Inoculum density of arbuscular mycorrhizal fungi needed to promote growth of *Hancornia speciosa* Gomes seedlings

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Abstract — Introduction. Arbuscular Mycorrhizal Fungi (AMF) can promote host growth and, among other benefits, alleviate the stress produced by transplanting seedlings from the nursery to the field. The objective of this work was to evaluate the effect of the amount of AMF inoculum on growth of *Hancornia speciosa* (Mangaba tree). **Materials and methods**. A greenhouse experiment was performed using a randomized experimental design in a factorial arrangement of 3 × 3 corresponding to: two AMF (*Gigaspora albida* Schenck & Smith and *Glomus etunicatum* Becker & Gerdeman) treatments plus one control with plants without AMF × three inoculum densities [(50, 100 and 300) spores per plant], with five replicates, in a methyl bromide sterilized soil. **Results**. After 120 d, seedlings associated with *G. albida* had greater height, shoot diameter and dry biomass of the aerial part and leaf area than those associated with *G. tunicatum*, independent of inoculum density. *G. etunicatum* did not influence growth of *H. speciosa*. Positive correlations occurred between plant growth parameters and root colonization promoted by *G. albida*. Regression analysis showed interaction between the inoculum density of *G. albida* and plant growth parameters. **Discussion and conclusion**. Increments in growth of *H. speciosa* can be obtained with inoculation of approximately 180 spores of *G. albida* per plant, which could reduce the necessary period of time for the transplanting of seedlings from the nursery to the field.

Brazil / *Hancornia speciosa* / fruit trees / arbuscular mycorrhizae / *Gigaspora albida* / *Glomus etunicatum* / growth

Densité d'inoculum de champignons mycorhiziens à arbuscules nécessaires pour favoriser la croissance de plantules de *Hancornia speciosa* Gomes.

Résumé — Introduction. Les champignons mycorhiziens à arbuscules (CMA) peuvent favoriser la croissance de la plante hôte et, entre autres avantages, atténuer le stress consécutif à la transplantation de la plantule de la pépinière au champ. L'objectif de nos travaux a été d'évaluer l'effet de la quantité d'inoculum de CMA sur la croissance de *Hancornia speciosa* (caoutchouc de Pernambouc). **Matériel et méthodes**. Une expérimentation a été effectuée en serre sur des germinations de *H. speciosa* selon un dispositif factoriel en randomisation totale de 3 × 3 facteurs: deux espèces de CMA (*Gigaspora albida* Schenck & Smith et *Glomus etunicatum* Becker & Gerdeman) et un traitement témoin sans CMA, et trois densités d'inoculum de [(50, 100 et 300) spores par plants], avec cinq réplications, sur un sol stérilisé au bromure de méthyle. **Résultats**. Après 120 jours, les plantules associées à *G. albida* ont eu une taille, un diamètre de tige, une biomasse sèche de la partie aérienne et une surface de feuille améliorés par rapport aux plants traités avec *G. etunicatum*. *G. etunicatum* n'a pas influencé la croissance de *H. speciosa*. Des corrélations positives ont été observées entre les paramètres de croissance des plantes et la colonisation des racines par *G. albida*. L'analyse de régression a révélé une interaction entre la densité d'inoculum de *G. albida* et les paramètres de croissance de plantes. **Discussion et conclusion**. La croissance de *H. speciosa* a pu être améliorée par une densité d'inoculum d'environ 180 spores de *G. albida* par plante, ce qui pourrait permettre de raccourcir la période de culture en pépinière.

Brésil / *Hancornia speciosa* / arbre fruitier / mycorhize à arbuscule / *Gigaspora albida* / *Glomus etunicatum* / croissance

1. Introduction

Hancornia speciosa Gomes, or Mangaba tree in English and Mangabeira in Brazilian, is one of the 29 species of Apocynaceae present in the Brazilian *cerrado*, and the only one producing edible, tasty and high proteic fruits. It is a native species from Brazil, growing spontaneously in the coastal areas with *restinga* vegetation, which are also called *tabuleiros* in the Northeast. The States of Sergipe and Minas Gerais are the most important producers in the Northeast and Southeast regions [1, 2].

