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Glomalin and Its Relationship with Inoculation, Fertilization and Soils with Different Sand Proportion

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Summary *A Healthy Agricultural Land Is A System With Resistance To Stress Due To Its Biological Diversity, An Active Cycling Of Nutrients And Energy And A High Physical Stability. The Activity Of Bacteria And Fungi Is Essential In The Humification Processes. One Of The Most Relevant Relevant Groups Of Soil Fungi Is The Arbuscular Mycorrhizals (AMF). AMF Synthesize A Glycoprotein Called Glomalin Or GRSP (Soil Protein Related To Glomalin), Which Is Not Secreted By The Mycelium, But Being The Release By Lysis Of Post Mortem Tissues The Main Route Of Its Deposition In The Soil. The Objective Of This Work Was To Evaluate The GRSP Content, The GRSP / Carbon Ratio, The Amount Of Dry Matter And The Foliar Phosphorus Content Of Festuca Arundinacea At Various Levels Of Inoculation, Fertilization And Soils With Different Sand Proportion In A Greenhouse Experiment. The Carbon Content Of The Soil Had A High Correlation With The GRSP Content (R^2 0.93). This Result Indicates That GRSP Could Be A Biological Indicator Of Soil, With The Accumulation Of Soil Carbon Simultaneously With GRSP Enrichment. The Amount Of Dry Matter And The Concentration Of Foliar Phosphorus Increased With The Practice Of Inoculation When Fertilizer Was Not Applied, But Before The Application Of Nitrogen And / Or Phosphorus The Differences In These Variables Between Inoculated And Non-Inoculated Treatments Were Diluted, Evidencing The Increased Activity Of AMF Inoculated In Soils With Low Nutrient Contents.*

Keywords*: GRSP, Inoculation, Fertilization*

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I. Introduction

A Healthy Agricultural Land Constitutes A System With Resilience To Stress Due To Its Biological Diversity, Active Cycling Of Nutrients And Energy And High Physical Stability. The Evaluation Of The Variables Related To The Cycling Of Soil Humus And Its Microbiological Processes, Is A Very Useful Tool To Detect Differences In The Management And Productivity Of Forages (Romaniuk Et Al., 2012; Romaniuk Et Al., 2011).

The Activity Of Bacteria And Fungi Are The Most Important Components In The Processes Of Humification And Physical Stability Of The Soil (Gomez Et Al., 2007; Romaniuk Et Al., 2012; Romaniuk Et Al., 2011). One Of The Most Relevant Groups Of Soil Fungi Is The Arbuscular Mycorrhizal Fungi (AMF). The AMF Belong To The Glomeromycota Phylogeny (Schüssler Et Al., 2001) Formerly Zygomycota. These Fungi Form Symbiotic Relationships With 80% Of Terrestrial Plants, Including The Main Production Species Such As Wheat, Maize, Sorghum And Forage Species.

The Plant-AMF Symbiosis Induces Physiological Changes By Increasing The Photosynthetic Rate, Redistributing Carbon And Increasing The Activity Of The Roots As A Destination, Favoring The Increase Of Carbonaceous Substances In The Soil (Rao And Staunton, 2012). Likewise, These Organisms Are Responsible For The Significant Increase In The Acquisition Of Nutrients (Smith And Read, 1997). At The Plant Community Level, It Has Been Shown That Amfs Are Mediators Of Competition Between Plants And Co-Factors That Determine The Diversity Of Plant Species (Van Der Heijden Et Al., 1998), And Finally At The Ecosystem Level, The AMF. They Are Of Recognized Importance In The Processes Of Nutrient Cycling And Soil Aggregation (Miller And Jastrow, 2000).

Mycorrhizal Fungi Synthesize A Glycoprotein Called Glomalin. Vijay Gadkar And Matthias Rillig (2006), In Vitro Tests, Discovered That It Was Similar To The Mitochondrial Protein Of Heat Shock 60 HSP60. The Glomalins Have Been Found In Relative Abundance (2-15 Mg G^{-1}) In A Wide Range Of Soils, Acidic Or Calcareous Soils (Schindler Et Al., 2007) And Under Diverse Crops, Such As Vegetables, Meadows, Cereals, Forest Species, Etc. (Purin And Rillig, 2008).

Using In Vitro Cultures Of AMF, *Glomus Intraradices* Species, It Was Observed That Glomalin Is Not Secreted Or Passively Released By The Growing Mycelium In Large Quantities, Only Small Portions Are Secreted, While Most (> 80%) Of The Glomalin Produced By The Fungus Is Strongly Retained And Firmly Incorporated Into The Walls Of The Hyphae And Spores, So The Main Route Of Its Deposition In The Soil Is Through The Process Of Decomposition Of The Hypha After Its Death (Pellegrini Et Al , 2009).

