DIFFERENTIAL MASS ACCUMULATION IN *PARAPIPTADENIA RIGIDA* (BENTH.) BRENAN AND *INGA MARGINATA* WILLD. GROWING IN SOIL WITH VOLUME CONTRASTING

Acúmulo diferencial de biomassa em *Parapiptadenia Rigida* (Benth.) E *Inga Marginata* Willd. crescendo em diferentes volumes de solo

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ABSTRACT: The soil volume influences seedling morphology and physiology during the initial growth in the nursery and under field conditions. The effect of soil volume on the initial growth and development of *Parapiptadenia rigida* and *Inga marginata* were evaluated by growing in containers of different sizes. Plants were grown in five sized containers: two polyethylene bags (PBL and PBS) and three polypropylene tubes of 175 cm^3, 100 cm^3 and 55 cm^3. *P. rigida* and *I. marginata* plants showed a general trend of reduction in stem diameter, root diameter, number of leaves, shoot, and roots dry mass with the decrease in depth and volume of containers. To both species, the main parameters accounting for separation of containers were respectively shoot biomass, total biomass and height (first axis) and DQI, R:S ratio and root biomass (second axis). Our results suggest that higher soil volume is best suited for large seedlings. However, a smaller soil volume (tubes with 175 and 100 cm^3) emerge as an interesting alternative for seedlings production, with morphological stability to acclimatization in natural conditions. The principal component analysis (PCA) showed that for both species DQI, R:S ratio and root biomass were the indices that best explained the observed differences in growth patterns in response to change in soil volume.

Keywords: Root System. Root: Shoot Ratio. Outplanting Performance. Containers.

RESUMO: O volume do solo pode influenciar aspectos morfológicos e fisiológicos das plântulas durante o crescimento inicial no viveiro e no campo. As espécies *Parapiptadenia rigida* e *Inga marginata* foram avaliadas quanto ao efeito da variação no volume do solo sobre o crescimento e desenvolvimento inicial, por meio do cultivo em recipientes de tamanhos diferentes. As plantas foram cultivadas em cinco tamanhos de recipientes: dois sacos de polietileno (PBL e PBS) e três tubos de polipropileno de 175 cm^3, com 100 cm^3 e 55 cm^3. Plantas de *P. rigida* e *I. marginata* mostraram uma tendência geral de redução no diâmetro do caule, diâmetro da raiz, número de folhas, massa seca das raízes com a diminuição da profundidade e volume de recipientes. Para ambas as espécies, os principais parâmetros explicando a separação dos recipientes foram respectivamente, massa seca da parte aérea, massa seca total e altura (primeiro eixo) e DQI, razão raiz: parte aérea e massa seca raiz (segundo eixo). Nossos resultados sugerem que o maior volume de solo é mais adequado para produção de plântulas com maior tamanho. No entanto, um menor volume de solo (tubos com 175 e 100 cm^3) representam alternativa interessante para a produção de mudas, com estabilidade morfológica para aclimatização em condições naturais. A análise de componentes principais (PCA) demonstrou que, para ambas as espécies, DQI, razão raiz:parte aérea e massa seca de raiz foram os índices que melhor explicaram as diferenças observadas nos padrões diferenciais de acúmulo de biomassa em resposta a alterações no volume de solo.

Palavras-chave: Sistema Radicular. Razão Raiz: Parte Aérea. Performance Após Plantio. Recipientes.

Introduction

The success in developing fast growing trees to forest restoration programs or timber management requires top quality seedlings in order to resist adverse field conditions. After planting, the growing behavior of a specific species is frequently related to an intricate correlation among morphological and physiological features that include height, diameter and biomass weight from aboveground and root systems (APHALO and RIKALA, 2003). Among several indexes designed to studying plant quality, the relationship between above and below ground biomass expresses the balance between losses due to transpiration and the capacity to maintain gas exchange level through the leaves, and the absorption of water and nutrients through the roots (JIMÉNEZ et al., 2005).