The fruits are used in the ice cream and juice industry. However, harvesting mainly concerns native plants, while planting is mostly conducted in small-scale exploitations. Investments directed to native plants in Brazil would benefit the country, increasing the participation in the internal and external market. Production is still small due to poor knowledge related to growth, management and cultural practices, which makes the commercial production of *H. speciosa* restricted to small producers [3].

The use of arbuscular mycorrhizal fungi (AMF) may constitute an alternative for efficient production of seedlings, with great potential for success, considering that the AMF can decrease the need for fertilizers and anticipate the time for field transplantation, with the production of more vigorous seedlings which are also more capable of withstanding the stress caused by transplantation [4]. Environmental factors have a strong effect on the colonization dynamic [5]. Besides that, the plant and fungus genome, as well as the type and density of propagules can interfere with the level of colonization, which originates different responses regarding mycorrhization and plant growth [6, 7]. Considering that the AMF are not produced in artificial medium, they are usually multiplied in susceptible hosts and applied as spore suspension or soil inoculum containing spores and root fragments [8]. A good inoculum would be that efficient to promote growth even when applied in small amounts. An increase in shoot dry mass was observed in strawberry (*Fragaria* sp.), associated with only 20 spores of *Gigaspora margarita* Becker & Hall per plant [9]. A similar response was observed in coffee (*Coffea arabica* L.), when 100 AMF spores were inoculated per plant [7]. In an experiment with passion fruit, three inoculum levels [(200, 300, and 400) spores per plant of *Gigaspora albida* Schenck & Smith, *G. margarita*, and *Glomus etunicatum* Becker & Gerdeman] were tested; the values of shoot dry mass and leaf area were reached in the treatments with 300 spores per plant [10].

Plant growth promotion induced by AMF has been evaluated for diverse fruit plants, such as citrus [11, 12], passion fruit [10], coffee [7], Barbados cherry (*Malpighia emarginata* D.C.) [13] and banana [14]. However, no information is available concerning the potential benefits of AMF for *H. speciosa,* even though improvement of seedling growth is an important aspect that should be considered to reduce the period in the nursery and to guarantee the establishment of plants in the field.

The objective of this research was to evaluate the effect of a minimal amount of AMF fungal propagules required to promote growth of *H. speciosa* seedlings.

2. Materials and methods

A greenhouse experiment was performed using a Red Yellow Podsoil [15], sterilized with Bromex (98% methyl bromide + 2% chloropicrin) 30 d before planting. The soil presented the following characteristics: 4,7 mg $P \cdot dm^{-3}$ of soil; (0.3, 0.046, 10.4 and 0.016) $mmol_c \cdot dm^{-3}$ of soil, of Al, Na, (Ca + Mg) and K, respectively; pH 5.24; 20.4 $g \cdot dm^{-3}$ of organic matter. Values of temperature and humidity were taken every day (TFA thermohydrometer, Germany) and varied from (23 to 32) °C and from (50 to 80)% during the experiment.

Spores of *Gigaspora albida* Schenck & Smith (UFPE 02) and *Glomus etunicatum* Becker & Gerdemann (UFPE 06), multiplied in Bahia grass (*Paspalum notatum* Flügge), were used after extraction from soil by wet sieving [16] and sucrose centrifugation [17].

Table I.
Effect of inoculation with arbuscular mycorrhizal fungi on plant growth parameters and respective increment of *Hancornia speciosa*, independent of inoculum density.

Arbuscular mycorrhizal fungi	Height (cm)		Increment (%)	Shoot diameter (mm)		Increment (%)	Shoot dry biomass (g)	Increment (%)	Leaf area (cm^2)	Increment (%)
	90 d	120 d	120 d	90 d	120 d	120 d	120 d	120 d	120 d	120 d
Gigaspora albida	23.23 a	31.15 a	102.27	0.20 a	0.24 a	26.31	1.06 a	158.53	134.31 a	259.98
Glomus etunicatum	15.47 b	17.15 b	11.36	0.19 b	0.19 b	0.00	0.42 b	2.43	46.38 b	24.30
Control	14.20 b	15.40 b	–	0.19 b	0.19 b	–	0.41 b	–	37.31 c	–

Data followed by the same letter in a column do not differ by the Tukey test at 5% probability.

