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Assessment of the Influence of Peach Production Systems on the Quantity and Quality of Root Collinization by Arbuscular Mycorrhizal Fungi

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Abstract

This work aimed to evaluate changes in arbuscular mycorrhizal fungi (AMF) communities in soils under different peach production systems in three municipalities in southern Brazil. Root and soil samples were collected in orchards under Integrated Production (IP) and Conventional Production (PB) management on municipal properties for three years. Soil samples of AMF spores were extracted, identified and counted. Root colonization and presence of mycorrhizal structures (hyphae, vesicles and arbuscules) present in the radicle. IP systems have always presented higher colonization levels than PC systems. The municipality of São Jerônimo presented the highest colonization rates in the summer, while Charqueadas presented the highest in the spring, all higher than the municipality of Pelotas. Colonization peaks occurred above 90% in São Jerônimo and Charqueadas for IP, while in Pelotas they did not reach levels above 61%. There were no differences between AMF communities regarding the number of species, between management systems in each production system. The municipality of Charqueadas presented a smaller number of species than Pelotas. The genus Glomus had the largest number of species (9), followed by *Acaulospora* (7), *Scutellospora* (2) and *Archaeospora*, *Entrophospora* and *Gigaspora* (1 each). *Archaeospora leptoticha* and *Gigaspora margarita* present in 97% of the samples.

Introduction

In Brazil, peach production is concentrated in the southern state of Rio Grande do Sul.1 Peach seedling production in southern Brazil takes place, the field,

at the risk of serious phytosanitary problems.² In order to avoid such problems, one alternative be the production of seedlings in environment shelter (greenhouses or nurseries). However, in such

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cases, it is necessary to disinfect of the substrate, to eliminate plant pathogens. That procedure eliminates almost all microorganisms, including beneficial ones, such as arbuscular mycorrhizal fungi (AMF).3

The inoculation of endomychorrhyzal fungi in substrates and disinfected soils in nurseries due to its ability to improve the radicular system, may increase to biotic and abiotic stresses. That can lead to production of high-quality plants in systems using lower amounts of agricultural inputs.⁴

The search for sustainable agriculture necessarily involves a substantial reduction of chemical inputs, including fertilizers, and Integrated Production system offers a possibility to increase farmers gains and consumer's access to high-quality products.¹

Those factors increase the relevance of understanding population changes in AMF in peach orchards, along seasons, once they are already adapted to the edaphocilmatic conditions of the region, propitiating a high potential of response when inoculated in plants to be cultivated in this region. Furthermore, it is crucial to assess the influence upon these populations of soil management systems used in the Integrated Production System and Conventional Production System. The aim of this work was to assess the influence of the management system (Conventional Production and Integrated Production), the locations, season upon the composition and diversity of species AMF peach orchards.

Material and Methods

The evaluations were carried out in the years 2021, 2022 and 2023, in commercial peach orchards in the municipalities of Charqueadas, São Jerônimo and Pelotas, in the state of Rio Grande do Sul, Brazil. The predominant soil in these locations is Typical Paleudult5, the climate is Cfa,⁶ corresponding to the humid subtropical climate with hot summer.⁷ The orchards were managed under the Integrated Production (IP) system, according to PI technical guidelines, and Conventional Production (PC), according to technical recommendations for peach cultivation.8

The samples (rhizospheric soil and roots collected in the four seasons of the year), composed of 20 subsamples, were collected in the upper 20 cm of the soil, under projections of the tree canopy. During the Spring-Summer period, samples were analyzed for pH, organic matter and macronutrient content9. The intensity of AMF colonization was evaluated in 96 segments of 1cm long rootlets, per orchard and season. These fragments were clarified and stained10 and examined under a microscope to evaluate the presence of hyphae, vesicles and arbuscules11. The percentage of colonized roots was obtained through the relationship: number of infected segments by the number of segments analyzed.

