AGE OF STOCK PLANTS, SEASONS AND IBA EFFECT ON VEGETATIVE PROPAGATION OF *Ilex paraguariensis*[1]

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ABSTRACT – The low germination of *Ilex paraguariensis* seeds and their long reproductive cycle make cuttings propagation a good alternative for its reproduction all year round, enabling to obtain genetically superior clones. Thus, we evaluated the influence of stock plants age, plant growth regulator indole-3-butyric acid (IBA) application and plant material collection in different seasons related to rooting of cuttings. From 12 and over 80-years-old trees shoots cuttings were made in four seasons, treated with IBA hydroalcoholic solution at concentrations of 0, 1500, 3000, 4500 and 6000 mg L^{-1}. The rooting was performed in plastic boxes filled with vermiculite and carbonized rice husk at a ratio of 1:1 (v/v) and, after 90 days in controlled greenhouse conditions were evaluated the rooting percentage, callus formation, survival, mortality, number of roots/cutting and length of three larger roots/cutting. Cuttings from 12-years-old stock plants have higher ability to form adventitious roots and the use of IBA did not increase rooting potential. Autumn proved to be the most favorable season for rooting, followed by spring and winter.

Keywords: Yerba mate; Vegetative rescue; Clonal forestry.

PROPAGAÇÃO VEGETATIVA DE ERVA-MATE EM FUNÇÃO DA IDADE DAS PLANTAS MATRIZES, ESTAÇÕES DO ANO E ÁCIDO INDOL BUTÍRICO

RESUMO – A baixa viabilidade na germinação das sementes de erva-mate e seu longo ciclo reprodutivo fazem com que a estaquia seja uma opção para produção de mudas durante o ano inteiro, possibilitando a obtenção de clones geneticamente superiores. Assim, avaliou-se a influência da idade das plantas matrizes, aplicação do regulador vegetal ácido indol butírico (AIB) e coleta de material vegetal em diferentes estações do ano no enraizamento de estacas caulinares de erva-mate. A partir de brotações de copa de árvores de 12 anos e de mais de 80 anos de idade, foram preparadas estacas 12±0,5 cm nas quatro estações do ano, as quais foram tratadas com AIB em solução hidroalcoólica nas concentrações de 0, 1500, 3000, 4500 e 6000 mg L^{-1}. O plantio foi realizado em caixas plásticas preenchidas com vermiculita e casca de arroz carbonizada na proporção de 1:1 (v/v) e, após 90 dias em casa de vegetação climatizada, foram avaliadas a porcentagem de estacas enraizadas, com calos, sobrevivência, mortalidade, número de raízes/estaca e comprimento das três maiores raízes por estaca. Estacas coletadas de matrizes de 12 anos apresentam maior aptidão à emissão de raízes adventícias e a aplicação de AIB não aumentou o enraizamento. O outono mostrou-se a estação mais favorável ao enraizamento, seguido da primavera e inverno.

*Palavras-chave: **Ilex paraguariensis**; Resgate vegetativo; Silvicultura clonal.*

http://dx.doi.org/10.1590/1806-90882017000200004

1. INTRODUCTION

Ilex paraguariensis A.St.-Hil. (Aquifoliaceae), also known as yerba mate, is a tree species that can reach 20 meters in height. It occurs naturally in South America (Carvalho, 2003) where it has great economic importance, since its leaves are used in medicine and consumed in the form of tea (Dartora et al., 2013).

The use of inadequate techniques of cultivation and management, and especially the low genetic quality of the seedlings, resulted in the low productivity of the currently plantations (Santin et al., 2014). In addition, the production of seedlings requires long periods of stratification, presenting reduced germination (Cuquel et al., 1994).

The slowness, coupled with the great phenotypic variation resulting from the sexual propagation, generates the need for more detailed studies with respect to other techniques for the production of yerba mate plants, making the conventional vegetative propagation interesting for obtaining genetically known material (Wendling et al., 2007; Bitencourt et al., 2009; Brondani et al., 2009). The challenge in this case is the success of rooting cuttings from selected adult plants, with the purpose of rescuing this genetic material without eliminating the stock plants (Wendling et al., 2013). For this, physiologically juvenile shoots are needed (Brondani et al., 2008; Bitencourt et al., 2009; Wendling et al., 2013; Kratz et al., 2015).