In The Soil Humus There Are Fractions Composed Of Proteins That Come From Diverse Microbiological Origins, But Until Now The Simple Separation And Quantification Of Glomalin From Other Humic Pseudoproteins, Has Not Been Possible. The Process Of Extracting Glomalin (Wright Et Al., 2006), Derives The Use Of The Term GRSP (Glomalin-Related Soil Protein) To Refer To The Generic Product Of The Pool Of Extracted And Quantified Soil Proteins. This Measurement Is Widely Used In AMF Studies At A Global Scale.

The Objective Of This Work Was To Evaluate The GRSP Content, The GRSP / Cox Ratio, The Amount Of Dry Matter And The Foliar Phosphorus Content Of *Festuca Arundinacea* Under Different Levels Of Inoculation, Fertilization And Soils With Different Sand Proportion.

II. Materials And Methods

A Test Was Carried Out In Greenhouse Conditions With DBCA Design (Design In Completely Randomized Blocks). The Experimental Units Were Plastic Pots Of 5 Liters Of Capacity Conditioned At The Bottom With A Plastic Net In Order To Avoid Loss Of Soil Content Through The Drainage Holes. The Species Planted Was *Festuca Arundinacea* Cultivar 1132.

The Soil Used For The Trial Was Collected From "San Claudio", A Farm Located In The Carlos Casares, In The West Of Buenos Aires Province, Argentina. Taxonomic Classification Of The Soil Series Is: Entic Hapludoll, Thick, Mixed, Thermal Family (USDA- Keys To Soil Taxonomy, 2006). The Soil Was Extracted Up To A Depth Of 0-20 Cm. In A Hill Position.

Treatments

The Design Of The Test Includes Three Factors: Soils With Different Sand Proportion, Inoculation And Fertilization. The Soils With Different Sand Proportion Factor Was Comprised Of Three Levels: 100% Soil, Soil Mixed With 50% Sand And 100% Sand. Tthe Sand Was Previously Washed And Rinsed Several Times With Distilled Water To Avoid High Electrical Conductivity Effects. The Inoculation Factor Had Two Levels: Inoculated With Spores Of AMF (*Glomus Intradices* Species) In A Dose Of 12.3 Gr. Of A Commercial Product Per Experimental Unit And Not Inoculated. The Fertilization Factor Presented 4 Levels: Unfertilized Control, Nitrogen Fertilization, Phosphorus Fertilization And Combined Nitrogen + Phosphorus Fertilization. For Nitrogen Fertilization, Urea (46-0-0) Was Usedin A Single Dose Equivalent To 100 Kg. Ha^{-1}. For Phosphorus Fertilization, Simple Superphosphate (0-21-0) Was Used, In A Single Dose Equivalent To 35 Mg Kg^{-1}. In The Case Of Nitrogen + Phosphorus Combined Fertilization Treatment, The Same Fertilizers And The Same Doses Were Used.

Measurements

Oxidizable Soil Carbon (Cox) (Walkley And Black, 1934); Extraction And Quantification Of Soil Proteins Related To Glomalin (GRSP) By Bicinchoninic Acid Method (Stoscheck, 1990), GRSP / Cox Ratio, Leaf P Content By Calcination Method And Determination By Colorimetry Of Phospho-Vanadomolybdate (Sadzawka Et Al., 2004) And Leaf Dry Matter (DM).

For The Studied Variables, The Assumptions Of Normality Of Data (Shapiro-Wilks Test) And Homogeneous Variances Were Checked. The Principle Of Independence Was Ensured Through Randomization In The Allocation Of Treatments In The Experimental Units. The Measured Response Variables Were Processed Statistically By ANOVA, Analyzing The Effects Of The Treatments And Performing The Corresponding Hypothesis Tests. Likewise, Correlation And Simple Regression Analyzes Were Carried Out Among The Different Variables To Evaluate Their Degree Of Association, Through The Statistical Package Infostat.

III. Results And Discussion

GRSP (Glomalin Relate Soil Protein)

The Soil Type, Inoculation And Fertilization Treatments Presented Double Effects And Interactions In GRSP Values ($P < 0.05$); The Triple Interaction Was Not Significant ($P > 0.05$).

The GRSP Values Of The Inoculated Treatments Were Significantly ($P < 0.05$) Higher Than Those Not Inoculated In All Soils With Different Sand Proportion (Figure 1).