Spores were isolated from 100 g of soil samples by wet sieving (105 μ m and 53 μ m sieves)^{12,13} followed by centrifugation and 50% sucrose gradient.¹⁴ Viable spores were counted under stereomicroscope and determined to species level.¹⁵

The data were subjected to analysis of variance, carried out using the Statistical Analysis System (SAS) software, and the means were compared using the Duncan test at a significance level of 5%. The results of presence and absence of species and chemical characteristics of the soil were subjected to correlation analysis (P<0.01 and P<0.05).

Results and Discussion

In the municipality of São Jerônimo, the percentage of root colonization by AMF was high (above 67%) in both production systems, with significant differences between them. The percentage of root colonization under IP was always higher than under CP, while in Charqueadas this occurred in winter and spring. Colonization reached peaks in the summers of 2021, 2022 and 2023, for São Jerônimo, and in the springs of 2021, 2022 and 2023 for Charqueadas, being greater than 90% in the IP. In the municipality of Pelotas, the percentage of root colonization by AMF was low in both production systems, always higher in the IP system. Throughout the year there were differences between the seasons, in both systems, always greater in spring. The comparison between regional averages shows that the percentage of root colonization by AMF was higher in the IP in the São Jerônimo and Charqueadas regions, when compared with the CP system there and with the results from the Pelotas systems (Table 1).

Table 1: Percentage of radicular colonization by AMF in peach-trees submitted to Integrates Production (IP) System and Conventional Production (CP) System in four orchards of three municipalities of Rio Grande do Sul, Brazil.

¹Means followed by the same low case letter in the column (seasons of the year) and capital letter in the line (production system) do not differ among themselves by the test of Duncan at 5% of significance. ² Means followed by the same capital letter in the line (municipalities and production system) do not differ from each other using the Duncan test at the 5% significance level.

The most likely cause for the variation in the percentage of root colonization between regions and treatments is related to the type of soil and its management used on each property and production system. The effective benefits of AMF in agricultural production are more promising in circumstances where the infection potential of native fungi is indirectly manipulated through soil management and crop rotation¹⁶ Thus, agricultural management is decisive in the establishment and performance of native AMF, since several factors (pH, fertility level, humidity, aeration, soil management, degree of mycorrhizal dependence of the plant, and the interactions between fungi and other soil microorganisms) interfere, positively or negatively, in the ineffectiveness of the fungus and in the efficiency of association.¹⁷

In the São Jerônimo and Charqueadas region, soil management in the IP area used winter cover with oats and herbicide between the rows, without the use of harrows and controlled use of agricultural inputs such as fertilizers and pesticides, in accordance with the Specific Technical Standards for Production Integrated Peach (NTEPIP). In the CP area, on the São Jerônimo property, soil management consisted of light harrowing with a depth varying between 4 and 5 cm between rows, incorporating fertilizer on the periphery of the tree. main. Subsequently, the farmer began to use only the brush cutter as a method of controlling the growth of natural cover between the rows.

On the Charqueadas property, the farmer only used the brushcutter, without harrowing in the CP. In IP, the use of a species for ground cover (oats) probably caused a decrease in biological diversity, which increased the possibility of root colonization in peach trees.

In the Pelotas region, oats were used as winter cover in IP and natural cover in CP. Until 2003, the producer used side harrows in the row and harrowing between CP lines to incorporate fertilizers, and natural coverage in IP. From the spring of 2004, the farmer started to use only natural cover in both production systems, without harrowing. The use of mulch or green manure promotes AMF colonization18. Furthermore, in agroecosystems with a low level of interference from agricultural practices, rates of mycorrhizal colonization are high.¹⁹ On the other hand, root colonization rates and AMF spore density are lower in soils subjected to mechanical disturbances or kept uncovered.²⁰ The use of natural cover in CP allowed an increase in the diversity of host plant species and the diversity of AMF species, which increased competition between them. Many species of autochthonous AMF could show lower colonization responses in the presence of a cultivated crop, which would lead to a decrease in the percentage of colonization of these cultivated plants.²¹ According to the same authors, species may be present in environments, but may not respond to the presence of roots of some plants. Furthermore, the diversity of host species promotes the establishment of a high number of colonization by AMF, due to the high number of hosts that can be colonized.²² Thus, the community of plants on which fungi depend to obtain photoassimilates can also influence the structure of the AMF community, which suggests that the diversity of spontaneous vegetation in agroecosystems alters the diversity of AMF species, increasing the number of species and competitions with each other.²³

