



## Growth and Nutrient Acquisition of Peach Plants CV. Maciel Grafted on CV. Okinawa Inoculated with Arbuscular Mycorrhizal Fungi under Polyhouse Conditions

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### Abstract

The study aimed to analyze the influence of arbuscular mycorrhizal fungi (AMF) on the acquisition of macronutrients, formation of reserve substances and vegetative growth of peach plants cv. Maciel grafted onto rootstocks of cv. Okinawa. The experiment lasted 240 days after grafting, when height (cm), diameter (mm), leaf area (cm<sup>2</sup>/plant), fresh and dry biomass (g), macronutrient content (% of plant) and reserve substances (% of plant) were evaluated. The experimental design was randomized block, consisting of 4 blocks with 4 plots per block, representative of each AMF treatment in each block, and 15 plants per plot, conducted in a controlled-environment polyhouse. The results obtained were subjected to analysis of variance using the SAS program and compared using the Duncan test, at a significance level of 5%. Correlations were analyzed using Pearson's correlation coefficient ( $r = 1$  and  $r = -1$ ). The plants were inoculated with the species *Acaulospora* sp., *Glomus clarum* and *G. etunicatum* and compared with non-inoculated control plants. All plants inoculated with AMF showed higher growth parameters, when compared with the control, with *Acaulospora* sp. promoting the best responses, while *G. clarum* and *G. etunicatum* induced superior growth than the control. Inoculated plants also showed greater absorption of nitrogen, phosphorus and potassium and greater formation of reserves, when compared with the control. The use of AMF from vineyards benefits plants of the Maciel cultivar grafted onto the Okinawa cultivar, accelerating their vegetative development and improving their nutritional status, especially when inoculated with the species *Acaulospora* sp.



### Article History

Received: 27 March 2025  
Accepted: 26 May 2025

### Keywords

*Acaulospora* Sp.;  
*Glomus Clarum*;  
*Glomus Etunicatum*;  
Maciel;  
Okinawa.

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Doi: <http://dx.doi.org/10.12944/CARJ.13.2.7>

### Abbreviations

AMF :	Arbuscular mycorrhizal fungi
Cv :	Cultivar
EEA :	Agronomic Experimental Station
SAS :	Statistical Analysis System
TCA :	Trichloroacetic acid
UFRGS :	Federal University of Rio Grande do Sul

### Introduction

The efficiency of arbuscular mycorrhizal fungi (AMF) is represented by their ability to stimulate plant growth and depends on the genetics and soil and climate factors involving both agents.<sup>1</sup> The AMF are obligate symbionts and colonized plants tend to have a greater capacity for absorption of macronutrients (nitrogen, phosphorus and potassium) and several micronutrients<sup>1,2</sup> apart from improved vegetative growth, when compared with non-colonized plants.

Fruit trees are naturally colonized, both in the field (late infection) and in nurseries (early infection). In this context, peach trees have been reported to respond well to colonization by AMF.<sup>3</sup> Similarly, hybrid between peach and almond plants (*Prunus persica* × *Prunus dulcis*), when transferred to the field, showed higher colonization, with excellent developmental responses in terms of nutrient acquisition and vegetative growth,<sup>4</sup> similar to the responses obtained with apple seedlings inoculated in nurseries, in the substrate.<sup>5</sup> Several reports<sup>6,7</sup> have shown that inoculated plants benefited from symbiosis, when transplanted to the field in areas with nutrient deficiencies or when subjected to prolonged drought. Other authors<sup>8,9,10</sup> observed that plants inoculated with AMF during seedling formation showed tolerance to salinity and resistance to attacks by soil-borne pests and diseases.

Knowledge about the benefits that inoculation with AMF provides in the preparation of peach tree seedlings and their dependence on this symbiosis is limited.

