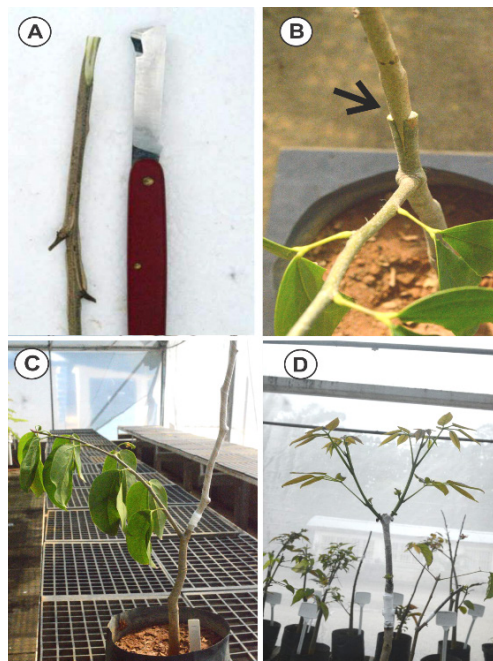
## Reproductive parameters

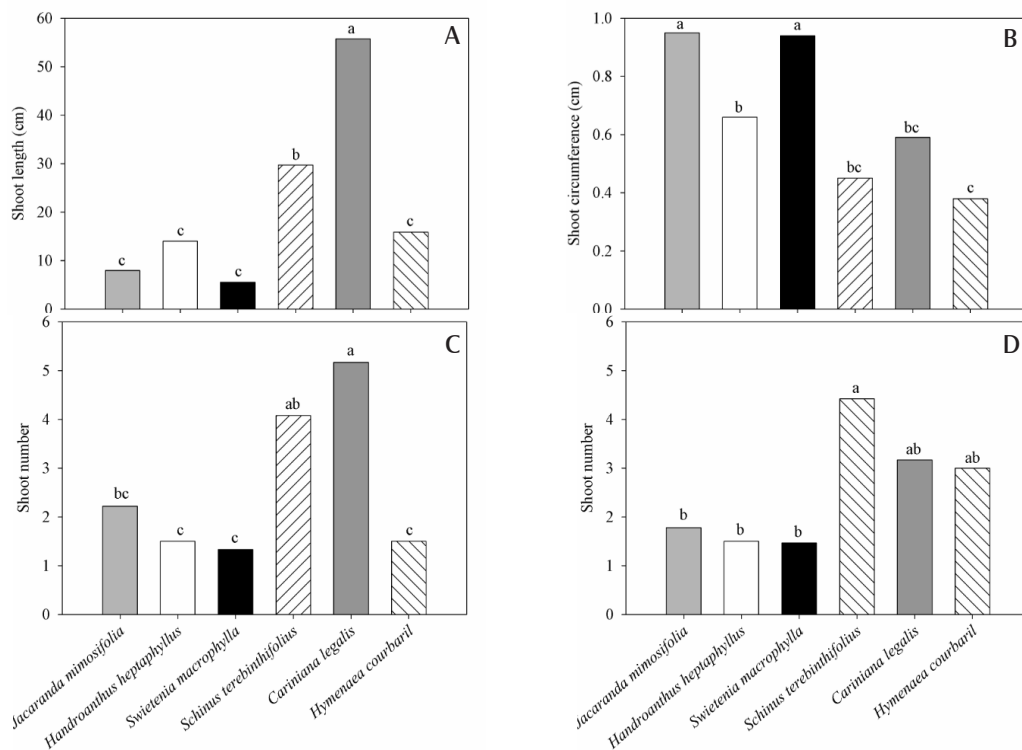
*J. mimosifolia* and *S. terebinthifolius* responded positively to flowering induction by PBZ (Fig. 7 and 8). At 50 days after PBZ application, *J. mimosifolia* plants grown outdoors and *S. terebinthifolius* grown in the greenhouse