Regions	Systems /year	Clay $(\%)$	pH	OM ³ $(\%)$	P^4 $(mq.dm-3)$	K ⁵	Ca ⁶ $(mg.dm-3)$ (cmolc.L ⁻¹)	Mg^7 $(cmolc.L^{-1})$
SJ ¹	IP2/21	26	6.6	1.8	27	123	4.5	0.9
	CP2/21	28	6.8	2.1	28	125	5.7	0.9
	IP2/22	32	6.3	1.7	22	145	10.7	0.7
	CP2/22	24	6.2	2.8	26	219	9.7	1.1
	IP2/23	22	6.1	2.2	15	113	5.8	1.1
	CP2/23	24	6.0	2.4	20	234	8.1	1.7
Ch ¹	IP2/21	32	6.3	2.4	61	324	6.9	2.1
	CP2/21	29	6.7	2.3	84	353	7.8	2.2
	IP2/22	30	6.5	2.5	47	344	6.3	1.9
	CP2/22	32	6.2	2.5	60	378	5.4	1.7
	IP2/23	34	6.2	2.4	31	357	6.9	2.1
	CP2/23	32	6.1	2.5	53	354	6.8	2.0
PeI ¹	IP2/21	22	4.5	2.5	14	132	1.5	0.5
	CP2/21	25	4.6	2.7	17	158	2.9	1.0
	IP2/22	22	5.2	2.3	16	104	2.7	1.0
	CP2/22	22	5.0	1.9	15	177	2.0	0.9
	IP2/23	23	5.1	2.1	15	114	2.4	1.0
	CP2/23	22	5.2	2.0	16	143	2.5	1.1

Table 2: Chemical Analysis of the soil samples collected in the years 2021, 2022, 2023 in peachorchards submitted to Integrates Production (IP) System and Conventional Production (CP) System in four orchards of three municipalities of Rio Grande do Sul, Brazil.

1 Ch - Charqueadas; SJ – Sao Jerônimo; Pel – Pelotas; 2 IP - Integrates Production; CP – Conventional Produtiction; $^3\rm{OM}$ – organic matter; $^4\rm{P}$ – Phosphorus; $^5\rm{K}$ – Potassium; $^6\rm{Ca}$ – Calcium; $^7\rm{Mg}$ – Magnesium.

It is worth noting that the high phosphorus content did not affect root colonization, especially in IP. Oats, under ideal pH conditions (around 6.0 to 6.5), extract large amounts of phosphorus from the soil, which would favor the action of mycorrhizae.²⁴ Furthermore, as there is a mixture of AMF in the soil of an orchard, it is plausible to assume that the different behaviors of these species may also have helped to ensure mycorrhizal colonization of the roots, regardless of the level of phosphorus present in the soil. This can be proven by the fact that, during the vegetative period of peach trees, the percentage of root colonization was higher in IP than in CP, even under similar fertility and pH conditions (Table 2).