Many factors influence the rooting process of cuttings; among them, the age of the mother tree (Altoé et al., 2011; Wendling et al., 2014b), the collection season (Zen et al., 2015) and the application of plant regulators (Stuepp et al., 2014; Fragoso et al., 2015). Thus, more juvenile branches and in good nutritional condition tend to present a better rooting (Wendling et al., 2014b).

Little is known about the effects of maturation on the rooting of yerba mate cuttings (Wendling et al., 2013; Stuepp et al., 2016). This results in a gap to be filled related to the regenerative and rescue capacity between adult and juvenile materials, especially to reestablish ontogenetic high age genotypes (Wendling et al., 2014b).

The application of plant regulators, mainly of the auxin group, has been cited as favorable to the adventitious rooting process in several species, and although indole-3-acetic acid (IAA) is the most abundant auxin in vegetables, the use of indole butyric acid (IBA) has been shown to be more stable and efficient (Ferreira et al., 2010).

The collection season may have a close relation with the physiological characteristics of the cuttings, being more herbaceous when collected during the period of vegetative growth (spring/summer) or more woody when collected during the period of greatest vegetative rest (autumn/winter) (Pizzatto et al., 2011).

The objective of the present study was to evaluate the influence of stock plants age, plant growth regulator IBA application and plant material collection in different seasons related to rooting of cuttings.

2. MATERIALS AND METHODS

The experiment was conducted between winter (May) 2006 and autumn (February) 2007 at the Laboratory of Forest Species Propagation, from Embrapa Florestas, located in Colombo, Paraná, Brazil (25°19'17" S and 49°09'39" W). According to the classification of Köppen, the climate of the region is temperate, of the type Cfb, with temperature of the coldest month between -3 and 18 °C, always humid, with rains well distributed throughout the year and temperature of the month warmer inferior to 22 °C.

Two groups of stock plants were used, the first one located in Bocaiúva do Sul - PR (25°12'22" S and 49°06'54'' W, 980 m), with 12 years of age, and the second located in Colombo - PR (25°20' S and 49°14' W, 950 m), with over 80 years of age. The branches of the year were collected in the morning and packed in polystyrene boxes, moistened and transported to the place of preparation. Woody stem cuttings 12 ± 0.5 cm long were cut bevel cut at the base and rectum above the last bud, with two leaves reduced to 50% of their original surface.

The experiments were carried out in the four seasons between 2006 and 2007 on the following dates: 05/05/2006 (autumn), 11/08/2006 (winter), 12/12/2006 (spring) and 08/02/2007 (summer).

The cuttings were disinfested with 0.5% sodium hypochlorite solution for 5 minutes (bactericidal action) and washed in tap water for 5 minutes, and then treated with 0.1% Benomyl® for 15 minutes (fungicidal action). Subsequently, the treatment of approximately 1 cm of the bases of the cuttings with IBA was carried out for 10 seconds, according to the following treatments (T): T1: 0 mg L^{-1} (witness); T2: 1500 mg L^{-1}; T3: 3000 mg L^{-1}; T4: 4500 mg L^{-1}; T5: 6000 mg L^{-1}. Subsequently, they were placed in plastic boxes of 13x30x37 cm (14.4 liters) filled with vermiculite of medium granulometry and carbonized rice husk (1:1 v/v), about 3 cm deep, conditioned in a greenhouse heated with intermittent misting (temperature between 20-30 °C and relative humidity higher than 85%) (Figure 1).

After 90 days of installation, the following variables were evaluated: rooting percentage (alive cuttings with roots of at least 1 mm in length); number of roots/cuttings; length of the three largest roots/cutting (mm); percentage of cuttings with callus (alive cuttings, without roots, with undifferentiated cell mass formation at the base); percentage of survival (alive cuttings that did not present root induction or callus formation) and; percentage of mortality (cuttings with necrotic tissues).

The experiments were carried out in a completely randomized design, with 2x5x4 factorial arrangement (2 ages of stock plants, 5 IBA concentrations and 4 seasons of the year), with 4 replications of 20 cuttings per experimental unit.