Few studies have addressed the nematode resistance capacity that AMF can confer on peach rootstocks. However, there seems to be a greater capacity of AMF to colonize and provide benefits to plants of the genus *Prunus* due to the cultivars themselves and not to the species itself.<sup>11</sup> Therefore, the ability of AMF to provide benefits to peach plants

should be investigated more specifically, even if there is evidence to suggest this.<sup>12</sup> This is because the conditions under which the association between AMF and plants is established, especially in relation to the correct type of AMF to be used, appear to be crucial for getting optimum benefits in plant development, nutritional status, tolerance to salinity, drought, soil borne diseases and nematodes.<sup>13</sup>

This study aimed to evaluate the influence of AMF species from vineyards on nutritional status and vegetative development of peach plants cv. Maciel grafted onto cv. Okinawa.

### Material and Methods

#### Study Site and Polyhouse Conditions

This study was conducted in a polyhouse with a modified irrigation system for forced sprinklers, located at EEA - UFRGS, municipality of Eldorado do Sul, State of Rio Grande do Sul, Brazil.

#### Plant Material, Substrate Preparation and AMF Inoculation

In order to break dormancy, peach seeds were stratified in sterilized sand (autoclaved at 120 °C for one hour) and placed in a refrigerator at 4 °C for a period of forty-five days. After this period, they were sown in the polyhouse, in soil composed of disinfected sand.

When the peach seedlings had an epicotyl measuring ca. 5 cm long, they were transplanted into 5-liter bags containing substrate i.e. dystrophic Red Argisol soil, 14 sand with an average grain size between 0.6 and 1.0 mm, and decomposed black acacia bark residue (1:1:1, v:v:v). The substrate was disinfested with a 10% v/v of formaldehyde solution and sieved through a 5-mm mesh prior to use.

Each polyethylene bag was inoculated immediately before transplanting the seedlings by adding 30 g of rhizosphere soil (soil, roots, and AMF structures)

after harvesting an oregano crop (*Origanum vulgare* Link), a plant used as a multiplier for AMF species, which was sown in a soil composed of clayey soil: sand (1:1, v:v) and harvested 90 days after sowing.

The polyethylene bags were filled to half their height with substrate, followed by the addition of a layer of AMF inoculum, and finally the bag was filled to the brim with substrate. Immediately afterwards, the rootstocks were transplanted from the sand bed into their respective polyethylene bags in order to conduct the studies described below.

### Experimental Design and Treatments

The statistical design used was randomized block design, consisting of 4 blocks with 4 plots per block, representative of each AMF treatment in each block, and 15 plants per plot, totaling 240 plants. After inoculation of AMF, the seedlings of the Okinawa rootstock were grafted with the Maciel scion cultivar, using the inverted "T" bud grafting method, at a height of 10 cm above the soil. The experiment lasted 240 days. Four treatments were tested, three with inoculation of AMF (*Acaulospora* sp., *Glomus clarum* and *G. etunicatum*) and one non-inoculated control treatment.

### Data Collection and Measurements

After grafting, the height from the grafting point to the apex of the main stem of peach plants was assessed monthly using a Cosmo 25 digital laser tape measure, and the stem diameter, just after the grafting point, was assessed using an MTX digital caliper. Five plants per replicate were also collected to determine the leaf area using a Li-Cor LI-3000

meter, and the fresh and dry biomass of leaves and stems by weighing and drying in an oven (65°C) until constant weight. After drying, the samples were ground and used to determine macronutrients by digestion, distillation and flame spectrometry of plant tissue.<sup>15</sup> In addition, in this process, one gram of each sample passed through a special sieve for filtering food (an instrument used to separate solid particles from a liquid or to sift dry ingredients), with pre- and post-digestion records in an aqueous solution of 5% v/v of trichloroacetic acid - TCA (99%) and 35% v/v of methanol (99%), aiming to extract the components of the plant tissue (carbohydrates, fats, etc.), except fibers (cellulose, hemicellulose and lignin) to determine the percentage of reserve substances.<sup>16</sup>

Secondary root segments were collected to determine root colonization of peach plants, through microscopy techniques, with the visualization of arbuscules and vesicles in the roots and application of the ratio between the number of infected segments to the total analyzed and by the presence indexes of colonization structures such as hyphae (0: absence; 1: weak; 2: moderate; 3: intense), vesicles and arbuscules (0: absence; 1: 1 to 50; 2: 51 to 100; 3: more than 100 structures/cm of rootlet).<sup>17</sup>

### Statistical Analysis

The results obtained were subjected to analysis of variance using the SAS program and compared using the Duncan's test,<sup>18</sup> at a significance level of 5%. Correlations were analyzed using Pearson's correlation coefficient ( $r = 1$  and  $r = -1$ ).