On the other hand, the average colonization of the IP, in the Pelotas region, was lower than that of São Jerônimo and Charqueadas, which can be explained by the soil pH, which is lower on the Pelotas properties, which could harm the development of oats. and phosphorus absorption (Table 2). Under low pH, it is assumed that colonization would be guaranteed by the different behavior of the different mycorrhizal species present in orchards, since many AMF species prefer acidic soils or are indifferent to acidity. However, in Pelotas, the use of natural cover in the IP, combined with the high phosphorus content and low pH, maintained the average percentage of colonization throughout the year at 48.02%. In CP, these values are 34.90%, largely due to the type of management used, with lateral harrowing towards the top of the tree, which would break the roots, damaging colonization (Table 1). Excessive soil disturbance reflects brightness in the AMF community and the establishment of the symbiotic association.25

The intensity of colonization, quantified by the presence of fungal structures (hyphae, vesicles and arbuscules) in the roots, was moderate for both systems in São Jerônimo. In PI, rates were higher than CP throughout the seasons of the years evaluated, except in the winter periods (Table 3).

Table 3: Presence of structures of colonization of AMF in roots of peachtrees submitted to Integrates Production (IP) System and Conventional Production (CP) System, collected in the years of 2021, 2022, and 2023, in the four seasons of the year at the property of Sao Jerônimo, RS, Brazil.

Means followed by the same low case letter in the column (seasons of the year) and capital letter in the line (production system) do not differ among themselves by the test of Duncan at 5% of significance.

In the case of the property in Charqueadas, the presence of fungal colonization structures was considered low to moderate in terms of the presence of hyphae and low in terms of vesicles and arbuscules, in both systems. In both systems, the presence of fungal structures was greater in spring, while the rate of root colonization by AMF was higher in the IP system compared to CP only in winter and spring of all years evaluated, this period also being the peak presence of structures (Table 4).

Table 4: Presence of structures of colonization of AMF in roots of peachtrees submitted to Integrates Production (IP) System and Conventional Production (CP) System, collected in the years of 2021, 2022, and 2023, in the four seasons of the year at the property of Charqueadas, RS, Brazil.

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Means followed by the same low case letter in the column (seasons of the year) and capital letter in the line (production system) do not differ among themselves by the test of Duncan at 5% of significance.

With regard to the municipality of Pelotas, the presence of colonization structures was considered scarce, both in systems and properties. The IP production system presented the highest rates in relation to CP, in all seasons of the years evaluated, always being higher in spring (Table 5).

Table 5: Presence of structures of colonization of AMF in roots of peachtrees submitted to Integrates Production (IP) system and Conventional (CP), collected in the years of 2021, 2022, and 2023, in the four seasons of the year at the property of Pelotas, RS, Brazil.

Means followed by the same low case letter in the column (seasons of the year) and capital letter in the line (production system) do not differ among themselves by the test of Duncan at 5% of significance.

Table 6: Number and relative occurrence (%) of species of AMF from peach-orchard submitted to Integrated Production (IP) and Conventional Production (CP) found in 84 samples (n) collected in the four-seasons of the years 2021, 2022, and 2023, in the municipalities of Charqueadas, Sao Jerônimo and Pelotas, Rio Grande do Sul State, Brazil.

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(1)Means followed by the same letter in the column do not differ among themselves by the test of Duncan at 5% of significance. ns Non-significative.

(2)Number of samples in which the species was found divided by the total of samples collected in the municipality multiplied by 100.

These results relating to the municipality of Pelotas can be explained by the high levels of phosphorus found in soil samples, which has hampered the colonization and development of AMF structures in the roots.^{26,27}

Were identified 21 species of AMF were identified in samples collected in orchards in both regions. There was no difference between the AMF communities in terms of the number of species,

when evaluating the influence of the soil management systems used in each production system and season (Table 6).

Several authors,²¹ when evaluating the influence of Conventional Production and Organic Production systems on the presence and composition of AMF species in citrus orchards, they realized that the organic soil management system, using conservation practices such as the use of plants to cover winter, presented more significant colonization rates, which coincides with the data obtained in this work. These authors also noted a greater number of AMF species under organic management, which disagrees with the results of this experiment. However, other research involving corn and soybeans maintained under conventional and organic soil management showed no differences in the number of AMF species between treatments, but found differences in the highest colonization rates in organic soil management,²⁸ which coincides with the results obtained in this work. When comparing regions, 13 species were found in the municipalities of Charqueadas and São Jerônimo, a value lower than that found in Pelotas, where 20 species of AMF were found.