The data were analyzed with an analysis of variance (ANOVA), the treatment variances were evaluated for homogeneity with the Bartlett's test, and the variables with significant differences in the F test had their means compared with the Tukey test at 5% probability. The data whose analysis involved comparison of two-year materials from two different sites were submitted to covariance analysis, using age as a covariant. The covariance analysis revealed that the plant cultivation site did not influence the results of the analyzed variables, at a 95% probability level, and did not require adjustment for this factor.

3. RESULTS

Regardless of IBA concentrations and seasons of the year, 12-year-old stock plants presented higher

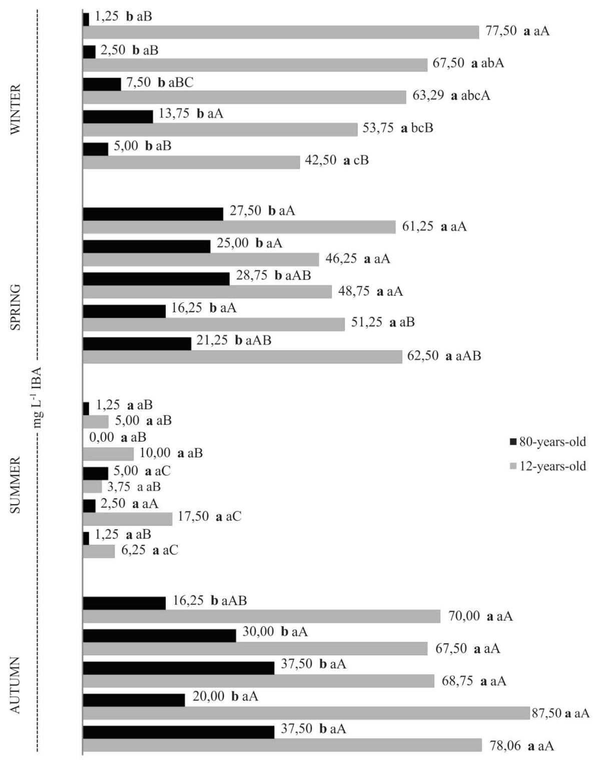

Figure 2 – Percentage of *Ilex paraguariensis* rooted cuttings from two age stock plants, four seasons and five concentrations of IBA. Bold letters for ages, small letters for treatments with IBA within each season and capital letters between the seasons in each treatment with IBA. Means followed by the same letter are not statistically different by Tukey test at 5% probability (p <0.05).

***Figura 2** – Porcentagem de estacas enraizadas de erva-mate, em duas idades de plantas matrizes, quatro estações do ano e cinco concentrações de AIB. Letras em negrito para as idades de matrizes, letras minúsculas para os tratamentos com AIB dentro de cada estação e letras maiúsculas entre as estações do ano em cada tratamento de AIB. Médias seguidas da mesma letra não diferem estatisticamente entre si pelo teste de Tukey ao nível de 5% de probabilidade (p<0,05).*

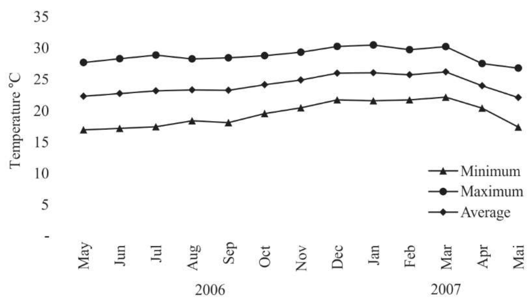

Figure 1 – Averages of maximum, medium and minimum temperatures in greenhouse during the experimental period.

***Figura 1** – Médias das temperaturas máximas, médias e mínimas da casa de vegetação, durante o período experimental.*

rooting, except for summer, in which the variables did not differ and the rooting rate was low. The highest percentage of rooting was observed in the fall, with application of 4500 mg L^{-1} of IBA (87.5%).

The application of IBA increased the number of roots per cutting collected from 12-year-old stock plants in the spring (concentrations of 4500 and 6000 mg L^{-1}) and summer (concentrations of 3000 and 4500 mg L^{-1}). For the concentrations of 0 and 1500 mg L^{-1}, winter was the best collection period (9.5 and 9.9 roots, respectively), while the concentration of 3000 mg L^{-1} of IBA was shown to be significantly higher only in the summer (3.9 roots). In autumn, the increase of roots number with IBA application for both stock plants ages was evident (Table 1).

