Growth and development of yerba mate seedlings associated with different winter cover species

Paula Rochelly De David[1], Felipe Adelio De David[1], Janaíne Oliveira Toso[1], Carla Pasinato[1], Caroline Müller[1], Leandro Galon[1] & Gismael Francisco Perin[1]

[1] Laboratory of Sustainable Management of Agricultural Systems, Federal University of Fronteira Sul, Erechim, Brazil

Correspondence: Caroline Müller, Laboratory of Sustainable Management of Agricultural Systems, Federal University of Fronteira Sul, Erechim, Brazil. E-mail: carolinemulleram@gmail.com

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Abstract

The lack of knowledge of management in the application of yerba mate is the reason for the present study, given the economic importance of this crop for family farming, the economy and also the culture in the southern region of Brazil. The objective of this work was to evaluate the effects of winter cover species on the morphophysiological characteristics of yerba mate seedlings. The experimental design was laid out in randomized blocks with four replicates. The weed species used were *Avena strigosa*, *Lolium multiflorum*, *Vicia sativa*, *Raphanus sativus*, *Lupinus albus*, *Axonopus catharinensis*, the consortium between *A. strigosa* and *V. sativa*, and the control treatment without cover. Physiological (photosynthesis, stomatal conductance, transpiration, internal CO_2 concentration, water use efficiency, carboxylative efficiency, and chlorophyll) and morphological (plant height, stem diameter, leaf area, shoot and of root dry matter, in addition to cover dry matter) were evaluated. There was competition between the crop and the cover plants for the factors of light and water, and the control without cultivation did not affect the morphophysiological characteristics of yerba mate. The species *A. catharinensis* proved to be the best cover plant because it competed less with yerba mate. The growth and development of yerba mate were affected by the presence of *A. strigosa*.

Keywords: *Ilex paraguariensis*, winter covers, plant interference.

Crescimento e desenvolvimento de mudas de erva-mate associadas a diferentes coberturas vegetais de inverno

Resumo

A escassez de conhecimento sobre o manejo na aplicação da erva-mate é a justificativa do presente estudo, haja vista a importância econômica desta cultura para a agricultura familiar, economia e também para a cultura da região sul do Brasil. O objetivo deste trabalho foi avaliar os efeitos de espécies de cobertura de inverno sobre as características morfofisiológicas de mudas de erva-mate. O delineamento experimental foi em blocos casualizados com quatro repetições. As espécies daninhas utilizadas foram *Avena strigosa*, *Lolium multiflorum*, *Vicia sativa*, *Raphanus sativus*, *Lupinus albus*, *Axonopus catharinensis*, o consórcio entre *A. strigosa* e *V. sativa*, e o tratamento controle sem cobertura. Foram avaliadas as características fisiológicas (fotossíntese, condutância estomática, transpiração, concentração interna de CO_2, eficiência do uso da água, eficiência carboxilativa e clorofila) e morfológicas (altura da planta, diâmetro do caule, área foliar, matéria seca da parte aérea e da raiz, além da matéria seca da cobertura) das plantas. Houve competição entre a cultura e as plantas de cobertura pelos fatores de luz e água, e o controle sem cultivo não afetou as características morfofisiológicas da erva-mate. A espécie *A. catharinensis* mostrou-se a melhor planta de cobertura por competir menos com a erva-mate. O crescimento e desenvolvimento da erva-mate foram afetados pela presença de *A. strigosa*.

Palavras-chave: *Ilex paraguariensis*, cobertura de inverno, interferência da planta.

1. Introduction

Yerba mate (*Ilex paraguariensis* St. Hil.) is classified as a forest species native to South America. Historically, the leaves of this plant were already used by indigenous tribes, especially the Guarani, who lived around the basins of the Paraná, Paraguay and Uruguay rivers. After colonization, the commercialization of yerba mate began and became an important economic activity (Ibramate, 2017) that continues today, mainly for small producers.

