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Review
INFLUENCE OF MYCORRHIZAL INOCULATION ON GREEN MANURES. EFFECT ON THE MAIN CROP. A CASE STUDY: CORN

Reseña bibliográfica
Influencia de la inoculación micorrízica en los abonos verdes. Efecto sobre el cultivo principal. Estudio de caso: el maíz

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ABSTRACT. The value of the green manures as nitrogen supply has been recognized during centuries by farmers. Another benefit associated to the use of these plant types is that they increase the activity and diversity of the soil microorganisms, such as the arbuscular mycorrhizical fungus. In the present bibliographical revision it summarizes some results obtained in the world and in Cuba in relation to the definition and importance of green manures, the evaluation of the use of nitrogen and the substitution of other nitrogen sources by these plants in the agricultural systems. Likewise, characteristics and benefits of the arbuscular mycorrhizical fungus (AMF) are approached, their function in the plant nutrition, factors that influence in this efficiency, the effect of the symbiosis establish in the optimization of the nutritious supply. Lastly, some considerations are offered about the mycorrhizical inoculation and rotation with green manures and some results obtained with corn. The use of these two nutritional alternatives for plants is inevitable, given the importance of the environment conservation, to obtain healthy foods and the higher price of fertilizers in the world market.

Key words: mycorrhizae, plant nutrition

RESUMEN. El valor de los abonos verdes como vía para el suministro de nitrógeno ha sido reconocido durante siglos por los agricultores. Otro beneficio asociado a la utilización de este tipo de plantas es que incrementan la actividad y diversidad de los microorganismos del suelo, como los hongos micorrízicos arbusculares (HMA). En la presente reseña bibliográfica se resumen algunos resultados obtenidos en el mundo y en Cuba en relación con la definición e importancia de los abonos verdes, la evaluación del aprovechamiento del nitrógeno y la sustitución de otras fuentes nitrogenadas por estos cultivos en los sistemas agrícolas. Así mismo, se abordan las características y beneficios de los HMA, su papel en la nutrición vegetal, los factores que influyen en su eficiencia y el efecto del establecimiento de la simbiosis sobre la optimización del suministro de nutrientes. Por último, se brindan algunas consideraciones acerca de la inoculación micorrízica y rotación con abonos verdes, y algunos resultados con el maíz. El empleo de alternativas nutricionales para los cultivos, como las dos mencionadas en este trabajo, es una necesidad impostergable, dada la importancia de la conservación del medio ambiente, obtener alimentos saludables y el precio cada vez más alto de los fertilizantes en el mercado mundial.

Palabras clave: micorrizas, nutrición de las plantas

INTRODUCTION

Adopting agroecological crop techniques is aimed at reducing external input dependence and promoting the biological processes of nitrogen (N) fixation and nutrient cycle. By using green manures, soil erosion control measures are favored and weed plant occurrence decreases (1).

Thus, the value of green manures as a way to supply nitrogen has been recognized by farmers for centuries. This effect mainly consists of N supplied by legumes in symbiosis with

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Rhizobium bacteria through biological nitrogen fixation (BNF) and subsequent N mineralization in the soil, which reduces crop requirements of nitrogen fertilizers (2).

Another benefit associated with green manures is that they increase soil microorganism activity and diversity, like N-fixing and arbuscular mycorrhizal fungi (AMF).

Arbuscular mycorrhizal fungi are obligatory biotrophic organisms, which are associated with roots from terrestrial vascular plants, epiphytes, aquatics, also with rhizoids and bryophyte thallus and other basal vegetables, forming a mutual symbiotic relationship called arbuscular mycorrhiza and mycothalia for vegetables with roots and without them, respectively. In recent decades, great attention has been paid due to their role in supplying nutrients to plants (3).

Mycorrhizal effectiveness is a symbiont that is able to positively influence plant growth, increasing propagule number in the soil or improving nutrient transfer to plants (4). By inoculating with efficient AMF strains, fertilization becomes more effective, reducing fertilizer doses to be applied to mycorrhized plants and, thus, decreasing contamination effects of soils and waters (5).