The average length of the three largest roots/cuttings was higher in 12-year stock plants collected in winter, autumn and spring, regardless of the IBA concentration, except for the control treatment (0 mg L^{-1} IBA) in the spring. For stock plants older than 80 years, the concentrations of 0, 3000, 4500 and 6000 mg L^{-1} applied in spring presented the best results for this variable (Table 1).

In general, 12-year-old stock plants showed the lowest percentage of cuttings with callus. The highest values were verified in autumn, regardless of IBA concentration, in stock plants of 80 years of age (Table 1). Summer showed the highest percentage of live cuttings, regardless of IBA concentrations, reaching 41.2% survival in 12-year stock plants (Table 1). With the exception of autumn, cuttings from 80-year-old stock plants showed high mortality rates. The highest percentages were verified in winter, at concentrations of 0 and 6000 mg L^{-1} IBA (Table 1).

4. DISCUSSION

Vegetative propagation has been an excellent method to support forest species genetic improvement, allowing the reproduction of genetically superior trees with high physiological maturation (Pijut et al., 2011; Wendling et al., 2014b). It is possible to observe in this study the viability to rescue genetic material of high chronological age, representing an advance in the propagation of yerba mate adult plants.

The success in the cuttings propagation of woody species is often related to the existence of reserve substances in the stem, mainly carbohydrates, which will supply the necessary energy to the rhizogenesis (Oliveira et al., 2012). The photosynthates translocation takes place during the dormancy period and, therefore, autumn is the time that provides these reserves immediately available (Denaxa et al., 2012), justifying the best rooting in this season.

Summer did not favor the increase in number and length of roots (Table 1), regardless of stock plants age and IBA concentrations, corroborating with the results observed by Brondani et al. (2009) in 12-years-old stock plants. In the case of a subtropical species (Santin et al., 2014), yerba mate tends to present greater limitations of growth and development when exposed to high temperature conditions, such as observed in the summer, reflecting not only the percentage of rooting, but also in other characteristics such as number and length of roots (Table 1). In addition, the metabolic and physiological activities in plants have their regulation, among other factors, through the temperature variation in the environment (Floss, 2004), and can act directly on the adventitious root emission (Brondani et al., 2007).

The use of plant regulators, specifically IBA, has been recommended for stimulating and accelerating the process of adventitious root formation (Guo et al., 2009; Pop et al., 2011), increasing the rooting index, the speed of formation, quality and uniformity of the root system (Pijut et al., 2011). However, only in the winter, in cuttings collected from 12-year-old stock plants, a significant difference was observed between IBA concentrations, with the highest concentrations resulting in the lowest rooting percentages (Figure 2) and root vigor, denoted by the number of roots and mean length of the three largest roots (Table 1).

The addition of auxins has been verified in many studies and the concentrations required vary from species to species (Abu-Zahra et al., 2013; Stuepp et al., 2015). The plants respond progressively to the stimulus to an optimal level of auxin, from which, the increase in concentration becomes inhibitory or even phytotoxic (Pop et al., 2011).

In general, the rooting percentage, number and length of roots had higher values in 12-years-old stock plants, a fact probably related to the greater vigor presented by these plants. Several authors recommend the use of juvenile materials for the propagation of woody species, justifying, above all, the improvements

Table 1 – General averages for number of roots per cutting, average length of three largest roots per cutting, percentage of cuttings with callus, survival and mortality in yerba mate cuttings from two stock plants age, four seasons and five concentrations of IBA .

Tabela 1 *– Médias gerais para número de raízes por estaca, comprimento médio das três maiores raízes por estaca, porcentagem de estacas com calos, sobrevivência e mortalidade em estacas de erva-mate, em duas idades de plantas matrizes, quatro estações do ano e cinco concentrações de AIB.*