A greenhouse experiment was performed using a randomized experimental design in a factorial arrangement of 3 × 3 corresponding to: 3 AMF treatments (*Gigaspora albida* and *Glomus etunicatum* plus a control) × 3 inoculum densities [(50, 100 and 300 spores) per plant], with 5 replicates.

The seeds of *H. speciosa* originated from a commercial plantation located in Sirinhaem, on the south coast of the State of Pernambuco, Northeast Brazil (8° 35' 30" S, 35° 07' 00" W). Seeds were taken from the fruits, separated from the pulp using dolomitic lime, and sown on cell platters with sterilized soil. Germination occurred 30 d after planting. Seedlings with two pairs of true leaves were transferred to plastic pots with 200 g of soil and inoculated with arbuscular mycorrhizal fungi. The seedlings were watered daily and after 15 d were transferred to containers with 3.5 kg of soil and watered every other day.

Measurements of plant height and shoot diameter (3 cm above ground level) were taken every 30 d. After 120 d, the shoot dry biomass, leaf area, root colonization and number of AMF spores in the rhizosphere were evaluated. Leaf area was evaluated using the SIARCS 3.0 program (EMBRAPA).

The increment in relation to the non-inoculated control was calculated using a modified model from Edginton *et al.* [18]: I (%) = [(Tr – T) / T] × 100, where I (%) = increment of the variable; Tr, average value of the treatment; and T, average value of the control.

Roots were clarified with 10% KOH, stained with Trypan blue [19], cut into 1-cm segments (100 segments per sample), and observed with a light microscope for evaluation of AMF colonization [20].

Data were submitted to analysis of variance using the SANEST program [21] and the averages were compared by the Tukey test at 5% significance. Single correlations were obtained in the NTIA program (EMBRAPA) according to the Karl Peterson Model [22].

3. Results and discussion

Seedlings associated with *G. albida* presented greater height, shoot diameter, shoot dry biomass and leaf area than those inoculated with *G. etunicatum*, which did not benefit plant growth (*table I*). Ninety days after inoculation these seedlings were high enough for transplanting to the field, considering that, without inoculation, it usually takes 120 d for seedlings to reach the transplanting stage, i.e., 15–20 cm in height [3]. Thus, AMF inoculation reduced by 30 d the period of maintenance of the seedlings in the nursery. Costa *et al.* [13] observed that inoculation with *G. margarita* and *G. etunicatum* reduced by 30 d the

time needed for production of seedlings of Barbados cherry - Miró genotype, but only *G. margarita* was efficient for improving seedling development of the Barbados genotype. Similar results were obtained with yellow passion fruit seedlings [10] which presented higher growth than the control when inoculated with the same AMF used in our work. Seedlings of cherimoya fruit (*Annona muricata* L.) also benefited from inoculation with *G. margarita* [23]. In citrus rootstocks, *G. etunicatum* was more efficient than other AMF for promoting plant growth, independent of the type of inoculum and of the rootstocks [6]. Silva and Siqueira [24] did not obtain any effect of inoculation with *G. etunicatum* in lemon Cravo, but Souza *et al.* [25] observed higher shoot diameter in citrus plants inoculated with *Glomus intraradices* Schenck & Smith. Similar results in improvement of biomass were observed in guava tree (*Psidium guajava* L.) inoculated with *Gigaspora margarita* and *Glomus clarum* [26] and in Barbados cherry - Barbados genotype [13].