As discussed by Puértolas et al. (2012) , plant quality is not only related to aboveground biomass, but it is also related to the development of its roots, which may reflect in a meaningful indicator of its physiological conditions. Bao et al. (2014) argue that the architecture of the root system and its branching pattern is the main determinant of vigor. Dickson et al. (1960) stated that plants with greater thickness and higher development of the root system would show greater survival capacity. The author described a methodology to infer plant quality, nowadays recognized as the Dickson Quality Index. On the other hand, the Robustness index evaluates the relationship between root collar diameters to the aboveground height and has been assigned to understand plant capacity to confront stress and to compete with existing vegetation (JIMÉNEZ et al., 2005).

According to Domínguez-Lerena et al. (2006) tubes and plastic bags are the most efficient containers for seedling production due to the direct relation between container size (soil volume) and production costs. The feasibility of a particular container is associated mainly to the amount of substrate, the nursery size and transportation to the field, beyond the influence on the amount of inputs and water during the initial growth (CHIRINO et al., 2008). The container size and volume influence seedling morphology and physiology during the growth in the nursery and after field planting (DOMÍNGUEZ-LERENA et al., 2006). On thus, way, defining proper parameters to evaluate seedling quality is quite important and frequently, such definition is still a matter of debate (DOMINGUEZ-LERENA et al., 2006; CHIRINO et al., 2008).

Parapiptadenia rigida (Benth.) Brenan (Fabaceae) is a deciduous tree widely distributed towards seasonal forests from southern Brazil and adjacencies and shows high abundance in secondary forest associations (JARENKOW and BUDKE, 2009). *Inga marginata* Willd. (Fabaceae) is a perennial tree also found in southeastern South America with high ecological importance due to abundant fresh fruit produced. Because of rapid growth and high annual seed production, both species have been recommended for forestation of degraded areas, mainly on riverine forests (BUDKE et al., 2010).

The aim of this study was to the evaluate the effect of soil volume using containers with different sizes on the initial seedling growth and development of two tree species, *Parapiptadenia rigida* and *Inga marginata*. The following questions were raised: (i) Is there a direct relationship between the soil volume and the roots and shoots height?; (ii) Do plants growing in different soil volumes (containers with distinct height and volume size) show different patterns of biomass accumulation? and (iii) Which growth indices do properly indicate the most suitable container for seedlings production?

Material and Methods

Seed collection and experiment design

Parapiptadenia rigida and *Inga marginata* seeds were collected in the Alto Uruguai region from southern Brazil. After processing, the seeds were placed to germinate in trays containing Ferric Aluminum Latosol and vermiculite in the ratio 3:1 and when 75% of germinated seeds reached five centimeters hign, the seedlings were located into different containers. The experiment was conducted in a glasshouse sited in Erechim, southern Brazil (27°36´31´´S and 52°15´54´´W), from October 17^{th} 2011 to January 26^{th} of 2012. Potted plants were kept under natural fluctuations of temperature and controlled irrigation.

To test the effect of the soil volume on growth and development, seedlings were transplanted to the following containers: polypropylene tubes of 175 cm^3 (T175) with dimensions of 5 x 13cm, polypropylene tubes of 100 cm^3 (T100) with dimensions of 3.5 x 13.5 cm, polypropylene tubes of 55 cm^3 (T55) with dimensions of 2.5 x 11.5 cm, polythene bag with a volume of 1962.5 cm^3 (PBL) and dimensions of 10 x 25 cm, and a polyethylene bag with a volume of 607.59 cm^3 (PBS) and 6 x 20 cm dimensions. All containers were filled with substrate prepared with subsoil (B horizon of Typic Ferric aluminum Latosol), cattle manure and vermiculite, in proportions of 3:2:1 by volume, respectively. Twenty-five experimental units (individual plants) were grown under each of five different size containers: PBL, PBS, T175, T100 and T55. A small mortality rate was observed in different treatments in the first weeks of the experiment, reaching a total of thirteen plants. The experiment was conducted with a total of twenty experimental units per treatment.

Plant parameters and statistical analysis

At the end of the experiment, ten plants were separated randomly of each treatment to evaluate growth and biomass accumulation. Initially, we measured shoot length, stem diameter and number of leaves. We further removed such seedlings from containers to verify the number of secondary roots, root diameter (two centimeters below the collar), length of primary root and dry masses (60 °C, until constant weight) of shoot and root. Growth allocation was approached by computing the following mass ratios = leaves: total mass ratio (L: TM), root biomass = total mass ratio (R:TM) and root: shoot ratio (R:S).

