



# Effect of planting density on wood anatomy in *Eucalyptus* and *Acacia* from Brazil

## Efecto de la densidad de plantación en la anatomía de madera de *Eucalyptus* y *Acacia* de Brasil

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### ABSTRACT

The effect of planting density on the anatomical characteristics of wood is still matter of debate. The aim of the study was to investigate the influence of planting density on the variation of wood anatomical elements in *Eucalyptus* and *Acacia* trees cultivated in areas of high planting density (monospecific) and low planting density (mixed) stands. The fiber length, fiber width, fiber lumen diameter, cell wall thickness, vessel diameter and vessel frequency were evaluated. The measurements were made from wood discs taken from five *Eucalyptus urophylla* x *E. grandis* trees and five *Acacia mangium* trees grown in high planting density (monospecific plantations, 1667 trees/ha) and in low planting density (mixed stands, 500 trees/ha), totalizing 20 trees at 4.5 years old. *Eucalyptus* and *Acacia* produced wood with shorter fibers and smaller vessels in high density stands, when compared to its equivalent in low density. *Acacia* presented more frequent vessels and fibers with walls thicker than its equivalent in low planting density. *Eucalyptus* presented longer and thinner fibers and vessels more frequent than *Acacia*, regardless of planting density. On the other hand, in high planting density, *Acacia* presented fibers with walls thicker than *Eucalyptus* and in low density of planting their vessels were larger.

KEYWORDS: cell wall thickness, fiber length, planting spacing, vessel diameter, vessel frequency.

### RESUMEN

El efecto de la densidad de plantación sobre las características anatómicas de la madera aún es tema de debate. El objetivo de este estudio fue investigar la influencia de la densidad de plantación sobre la variación de elementos anatómicos de la madera de árboles de *Eucalyptus* y *Acacia*, creciendo en áreas de alta densidad de plantación (monoespecífica) y en rodales de baja densidad de plantación (mixta). Se evaluaron: longitud, ancho y diámetro de lumen de la fibra, grosor de pared celular, diámetro y frecuencia de vasos. Las mediciones se hicieron en rodajas de madera tomadas de cinco árboles de *Eucalyptus urophylla* x *E. grandis* y cinco de *Acacia mangium*, creciendo en alta densidad de plantación (plantaciones monoespecíficas, 1667 árb/ha) y de otro tanto igual de árboles creciendo en baja densidad de plantación (rodales mixtos, 500 árb/ha), lo que hace un total de 20 árboles de 4.5 años de edad. *Eucalyptus* y *Acacia* de rodales de alta densidad produjeron madera con fibras más cortas y vasos más pequeños en comparación con sus equivalentes en baja densidad de plantación. *Eucalyptus* presentó fibras más largas y delgadas y vasos más frecuentes que *Acacia*, independientemente de la densidad de plantación. *Acacia* presentó vasos más frecuentes y fibras con paredes más gruesas que su equivalente en plantación de baja densidad. Por otro lado, en alta densidad de plantación, *Acacia* presentó fibras con paredes más gruesas que *Eucalyptus* y en baja densidad de plantación sus vasos fueron mayores.

PALABRAS CLAVE: grosor de pared celular, longitud de fibra, espaciamiento de plantación, diámetro de vaso, frecuencia de vasos.

### INTRODUCTION

*Eucalyptus* plantations in Brazil have grown at a rate of 36 m<sup>3</sup>/ha/year, the planted surface occupied 5.6 million hectares in 2015 and the biomass produced has met the demand for wood from industries producing pulp and paper, charcoal and firewood (Indústria Brasileira de Árvores [IBA], 2016). At the same time, the *Acacia* genus has been devoted to multiple uses, once the trees have the ability to restore damaged areas, produce tannin, fix

nitrogen, supply energy and present a yield ranging from 10 to 25 m<sup>3</sup>/ha/year in Indonesia (Sein and Mitlohner, 2011). Grigoletti *et al.* (2003) suggest that the use of *Acacia* in forest systems of consortium can further optimize biomass productivity. Several studies have shown promissory findings when *Acacia* and *Eucalyptus* are planted together (Bouillet *et al.*, 2008; Forrester, Theiveyanathan, Collopy and Marcar, 2010; Gonçalves and Lelis, 2012). In general, the planting density in mixed stands

is lower (~400 trees/ha - 600 trees/ha, low density) than those adopted in high monocultures (~1000 trees/ha - 1800 trees/ha, high density), especially in Brazil (Associação Brasileira de Produtores de Florestas Plantadas [ABRAF], 2013). The wood products industry is increasingly attentive to the final quality of their products. The mixed stands have been indicated as a more sustainable option of forest production (Bouillet *et al.*, 2008) and, therefore, such raw material has been recommended for a range of applications in which high quality products are required.