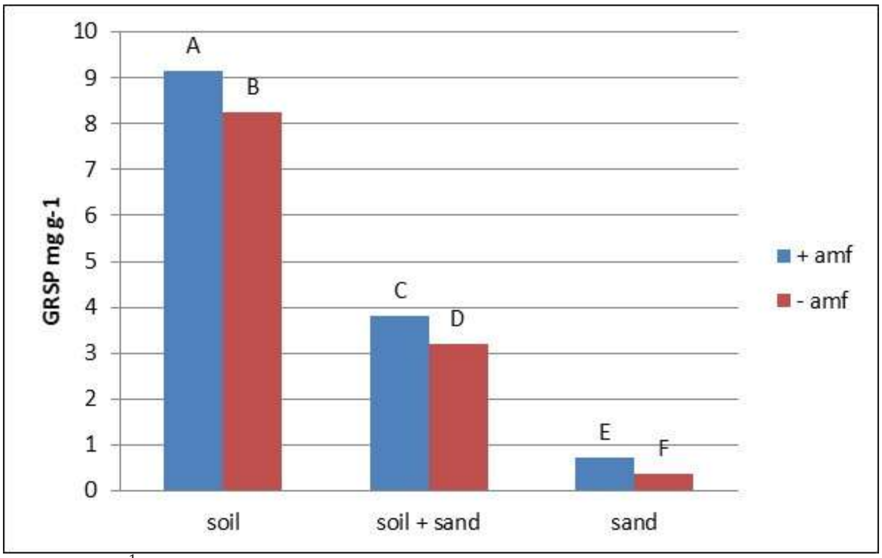

Figure 1. GRSP (Mg G^{-1}) Depending On The Soils With Different Sand Proportion And AMF Inoculation. Different Letters Indicate Significant Differences (P <0.05). Treatment Inoculated With AMF (+ Amf) And Treatment Not Inoculated With AMF (- Amf).

In The Inoculated Treatments, GRSP Increased 9.83% In The Soil, 16.23% In The Soil + Sand And 49.3% In The Sand With Respect To The Non-Inoculated Treatments. The Effect Of The Inoculation With AMF Was Proportionally Higher In The Sand, Evidencing The Expression And Synthesis Of Glomalin By AMF Product Of The Inoculum Addition Under Short-Term Conditions. Gang Wang And Others (2016) Determined In Carbon Rich Soils That The Inoculation With AMF Significantly Increased The GRSP And Carbon Contents Of The Soil With Respect To The Control Treatments.

The GRSP Values Were Significantly Different (P <0.05) Among The Three Soils With Different Sand Proportion At All Fertilization Levels (Figure 2).

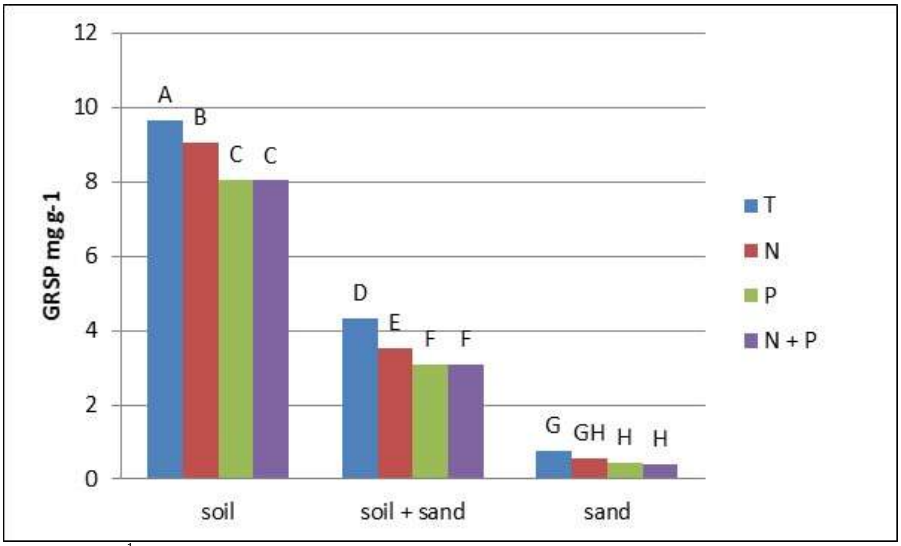

Figure 2. GRSP (Mg G^{-1}) Depending On Soils With Different Sand Proportion And Fertilization Treatments. Different Letters Indicate Significant Differences (P <0.05). T: Unfertilized Control, N: Treatment Fertilized With Nitrogen, P: Treatment Fertilized With Phosphorus And N+P: Treatment Fertilized With Nitrogen Plus Phosphorus.

In The Soil And Soil + Sand Mixture The GRSP Values Were Significantly Higher (P <0.05) In The Unfertilized Treatment Compared To The Nitrogen Treatment, And Both (T And N) Were Significantly Higher Than The Phosphorus And Nitrogen Plus Phosphorus Treatments; The Latter (P And N + P) Did Not Differ Significantly From Each Other. In The Sand The Differences Were Less Marked But There Were Still Significant Differences Between The Unfertilized Control With Respect To The Phosphorus And Nitrogen Plus Phosphorus Treatments, The Latter (P And N + P) Did Not Differ Significantly From Each Other; The

Unfertilized Treatment Did Not Differ From That Fertilized With Nitrogen And This (N) Also Did Not Differ From The Phosphorus And Nitrogen Plus Phosphorus Treatments (Figure 2).
Within Each Fertilization Level, The GRSP Values Were Significantly Higher (P <0.05) In The Treatments Inoculated Than In The Non-Inoculated Ones (Figure 3).