Table 7: Correlation between the occurrence of species of AMF and the chemical characteristics of the soil belonging to the samples collected in the peach-orchards submitted to Integrated Production (IP) System and Conventional Production (CP) System during Spring-Summer period in 2021, 2022 and 2023 in the municipalities of Charqueadas, Sao Jerônimo and Pelotas, Rio Grande do Sul State, Brazil.

Species of AMF	рH	M.O.	P	K	Ca	Mg
Acaulospora bireticulata	0.11^{ns}	$-0.13ns$	$-0.51*$	-0.09^{ns}	0.06 ^{ns}	0.03 ^{ns}
Acaulospora denticulata	0.02 ^{ns}	0.15^{ns}	0.10^{ns}	0.13 ^{ns}	-0.11^{ns}	-0.14^{ns}
Acaulospora foveata	0.04 ^{ns}	$-0.12ns$	$-0.48*$	$-0.06ns$	0.14^{ns}	0.08 ^{ns}
Acaulospora laevis	0.01 ^{ns}	$-0.51*$	$-0.64**$	$-0.50*$	0.06 ^{ns}	0.14^{ns}
Acaulospora mellea	0.02 ^{ns}	$-0.53*$	$-0.51*$	$-0.49*$	0.08 ^{ns}	0.05^{ns}
Acaulospora scrobiculata	$-0.09ns$	0.14^{ns}	0.12^{ns}	0.10 ^{ns}	-0.11^{ns}	$-0.18ns$
Acaulospora tuberculata	0.02 ^{ns}	$-0.41*$	$-0.49*$	$-0.18ns$	0.05^{ns}	0.04 ^{ns}
Archaeospora leptoticha	0.05 ^{ns}	0.12 ^{ns}	0.19 ^{ns}	0.08 ^{ns}	$-0.07ns$	-0.10^{ns}
Entrophospora colombiana	$-0.09ns$	0.09 ^{ns}	$-0.50*$	$-0.11ns$	0.06 ^{ns}	0.07 ^{ns}
Gigaspora margarita	0.15^{ns}	0.18 ^{ns}	0.14^{ns}	0.16 ^{ns}	-0.20 ^{ns}	-0.19^{ns}
Glomus coremioides	$-0.59**$	$-0.02ns$	-0.10^{ns}	$-0.08ns$	0.13^{ns}	0.09 _{ns}
Glomus claroideum	0.15^{ns}	$-0.18ns$	$-0.46*$	$-0.17ns$	0.03 ^{ns}	0.01 ^{ns}
Glomus clarum	$0.50*$	0.11 ^{ns}	0.08 ^{ns}	0.13^{ns}	-0.05^{ns}	$-0.07ns$
Glomus etunicatum	0.06 ^{ns}	$-0.58*$	$-0.64**$	$-0.50*$	0.12 ^{ns}	0.15^{ns}
Glomus geosporum	$0.51*$	0.06 ^{ns}	$-0.07ns$	0.11 ^{ns}	0.03 ^{ns}	0.01 ^{ns}
Glomus glomerulatum	0.05 ^{ns}	$-0.47*$	$-0.52*$	-0.11^{ns}	0.05^{ns}	0.07^{ns}
Glomus macrocarpum	0.09 _{ns}	0.12 ^{ns}	0.05 ^{ns}	0.04^{ns}	$-0.06ns$	$-0.02ns$
Glomus microaggregatum	0.06 ^{ns}	$-0.11ns$	$-0.48*$	-0.15^{ns}	0.14^{ns}	0.12^{ns}
Glomus tortuosum	0.05 ^{ns}	$-0.52*$	$-0.56*$	$-0.47*$	0.08 ^{ns}	0.11 ^{ns}
Scutellospora heterogama	0.08 ^{ns}	0.10^{ns}	0.08 ^{ns}	0.06 ^{ns}	$-0.07ns$	$-0.09ns$
Scutellospora weresubiae	0.05^{ns}	$-0.18ns$	$-0.51*$	0.05^{ns}	0.06 ^{ns}	0.04 ^{ns}

* and **Significative at 5% and at 1% of probability, respectively, by the test of Duncan. nsNon-significative.