In forests, any change that would alter the growth pattern of a tree will likely result in variation in the technological properties of the wood. However, the response of the cambial activity of the tree to these environmental changes, and therefore the resultant wood properties, is not clear and the results are controversial (Zobel, 1992). Most of studies on the relationship of wood properties variation with growth conditions are frequently contrasting, as pointed out by Gonçalves, Stape, Laclau, Smethurst and Gava (2004) in their literature review.

Reduced spacing, such as those adopted in *Eucalyptus* monocultures, usually induce greater competition for light, water and nutrients between trees, causing variations in height and diameter (Zobel and Van Buijtenen, 1989). Thus, considering that trees planted with larger spacing have a greater availability of water and nutrients, allowing the formation of larger conduits, we hypothesized that the supposed greater amount of assimilates cause variation in the anatomical structure of the timber. Furthermore, lower plantation density is usually adopted in mixed stands (Viera, Schumacher and Liberalesso, 2011), possibly causing variation on the quality of the wood produced and potentially generating considerable impact in their multiple forms of use.

The variation in anatomical characters of this material is important as it influences the final properties of the wood, such as its density and mechanical strength. When the fiber is intended for the manufacture of paper, for example, the fiber length affects the resistance to tear or folding test (Santos, 2005). According Nisgoski, de Muñiz, Trianoski, de Matos and Venson (2012) the lumen and fiber width are also important characteristics because they determine the fiber flexibility coefficient (lumen width / fiber width). The coefficient of flexibility is a parameter used in pulp and paper industry to determine the probability of collapse

between fibers and the degree of union between them on a paper sheet.

Many studies have evaluated the effect of growth conditions on the anatomical features of the wood from fast-growing species, as *Eucalyptus* (Pirralho *et al.* 2014; Monteoliva, Barotto and Fernandez, 2015) and *Acacia* (Igartúa and Monteoliva, 2010), however, few articles (Forrester *et al.*, 2010) have deal with mixed stands composed by these two species.

## OBJECTIVES

The aim of this study was to investigate whether the planting density has an influence on anatomical elements of wood from *Eucalyptus urophylla* × *E. grandis* and *Acacia mangium*.

## MATERIAL AND METHODS

### Characterization of the study area

The experiment was established in January 2009 at the Instituto de Ciências Agrárias of the Federal University of Minas Gerais, in Montes Claros city, Minas Gerais, Brazil (16°40'03.60"S; 43°50'41.52"O; 598 meters above sea level) as described by Silva *et al.* (2018). The climate is Aw-Tropical Savanna, characterized by high annual temperatures and rainy regime marked by two distinct seasons, rainy summer and dry winter (Köppen and Geiger, 1928). The soil is characterized as a eutrophic red-yellow argisil (Claessen, Barreto, de Paula and Duarte, 2006).

### Experimental design and sampling

The trees were planted in high density and low density stand systems with *Eucalyptus urophylla* × *E. grandis* hybrid (hereafter *Eucalyptus*) and *Acacia mangium* Willd (hereafter *Acacia*) as shown in figure 1. The plantation density in the monoculture system (high density) was 2 m × 3 m, while in the mixed stand (low density) the spacing was 10 m × 2 m. The experiment was conducted in a completely randomized design composed by four treatments, represented in this study by five replications. The four treatments were considered as: i) *Eucalyptus* in high planting density (monospecific plantations, 1667 trees/ha), ii) *Eucalyptus* in low planting density (mixed stands, 500 trees/ha), iii) *Acacia* in high planting density (monospecific plantations, 1667 trees/ha), iv) *Acacia* in low planting density (mixed stands, 500 trees/ha). Ten trees of *Acacia* and ten of *Eucalyptus* were selected (5 of each



type of planting density) and harvested in August 2013, when the trees achieved four years and six months of age. The selection of these 20 individuals (2 species  $\times$  2 planting density  $\times$  5 repetitions) was based on the uprightness of the stem and absence of visible diseases.