Brazil is the world´s largest producer of yerba mate with a production of 1,064,121 thousand tons in 2021, followed by Argentina (882,095 thousand tons) and Paraguay (~170 thousand tons) (Deral, 2022). In the national territory, the state of Rio Grande do Sul is the largest producer with an average productivity of 9,753 kg ha^{-1} higher than the national average (~8,000 kg ha^{-1}). In March 2023 alone, Brazil exported 3,390,583 kg of yerba mate, generating US$ 7,303,162 in revenues, 85.4% of which came from the state alone RS (Fundomate, 2023). The high productivity in the state can be explained by the greater investment in herbals and improvement in management practices, resulting in higher profitability for producers (Ibramate, 2018). Currently, the extraction of yerba mate is once again considered an attractive source of income for producers, due to new public policies aimed at strengthening the production chain and increasing the price paid for the arroba, or even enhancing the value of the by-products obtained from yerba mate, such as teas.

Perennial cultivation of yerba mate is usually done on small plots of land, mostly in family farms, as an alternative to other activities. About 84% of yerba mate obtained for processing in the state RS is grown on properties of up to ten hectares (Oliveira & Waquil, 2017). In this way, yerba mate makes an important economic contribution to the livelihoods of small-scale producers who grow this crop on previously underutilized land or even combined with other crops, such as maize, soybeans, beans, pasture (livestock), and others (Antoniazzi, 2018).

The decline in yerba mate production over the years is related to several factors, one of which is the adopted conventional system model, which has proven to be flawed due to several errors in cultivation. These failures include the lack of knowledge, of appropriate technologies, and of greater interaction between growers and research in the sector (Pasinato; Valter, 2012). Over the years, it has been observed that farmers are changing the way they grow yerba mate, trying to improve the production system to reduce costs by controlling diseases, insects, weeds, correcting fertility, pruning, etc.

Implementing a cropping system requires practices that allow for minimizing the impact of production on the agroecosystem, with less risk of erosion and keeping the soil covered for a long period of time. The use of soil covers not only protects against erosion, but also conserves soil moisture, promotes weed control, provides nutrients to plants, and maintains soil fauna. To determine which soil cover is most suitable, it is important to evaluate the production of its dry matter, the production of allelopathic substances to prevent the germination of weeds, which are easy to manage, have a deep root system, have the ability to recycle nutrients and have a high C/N ratio (Isga, 2010).

The definition of competition is the struggle between one plant and another for resources available in the environment such as water, light, and nutrients (Haring; Hanson, 2022). When intercropping between two or more crops, boundaries must be established so that species do not interfere with each other (Wilson, 1988; Haring & Hanson, 2022). The use of winter cover plants between yerba mate rows continues to raise questions among producers because they can affect the root development of the crop and have a negative impact on productivity, i.e., preventing these species from competing with the crop of agricultural interest (Philipovsky et al., 2004).

Competition between plants, especially those sown on the same area, occurs through the effect of genetic improvement, which seeks to increase the productivity of cultivated crops, which have lower vegetative growth and are less able to compete with crops that are not genetically improved or naturally occurring in that environment (Haring; Hanson, 2022). Best management practices for agricultural crops can be defined based on studies of their competition in the presence of weeds or even cover crops (Forte et al., 2018; Haring; Hanson, 2022). Martins et al. (2015) point out that even when quantifying the benefits of soil cover, attention should be paid to whether intercropping does not lead to competition with the crop in question.

The hypothesis of this work is that winter cover species affect the growth and initial development of yerba mate seedlings, negatively affecting the morphophysiological characteristics of the crop. Therefore, the objective of this work was to evaluate the effects of winter cover species on the morphophysiological characteristics of the yerba mate crop.

2. Materials and Methods

2.1. Experimental design and growth conditions

The experiment was conducted in a greenhouse at the Federal University of Fronteira Sul – UFFS, Erechim campus (27º43'47"S latitude and 52º17'37"W longitude, altitude of 670 m) in a completely randomized block design with four replicates. Native yerba mate seedlings were obtained from seeds derived from clonal plants grown in the same lot. Seedlings were transplanted in early May (late fall) into plastic pots with a capacity of 18 dm3 or 0.09 m^2 area filled with soil classified as Humic Aluminoferric Red Latosol (Streck et al., 2018). The chemical and physical properties of the soil (0-20 cm depth) were: pH 5.0, organic matter 2.35%, P 2.1 mg dm^{-3}, K 48 mg dm^{-3}, Al 0.5 cmolc dm^{-3}, Ca 4.5 cmolc dm^{-3}, Mg 1.6 cmolc dm^{-3}, CEC (ef) 6.8 cmolc dm^{-3}, CECpH=7 12.5 cmolc dm^{-3}, H+Al 6.2 cmolc dm^{-3}, SB 6.2 cmolc dm^{-3}, V 51%, and clay 50%.