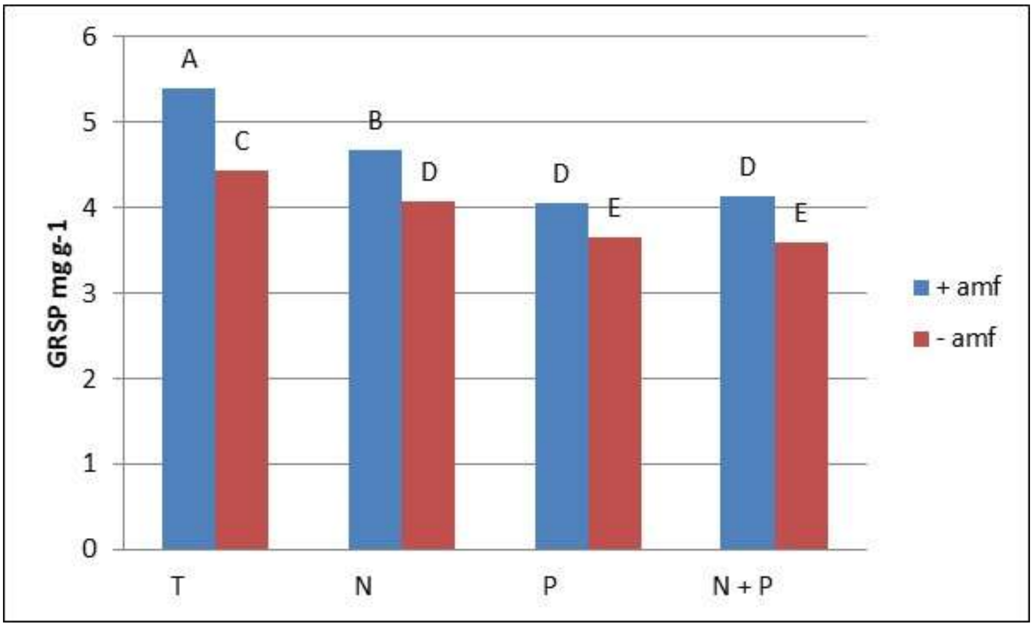

Figure 3. GRSP (Mg G^{-1}) Depending On The Interaction Inoculation By Fertilization. Different Letters Indicate Significant Differences (P<0.05). Treatment Without Fertilization (T), Treatment Fertilized With Nitrogen (N), Treatment Fertilized With Phosphorus (P) And Treatment Fertilized With Nitrogen Plus Phosphorus (N + P). Treatment Inoculated With AMF (+ Amf) And Treatment Not Inoculated With AMF (- Amf).\

Inoculation Had Different Effects In GRSP Contents According To The Levels Of Fertilization (Figure 3). The Largest Increments Of GRSP 18% Were Observed In The Unfertilized Treatment. Among The Fertilized Treatments, Increases In GRSP Were 12.85% In N Treatment, 9.85% In P Treatment And 12.83% In N+P Treatment.

In Figure 4, The Values Of GRSP That Presented A Positive Linear Behavior With Respect To Cox (P <0.0001) Are Shown, Independently Of The Treatments Applied, Being The Adjustment Of The Regression R2 0.93. These Results Coincide With Other Authors Regarding The Positive Linear Association Between GRSP In Terms Of Soil Carbon (Ferrero Holtz Et Al., 2016, Nobre Et Al., 2015, Wu Et Al., 2014), Indicating That The Factors Involved In Soil Carbon Accumulation Simultaneously Encourage The Proliferation Of AMF And The Enrichment Of GRSP (Singh Et Al., 2016).

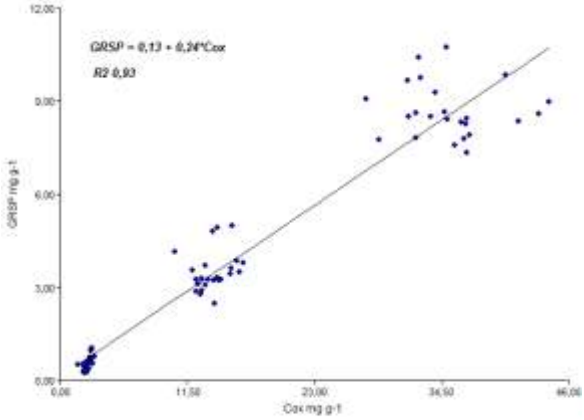

Figure 4. GRSP (Mg Gr^{-1}) As A Function Of Cox (Mg Gr^{-1}). The Points Represent All The Test Data.

GRSP / Cox Ratio

The Soils With Different Sand Proportion Treatments And Inoculation Presented Interactive Effects On The GRSP / Cox Ratio ($P < 0.05$); Interaction Of Fertilization With Soils With Different Sand Proportion Or Inoculation Were Not Significant ($P > 0.05$) And The Simple Effect Of Fertilization On GRSP/Cox Could Be Observed.