After obtaining growth and biomass measures we calculated the Dickson Quality Index (DQI) according to Dickson *et al.* (1960), and the Robustness Index (RI) (SCHMIDT-VOGT 1990), expressed as:

$$DQI = \frac{Bt}{\left(\frac{Hs}{Ds}\right) + \left(\frac{Ba}{Br}\right)}$$

$$RI = \frac{Ds}{\left(\frac{Hs}{10}\right) + 2}$$

Where Bt is total biomass (g); Hs is stem height (cm); Ds is stem diameter, Ba is aboveground biomass (g) and Br is root biomass (g).

Data analysis

The morphological features, mass accumulation parameters and seedlings quality indexes were submitted to analysis of variance (ANOVA), among all treatments and for each of the types of containers (tubes or plastic bags). We also performed a principal components analysis (PCA) to evaluate the ordination of recipients regarding all analyzed parameters. Statistical significance was attributed to mean differences associated to $p \leq 0.05$. Data analyses were performed with SigmaPlot 2011 (Stat Software Inc.).

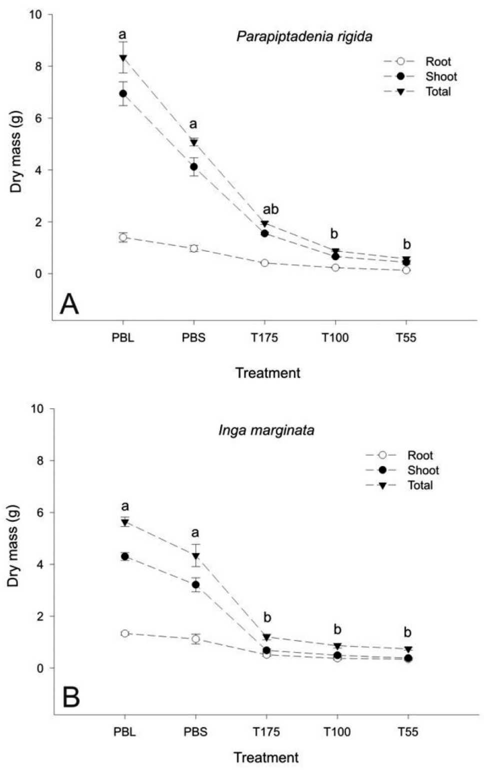
Results

Parapiptadenia rigida and *Inga marginata* plants grown in five different containers showed a general trend of reduction in growth with the decrease in soil volume. This trend was observed for the stem diameter, root diameter, number of leaves and shoot and roots dry mass (Figure 1, Tables I and II), despite the absence of differences between PBL and PBS for the stem diameter and leaf number in both species.

However, the species showed subtle differences in relation to soil volume (container size) effect on the shoot height (Figure 1). *Parapiptadenia rigida* showed a positive correlation between shoot height and containers size, despite the absence of differences between PBL and PBS, but with a growing effect among tubes (T175, T100 and T55) (Figure 1). On the other hand, in *Inga marginata*, higher shoot heights were observed in PBS, followed by PBL, while the tubes showed no differences among them (Figure 1).

Regarding to root growth, we observed that from *Parapiptadenia rigida*, variations in the soil volume did not lead to differences in length of primary root, but plants in PBS

Figure 1 - Root, shoot and total dry mass accumulation of *Parapiptadenia rigida* (A) and *Inga marginata* (B) plants at varying containers sizes. Values are means (n = 10), and vertical bars are the pooled standard errors of the mean from ANOVA. Different letters indicate significant differences (P ≤ 0.05).