Inherent to the use of green manures is their association with native AMF populations, to provoke qualitative and quantitative changes in soil fungal population and the subsequent crop colonization[A] (6), although such mycorrhization does not usually become fully effective and crops respond to inoculation with efficient AMF strains (7).

In Cuba, there are some recent studies approaching both the effect of different green manure species on AMF propagule multiplication in the soil and the establishment of subsequent crop symbiosis (8, 9, 10, 11), as well as mycorrhizal inoculation of legume species, which consequently encourages not only plant growth and development but also a direct benefit on the main crop grown in sequence (12).

Indeed, one of the challenges in sustainable agriculture is to assure an adequate nutrient supply to secure high yields, so that the use of green manures, their inoculation with efficient strains and integration with the effective management of mycorrhizal symbiosis are remarkable for their facilities and benefits. This review summarizes some results related to these aspects obtained worldwide and in Cuba.

GREEN MANURES. DEFINITION AND SIGNIFICANCE

Green manures constitute an agronomic practice that consists of incorporating a non-decomposed vegetal mass of cultivated plants, with the aim of improving nutrient availability and soil properties. At present, the definition of plants grown to cover the soil, protect it from erosion, control weeds and use them as animal and human food has been expanded.

Legumes have great value as green manures, because they provide N through BNF process associated with *Rhizobium* bacteria, although other fast-growing species with good green mass production can be cultivated, such as grasses and the combinations of some of them as a mixture (13).

The functions of green manures are associated with soil protection against erosion and reduction of temperature and water evaporation. They improve physical, chemical and biological soil properties, increase soil organic matter content as well as nutrient supply, recycling and mobilization; they also help the natural control against nematodes, pests, diseases and weeds[B].

Among the main benefits of green manures are the reduced use of herbicides and pesticides, increased crop yield and quality, preserved soil moisture and organic matter, decreased fertilization costs and increased seed sale inputs[B] (14).

The selection of species and how to use green manures must meet certain requirements, as to adopt the production system used by the farmer, with low cost of implementation and driving, to be resistant against pests and diseases, to have rapid growth, to produce a favorable effect for increasing crop yields and to have higher returns than the traditional system (15).

[A] Sánchez, C. Manejo de las asociaciones micorrízicas arbusculares y abonos verdes en la producción de posturas de cafeto en algunos tipos de suelo. Tesis de Doctorado, INCA, La Habana, Cuba, 2001, 105 p.

[B] Florentín, M. A.; Peñalva, M.; Calegari, A. y Derpsch, R. Abonos verdes y rotación de cultivos en siembra directa. Pequeñas propiedades. (ser. Conservación de suelos), Inst. MAG–GTZ San Lorenzo, Paraguay, 2001, 84 p.

The main constraint in adopting green manures is that, according to some growers, to produce enough green mass, they should receive nutrients and cultural care while the occupied area is not producing food during this stage. This is a misconception of their use. The correct analysis should be that by improving the soil, higher yields can be obtained, fertilizers are saved and the main natural soil resource available is preserved[C].

Other limitations of using green manures on a large scale are the problems associated with high seed cost and the difficulty of reaching a synchrony between plant nutrient release and the main crop demand (16).

Among the practical options is to plant them in succession or bands inside cultivated fields, or in association with short-cycle or perennial crops. If they are inserted within species present in grasslands, they will fulfill dual roles as soil improvers and as animal feeding[B].

Therefore, to adopt green manures, growers should observe that they can get more than a benefit of this crop. Hence, farmers' innovation is encouraged[D].

INFLUENCE ON PHYSICAL AND CHEMICAL SOIL CHARACTERISTICS

The application of green manures improves soil properties, although this effect is not always quantifiable in the short term on tropical areas, because plants rapidly breakdown under high temperature and moisture conditions (17).

Physical effects

It was shown that green manures/cover crops have a direct influence on physical soil characteristics, because they add organic matter and increase root proliferation. The most influenced physical characteristics by green manures/cover crops are: aggregation, water-holding capacity, density, infiltration and ventilation rates (18). These effects depend on the quality, quantity and type of management provided to the added material, as well as climatic factors and soil properties.