**Table 1** Mean shoot number, length, and circumference of grafted seedlings grown under greenhouse or outdoor conditions for 50 days after paclobutrazol application

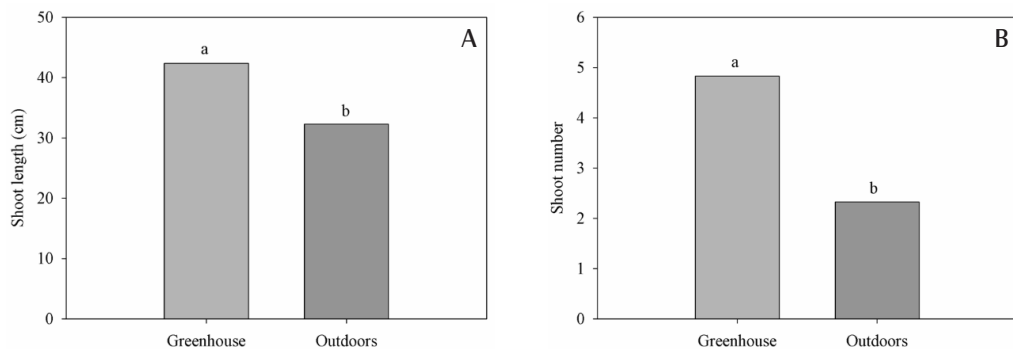
Parameter	Greenhouse	Outdoors
Shoot length (cm)	21.47 <sup>a</sup>	16.23 <sup>b</sup>
Shoot circumference (cm)	0.78 <sup>a</sup>	0.66 <sup>b</sup>
Shoot number	5.17 <sup>a</sup>	3.17 <sup>b</sup>

**Figure 1** Representative photographs of (a) wedge-cut scion, (b) scion inserted into the rootstock (scion/stock junction indicated by an arrow), (c) graft treated with sealant and secured with parafilm, and (d) grafted plants inside a screened greenhouse





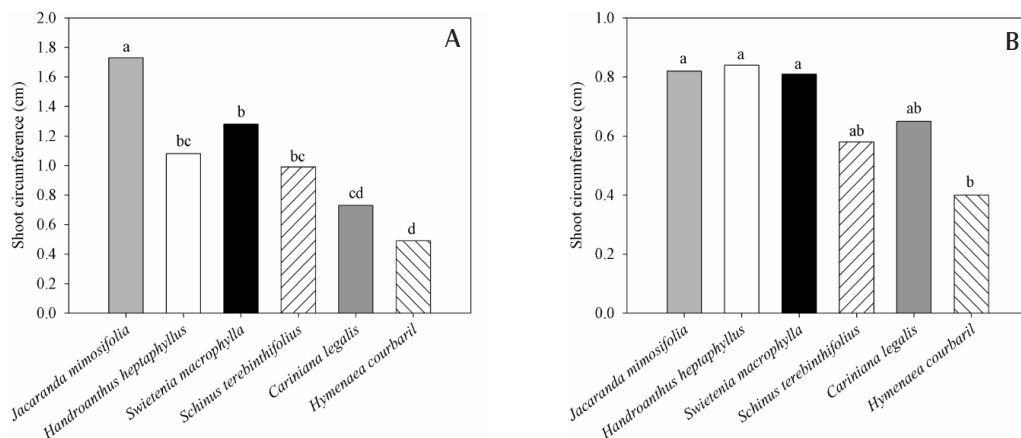
**Figure 2** Shoot length (A), shoot circumference (B), shoot number of grafted seedlings grown under greenhouse (C) and outdoor (D) conditions of grafted seedlings at 50 days after application of paclobutrazol. Different letters indicate significant differences at  $P \leq 0.05$  by Tukey's test.