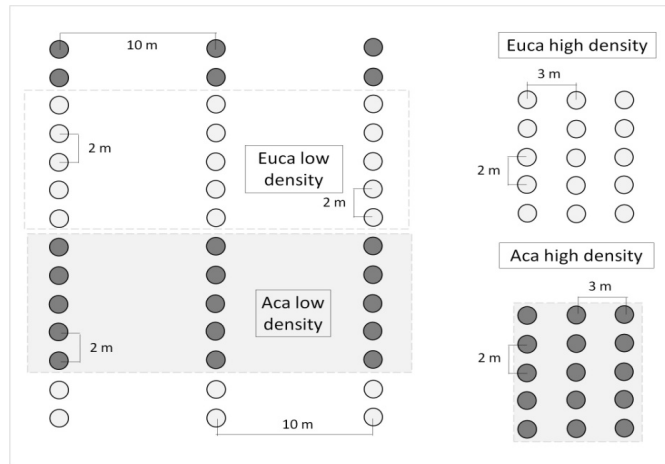


FIGURE 1. Plantation scheme showing high and low density stands with *Eucalyptus* (white circles) and *Acacia* (gray circles) trees.

In regard to the growing characteristics of trees, *Eucalyptus* presented diameter at breast height (DBH) of 18.92 cm  $\pm$  0.93 cm in monospecific plantations and 15.01 cm  $\pm$  1.56 cm in mixed stands, while *Acacia* had DBH of 14.64 cm  $\pm$  1.73 cm (monospecific plantation) and 11.11 cm  $\pm$  1.09 cm (mixed stands), as reported by Silva, Roldão, Santos, Hein (2018).

Wood discs (thickness: 30 mm) were cut at breast height, properly identified, placed in plastic bags to keep moisture and then divided into four wedges, free from defects. Sixty-eight cubic specimens measuring 20 mm  $\times$  20 mm  $\times$  20 mm used in this study were cut from the wedges. For each wedge, cubic specimens were cut near the pith, at intermediate radial position and near the cambium.

### Anatomical characterization of wood

Histological sections were made from the specimens in order to study the variation in the anatomical features of the wood. For preparing the slides, the wood specimens were immersed in water for 24 hours and then placed in a pressure cooker with water and glycerin during 15 minutes, to soften the wood.

The sections were produced using a sliding microtome with nominal thickness of 30 microns and semi-permanent sections were stored with glycerin. The thin sections were placed in bleach until they become clear and subsequently in distilled water, to remove traces of bleach. The sections were stained with Safranin and passed through an alcoholic series, from lower concentration (20%) to absolute ethanol, in order to remove the dye excess and dehydrate them, as described by Johansen (1945). The slides were mounted with the thin sections, fixed with Entellan and covered with coverslips.

For measuring fiber diameter, wall thickness and fibers length, small fragments of wood were removed from specimens, placed in macerate solution of hydrogen peroxide and 1 N acetic acid at a ratio of 1:1 and conditioned in an oven at temperature of 60 °C for 48 hours, in order to promote fiber individualization, as suggested by Franklin (1945). After this period, the fibers were washed in water, stained with diluted safranin and mounted in histological slides. The temporary mounts were prepared for the measurements on optical microscope equipped with an ocular micrometer and a graduated lamina with 1 mm of scale. The average fiber length and diameter was based on the observation of 30 fibers per lamina using a light microscope with 10x objective lens. The cell wall thickness was measured by observing 30 fibers per sample from the macerated material through the 40x objective lens, as experimental procedure adopted by Monteiro *et al.* (2017).

To measure the frequency of vessels, the mounts were analyzed using a 10x objective lens. Vessel diameter was observed and accounted for twin or multiple vessels; each unit was individually measured in tangential and radial direction and the average values were calculated for each unit, according to an adaptation of International Association of Wood Anatomy [IAWA] (1989). The vessels were randomized including different sizes found in the sections.