Each pot with one seedling represented one experimental unit. Fertilization was done according to the recommendations for the cultivation of yerba mate (SBCS, 2016). The yerba mate seedlings were acclimated for 45 days before being sown as winter cover crop.

The following species were used as winter cover: *Avena strigosa* Schreb. (black oat) (65 kg ha^{-1}), *Lolium multiflorum* Lam. (ryegrass) (25 kg ha^{-1}), *Vicia sativa* L. (vetch) (35 kg ha^{-1}), *Raphanus sativus* L. (forage turnip) (25 kg ha^{-1}), *Lupinus albus* L. (white lupin) (65 kg ha^{-1}), *Axonopus catharinensis* Valls (47 kg ha^{-1}) (giant missionary grass) and the consortium between *A. strigosa* and *V. sativa* (3:1). The species were sown with a spike in plastic pots, according to their respective recommendations (Calegari; Donizete-Carlos, 2014). Yerba mate seedlings without any vegetative cover were treated as control. Cover crops were sown 45 days after transplanting the yerba mate seedlings. In order to sow the required amount of seeds per pot, the densities described in the technical specifications for each cover species were considered, but transformed for the experimental units (plastic pots of 18 dm-3 or 0.09 m^2 area). All cover species were kept in the pots together with the yerba mate depending on the treatment, without pruning throughout the experimental period.

2.2. Physiological traits

Gas exchange measurements of yerba mate plants were evaluated at 145 DAT to determine photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_S, mol H_2O m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}), and internal CO_2 concentration (Ci, μmol CO_2 mol^{-1}), and water use efficiency (WUE, mol CO_2 mol H_2O^{-1}), using the infrared gas analyzer (IRGA, LCA PRO, Analytical Development Co. Ltd, Hoddesdon, UK). Measurements were performed in the morning between 8:00 and 10:00 am under the conditions of ambient light (~ 570 μmol m^{-2} s^{-1}), temperature (~ 25.5 ºC), and CO_2 concentration (Ca, ~400 μmol mol^{-1}).

Chlorophyll (Chl) content was analyzed in different leaves of mate plants, with a total of 5 measurements per plant using a portable chlorophyll meter (SPAD 502 Plus, Konica Minolta Inc., USA).

2.3. Morphological traits

At 145 DAT, plant height (PH, cm), stem diameter (SD, mm), leaf area (LA, cm^2), and shoot dry matter (SDM, g) and root dry matter (RDM, g) of all yerba mate plants and also SDM of cover plants were evaluated. The LA was determined using a portable leaf area meter (CI-203, CID Bio-Science, Inc., Camas, USA). To determine DM, plants were cut open near the ground, placed in paper bags, and dried in a forced-circulation oven (60±5 ºC) until they reach constant matter.

2.4. Statistical analysis

Data were analyzed using the one-way method ANOVA and means were compared using Tukey´s test at $p \leqslant 0.05$. Statistical analyzes were performed using the analysis of variance system (SISVAR® v. 5.4, Lavras, Brazil).

3. Results

Yerba mate plants in the presence of cover plants altered the physiological and morphological characteristics of the crop. A decrease in photosynthetic rate (A) of yerba mate plants was observed, especially in the presence of black oat (55.1%), white lupin (42.7%), forage turnip (41.3%), ryegrass (36.5%), and a consortium of black oat + vetch (28.2%) (Table 1). These lower photosynthetic rates were accompanied by an average decrease in stomatal conductance (g_S) (~47.9%) and transpiration (E) (~34.1%) of yerba mate plants, but with no change in internal CO_2 concentration (Ci) (Table 1).

Table 1. Photosynthetic rate (*A*), stomatal conductance (g_S), transpiration (*E*), internal CO_2 concentration (*Ci*), water use efficiency (WUE), carboxylative efficiency (*A*/*Ci*), and chlorophyll index (Chl) in yerba mate plants grown for 145 days in the presence of different cover crops.