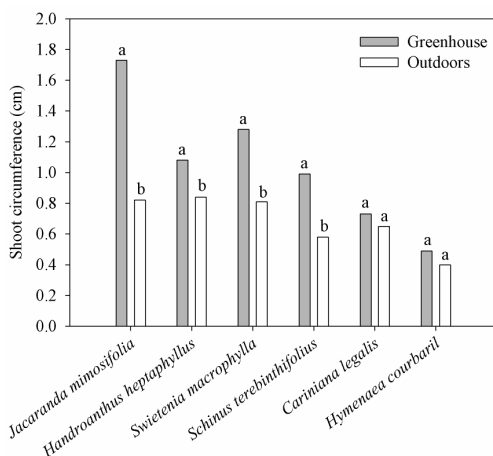
**Figure 3** Shoot length (A) and shoot number (B) of Brazilian tree seedlings grown in a greenhouse and outdoors for 90 days after PBZ application. Different letters indicate significant differences at  $P \leq 0.05$  by Tukey's test.

showed the largest number of flowers (Fig. 9A). *S. terebinthifolius* was the only plant with fruits at 50 days after PBZ application. The

number of fruits was significantly higher in seedlings grown outdoors than in those grown in the greenhouse (Fig. 9B).



**Figure 4** Shoot circumference of Brazilian tree seedlings grown under greenhouse (A) and outdoor (B) conditions for 90 days after PBZ application. Different letters indicate significant differences at  $P \leq 0.05$  by Tukey's test.



**Figure 5** Shoot circumference of Brazilian tree seedlings grown under greenhouse and outdoor conditions for 90 days after paclobutrazol application. Different letters indicate significant differences at  $P \leq 0.05$  by Tukey's test.

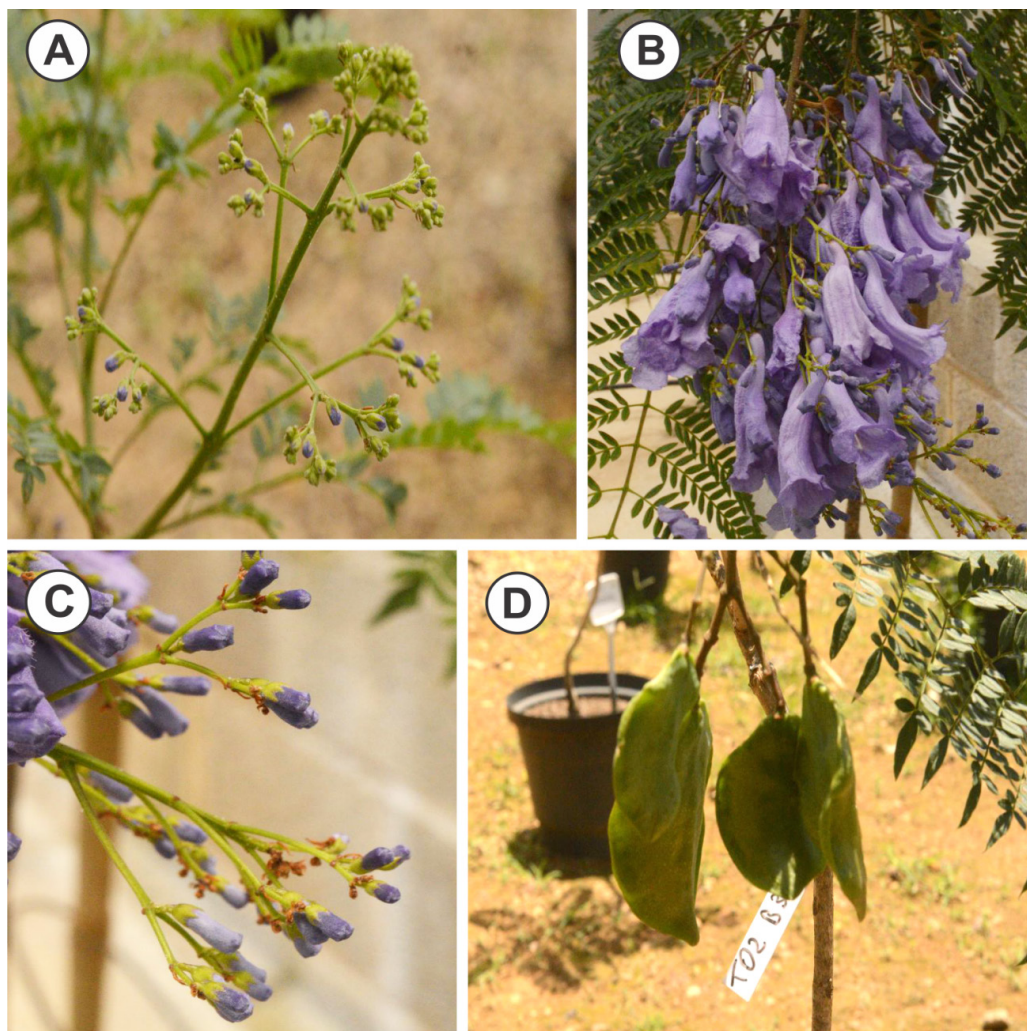
At 90 days after PBZ application, only *S. terebinthifolius* seedlings were flowering. The number of flowers was higher in seedlings grown in the greenhouse (Fig. 10A). *J. mimosifolia* produced fruits when grown under outdoors conditions, and *S. terebinthifolius* produced fruits in both environments (Fig. 10B).