IBA	Winter		Spring		Summer		Autumn	
mg L^{-1}	12-years-old	80-years-old	12-years-old	80-years-old	12-years-old	80-years-old	12-years-old	80-years-old
Number of roots per cutting (n)								
0	9,48 **a** aA	0,50 **b** aB	4,05 **a** bBC	5,67 **a** abA	1,88 **a** aC	0,25 **a** aB	4,93 **a** bB	1,92 **b** aB
1500	9,86 **a** aA	1,75 **b** aAB	5,50 **a** bB	3,14 **a** bA	1,63 **a** aC	0,00 **a** aB	6,24 **a** abB	3,27 **b** aA
3000	8,45 **a** aA	1,63 **b** aB	6,46 **a** bAB	6,81 **a** aA	3,88 **a** aB	0,67 **b** aB	6,77 **a** abAB	2,86 **b** aB
4500	8,50 **a** aB	2,29 **b** aB	13,25 **a** aA	5,55 **b** abA	2,80 **a** aC	0,75 **a** aB	8,30 **a** aB	2,60 **b** aB
6000	10,12 **a** aAB	1,88 **b** aBC	12,38 **a** aA	5,09 **b** abA	1,25 **a** aC	0,75 **a** aC	8,89 **a** aB	3,90 **b** aAB
Average length of three largest roots per cutting (cm)								
0	26,76 **a** aA	2,25 **b** aB	23,64 **a** cdAB	19,47 **a** abA	2,11 **a** aC	2,25 **a** aB	18,51 **a** cB	6,21 **b** aB
1500	31,32 **a** aA	5,75 **b** aAB	18,92 **a** dB	11,19 **b** bA	2,08 **a** aC	0,00 **a** aB	21,16 **a** bcB	11,66 **b** aA
3000	31,37 **a** aA	5,29 **b** aBC	30,68 **a** bcA	20,61 **b** aA	2,40 **a** aB	2,20 **a** aC	26,15 **a** abcA	11,85 **b** aB
4500	28,30 **a** aB	6,59 **b** aBC	41,85 **a** aA	19,10 **b** abA	7,97 **a** aC	2,00 **a** aC	30,88 **a** aB	10,59 **b** aB
6000	24,83 **a** aB	3,54 **b** aBC	37,17 **a** abA	17,47 **b** abA	7,69 **a** aC	1,17 **a** aC	27,52 **a** abB	10,32 **b** aAB
Callus (%)								
0	7,50 **a** aB	5,00 **a** cB	10,00 **a** aB	17,50 **a** aB	17,50 **a** aAB	15,00 **a** aB	26,25 **b** aA	82,50 **a** aA
1500	13,75 **b** aAB	31,25 **a** aB	1,25 **a** aB	7,50 **a** abC	23,75 **a** aA	2,50 **b** aC	27,50 **b** aA	68,75 **a** abA
3000	7,63 **b** aAB	23,75 **a** abB	5,00 **a** aB	1,25 **a** abC	17,50 **a** aAB	7,50 **a** aC	22,50 **b** abA	57,50 **a** bA
4500	2,64 **b** aA	38,75 **a** aB	5,00 **a** aA	3,75 **a** abC	17,50 **a** aA	8,75 **a** aC	8,75 **b** bA	73,75 **a** abA
6000	8,75 **a** aA	8,75 **a** bcB	5,00 **a** aA	0,00 **a** bB	12,50 **a** aA	8,75 **a** aB	16,67 **b** abA	61,25 **a** bA
Survival (%)								
0	0,00 **a** aC	0,00 **a** aA	11,25 **a** abB	5,00 **a** aA	41,25 **a** aA	8,75 **b** aA	2,50 **a** aBC	0,00 **a** aA
1500	6,25 **a** aC	3,75 **a** aC	20,00 **a** aB	6,25 **b** aA	31,25 **a** bcA	2,50 **b** aA	3,75 **a** aC	1,25 **a** aA
3000	2,57 **a** aC	1,25 **a** aC	12,50 **a** abB	2,50 **b** aA	30,00 **a** bcA	5,00 **b** aA	7,50 **a** aBC	0,00 **a** aA
4500	7,64 **a** BC	1,25 **a** aA	11,25 **a** abB	2,50 **b** aA	25,00 **a** cA	7,50 **b** aA	1,25 **a** aC	2,50 **a** aA
6000	8,75 **a** aB	2,50 **a** aA	3,75 **a** bB	2,50 **a** aA	35,00 **a** abA	5,00 **b** aA	2,78 **a** aB	0,00 **a** aA
Mortality (%)								
0	15,00 **b** bB	93,75 **a** aA	17,50 **b** aB	50,00 **a** bC	36,25 **b** aA	75,00 **a** bB	1,25 **a** aB	1,25 **a** aD
1500	12,50 **b** bB	62,50 **a** cdB	32,50 **b** aA	61,25 **a** bB	35,00 **b** aA	95,00 **a** aA	1,25 **a** aB	0,00 **a** aC
3000	26,51 **b** abB	67,50 **a** bcA	33,75 **b** aAB	67,50 **a** aA	48,75 **b** aA	82,50 **a** abA	1,20 **a** aC	5,00 **a** aB
4500	35,98 **a** aA	46,25 **a** dB	32,50 **b** aA	77,50 **a** aA	40,00 **b** aA	81,25 **a** abA	2,50 **a** aB	2,50 **a** aC
6000	40,00 **b** aAB	83,75 **a** abA	28,75 **b** aB	76,25 **a** aA	46,25 **b** aA	85,00 **a** abA	2,50 **a** aC	2,50 **a** aB