**Table1: Development parameters of leaves, branches and stems of peach plants inoculated with AMF (*Acaulospora* sp., *G. clarum* and *G. etunicatum*) and without inoculation (Control), collected at 240 days.**

Control	Height cm	Diameter mm	Leaf area cm <sup>2</sup> /plant	Fresh biomass g	Dry biomass g
<i>Acaulospora</i> sp.	90.33a	5.80a	745.95a	201.50a	121.00a
<i>G. clarum</i>	70.44c	4.49c	602.31c	171.25b	88.25b
<i>G. etunicatum</i>	78.05b	5.11b	660.91b	171.75b	88.50b
Testemunha	65.35d	4.00d	521.82d	132.75c	76.00c
C.V. (%)	7.74	6.52	11.75	8.54	8.04

The same letter in the means indicates equality at the 5% significance level by Duncan's test.

**Results**

*Acaulospora* sp. provided greater increase in all the parameters evaluated, while *G. etunicatum* was superior to *G. clarum* in the parameters such as height, diameter and leaf area. All the inoculated treatments were statistically superior to the control (Table 1).

*Acaulospora* sp. provided the best results for nitrogen and potassium, with the other species being statistically similar to each other and superior over the control. For phosphorus, *G. etunicatum*

provided the best results, while the other AMF were statistically similar to each other and superior to the uninoculated control. For calcium and magnesium, the control plants exhibited the highest values, superior to the treatments inoculated with AMF, which presented similar behavior. It was also found that the plants inoculated with *Acaulospora* sp. presented the highest levels of reserved substances (carbohydrates, fats, fatty acids, etc.) in the leaves and stems, while the other species were statistically similar to each other and superior over the control (Table 2).

**Table 2: Macronutrients and reserved substances present in the leaves and stems of peach plants inoculated with three species of AMF (*Acaulospora* sp., *G. clarum* and *G. etunicatum*) and without inoculation (Control), collected at 240 days.**

Control	N	P	K	Ca	Mg	Reserved substances
	% of plant					
<i>Acaulospora</i> sp.	3.46a	0.19b	2.85a	1.37b	0.49b	41.02a
<i>G. clarum</i>	3.01b	0.19b	2.56b	1.44b	0.48b	32.91b
<i>G. etunicatum</i>	3.05b	0.21a	2.53b	1.49b	0.48b	36.69b
Testemunha	2.71c	0.16c	2.35c	1.74a	0.62a	28.00c
C.V.(%)	7.35	4.56	6.82	9.31	5.71	6.44

The same letter in the means indicates equality at the 5% significance level by Duncan's test.

**Table 3: Percentage of colonization, number of spores in the substrates and presence of colonization structures in the roots (hyphae, vesicles and arbuscules<sup>17</sup>) in peach plants inoculated with three species of AMF (*Acaulospora* sp., *G. clarum* and *G. etunicatum*) and without inoculation (Control), collected at the time of transplantation to the field.**

Control	Colonization	Spores	Presence of AMF structures		
	(%)	(n° middle/ 100g dry soil)	Hyphae <sup>1</sup>	Vesicles <sup>2</sup>	Arbuscule <sup>2</sup>
<i>Acaulospora</i> sp.	98.25a	228.00a	2.19a	1.92a	2.12a
<i>G. clarum</i>	93.13b	171.00b	1.66c	1.57b	1.72b
<i>G. etunicatum</i>	97.00a	211.00a	1.92b	1.86a	2.08a
Testemunha	0.00d	0.00d	0.00d	0.00d	0.00d
C.V. (%)	13.59	12.75	6.51	6.47	6.67

Plants inoculated with *Acaulospora* sp. and *G. etunicatum* showed high colonization percentages, always above 90%, similar to each other and higher than the other treatments. The same occurred with the average number of spores present in the

substrate. Regarding the presence of colonization structures, all treatments presented colonization rates with hyphae, vesicles and arbuscules that were considered average. For hyphae, the treatment with *Acaulospora* sp. was superior to *G. etunicatum*

and this one to *G. clarum*, while, for vesicles and arbuscules, the treatments with *Acaulospora* sp. and *G. etunicatum* presented the highest results, superior to *G. clarum* (Table 3).