The fruit yield of *S. terebinthifolius* was slightly higher under greenhouse conditions (Fig. 10B).

## Discussion

The improved vegetative development observed in greenhouse plants may be due to their enhanced nutritional status; under greenhouse conditions, soils are not exposed to rain and are less prone to nutrient leaching (Souza et al. 2012).

Other factors known to affect plant growth are a potent inhibitor of GA synthesis. It acts as an antagonist of *ent*-kaurene oxidase, an enzyme that catalyzes the oxidation of *ent*-kaurene to *ent*-kaurenoic acid. By inhibiting GA synthesis, PBZ leads to reduced shoot height and leaf length but increased shoot and leaf diameters (Wang et al. 2019, Xia et al. 2018). Chlorophyll content (Wang et al. 2019) and stomatal conductance (Xia et al. 2018) are improved by PBZ treatment, which in turn enhances photosynthetic performance and carbon uptake. In this study, PBZ application may have increased the photosynthetic rate of plants, as previously reported in *Citrus*

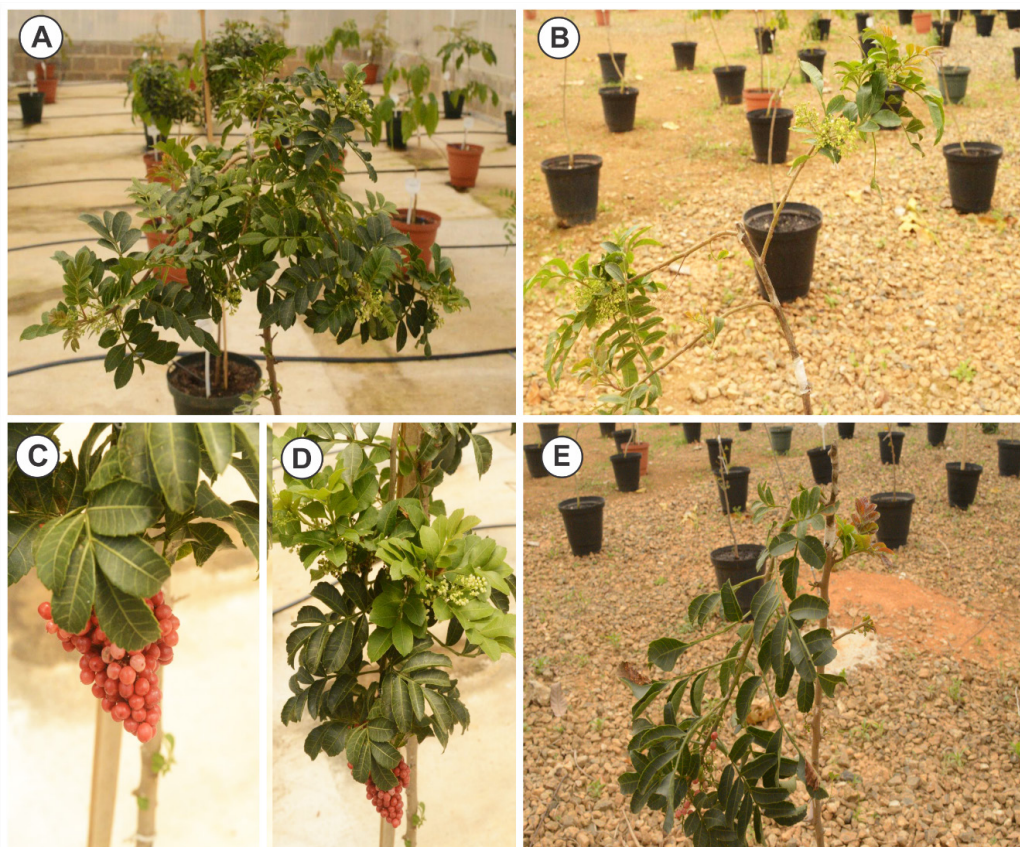