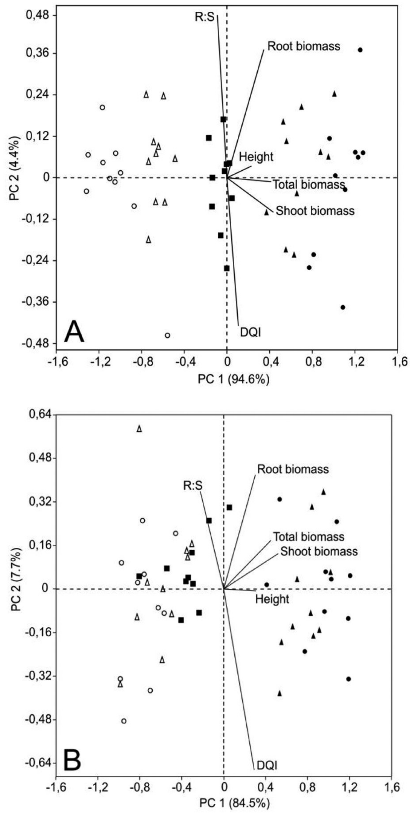
had greater number of secondary roots than PBL (Table I). *Inga marginata* showed greater length of primary root on PBL in relation to PBS, but no differences for the number of secondary roots. Interestingly, when these parameters were compared in relation to different tube sizes, both species showed greater length of main root in T55 in relation to T100 and T175 (Table II). To *Parapiptadenia rigida*, the same effect has been noted for the number of secondary roots (Table I).

From *Parapiptadenia rigida*, the first two ordination axes explained 99% of total variation (PC1 = 94.6%; PC2 = 4.6%), while, from *Inga marginata*, the first two ordination axes explained 92.2 % of total variation (PC1 = 84.5%; PC2 = 7.7%). To both species, the main parameters accounting for separation of containers with different soil volume were respectively shoot biomass, total biomass and height (first axis) and DQI, R:S and root biomass (second axis) (Figure 2).

Table I - Growth parameters in *Parapiptadenia rigida* plants under different containers sizes, measured at the end of the experiment. Values are means followed by the standard errors from ANOVA. Values followed by different letters indicate significant differences (P ≤ 0.05).

PARAMETER	PBL	PBS	T175	T100	T55
Leaf number	*20,3 ± 6,4 a*	15,7± 5 a	10,3± 2,3 b	10± 1,2 b	9,6± 0,7 b
Root length (cm)	34,7± 8 a	28,2± 4,2 a	13,5± 1,2 c	13,5± 2,4 c	15,6± 1,1 b
Root diameter (mm)	4,5± 1 a	3,9± 1 a	2,4± 0,3 b	1,5± 0,2 b	1,2± 0,2 c
Lateral root number	68,3± 10,2 b	78,1± 8,5 a	33,6± 7,6 b	24,4± 8,4 c	29,1± 5,4 b
R:S ratio	0,2± 0,1 b	0,2± 0,1 b	0,3± 0,05 b	0,3± 0,1 b	0,4± 0,1 a
Hs: Ds ratio	12,7± 2 a	10,6± 1,7 a	8,6± 1,7 b	8,2± 0,6 b	7,1± 0,5 c
Root: total mass ratio (R:TM)	0,2± 0,04 a	0,2 0,03 a	0,2± 0,03 a	0,3± 0,04 a	0,3± 0,09 a
Leaf:total mass ratio (L:TM)	0,5± 0,05 a	0,5± 0,08 a	0,5± 0,03 a	0,5± 0,03 a	0,4± 0,09 a
RI	2,8± 0,2 a	3 ± 0,2 a	3,2± 0,3 b	3,2± 0,1 b	3,4± 0,1 c
DQI	6,1± 1,5 a	5± 0,9 a	4,1± 0,8 b	3,1± 0,6 c	3,4± 2 c

Figure 2 - Principal component analysis of different morphological features, mass accumulation parameters and seedlings quality indexes from *Parapiptadenia rigida* (A) and *Inga marginata* (B) growing in different container sizes.



Discussion

The results of this study demonstrated that *Parapiptadenia rigida* and *Inga marginata* plants grown in containers with larger soil volume showed higher growth and mass accumulation. Similar to the results of this study, Gomes et al. (2003) noted that *Eucalyptus grandis* seedlings growing in different containers sizes were significantly larger as one increased the volume of containers, probably due to increased availability of nutrients and substrate to the plant. Kozlowski et al. (1991) points out that larger plant in the same chronological age have larger reserves, most likely due to increased availability of resources in larger containers, leading to greater growth and mass accumulation.

Cunha et al. (2005) found that, in addition to the effect on the plant height, container dimensions also influenced the increase in stem diameter, mainly in larger containers, regardless of substrate used, similar to our results. These authors reinforce that seedlings with low stem diameter may be injured in the field, first by having trouble staying upright

Table II - Growth parameters in Inga marginata plants under different containers sizes, measured at the end of the experiment. Values are means followed by the standard errors from ANOVA. Values followed by different letters indicate significant differences (P ≤ 0.05).