The capture of images was performed using a digital camera coupled to an optical microscope (Ken-Vision, TT-1010 model) and the software WinCellPro was used for the anatomical measurements of the wood sections, which followed the IAWA (1989) procedure. The following quantitative anatomical features were evaluated: vessel lumen diameter (VD), vessel frequency (VF), fiber length (FL), fiber width (FW), fiber lumen diameter

(LD) and fiber cell wall thickness (CWT). Each slide was composed by three replicates of cross section. Thirty measurements per slide were made for each anatomical feature.

### Analysis of results

The statistical software "SPSS v.19" was used to calculate the descriptive statistics, analysis of variance (Oneway), multiple comparison among means (*Tukey*) and t-test. For the analysis of variance were considered four variation sources: 1) high density plantation of *Eucalyptus* hybrid; 2) high density plantation of *Acacia mangium*; 3) low planting density stands of *Eucalyptus* hybrid and 4) high planting density stands of *Acacia mangium*. The normality of the residuals and the homogeneity of variances were tested and the averages compared by *Tukey* test at 5% significance level.

## RESULTS AND DISCUSSION

### Anatomical variability

The mean, values of the anatomical features observed in *Eucalyptus grandis* x *E. urophylla* hybrid and *Acacia mangium* grown in high and low density stands are shown in table 1. The *Eucalyptus* trees grown in low or high plantation density did not present significant differences between values of fiber width (FW), fiber lumen diameter (LD) and cell wall thickness (CWT). However, there was a significant effect of planting density on vessel diameter (VD), frequency (VF) and fiber length (FL) of *Eucalyptus* wood cells.

*Acacia* trees grown in low density stand (spacing: 10 m × 2 m) presented higher values in most anatomical parameters, but the CWT and VF were higher when the *Acacia* was grown in high density plantations.

### Effect of planting density on vessels diameter and frequency

The two species produced wood with larger vessel diameters when cultivated in low density plantations (Table 1). The diameter of vessels found in this study for 4.5 years-old *Eucalyptus* wood in low density stand (125.9 $\mu$ m) was significantly higher than in high density plantation (120.1 $\mu$ m). The vessel diameters of these woods were slightly higher than those reported by Alzate (2004), who found an average vessel diameter of 103.63  $\mu$ m for *Eucalyptus grandis* x *E. urophylla* with 8 years old, and Lima de Oliveira *et al.* (2012) who found average values of 99.21  $\mu$ m in several species of *Eucalyptus* with 64 months of age. Here, the average vessel diameter of *Acacia* wood was 133.7  $\mu$ m and 119.2  $\mu$ m for the low density stand and high density (Table 1), respectively (the VD of *Acacia* wood were significantly different by t-test). These values are consistent with those reported by Antunes (2009), who found vessels with diameters ranging from 120 microns to 160 microns in *Acacia crassicarpa* and *Acacia mangium* wood.

The variation in vessel frequency of the *Eucalyptus* and *Acacia* wood grown in high and in low density stands are shown in figure 2.

TABLE 1. Descriptive statistics of the growing and anatomical characteristics of *Eucalyptus grandis* x *E. urophylla* and *Acacia mangium* woods produced in high and low density plantations.

	VD ( $\mu$ m)	VF (mm <sup>-2</sup> )	FL ( $\mu$ m)	FW ( $\mu$ m)	LD ( $\mu$ m)	CWT ( $\mu$ m)
<i>Eucalyptus</i> high density	120.1C ±23.2	15.2B	864.6B	16.,13B	9.31C	3.18B
<i>Eucalyptus</i> high density	120.1C ±23.2	15.2B	864.6B	16.,13B	9.31C	3.18B
<i>Eucalyptus</i> low density	125.9B ±26.1	16.1A	906.9A	16.48B	10.09C	3.22B
<i>Acacia</i> high density	119.2C	11.08C	744.3D	19.19A	12.32B	3.42A
<i>Acacia</i> low density	133.7A	9.28D	789.9C	19.88A	13.56A	3.14B

VD - vessel diameter, VF - vessel frequency, FL - fiber length, FW - fiber width, LD - fiber lumen diameter and CWT - cell wall thickness. The anatomical characteristics were compared among treatments by the *Tukey* test at p = 0.01 threshold (means followed by same letter do not significantly differ).