**Figure 6** Shoot length (A), shoot circumference (B), shoot number of grafted seedlings grown under greenhouse (C) and outdoor (D) conditions of grafted seedlings at 50 days after application of paclobutrazol. Different letters indicate significant differences at  $P \leq 0.05$  by Tukey's test.

*luslanatus* (Baninasab 2009), *Cucumisativus* (Baninasab & Ghobadi 2011), and *Punicagranatum* (Moradi et al. 2016). PBZ-treated *Stevia rebaudiana* exhibited enhanced tolerance to biotic and abiotic stresses as well as increased concentrations of photosynthetic metabolites, carbohydrates, and antioxidant compounds (Hajihashemi 2018). Root biomass responds positively to PBZ (Tanis et al. 2015, Abod

& Aminor 2000), indicating that this plant growth regulator also contributes to water and nutrient uptake.

The success of breeding programs for genetic conservation of native tree species depends on the development of artificial breeding strategies that reduce the number of inbred progenies and the loss of diversity through genetic drift (Higa & Silva 2006). Flowering induc-



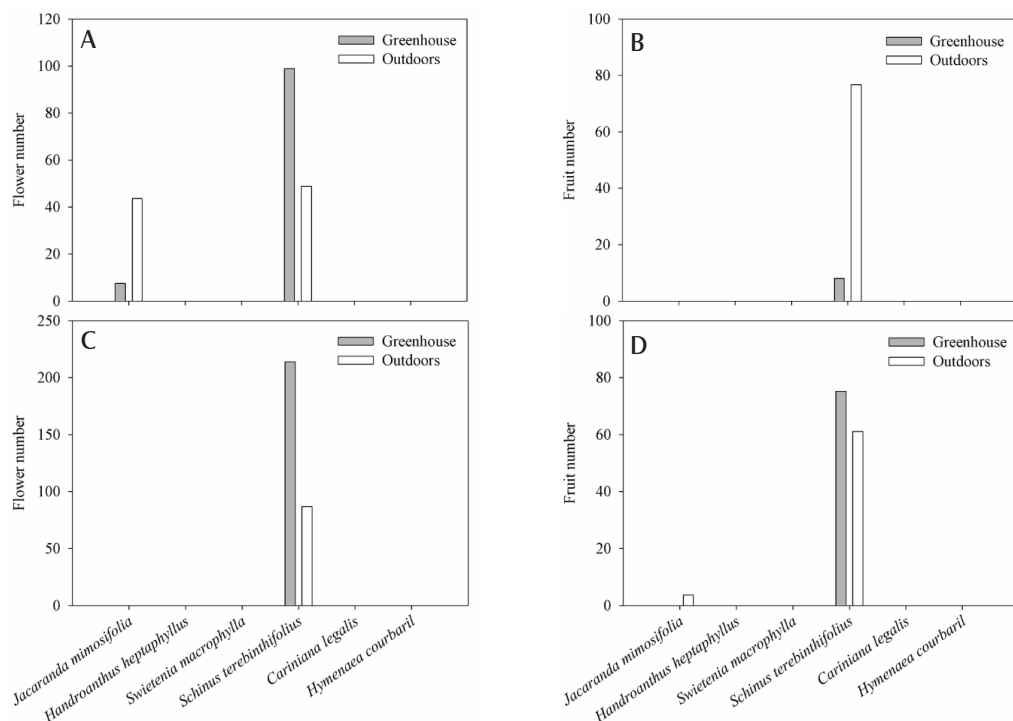
**Figure 7** *Schinus terebinthifolius* plants treated with paclobutrazol showing inflorescences and fruits of plants grown in a greenhouse (A, C, and D) and outdoors (B and E)