The Non-Inoculated Treatment On Sand Substrate Was Significantly Lower Than The Other Treatments ($P < 0.05$, Fig.5). This Variation Could Be Linked To The Expression And Differential Synthesis Of Glomalin In The Short Term By Arbuscular Mycorrhizal Fungi (AMF) Under Low Levels Of Soil Carbon With The Addition Of Inoculum And Its Naturally Low Presence In The Sand.

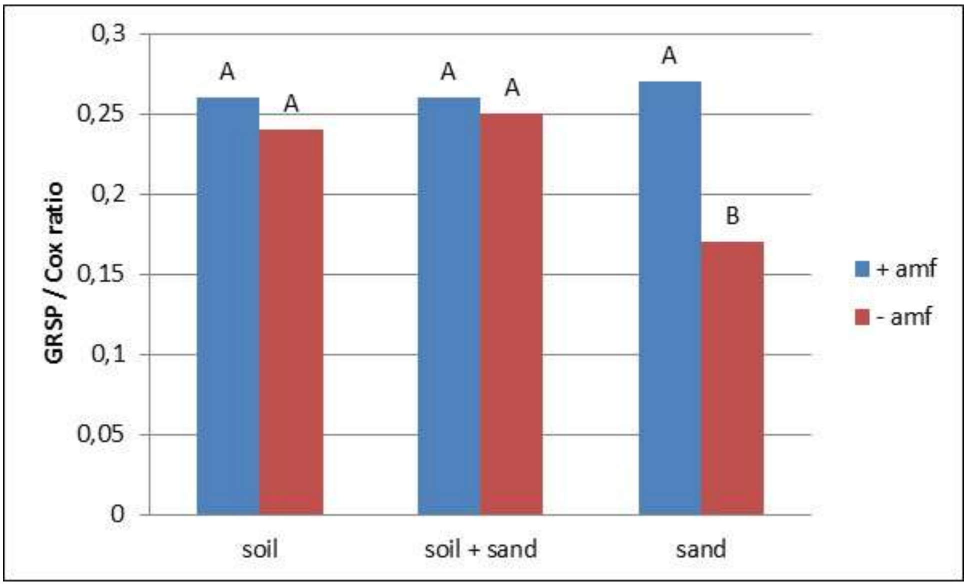

Figure 5. GRSP / Cox Ratio Depending On Soils With Different Sand Proportion And Inoculation. Different Letters Indicate Significant Differences ($P < 0.05$). Treatment Inoculated With AMF (+ Amf) And Treatment Not Inoculated With AMF (- Amf).

The GRSP / Cox Ratio Represented 31% For The Unfertilized Level, 23% For The N Treatment, 22% For The P Treatment And 20% For The Combined Fertilization Treatment (Figure 6). The Unfertilized Level Had Significantly Higher GRSP/Cox Ratio ($P < 0.05$) Compared To The Other Levels Of Fertilization. The Reduction Of GRSP / Cox In The Presence Of Fertilization In Its Three Levels Could Indicate A Lower Symbiotic Mutualistic Activity Between Arbuscular Mycorrhizal Fungi And *Festuca Arundinacea* Due To An Increased Availability Of Nutrients (Aguilera Gómez Et Al., 2007).

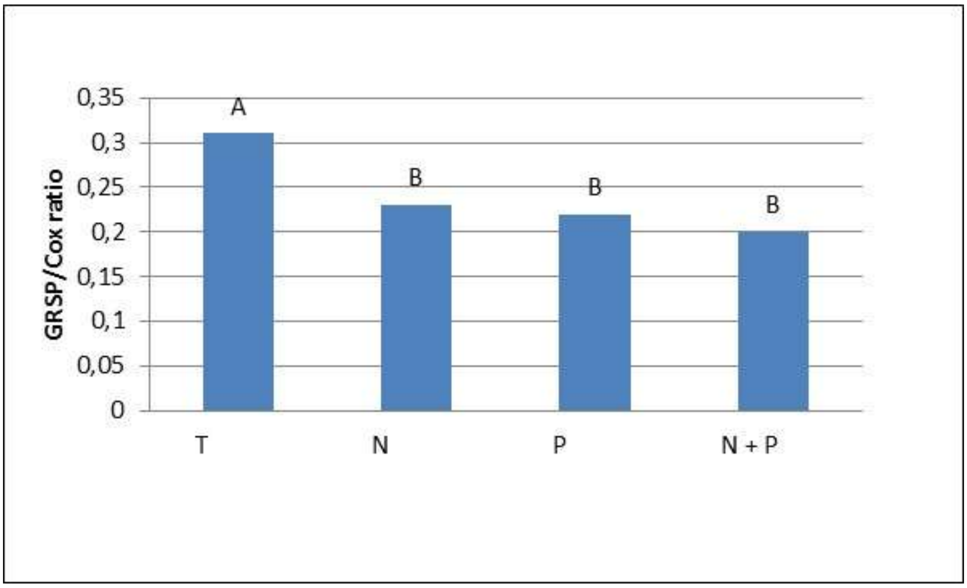

Figure 6. GRSP / Cox Ratio Relationship With Fertilization. Different Letters Indicate Significant Differences ($P < 0.05$).