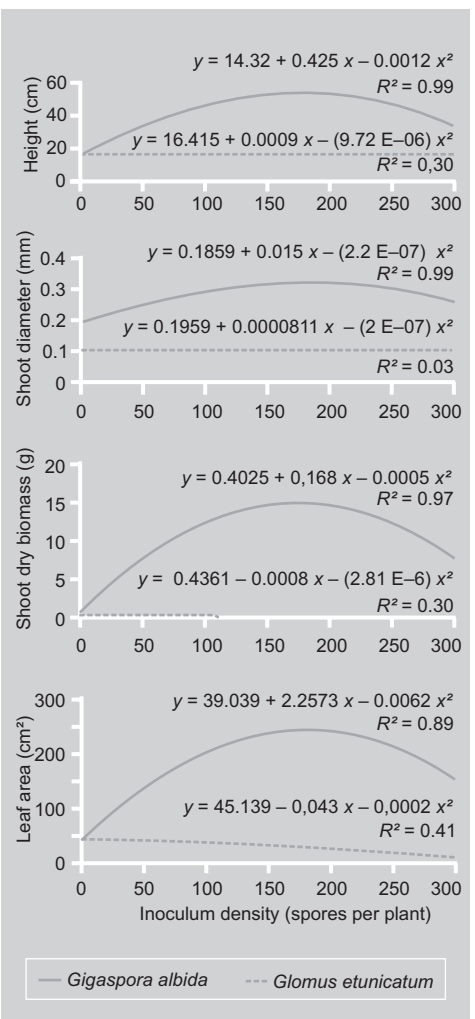

Figure 1.
Effect of inoculum density of *Gigaspora albida* and *Glomus etunicatum* on plant growth parameters of *Hancornia speciosa* seedlings, 120 d after inoculation.

The leaf area of seedlings inoculated with *G. albida* differed statistically from that associated with *G. etunicatum,* which did not differ from the control (*table I*). Seedlings of Barbados cherry - Miró genotype also benefited from inoculation with *G. margarita* [13]. In studies on *H. speciosa* inoculated with native AMF, the values of the leaf area, 12 months after planting, were 50% lower [27] than those obtained here after 120 d in seedlings associated with *G. albida*.

There was significant interaction, with square regression, between inoculation with *G. albida* and inoculum density for height, shoot diameter, shoot dry biomass and leaf area (*figure 1*), with approximately 180 spores of *G. albida* per plant being needed to promote growth of *H. speciosa*. The benefit of the association was not proportional to the increase in spore density, which was also observed in coffee seedlings [7] and indicated that, in our study conditions, addition of more than 180 spores per plant is not necessary for growth promotion. To improve the height of Barbados cherry (Barbados genotype), inoculation with 200 spores of *G. margarita* per plant is enough, while for the Miró genotype, using the same inoculum density, both *G. etunicatum* and *G. margarita,* are efficient at promoting plant growth [13]. In yellow passion fruit, growth was improved with inoculation of 300 spores of AMF per plant [10]. On the other hand, the inoculation of 500 spores of *G. etunicatum* was not enough to promote plant growth of citrus rootstocks [11].

Greater shoot diameter and production of shoot dry biomass were obtained with inoculation of approximately 175 spores per plant (*figure 1*). For yellow passion fruit, densities of (200 to 400) spores per plant did not improve shoot diameter, while, for *G. etunicatum*, *G. albida* and

Table II.
Increment (%) in shoot dry biomass, height, shoot diameter and leaf area, in relation to control, of *Hancornia speciosa* seedlings, 120 days after inoculation with different spore densities of *Gigaspora albida*.

Inoculum density (spore per plant)	Shoot dry biomass (g)	Height (cm)	Shoot diameter (mm)	Leaf area (cm^2)
50	246.62	146.90	33.33	551.81
100	330.33	219.44	61.11	601.10
300	214.60	140.78	38.38	503.30

Table III.
Correlation coefficient (r^2) between plant growth parameters of *Hancornia speciosa* and those related to arbuscular mycorrhizal fungi (AMF), independent of the spore density, 120 d after inoculation of seedlings.

Plant growth parameter	*Gigaspora albida*	*Glomus etunicatum*
Shoot diameter × root colonization	0.79 **	0.55 *
Height × number of AMF spores	0.73 **	– 0.18 ^{ns}
Height × root colonization	0.86 **	0.63 **
Leaf area × number of AMF spores	0.66 **	– 0.14 ^{ns}
Leaf area × root colonization	0.82 **	0.75 **
Number of AMF spores × shoot dry biomass	0.73 **	– 0.32 ^{ns}
Root colonization × shoot dry biomass	0.86 **	0.55 *
Number of AMF spores × root colonization	0.69 **	– 0.11 ^{ns}

ns: not significant; * significant at $0.01 < p < 0.05$; ** significant at $p < 0.01$.

G. margarita, 300 spores per plant were indicated for improving dry biomass of the aerial part [10]. In soybean, positive growth responses were obtained only with inoculation of 1000 spores of *G. macrocarpus* per plant [28].

Maximum leaf growth was obtained with inoculation of 180 spores of *G. albida* per plant (*figure 1*). In Barbados cherry (Barbados genotype), 200 AMF spores per plant were needed to increase leaf growth [13]. The benefit of mycorrhizal association in the leaf area of citrus occurred only with inoculation of 500 spores per plant of *G. etunicatum* [11]; the same was observed in associations with *G. intraradices* [25, 29].