The same letter in the means indicates equality at the 5% significance level by Duncan's test. 1 Presence of hyphae - 0: absence of structures; 1: weak presence; 2: moderate presence; 3: intense presence. 2 Presence of vesicles or arbuscules - 0:

absence of structures; 1: 1 to 50 structures; 2: 51 to 100 structures; 3: more than 100 structures per centimeter of radicle.

When evaluating the degree of association between the various parameters of plant growth and mineral nutrition and the percentage of root colonization by AMF, it was found that there was a positive or negative correlation between them, regardless of the species of AMF used (Table 4).

**Table 4: Correlation coefficient (r) between root colonization and growth and nutrition parameters of peach plants inoculated with AMF, collected at the time of transplantation to the field.**

Parameter	r	Probability
%Colonization x height	0.65**	0.0004
%Colonization x diameter	0.80**	0.0001
%Colonization x leaf area	0.60*	0.0389
%Colonization x fresh biomass from stems and leaves	0.65**	0.0001
%Colonization x dry biomass from stems and leaves	0.61**	0.0024
%Colonization x % nitrogen from stems and leaves	0.81**	0.0001
%Colonization x % phosphorus of stems and leaves	0.71**	0.0011
%Colonization x %potassium from stems and leaves	0.70**	0.0001
%Colonization x %calcium from stems and leaves	-0.78**	0.0001
%Colonization x %magnesium from stems and leaves	-0.54*	0.0341
%Colonization x %reserve substances stems and leaves	0.74**	0.0003

Probability of 5% (\*) and 1% (\*\*).

### Discussion

Mycorrhizal symbioses do not present specificity between fungus and plant.<sup>19</sup> However, several authors<sup>20</sup> observed that AMF species responded differently to climatic factors, and chemical and physical characteristics of the soil. Furthermore, differences between AMF species in providing an increase in the growth parameters of the same plant species have been reported, being interpreted as functional specificity.<sup>21</sup> Under the conditions of the present study, where the environment and substrate were similar, the differentiated responses observed in the canopy cultivar, according to the AMF species inoculated in the plants of cv. Okinawa, were, therefore, due to this functional compatibility between the symbionts.

The production of photo-assimilates is directly related to the leaf area, since this is one of the factors that defines the plant's photosynthesis rate.<sup>22</sup>

Several authors<sup>23,24</sup> have reported that the symbiosis established between fruit plants and AMF could provide an increase in leaf area due to the increase in nutrient absorption. In addition, some AMF species have the ability to increase the size and number of fruit leaves, which favors the increase in the photosynthetically active area.<sup>25</sup> In addition, due to the ability of AMF to promote greater absorption and water content in colonized plants, these tend to have greater fresh and dry biomass compared to non-colonized plants.<sup>21,26,27</sup> Other studies have shown that mycorrhizal symbiosis caused changes in water absorption rates in host plants, with consequent effects on tissue hydration, fresh biomass and leaf physiology.<sup>28,29</sup> These reports corroborate the results obtained in this study.

Dry biomass may present similar behavior between inoculated and non-inoculated plants,<sup>21</sup> but there is evidence that plant inoculation can promote dry

biomass accumulation, which can vary from 10% to 800%, with the largest and most consistent responses observed in young plants in the nursery phase.<sup>30</sup> In this study, the results were not the same among all treatments, with the colonized seedlings presenting superior behavior to the control, probably due to the larger leaf surface that, combined with the height, contributed to the accumulation of this parameter, in response to the greater production of photoassimilates.<sup>31</sup> The greater nitrogen absorption by the inoculated plants contributed to these treatments presenting greater responses in terms of growth parameters, when compared with the control. The contribution of mycorrhizae to the increase in nitrogen absorption can be estimated between 10% and 15%,<sup>32</sup> and may reach 25%,<sup>33,34</sup> due to the ability of the hyphae to grow beyond the depletion zone that forms close to the surface of the absorbent roots.