The genus Glomus had the largest number of species (nine) followed by the genera *Acaulospora* (seven), *Scutellospora* (two), *Archaeospora, Entrophospora* and *Gigaspora* (one each) (Table 6). Regarding the species found, Archaeospora leptoticha and *Gigaspora margarita* appeared in 96.55% of the 58 samples analyzed, *Acaulospora denticulate* in 79.31%, *Glomus macrocarpum* and *Scutellospora heterogama* in 75.86%, *Glomus clarum* in 65.81%, *Glomus geosporum* in 63.79%, Glomus tortuosum in 43.10%, *Acaulospora scrobiculata* in 36.20%, Glomus etunicatum in 5.17%, *Acaulospora bireticulata*, *Acaulospora laevis*, *Glomus glomerulatum* and *Glomus claraideum* in 3.45% and *Acaulospora foveata* in 1.72% of the samples. Authors found that the most frequent species found in all samples collected in citrus groves under organic and conventional production were Glomus microaregtum and Acaulospora scrobiculata, 21 while others showed the presence of ten species in the samples, with Glomus macrocarpum being the most frequent (in 95% of samples) followed by Scutellospora heterogama (in 86%).³

Some AMF species showed a significant correlation with soil chemical characteristics (Table 7).

The species *Acaulospora bireticulata*, *Acaulospora foveata*, *Acaulospora laevis*, *Acaulospora mellea*, *Acaulospora tuberculata*, *Entrophospora colombiana*, *Glomus claroidium*, *Glomus etunicatum*, *Glomus glomerulatum*, *Glomus microaggregatum*, *Glomus tortuosum*, and Scutellospora Weresubiae occurred in environment with lower contents of phosphorus, while Acaulospora laevis, Acaulospora mellea, Glomus etunicatum, and Glomus tortuosum have shown themselves very sensitive also to the presence of high contents of organic matter and potassium. Several studies 21 show the same correlation between the species Acaulospora mellea, Entrophospora colombiana, Glomus etunicatum and Glomus geosporum with phosphorus, in the same way²⁹ that Acaulospora mellea and Glomus etunicatum prefer environments with lower phosphorus levels.

The species *Acaulospora denticulata*, *Acaulospora scrobiculata*, *Archaeospora leptoticha*, *Gigaspora margarita*, *Glomus macrocarpum*, and *Scutellospora heterogama* were indifferent to the soil characteristics. Glomus coremioides has shown itself sensitive to high pH, while Glomus clarum and *Glomus geosporum* have shown their preference for little acidic or neutral soils. Some species of the genus Gigaspora occurs in environments with pH lower than six,29,30 while many species of the genera

Acaulospora, Gigaspora and the species Glomus manihotis are particularly tolerant to conditions of high acidity.³¹

Conclusion

There is variation in the percentage of colonization of roots by AMF in function of the time of the year and system of production, where the Integrated Production presents higher colonization than the Conventional Production.

The type of soil and management used interfere in the colonization of the roots by AMFs and in the formation of their structures of colonization.

The use of oat as winter cover increases the possibility of colonization of plants cultivated.

There are not differences among the AMF communities regarding the number and composition of the species when assessing the influence of the systems of managements of soil employed in each system of production.

The chemical characteristics such as organic matter, phosphorus content, and the pH are determinant for the colonization of roots and for the formation of the spores, therefore influencing in the presence and response of the species of AMF.

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Conflict of Interests

The author has no conflicts of interest.

Authors' Contribution

The author, Dr. José Luis da Silva Nunes, was responsible for the conception and design of the study, data acquisition, analysis and interpretation of the data and writing of the article.

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