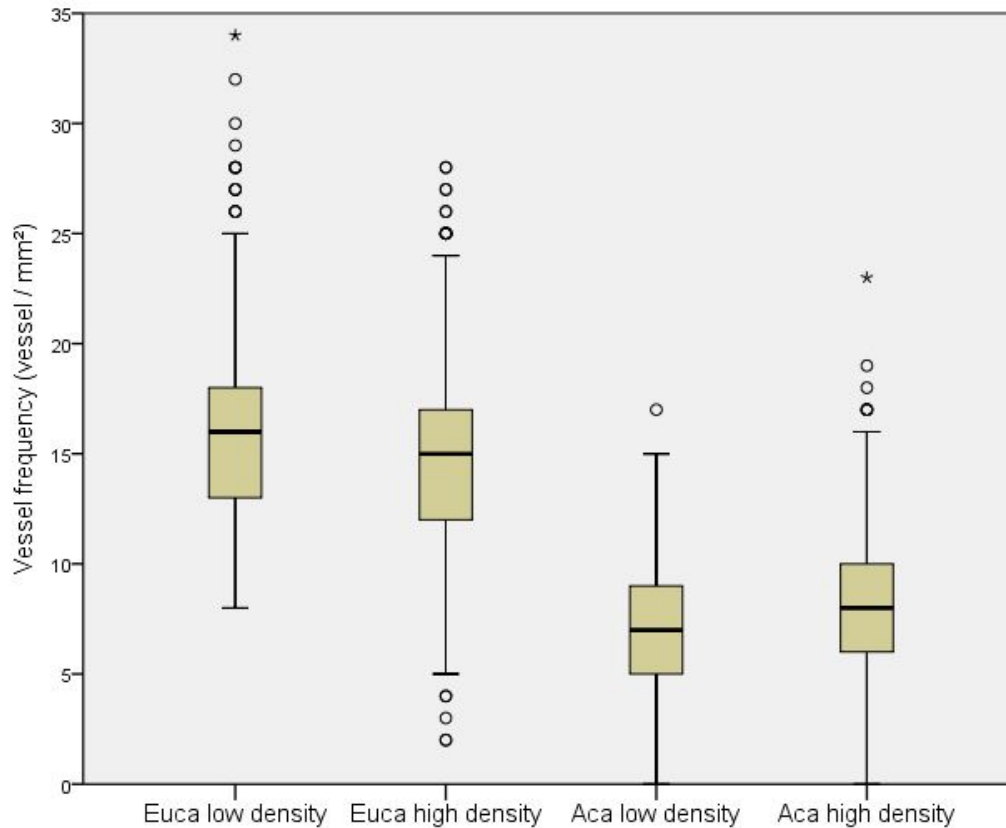


FIGURE 2. Variation in vessel frequency of *Eucalyptus grandis* x *E. urophylla* and *Acacia mangium* grown in high and low density stands.

In regard to the vessels frequency, there was a significant difference between treatments (Table 1). Figure 2 reveals there was effect within each density system. *Eucalyptus* wood planted in low density system produces significantly more vessels per unit area than the wood produced in high density. In *Acacia* plantations, the reverse trend was observed.

The frequency of vessels observed by Alzate (2004) was of 8-13 vessels/mm<sup>2</sup> in 8-year-old *Eucalyptus grandis* x *E. urophylla* hybrids. Monteiro *et al.* (2017) reported frequency of 16 vessels per mm<sup>2</sup> and Evangelista, Silva, Valle and Xavier (2010) found 9.9 vessels/mm<sup>2</sup> in *E. urophylla* wood. Antunes (2009) reported vessels frequency from 4 to 9 vessels/mm<sup>2</sup> in *A. mangium* wood. Sousa Junior (2004) has evaluated the differences in diameter and frequency of vessels from *E. urophylla* wood grown in distinct spacing plantations (6 m × 6 m and 3 m × 2 m), reporting lower vessel frequency (10,89 mm<sup>-2</sup>) and higher vessel diameter (105,85 μm) in the wood produced in lower planting density than the wood from the higher planting density (VF of 11,37 mm<sup>-2</sup>, VD

of 96,09 μm). However, the trees growing at different planting density had different ages and provenance: trees planted at 6 m × 6 m had 15 years old and came from Paraopeba city, while the other trees had 25 years old and were cultivated at Turmalina city, Brazil. To our knowledge, the study conducted by Sousa Junior (2004) is the closer one to our findings on *Eucalyptus*, but it is difficult to make comparisons because of such differences in provenance and age.