Corn plants colonized by *G. intraradices* presented nitrogen levels in the leaves up to 28% higher than non-colonized plants, when subjected to water shortages, and up to 33% higher, when subjected to regular irrigation periods,<sup>35</sup> which corroborates the results obtained in this experiment, since plants inoculated with AMF increased nitrogen levels in the aerial part, when compared with control plants.

According to several reports,<sup>31</sup> AMF are vital for plants, as they provide an estimated increase of up to 80% in the absorption of phosphorus<sup>32</sup> and 60% for potassium.<sup>33</sup> The greater absorption of nutrients results from the increase in the surface area and absorption capacity of the roots provided by the hyphae and external mycelium of AMF, allowing greater accessibility to nutrients, in addition to enabling the use of forms not available to non-colonized roots, by solubilizing and mineralizing nutrients in the rhizosphere.<sup>33</sup> If the chemical diffusion of nutrients is limiting, hyphae can increase the assimilation area of the root system by up to 700%, due to their small size and large subdivision in the soil.<sup>31</sup>

In experiments with cupuaçu plants colonized by AMF native to soils in agroforestry systems<sup>36</sup> and in studies with five banana cultivars inoculated with AMF isolates native to an Amazonian latosol,<sup>37</sup> it was observed that the mycorrhizal association

correlated positively with high levels of phosphorus and potassium in the leaves of the crops. This report coincides with those obtained in this study, since the plants of cv. Maciel presented high percentages of macronutrients in the plant tissues, always higher than the control plants.

The decrease in the percentage of calcium and magnesium may be due to their dilution in the tissues, due to the increase in the vegetative growth of colonized plants,<sup>21</sup> or to the ability of AMF to reduce the absorption of these elements,<sup>38</sup> due to a buffering effect provided by the fungi,<sup>39</sup> which is in agreement with the results obtained in this work. Studies indicate<sup>26</sup> that, in mandarin plants cv. Marisol grafted onto Troyer citrange, a reduction in calcium and magnesium absorption was also observed, while others<sup>22</sup> show that plants colonized with *Gigaspora margarita* presented lower calcium and magnesium contents than non-colonized plants, while plants colonized with *A. scrobiculata*, *G. clarum*, *G. etunicatum* and *Scutellospora heterogama* presented similar results. On the other hand, there are reports<sup>40</sup> that plants of grapevine rootstock 101-14 inoculated with *G. clarum* showed a decrease in calcium concentration compared to non-inoculated plants, but no differences were observed in relation to magnesium. On the other hand, in plants of grapevine rootstock P1103 inoculated with several species of AMF, it was observed that the calcium concentration was below normal, which differs from the results obtained in this study.<sup>41</sup>

The greater development in height and leaf area of colonized plants allows them to present larger photosynthetic areas and, as a consequence, greater production of photoassimilates and accumulation of biomass.<sup>42</sup> Furthermore, by inducing greater development of stem diameter, AMF give plants the ability to translocate a greater volume of nutrients and water to the aerial part, which would be used in vegetative growth, biomass accumulation and in the plant's metabolic and photosynthetic processes.<sup>43</sup>

The accumulation of reserve substances is directly related to the greater or lesser production of photoassimilates and these, in turn, to larger or smaller leaf areas.<sup>44</sup> Larger leaf areas provide greater light capture and, consequently, greater photosynthesis. Studies have indicated that coffee plants grown

in different agroecosystems and whose seedlings had been inoculated with AMF, presented greater accumulation of reserve substances than plants grown in the same system, but without inoculation.<sup>45</sup> While analyzing the use of different substrates associated with AMF inoculation in the development of the Flying Dragon citrus rootstock (*Poncirus trifoliata*, var. *monstruosa* Swing.), it was observed that the efficiency of the symbiosis in accumulating reserves was only noted when the substrate used was nutritionally poor, and the most efficient AMF species was *A. scrobiculata*.<sup>39</sup> These results coincide with those observed in this study. Many mechanisms of action of AMF are directly or indirectly responsible for the benefits provided to plants,<sup>46</sup> such as increased absorption of nutrients that are slow to diffuse in the soil, such as phosphorus and potassium,<sup>47,15,38,32</sup> thus improving the nutritional content of plants.<sup>48,49</sup>