Bold letters for stock plants ages, small letters for treatments with IBA within each season and capital letters between the seasons in each treatment with IBA. Means followed by the same letter are not statistically different by Tukey test at 5% probability ($p < 0.05$).
Letras em negrito para as idades de matrizes, letras minúsculas para os tratamentos com AIB dentro de cada estação e letras maiúsculas entre as estações do ano em cada tratamento de AIB. Médias seguidas da mesma letra não diferem estatisticamente entre si pelo teste de Tukey ao nível de 5% de probabilidade (p<0,05).

related to the rooting and vigor of clonal plants (Wendling et al., 2007; Pijut et al., 2011; Wendling et al., 2014b). As the chronological age of the stock plants increases, the cuttings from the branches of the year tend to be less responsive to the application of IBA (Table 1), while those from younger plants show a greater responsiveness in root length when submitted to its application, which may, to a certain extent, be related to the absence of cofactors, or even the presence of inhibitors of rooting in adult material (Pijut et al., 2011).

The decline in rooting capacity in woody species is often linked to the effects of maturation (Ferreira et al., 2010) where cuttings from more juvenile plants or branches tend to have a better rooting (Wendling et al., 2013; Stuepp et al., 2014; Wendling et al., 2014b). Thus, cuttings collected from younger stock plants have greater vigor and greater easiness to root, justifying the best results observed in the present work, for cuttings collected from 12-years-old stock plants.

The high percentage of callus formation is another indicative of the high maturation of the used material (Wendling et al., 2014b), since the highest percentages of callus formation were observed in the fall, in cuttings coming from stock plants with more than 80 years of age (Table 1). In addition, autumn was one of the seasons that presented the highest rooting percentages of the youngest stock plants. These results support the hypothesis that callus formation is detrimental to the rooting of yerba mate cuttings, indicating that the rhizogenesis is direct, without the dependence of the previous formation of callus.

The high survival (41.2%) and mortality (95.0%) of cuttings verified in 12 and 80-years-old stock plants, respectively, show the recalcitrant characteristic of the species, responding differently for the two ages, concentrations of IBA and evaluated seasons (Table 1). It should be emphasized that the high survival observed in the younger stock plants, could reflect in a higher percentage of rooting, if exposed to a longer period of permanence in greenhouse.

The high mortality seems to be related mainly to the harvesting season, since in the autumn the mortality was low for the two plants ages, as well as for all the concentrations of IBA used. This result may be a consequence of the branches physiological condition at the time of collection, because it is during this period that plants begin to store reserves in the stem, being readily available to the cuttings during the periods of greatest growth, decreasing, consequently the mortality (Oliveira et al., 2012).

The higher summer mortality (95%) in 80-year-old stock plants submitted to treatments with 1500 mg L^{-1} IBA and zero mortality in autumn, evidenced the importance of the time of year for the collection of yerba mate cuttings (Table 1). It is important to note that the loss of vegetative vigor is one of the responses to maturation in forest species (Wendling et al., 2014a), evidenced by the high mortality percentages in cuttings from stock plants over 80 years of age.

4. CONCLUSIONS

Cuttings from younger stock plants are more responsive for adventitious roots formation, as well as result in the formation of a more vigorous root system. Autumn is the most favorable season to root induction, independently of the two studied stock plant ages. The use of IBA does not increase rooting percentages, regardless of stock plants age, and callus formation does not favor rooting in yerba mate cuttings.

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