Leaf Dry Matter (DM)

Fertilization Presented Double Interactions With The Soils With Different Sand Proportion And Inoculation Treatments On The Production Of Leaf Dry Matter. (P <0.05). The Double Interaction Soils With Different Sand Proportion By Inoculation And The Triple Interaction Were Not Significant (P>0.05).

The Interaction Soils With Different Sand Proportion By Fertilization Led To Different Significant Effects On The Leaf Dry Matter According To The Levels Considered (P <0.05) (Figure 7).

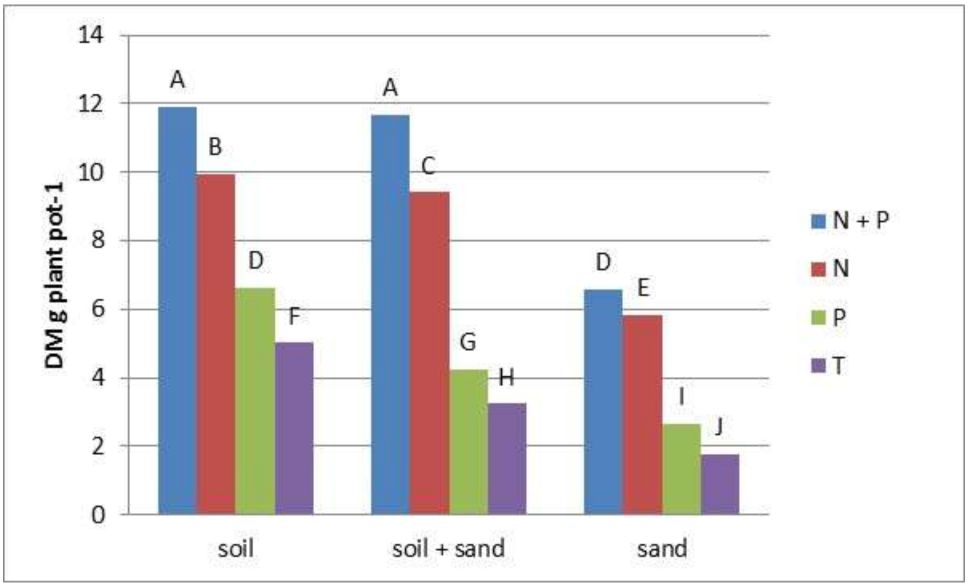

Figure 7. Leaf Dry Matter (G Plant Pot-1) As A Function Of The Soils With Different Sand Proportion By Fertilization Interaction. Different Letters Indicate Significant Differences (P<0.05). Treatment Without Fertilization (T), Treatment Fertilized With Nitrogen (N), Treatment Fertilized With Phosphorus (P) And Treatment Fertilized With Nitrogen Plus Phosphorus (N + P).

The N + P Treatment Yielded The Highest Results Of DM. In The Three Soils With Different Sand Proportion Levels, Followed In Decreasing Order By N, P And T Treatments, With Significant Differences ($P \leq 0.05$). Leaf Dry Matter Was Similar At Soil Or At Soil + Sand Pots When Fertilized With N+P (Figure 7).
Inoculation Had Different Effects On Leaf Dry Matter According To The Considered Levels Of Fertilization (P <0.05) (Figure 8).

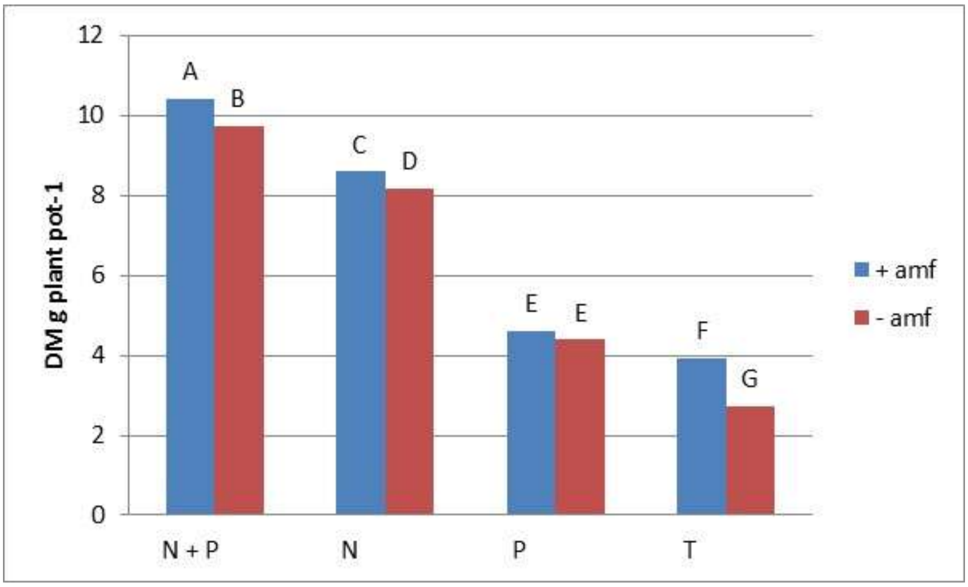

Figure 8. Ms. According To The Interaction Inoculation By Fertilization. Different Letters Indicate Significant Differences (P <0.05). Treatment Inoculated With AMF (+ Amf) And Treatment Not Inoculated With AMF (-