Higher increments, especially in shoot dry biomass and leaf area, were obtained when the seedlings were inoculated with 100 spores of *G. albida* per plant (*table II*). Similar results were reported for yellow passion fruit [10] and Barbados cherry [13] inoculated with *Gigaspora* spp. Some reports have shown that this growth increment varies according to plant and fungus genotypes [13, 30, 31].

The degree of correlation indicates when characteristics of the plant and fungus are dependent, those characteristics that are not correlated being completely independent [32]. Correlation between root colonization and AMF spore density are not dependent, although might be associated due to the symbionts or environmental conditions [33]. Positive correlation between plant and fungus growth parameters were observed mostly with *G. albida* and also with *G. etunicatum* (*table III*).

Strong significant correlation occurred ($p < 0.01$) between plant growth parameters and root colonization by *G. albida*. Similar results were obtained in *Euterpe oleraceae* Mart. independently of the AMF associated [31], but the same correlation did not occur in the interaction between Barbados cherry - Barbados genotype and *G. etunicatum* [13], nor in grapes [34].

In all growth parameters studied, plants associated with *G. albida* presented higher development, which suggests that, even though the AMF are not host-specific, "host preferences" do exist, considering that the efficiency of the association is genetically controlled by both symbionts. Besides that, it seems that *G. albida* colonizes *H. speciosa* roots faster than *G. etunicatum*; this fungus probably needs a longer period of time, as observed on yellow passion fruit [10], or higher density of inoculum for establishing the symbiosis; root colonization of *H. speciosa* (3.49%) was observed only after inoculation with 300 spores (data not shown).

4. Conclusion

The association of *Hancornia speciosa* with *Gigaspora albida* promoted greater plant development than that with *Glomus etunicatum*, which was not different from a non-inoculated control. This mycorrhizal association resulted in a reduction of 30 d [from (120 to 90) d] in the time for seedling development in the greenhouse. Optimum inoculation was estimated to be with 180 spores of *G. albida* per plant. Thus, association of *H. speciosa* with *G. albida* constitutes a viable alternative for production of seedlings in a shorter period of time.

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Densidad de inóculo de hongos micorrícicos arbusculares necesaria para favorecer el crecimiento de plántulas de *Hancornia speciosa* Gomes.

Resumen — Introducción. Los hongos micorrícicos arbusculares (HMA) pueden favorecer el crecimiento de la planta hospedadora y, entre otras ventajas, disminuir el estrés derivado del trasplante de la plántula del vivero al campo. El objetivo de nuestro trabajo consistió en evaluar el efecto de la cantidad de inóculo de HMA en el crecimiento de *Hancornia speciosa* (mangaba). **Material y métodos**. Se efectuó un experimento en invernadero sobre germinaciones de *H. speciosa* con un diseño factorial al azar de 3 × 3 factores: dos especies de HMA (*Gigaspora albida* Schenck y Smith y *Glomus etunicatum* Becker y Gerdeman) y un tratamiento testigo sin HMA y tres densidades de inóculo de [(50, 100 y 300) esporas por planta] con cinco repeticiones en un suelo esterilizado con bromuro de metilo. **Resultados**. Tras 120 días, las plántulas asociadas a *G. albida* presentaban un tamaño, un diámetro de tallo, una biomasa seca de la parte aérea y una superficie foliar mejores que las plantas tratadas con *G. etunicatum*. *G. etunicatum* no influyo en el crecimiento de *H. speciosa*. Se observaron correlaciones positivas entre los parámetros de crecimiento de las plantas y la colonización de las raíces por *G. albida*. El análisis de regresión reveló una interacción entre la densidad de inóculo de *G. albida* y los parámetros de crecimiento de las plantas. **Discusión y conclusión**. Se pudo mejorar el crecimiento de *H. especiosa* con una densidad de aproximadamente 180 esporas de *G. albida* por planta, esto podría permitir la reducción del período de cultivo en vivero.

Brasil / *Hancornia speciosa* / árboles frutales / micorrizas arbusculares / *Gigaspora albida* / *Glomus etunicatum* / crecimiento