Treatment	*A* (µmol mol^{-1})	g_S (mol m^{-1} s^{-1})	*E* (mol m^{-2} s^{-1})	*Ci* (µmol mol^{-1})	WUE (mol mol^{-1})	*A*/Ci (µmol mol^{-1})	Chl (SPAD)
Control	8.71±0.53 a	0.233±0.035 a	1.75±0.32 a	313.6±7.6 a	5.72±1.40 a	0.028±0.002 a	57.9±0.3 a
Black oat	3.91±0.29 b	0.108±0.017 b	1.10±0.26 a	327.3±19.5 a	4.07±0.72 a	0.012±0.001 b	45.5±1.2 b
Ryegrass	5.53±0.65 ab	0.114±0.017 b	1.21±0.23 a	315.2±8.7 a	5.21±1.20 a	0.017±0.002 ab	52.9±0.4a
Vetch	7.69±0.61 ab	0.172±0.024 ab	1.68±0.34 a	302.3±13.6 a	5.10±1.00 a	0.026±0.003 ab	55.4±1.2 a
Forage turnip	5.11±1.11 ab	0.153±0.014 ab	1.19±0.18 a	340.5±9.3 a	5.04±1.72 a	0.015±0.004 ab	49.5±0.8 bcd
White lupin	4.99±0.81 ab	0.123±0.020 ab	1.21±0.20 a	319.8±10.4 a	4.63±1.08 a	0.016±0.003 ab	54.0±1.3 abc
Giant missionary grass	7.89±0.63 ab	0.200±0.013 ab	1.82±0.46 a	315.3±7.6 a	5.36±1.40 a	0.025±0.003 ab	57.1±1.6 ab
Black oat + vetch	6.25±1.57 ab	0.109±0.027 b	1.07±0.15 a	318.8±17.3 a	5.85±1.00 a	0.020±0.005 ab	45.5±1.2 bd

Data represent means ± SEM (n = 4). Means following the same letter do not differ by Tukey's test ($p \leq 0.05$).

Table 2. Plant height (PH), stem diameter (SD), leaf area (LA), shoot dry matter (SDM) and root dry matter (RDM) of yerba mate and dry matter (DM) of cover species after 145 days of yerba mate cultivation in the presence of different cover crops.

Treatment	PH (cm)	SD (mm)	LA (cm^2 $plant^{-1}$)	SDM (g $plant^{-1}$)	RDM (g $plant^{-1}$)	DM (g pot^{-1})
Control	30.9±3.8 ab	4.63±0.35 a	801.3±87.8 a	8.16±0.68 a	3.64±0.36 a	0.00 e
Black oat	22.5±1.3 b	3.94±0.16 a	422.3±59.4 ab	4.15±0.50 b	2.33±0.27 b	48.91±2.81 a
Ryegrass	29.6±3.8 ab	4.57±0.33 a	399.4±18.4 b	4.96±0.74 ab	2.64±0.30 ab	40.48±3.10 ab
Vetch	37.5±4.3 a	4.91±0.47 a	559.6±124.6 ab	6.83±1.07 ab	2.91±0.13 ab	15.67±1.76 d
Forage turnip	31.6±2.8 ab	4.73±0.15 a	452.2±13.1 ab	5.71±0.35 ab	3.12±0.14 ab	41.04±2.84 ab
White lupin	31.2±2.3 ab	4.72±0.25 a	593.3±138.3 ab	6.36±0.92 ab	3.30±0.12 ab	23.41±1.56 cd
Giant missionary grass	36.3±3.3 a	5.01±0.029 a	597.9±56.6 ab	6.81±0.78 ab	3.52±0.33 a	32.61±3.34 bc
Black oat + vetch	27.2±1.9 ab	4.28±0.20 a	484.4±23.7 ab	5.67±0.41 ab	2.83±0.24 ab	44.47±2.68 ab

Data represent means ± SEM (n = 4). Means following the same letter do not differ by Tukey's test ($p \leq 0.05$).