PARAMETER	PBL	PBS	T175	T100	T55
Leaf number	*14.3 ± 0.59 a*	10 ± 0.92 a	8.8± 0.7 b	9.9 ± 1.81 b	6 ± 0.35 b
Root length (cm)	34.6 ± 0.47 a	29.6 ± 1.73 a	14.8 ± 1.3 b	15.6 ± 1.3 b	20 ± 1.94 ab
Root diameter (mm)	0.15 ±0.02 a	0.14 ±0.02 a	0.09± 0.01 b	0.07± 0.02 b	0.07 ± 0.01 c
Lateral root number	38.1 ± 1.43 a	38.5 ± 3.86 a	25± 2.28 b	21.4± 1.59 b	23.8 ± 1.18 b
R:S ratio	0.32 ± 0.01 a	0.34 ± 0.04 a	0.75 ± 0.04 b	0.75 ±0.05 b	0.94 ± 0.13 b
Hs:Ds ratio	7.82 ± 0.09 a	8.15 ±1.0 a	5.18 ± 0.21 b	6.3 ± 0.97 b	4.41 ± 0.42 b
Root: total mass ratio (R:TM)	0.24 ± 0.01 b	0.25 ± 0.02 b	0.42 ± 0.01 a	0.42 ± 0.02 a	0.47 ± 0.03 a
Leaf:total mass ratio (L:TM)	0.55 ± 0.01a	0.53 ± 0.02 a	0.38 ± 0.02 b	0.39 ± 0.02 b	0.39 ± 0.04 b
RI	3.3 ± 0.01 b	3.3 ± 0.14 b	3.96 ± 0.08 ab	3.98 ± 0.17 a	4.43 ± 0.16 a
DQI	4.26 ± 0.15 a	3.84 ± 0.22 a	1.51 ± 0.12 b	1.35 ± 0.18 b	1.42 ± 0.16 b

and second by the tumbling effect, which can result in death or morphological deformations that compromise the silviculture value. Niklas and Speck (2001) point out that the critical tree height, the one above which commits its support, is generally proportional to its diameter.

Containers with larger soil volume support not only the development in roots length, but they are also associated to a better distribution of root system (SCHWENGBER et al. 2002). Furthermore, Bao et al. (2014) observed that eudicot species developed mechanisms to interpret and respond to changes in the microscale differences in the environment and that the establishment of root architecture is associated with the distribution of water near the root tip. This pattern of root development dependent on water availability is particularly important when interpreting the responses of plants in natural soils or, in the case of this study, the soil volume present in the containers with different sizes.

The length and diameter of the main root in *Parapiptadenia rigida* and *Inga marginata* were higher in plastic bags, with lower values compared to the tubes, a fact explained by the smaller dimensions of the tubes, both in relation to the volume of substrate to root development. Thus it, is noteworthy that containers with less depth for rooting may restrict the main root elongation, then limiting shoot growth. However, when the different tubes sizes were compared, we found an inverse relationship between the soil volume and root growth, since plants growing in T55 had higher root length and higher number of lateral roots compared to tubes with larger soil volume. Similar results were observed by Domingues-Lerena et al. (2006) in *Pinus pinea* seedlings. The largest root growth and lateral roots formation in tubes with smaller dimension may be associated with the mechanical effect imposed by the side walls from tubes causing greater growth in length and higher number of roots, which rapidly explore the depth and volume available. Falik et al. (2005) found that roots tend to avoid physical obstacles such as rocks or roots or nearby, in the case of this study, the walls of containers, changing its growth direction to a region without physical impediment.

The main root works as a stake that penetrates the soil and sustains the shoot, and its role in anchoring is more significant than the lateral roots. However, when the main root is unable to stretch, compensation occurs in the lateral roots (DANJON et al. 2005; KHUDER et al. 2007). Thus, the lateral roots do not play only the role of absorption of nutrients, but may also assist in the process of the plant in the soil support (DANJON et al. 2005; DUPUY et al. 2005; KHUDER et al. 2007).