In addition, other study<sup>50</sup> has indicated that AMF can influence the conductive tissue of colonized plants, showing a thinner cortex than in non-inoculated plants, a greater number of primary xylem cells, and a greater diameter, perimeter and area of cells. The changes promoted by colonization in the structure of the primary xylem, with an expansion in the number and caliber of cells that make up the metaxylem and a decrease in the cortex,<sup>51,38</sup> increasing water and nutritional translocation to the upper part of the plant. The increase in the amount of water displaced to the upper part of the plants, which favors the water-plant relationship, as it allows plants to be more resistant to drought, as a consequence of several changes that occur in their physiology.<sup>52</sup> The increase in the volume of absorption and transport of nutrients, such as nitrogen, a constituent of proteins and vital for vegetative growth,<sup>15</sup> phosphorus, essential for cell division, reproduction and metabolism of photosynthesis, respiration and synthesis of organic substances<sup>22</sup> and potassium, which acts on the electrical balance of cells and the opening and closing of stomata, whose functioning interferes with photosynthesis,<sup>15</sup> contributes to greater plant responses in terms of plant growth parameters. Such reports corroborate the responses found in this study.

According to reports,<sup>26</sup> plant growth responses are directly related to the genotype of the plant and

the inoculated AMF. Furthermore, the affinity of AMF is more related to the root system than to the aerial part of the plants.<sup>52</sup> The results obtained in this experiment indicated a greater affinity between the genotypes of the AMF species *Acaulospora* sp. and *G. etinicum*, when inoculated in plants of cv. Maciel grafted onto the rootstock cv. Okinawa, as they provided the plants with the greatest responses in vegetative growth, when compared with *G. clarum* and the control plants.

Positive correlations indicate a direct relationship between the variables studied, while negative correlations indicate inverse relationships between the variables evaluated.<sup>22</sup> In the case of this study, high values of root colonization were responsible for high values of the parameters evaluated. Calcium and magnesium showed results indicating that the greater the root colonization, the lower the absorption of these elements, since root colonization allows for reduced absorption of these elements, varying according to the species of fungus inoculated and the plant species used.<sup>22,45</sup> In several studies,<sup>23,47</sup> significant correlations were observed between colonization rates and the development parameters and nutritional content of the plants. Studies carried out with banana<sup>53</sup> plants showed significant correlations between root colonization data and seedling growth, indicating that high colonization percentages provide high growth. Similarly, in studies carried out with three banana cultivars inoculated with AMF and planted in the Amazon Yellow Latosol, significant positive correlations were reported between potassium levels, and negative correlations for calcium for the cultivar apple and silve banana cultivars, which allowed the authors to infer that the mycorrhizal association was important for stimulating potassium absorption and decreasing calcium absorption.<sup>53</sup> Such reports coincide with the results obtained in this study.

In studies carried out with acerola,<sup>54</sup> cupuaçu,<sup>36</sup> guarana<sup>36</sup> and sweet passion fruit<sup>55</sup> plants, very significant positive correlations were observed between the colonization of plant roots and the growth parameters and macronutrient levels found in the leaf tissues of these plants, similar to the results found in this study.

**Conclusion**

The use of AMF from vineyards benefitted the peach plants of cv. Maciel grafted onto cv. Okinawa, accelerating their vegetative development and improving their nutritional status, especially when inoculated with the species *Acaulospora* sp.

**Acknowledgement**

The author thanks the Department of Horticulture and Forestry of the Faculty of Agronomy of the Federal University of Rio Grande do Sul (UFRGS) and the National Research Council (CNPq).

**Funding Sources**

Conselho Nacional de Pesquisa (CNPq).

**Conflict of Interest**

Declare if any economic interest or any conflict of interest exists.

**Data Availability Statement**

The manuscript incorporates all datasets produced or examined throughout this research study.

**Ethics Statement**

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

**Author Contributions**

The sole author was responsible for the conceptualization, methodology, data collection, analysis, writing, and final approval of the manuscript.

**Permission to Reproduce Material from other Sources**

Not Applicable.

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