tion is widely used in breeding strategies for selection of superior and diverse genotypes. PBZ application proved to be efficient in stimulating flower bud formation and reducing the time to flowering and fruiting in *J. mimosifolia* and *S. terebinthifolius*. These effects are of great importance in breeding programs for slow-growth, long-lived trees.

*S. terebinthifolius* and *C. legalis* show a shoot length, and shoot number significantly higher under greenhouse and outdoor condition when compared with other species. This behaviour can be attributed to the species's characteristics. Considering that *C. legalis*, according to the classification of Budowski (1965), is a late secondary species that toler-

ates shade in the seedling stage and becomes an emerging tree as an adult (Costa et al., 2019, Sanches et al., 2010). And *S. terebinthifolius* has several leaf anatomical aspects found in xerophytes. The multiple layers of the epidermis in the leaf adaxial face protected or mesophilic from excessive desiccation, as well as the presence of a cuticle thick (Azevedo et al. 2015), as occurs in other species of semi-arid environments (Dickson 2000). The presence of stomata in large amount is also a resource shared by most plants in dry environments, and examples are of *M. urundeuva* (Silva et al. 2011) and *Agave sisalana* (Cunha Neto & Martins 2012). According to Dickison (2000), as leaves of these plants often used in





**Figure 8** Number of flowers (A, C) and fruits (B, D) of Brazilian tree species at 50 and 90 days after paclobutrazol application, respectively

quite abigger number types of plants than other types of plants are due to the need to control sweating. Besides that, in most species, phloem is facing the abaxial face and the xylem is facing to the adaxial face in the vascular bundle of the rib central, in continuity with these tissues in the stem (Esau 1998). However, in *S. terebinthifolius*, the xylem is arranged radially toaxis, and the phloem is also arranged radially (Azevedo et al. 2015). Giving species an advantage to tolerate and thrive under adverses conditions. Following the same reasoning, *J. mimosifolius* and *S. macrophylla* presented the higher circumferences. This behaviour can be attributed to the evolutionary characteristics of these species. One is a pioneer and the other is secondary, respectively. Therefore, their growth potential in the shortest time is greater, as they have a more robust root system. Thus, justifying or increasing the circumference of

the aerial part.

*S. terebinthifolius* and *J. mimosifolia* produced flowers and fruits both outdoors and in the greenhouse, indicating that climatic conditions were favorable for the reproduction of these species. PBZ probably contributed to these results by decreasing GA concentrations (Zhang et al. 2019) and increasing abscisic acid and cytokine concentrations in flower buds (Srivastav et al. 2010, Gardner et al. 2016), thereby redirecting the available resources to flower development (Yuceeret et al. 2003, Upreti et al. 2014, Srilatha et al. 2016).