In addition to the effects on seedlings growth, it was observed that plants growing in larger soil volume also showed higher shoot and roots mass accumulation. A drastic reduction in dry mass accumulation was observed when plastic bags were compared to tubes. However, these differences become less pronounced or even absent when the plastic bags and tubes were compared to each other, indicating that the changes in the volume seem to exert greater influence than differences in diameter and depth of containers, since plastic bags and tubes feature a minor variation in these parameters than in relation to the volume, similarly to Korndörfer et al. (2008).

Seedlings growing in containers with smaller volumes (T55) presented a root: shoot ratio significantly higher compared to the other tubes (T100 and T175) and plastic bags (PBS and PBL). The allocation of resources to the roots or to shoots is considered a key factor in the plant strategy for the acquisition of water (LEYVA; FERNÁNDEZ, 1998), which is extremely important to survive and develop in field conditions (SOUTH, 2000). The relationship between root and shoot (R:S ratio) is one of the parameters largely used to determine the stability of forest seedlings.

Ferraz and Engel (2011) observed that very low R:S ratios may compromise the establishment of seedlings into the field, occasionally occurring tipping due to a poorly developed root system against a prominent shoots. In this study, the values observed in different containers were lower than those observed by Chirino et al. (2008) suggesting that these plants showed an excessive investment in shoot growth at the expense of the root system, which could adversely affect their initial establishment under field conditions. On the other hand, *Inga marginata* plants showed values close to 1, especially in the smaller tubes, leading to a better proportional rate when compared to *Parapiptadenia rigida*.

Similar to the results observed in plants growing T55, Figueiredo et al. (2014) observed that root deformations in clonal seedlings of hybrid *Eucalyptus grandis* x *Eucalyptus urophylla* grown in tubes of 53 cm^3 caused a reduction in the root system hydraulic conductivity and a decrease in the photosynthetic rate, indicating that the physical deformations caused by the cultivation container can compromise the plant water status.

Given that the effects of soil volume and container size on plant growth and development cause direct consequences on the acclimatization of these plants into the field, it is extremely important to define parameters that can indicate the most suitable containers for seedling production. Different indexes are useful to evaluate seedlings morphophysiological quality as the ratio of shoot height and stem diameter (H: Ds), the robustness index (RI) and Dickson quality index (DQI). Luca et al. (2010) suggested that the RI would be the most suitable parameter for the choice of container cultivation of *Cedrela fissilis* (Meliaceae) seedlings. However, the RI considers only the relationship between stem diameters and shoots height, with no information on mass allocation patterns, which therefore does not allow one to precisely identify the morphological features from plants.

Sturion and Antunes (2000) define the H:Ds as one of the main parameters to assess forest seedlings quality because it reflects both reserves accumulation and also ensures greater strength and better grip into the soil. The highest values for the H: Ds ratio and DQI were observed in plastic bags followed by tubes, despite minor variations in *I. marginata* plants, mainly between three tube sizes and the two bag sizes. In this study, both species showed a similar pattern to the DQI and the H:Ds ratio in response to variation in soil volume. In contrast, when the RI was assessed, an inverse correlation between the soil volume and this index was observed to both species.

Our results suggest that recipients with higher soil volume, in the case of this study, plastic bags are best suited for producing large seedlings. However, when analyzing the relationship between investment in aboveground and belowground mass, tubes with 175 and 100 cm^3 emerge as an interesting alternative for seedlings production, with morphological stability to acclimatization in natural conditions. Furthermore, when variations in growth parameters among different soil volume were evaluated by a principal component analysis (PCA) showed that for both species DQI and R: S ratio were the indices that best explained the observed differences in growth patterns in response to change in soil volume and containers size. So, our results indicate that the Dickson quality index (DQI) and, mainly, root biomass and the root: shoot ratio (R:S) provide distinct evaluations of morphological features and thus they can be useful to characterize the initial seedlings growth in containers or environments with differences in size, but especially on the soil volume.

Conclusions

The results of this study showed the relationship between plant architecture, especially the relationship between growth of shoots and roots and the soil volume, through an experiment growing seedlings in containers of different sizes. Seedlings growing in containers with larger volume showed a higher shoot height. However, when investing in root biomass and root length was analyzed, this trend was not observed, because containers with smaller volume had increased root development. The root: shoot as the, by principal component analysis, seems to be an important parameter to select the seedlings and especially the cultivation containers, to be used in the initial growth of plants intended for forest restoration programs in environments varying the soil characteristics.

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