As a result of the lower photosynthetic rate, a drastic reduction in carboxylation efficiency (*A*/Ci) (56.2%) and lower water use efficiency (WUE) (28.8%) was observed in the crop plants in the presence of black oat compared to the control plants, i.e., in the absence of the cover species. In addition, lower chlorophyll content was observed in yerba mate plants in the presence of black oat (21.4%), black oat + vetch (21.5%), and forage turnip (14.6%) (Table 1).

Analysis of morphological variables revealed a reduction in height of yerba mate plants (27.3%) when grown in the presence of black oat and lower leaf area values (~48.7%) when grown in the presence of black oat and ryegrass compared to control plants (Table 2). The presence of vetch and giant missionary grass increased yerba mate plant height by 21.4% and 17.6%, respectively (Table 2). Yerba mate plants did not show significant differences after 175 days of cultivation, regardless of the presence of cover crops.

Shoot dry matter (SDM) of yerba mate plants was reduced in all treatments evaluated, with a greater decrease in plants grown in the presence of black oat (49.1%) than in control plants (Table 2). Similarly, the greatest decrease in root dry matter (RDM) of yerba mate was observed in the black oat treatment (36.1%), while the presence of giant missionary grass had no effect on RDM of yerba mate plants (Table 2).

At 145 days after cultivation, the dry matters of the aerial parts of the cover crops differed significantly from each other. Vetch (15.67 g pot^{-1}), white lupin (23.40 g pot^{-1}), and giant missionary grass (32.60 g pot^{-1}) species had the lowest DM values, while the other species ranged from 40.47 g pot-1 (ryegrass) to 48.90 g pot^{-1} (black oat) (Table 2).

4. Discussion

The growth and development of a plant depends largely on the availability of external factors such as water, light, nutrients and CO_2 (Engineer et al., 2016). All physiological, morphological and nutritional variables are closely related to the limitation or availability of these factors in the environment (Atay et al., 2017; Abdelaal et al., 2022).

The reduction in the photosynthetic rate of yerba mate intercropped with black oat represents competition for water and light factors, as previously observed when yerba mate was grown in the presence of the weed species *Bidens pilosa* and *Ipomoea indivisa* (David et al., 2018). The photosynthetic rate is directly related to the availability of water and light incidence on the plants (Devin et al., 2023). The black oat, ryegrass, and black oat + vetch treatments showed similar results for stomatal conductance compared to the other cover plants, marking the occurrence of competition for the water resource. Water deficiency leads to stomatal closure to maintain plant water potential, but can reduce CO_2 assimilation and affect plant growth (Engineer et al., 2016; Jia et al., 2022).

In terms of light competition, artificial shading of 50% to 70% resulted in a reduction in photosynthetic rate, stomatal conductance, and growth of *Cupania vernalis* (Lima Junior et al., 2005), which is not observed when the tree is cultivated in full sun and low shading (30%). Also, *Swietenia macrophylla* seedlings developed better when exposed to full sun than when cultivated in shade (Gonçalves et al., 2012). Plants used in high light intensity environments have multiple layers of mesophyll cells and a large amount of chloroplasts in the cells (Wendling; Santin, 2015), which could be related to the chlorophyll content observed in the control treatment.

On the other hand, the greater amount of dry matter produced by the black oat resulted in shading of the yerba mate seedlings and consequently a reduction in chlorophyll content, growth, and development. In conjunction with a lower photosynthetic rate and chlorophyll content, a reduction in the carboxylative efficiency of yerba mate competing with black oats was observed. The reduction in carboxyl efficiency may indicate biochemical limitations associated with damage to the Calvin cycle (Müller et al., 2020) and production of reactive oxygen species (Abdelaal et al., 2022), which can cause oxidation of photosynthetic components and chlorophylls. Water limitation can also affect chlorophyll synthesis and degradation by inhibiting genes associated with the chlorophyll cycle (Peloso et al., 2017; Li et al., 2020). In the black oat and black oat + vetch treatments, assuming water competition, it was observed that chlorophyll content was lower in yerba mate than in the other treatments, which was related to the impairment of light intensity on the crop.