The high number of flowers observed in *S. terebinthifolius* plants grown under greenhouse conditions may be due to the high ambient temperature (Cesário & Gaglianone 2008). Fruit number was higher in *S. terebinthifolius* plants grown outdoors at 50 days after PBZ application, possibly because of a fast initial

response to the growth regulator. Such differences were not observed at 90 days after PBZ treatment. Different from *S. terebinthifolius*, *J. mimosifolia* produced a greater number of flowers and fruits under outdoor conditions, confirming the preference of the species for mild temperatures (Carón et al. 2018). *J. mimosifolia* trees bloom when the temperature is between 20 and 30 °C and the relative humidity is about 70% (Sun et al. 2013). The lack of fruiting in *J. mimosifolia* grown in the greenhouse may be due to late-acting self-incompatibility. *J. mimosifolia* flowers are protandrous to prevent self-pollination (Alves et al. 2012); that is, their anthers open before the stigma is receptive (Bittencourt & Semir 2006, Yanagizawa & Maimoni-Rodella 2007, Guimarães et al. 2008). *J. mimosifolia* is preferably pollinated by medium- and large-sized bees (Bittencourt & Semir 2006, Guimarães et al. 2008), and the enclosed environment of the greenhouse probably limited visitation by these natural pollinators.

In this study, we found that PBZ application induced early flowering and fruiting, in agreement with its effects on grafted *Eucalyptus nitens* (Gardner et al. 2013), *E. smithii* (Gardner et al. 2016), *E. globulus* (Hasan & Reid 1995), *E. camaldulensis*, and *E. tereticornis* (Varghese et al. 2009) seedlings, confirming the efficacy of this growth regulator in accelerating the transition to adulthood in perennial tree species.

Excepting *S. terebinthifolius* and *J. mimosifolia*, the other tree species analyzed in this study did not flower. This can be attributed to the following factors: (i) existence of interspecific differences in susceptibility to flowering induction (Gardner et al. 2016), (ii) use of insufficient PBZ concentrations (Yuceer et al. 2003), (iii) inadequate timing of PBZ application (Sarker & Rahim 2012, Martínez-Fuentes et al. 2013), (iv) transfer of juvenile hormones from the rootstock to the scion (Gardner et al. 2016), and (v) seedling immaturity, as observed in *Populus deltoides* (Yuceer et al. 2003) and *E. nitens* (Gardner et al. 2013). Wil-

liams et al. (1999), studying the effect of PBZ on *E. nitens* flowering, found that the plants have high levels of reproductive inhibitors and few individuals can be induced to flower early, indicating that a complex set of conditions must be met for the onset of reproductive development. Thus, the phenological differences observed between the six native tree species after PBZ application and cultivation under greenhouse and outdoor conditions may be of physiological origin.

Clonal seed orchards can produce seeds with wide genetic variability for reforestation and ecosystem restoration programs. For genetic conservation programs, it is important to maintain as many alleles as possible and maximize the degree of genetic dissimilarity by crossing. This strategy can reduce the negative effects of inbreeding and genetic drift (El-Kassaby et al. 2019). An effective population size of 175 to 200 (Resende 2002) is preferred and should be obtained by crossing dissimilar individuals. Genetic dissimilarity can be promoted by introducing pollen from other populations.

## Conclusion

Shoot number, length, and circumference were greater in plants grown under greenhouse conditions. The vegetative developments of *S. terebinthifolius* and *C. legalis* are superior to the rest of the species studied under cultivation conditions. The combined use of grafting and PBZ application was effective in inducing early flowering in *J. mimosifolia* and *S. terebinthifolius*, an important advance for the conservation and genetic improvement of these species. This method can allow the production of seeds with good genetic value from full-sib progeny tests, genetic rescue of plants at risk of extinction, and rescue of trees damaged by environmental accidents. Further studies can be carried out to hybridize *J. mimosifolia* and *S. terebinthifolius* with related species for the production of interspecific, heterotic hybrids.

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