Amf). Treatment Without Fertilization (T), Treatment Fertilized With Nitrogen (N), Treatment Fertilized With Phosphorus (P) And Treatment Fertilized With Nitrogen Plus Phosphorus (N + P).

In The Treatments Without Fertilization, With N Fertilization And Combined N+P Fertilization It Was Observed That In Combination With Inoculation With AMF, The Dry Matter Was Significantly Higher ($P <0.05$) Than In The Non-Inoculated Treatments. In The P Fertilization Treatment There Were No Significant Differences Between Inoculated And Non-Inoculated Plants (Figure 8). Wu Et Al. (2014) Working On Plants Of Citrus *Tangerina, Fortunella Margarita* And *Poncirus Trifoliata* Inoculated With AMF, Found That Colonization With AMF Significantly Improved All Plant Growth And Morphological Traits Of The Roots; In The Mycosphere Of These Citrus Plants, Increases In GRSP And Soil Organic Carbon Were Also Found.

Foliar Phosphorus (P)

Fertilization Presented Double Interactions With The Soils With Different Sand Proportion And Inoculation Treatments On The Leaf P Content ($P <0.05$). The Double Interaction Soils With Different Sand Proportion By Inoculation And The Triple Interaction Were Not Significant ($P>0.05$).

Fertilization Treatments Had Different Significant Effects On Leaf P In Each Soils With Different Sand Proportion ($P <0.05$) (Figure 9).

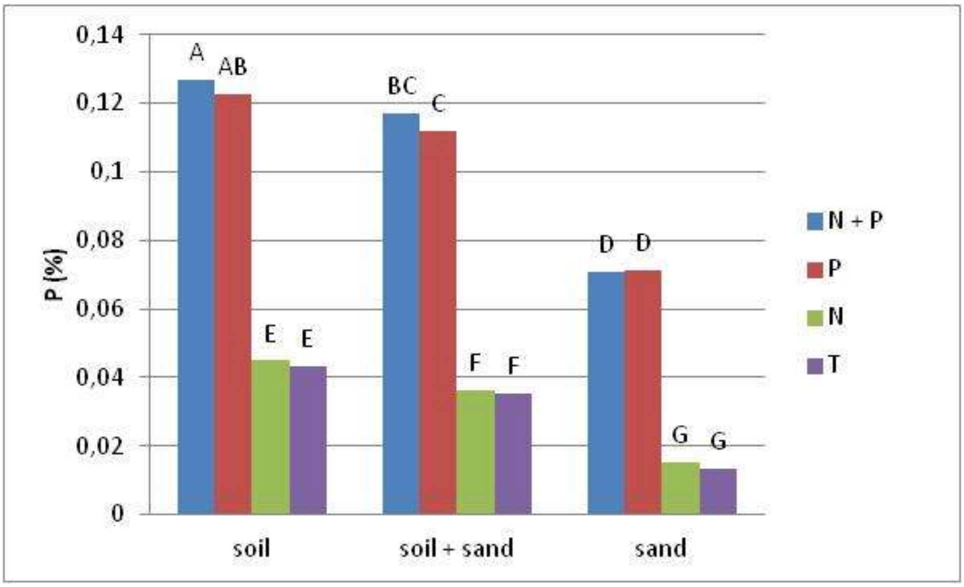

Figure 9. Leaf P Content (%) As Affected By Fertilization At Different Soils With Different Sand Proportion. Different Letters Indicate Significant Differences ($P<0.05$). Treatment Without Fertilization (T), Treatment Fertilized With Nitrogen (N), Treatment Fertilized With Phosphorus (P) And Treatment Fertilized With Nitrogen Plus Phosphorus (N + P).

The Highest Percentages Of Foliar Phosphorus Were Observed For The Three Soils With Different Sand Proportion Levels Considered In The N+P Combined Fertilization Treatment ($P <0.05$) (Figure 9). The Fertilization With P Had Significantly Lower P Contents Compared To The Combined Fertilization Treatment At The Soil And Soil Plus Sand Levels, But Did Not Differ At The Sand Level. The Treatments Without Fertilizing And Fertilizing With Nitrogen Had The Lowest Leaf Phosphorus Values And Differed Significantly Between Each Soil With Different Proportions Of Sand Considered ($P <0.05$) (Figure 12).

The Inoculation Had Different Significant Effects On Leaf P Content According To The Levels Of Fertilization ($P <0.05$), Which Is Shown In Figure 10.