#### Fiber biometric changes according to planting density

The results shown in Table 1 indicate that both *Eucalyptus* and *Acacia* trees planted in low density system produce significantly longer fibers. This finding agrees with those found by Teago (2012), who studied *Acacia mangium* and *Eucalyptus* woods and found that in monoculture these trees produce wood with shorter fiber, besides having greater radial heterogeneity for the fiber width, lumen diameter and wall thickness of the fiber.

The length of *Eucalyptus* fibers found in this study are in agreement with the literature (Alzate, 2004; Alencar, Barrichelo and Silva 2002; Brisolal and Demarco 2011). But the *Acacia* fibers are longer than those reported in similar studies. Alencar *et al.* (2002) reported fibers 1.18 mm long in 4 year-old *E. grandis* x *E. urophylla* wood while Brisolal and Demarco (2011) analyzed 6 year-old *E. grandis* x *E. urophylla* with fiber length of 1.1 mm. Tomazello-Filho (1983) reported 10 year-old *Eucalyptus grandis* with fiber length of 1.03 mm. Regarding the fiber length of *Acacia* wood, Alencar *et al.* (2002) found fibers of 0.89 mm and Rossi, Azevedo and Souza (2003) found fibers measuring from 1.0 mm to 1.2 mm, a little longer than the fibers of the wood investigated in this study.

The fibers width does not seem to be sensitive to planting space. Table 1 shows no significant difference between the planting density for both species. However, there was a species effect on the variation of this fiber feature. The *Acacia* wood has wider fibers (~ 20 µm) than *Eucalyptus* (~ 16 µm). The fibers width of the *E. grandis* x *E. urophylla* hybrid is in agreement with the literature: Alencar *et al.* (2002) found fibers with 19.95 µm in width in 4-year-old *E. grandis* x *E. urophylla*; Brisolal and Demarco (2011) reported fibers of 20 µm in width and Alzate (2004) evaluated wide fibers, with average of 19.7µm. In *Acacia*, the literature indicates a larger variation between fiber widths: 24.7 µm in Alencar *et al.* (2002) and 16 mm in Antunes (2009).

The lumen diameter of *Eucalyptus* fiber was not influenced by planting density while there was a significant difference between the mean lumen diameters of the *A. mangium* samples. The *Acacia mangium* wood produced in low density stand had fibers with bigger lumen diameter when compared to fibers produced in high density plantation (Table 1). This characteristic is within the range found in the literature for *Eucalyptus* (Alencar *et al.*, 2002; Brisolal and Demarco, 2011; Alzate, 2004) and *Acacia* (Alencar *et al.*, 2002; Antunes, 2009).

There was no variation in cell wall thickness of the fibers of *Eucalyptus* trees grown in high and low density stands. However, when *Acacia* is planted in high density, the cell wall thickness of the fibers is higher than when it grows in low density stands (Table 1).

The cell wall thickness varies greatly in *Eucalyptus* literature: 4.31 mm in Alencar *et al.* (2002); 6.1 µm in Brisolal and Demarco

(2011); and 5.01 µm in Alzate (2004). The cell wall thickness of the *Eucalyptus* wood in this study had lower values (~3.2 µm). For *Acacia*, the cell wall thicknesses found in this study (3.1 µm - 3.4 µm) were close to the literature: 3.59 µm in Alencar *et al.* (2002) and 3.2 µm in Antunes (2009). Cell wall thickness is important because it is highly correlated to the density of the wood and also influences its hardness (Cutler, Botha and Stevenson (2008).

## CONCLUSIONS

The findings of this study indicate that the planting density significantly influences the anatomical features of the *Eucalyptus* and *Acacia* wood.

Within species: *Eucalyptus* and *Acacia* produced wood with shorter fibers and smaller vessels in high density stands, when compared to its equivalent in low density. However, *Acacia* presented more frequent vessels and fibers with walls thicker than its equivalent in low planting density.

Among species: *Eucalyptus* presented longer and thinner fibers and vessels more frequent than *Acacia*, regardless of planting density. On the other hand, in high planting density, *Acacia* presented fibers with walls thicker than *Eucalyptus* and in low density of planting their vessels were larger.

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