The morphology of yerba mate is mainly related to the genetic characteristics of the plant and its physiology. The positive variation in plant height in the vetch and giant missionary grass treatments indicates that these two cover plants competed least with yerba mate. This could be due to the slow development of these two weed species. On the other hand, black oat resulted in a reduction in the height of yerba mate plants, which may be due to the rapid development of the cover that may have shaded the yerba mate plants. Competing plants tend to be more aggressive in their early stages, growing stronger and taking resources from the environment (Clements; Jones, 2021). The differential sensitivity of weed species in competition with tree plants was also confirmed by Martins et al. (2015), who observed that the olive tree cultivar Arbequina was more sensitive than cultivar Koroneiki to the weeds *Brachiaria brizantha*, *Canavalia ensiformis*, and *Cenchrus echinatus*.

Leaf area of yerba mate plants was reduced when grown in the presence of all weeds evaluated, but significantly in the presence of ryegrass. Ryegrass is not only a plant used for winter cover and forage, but also a weed that attacks crop such as wheat and barley, making it highly competitive (Pies et al., 2019; Galon et al., 2023). On the other hand, the dry matter of the aerial part and roots of the yerba mate was drastically affected by the presence of black oat, which, combined with the reduction in physiological traits, could be due to the greater development of the weed observed due to the greater dry matter of black oat. The difference in weed competitiveness was also observed in the competition of *Brachiaria plantaginea* and *B. decumbens* with coffee plants, where a lower accumulation of dry matter was observed 180 days after transplanting the seedlings of the crop associated with *B. plantaginea* (Fialho et al., 2010). The establishment and growth of competing species also affected the availability of resources for *Eucalyptus globulus* plants, resulting in a loss of production volume after 9 years of cultivation (Vargas et al., 2018).

Following the productivity losses, producers have recommended the associated use of herbicides to control weeds with resistant biotypes (Mendes; Silva, 2022) or focused on integrated weed management (Fontes et al., 2023). Weeds can also adapt to the management method used, so more than one integrated method must be combined for an effective control program, such as limiting the spread of weeds and helping crops compete with them, and using practices that prevent weeds from adapting (Abdelaal et al., 2022). On the other hand, yerba mate associated with giant missionary grass showed the same effect as the non-cultivated control plant, with little or no interference in the accumulation of substances in the crop roots. It can be observed that the cultivation of giant missionary grass in association with yerba mate is advantageous because the crop has a root system that reaches a depth of about 45 cm, while the root system of the grass is superficial, which reduces competition to an unprecedented level.

The comparison between the dry matter of the winter covers and the other morphological variables shows that the increase in the dry matter of the covers affects the growth and development of the yerba mate plants. In conjunction with the higher dry matter, weed competitiveness is also responsible for influencing the physiological and morphological characteristics of yerba mate plants. While black oat showed greater competitive ability by affecting the physiological and morphological traits of yerba mate, vetch, forage turnip, white lupin and giant missionary grass showed lower competitive ability with yerba mate plants for environmental resources such as water, light, and CO_2. The plant architecture and leaf shape of the forage turnip and white lupin may have allowed better availability of light to yerba mate plants, which, in combination with prior establishment of yerba mate seedlings, favored the growth and physiological performance of the crop. In addition, Philipovsky et al. (2004) found that the use of white lupin as a winter cover increased the productivity of yerba mate during the three years of evaluation; in contrast, the use of oats and the non-use of covers where spontaneous vegetation occurred resulted in lower productivity of the herb. Thus, it can be observed that the effects on the physiological and morphological performance of yerba mate depend on winter cover. The morphophysiological responses of the crop in competition with other species may be very different, since yerba mate has a perennial cycle and a slow initial development. Therefore, these studies are extremely important for improving crop management.

5. Conclusions

In general, it is observed that the control treatment without cultivation stands out in the morphophysiological variables of yerba mate. Black oat proved to be the most damaging winter cover, affecting the growth and development of the crop. Among the covers, giant missionary grass showed more positive results compared to the other treatments, with yerba mate having less negative effects on morphophysiological traits in the presence of this species.

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6. Authors' Contributions

Paula Rochelly de David: database, analysis and writing, *Felipe Adelio de David*: analysis, *Janaína de Oliveira Toso*: analysis, *Carla Pasinato*: analysis, *Caroline Müller*: processing and writing, *Leandro Galon*: project idea, funding, processing and review and *Gismael Francisco Perin*: processing and statistical analysis.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

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