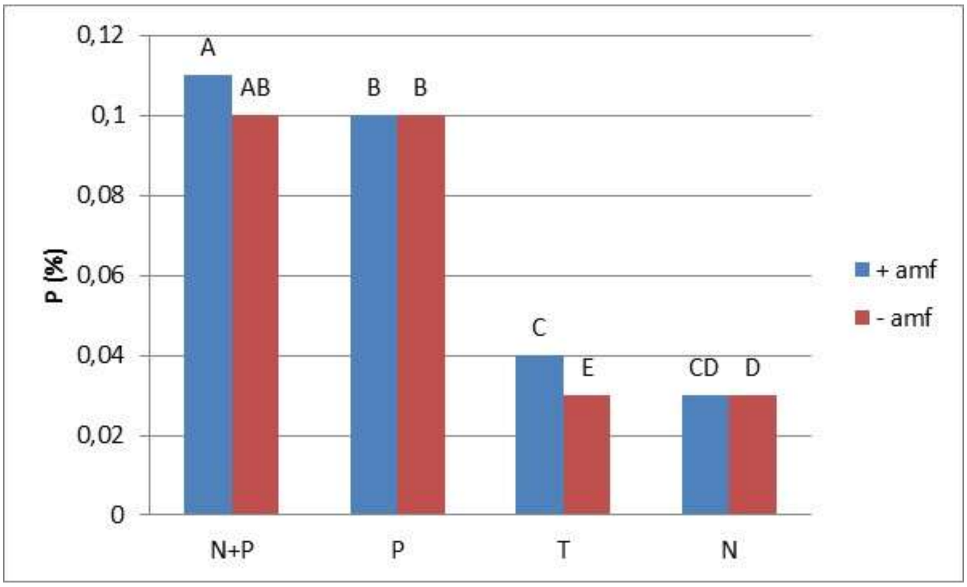

Figure 10. Leaf P Content (%) As Affected By Inoculation At Different Fertilization Treatments. Different Letters Indicate Significant Differences ($P<0.05$). Treatment Inoculated With AMF (+ Amf) And Treatment Not Inoculated With AMF (- Amf). Treatment Without Fertilization (T), Treatment Fertilized With Nitrogen (N), Treatment Fertilized With Phosphorus (P) And Treatment Fertilized With Nitrogen Plus Phosphorus (N + P).

In The Fertilized Treatments No Significant Differences Were Observed Between Inoculated And Non-Inoculated With Respect To Foliar P Content. One Of The Nutrients That Has Been Most Studied In Relation To Its Uptake Mediated By Arbuscular Mycorrhizae Is P, Because Plants Require It In Relatively Large Quantities, But Phosphate Is Also A Quite Immobile Ion Found In Very Low Concentrations In The Soil Solution (Yong -Guan Et Al., 2003); When P Is Available AMF Activity Is Usually Decreased. This Effect Was Also Shown In This Test At N Fertilized Plants.

When No Fertilization Was Added, It Could Be Observed That In The Inoculated Treatment The P Leaf Content Was Significantly Higher ($P <0.05$) Compared To The Non-Inoculated Treatment (Figure 10). In This Test, The Lack Of Response In Foliar Phosphorus Between Inoculated And Non-Inoculated Treatments When Fertilized Could Be Linked To The Availability Of Nutrients From The Application Of Fertilizers.

IV. Conclusions

The Carbon Content Of The Soil Had A High Correlation With The GRSP Content (R^2 0.93). This Result Indicates That GRSP Could Be Considered A Biological Indicator Of Soil, With The Accumulation Of Soil Carbon Simultaneously With GRSP Enrichment.

The Effect Of The Inoculation With AMF Resulted In Increases Of GRSP, Which Was More Relevant At The Sand Proportion, Possibly Due To The Lack Of Native Inoculum.

The GRSP / Cox Ratio Was Significantly Higher In The Unfertilized Treatment With Respect To The Fertilized Treatments (Nitrogen, Phosphorus And Nitrogen Plus Phosphorus), Showing Less AMF Symbiotic Activity In The Fertilized Situations Due To A Higher Availability Of Nutrients.

The Amount Of Dry Matter And The Concentration Of Foliar Phosphorus Increased With The Agronomic Practice Of Inoculation When Fertilizer Was Not Applied, But With Nitrogen And / Or Phosphorus Showed That The Differences In These Variables Between Inoculated And Non-Inoculated Treatments Were Diluted, Evidencing The Greater Activity Of AMF Inoculated In Nutrient Poor Soils. However, The Glomalin Synthesis Was Not A Sensitive Indicator Regarding The DM And Leaf P Variables. Considering That There Are Properties Of The Soils Whose Expression Is Evident Only In Prolonged Periods Of Time, The Absence Of Marked Effects In The Case Of Leaf DM And P Could Be Explained By The Short Period Of The Trial, Which Would Not Allow The Expression Of Direct Effects On These Variables.

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