Biological means is the best way to loosen soils. It is the presence of aggressive root systems within its growing process that activate microorganisms, create spaces between particles, make porous aggregates, become into preferential paths of other root systems, improve air and water dynamics in the soil.

Table I shows the results of evaluating bulk density of an Eutric Rhodic Nitisol at the beginning of the experiment and after planting the green manure species *Canavalia ensiformis*, grown for nine months[E].

At first, soil compaction was observed at 20 cm deep, with values of 1,28 up to 1,40 mg m^{-3}. When green manures were planted, the values of soil compaction decreased up to 70 cm deep and the authors reported that it could have been because *Canavalia ensiformis* taproot reaches 50-60 cm deep, so having a very strong biological decompacting tillage effect.

Several studies have shown that by incorporating green manures/cover crops to the soil, significant increases in aggregate number and size are achieved under different ecosystems. In this sense, when using *Vicia villosa*-corn (*Zea mays*) rotation, this association was the most effective in stabilizing aggregates, which was observed in a higher corn productivity (24).

In this regard, it is reported that rye (*Secale cereale*) root and stalk decomposition and the substances released from this process effectively contribute to soil nutrient cycling, aggregate formation and its stabilization. The latter are important in keeping soil structure and productivity, since they improve soil characteristics, such as moisture retention, gas diffusion, hydraulic conductivity and erodibility reduction (25).

Biological soil preparation through roots of green manures/cover crops helps its aggregate formation[F].

[C] Martín, G. M. Manejo de la inoculación micorrízica arbuscular, la *Canavalia ensiformis* y la fertilización nitrogenada en plantas de maíz (*Zea mays*) cultivadas sobre suelos Ferralíticos Rojos de La Habana. Tesis de Doctorado, Universidad Agraria de la Habana, La Habana, Cuba, 2009, 101 p.

[D] CIDICCO. Catálogo de abonos verdes/cultivos de cobertura (AVCC) empleados por pequeños productores de los trópicos. Informe Final del Proyecto 2000 FS 125 "Catálogo de sistemas de cultivos de cobertura y abonos verdes (CCAV) empleados por pequeños agricultores de los trópicos, Inst. CIDICCO, Honduras, 2003, 17 p.

[E] Borges, M. Influencia de la *Canavalia ensiformis* (L) en algunas propiedades de un suelo Ferralítico Rojo. Tesis de Maestría, Universidad Agraria de La Habana, La Habana, Cuba, 2009, 60 p.

[F] Vallejos, F.; Kliever, I.; Florentín, M. A.; Casaccia, J.; Calegari, A. y Derpsch, R. Abonos verdes y rotación de cultivos en siembra directa. Sistemas de producción tractorizados. (ser. conservación de suelos), Inst. MAG–GTZ San Lorenzo, Paraguay, 2001, p. 92.

Table I. Behavior of bulk density (mg m^{-3}) in an Eutric Rhodic Nitisol at the beginning of the experiment and nine months after planting *Canavalia ensiformis*[E]

Treatmens	Depth (cm)				
	0-10	10-20	20-40	40-70	70-100
Control (beginning of the experiment)	1,23	1,25	1,28	1,35	1,40
Canavalia ensiformis	0,94	1,04	1,18	1,22	1,26
Canavalia + AMF	1,03	1,14	1,23	1,20	1,27
Canavalia + cattle manure	1,00	1,10	1,20	1,18	1,30
Canavalia + AMF + cattle manure	1,06	1,11	1,23	1,21	1,28

AMF: arbuscular mycorrhizal fungi, *Glomus* genre

For example: in Paraguay, the use of forage turnip (*Raphanus sativus* var *oleiferus*) with deep taproots capable of breaking soil compact layers; after its decomposition, these roots leave channels favoring water infiltration and subsequent crop root penetration[B].

Other studies have shown the positive influence of *Crotalaria juncea* and *Sesbania rostrata* species on organic matter, natural moisture, soil aggregate distribution and stability. The most effective behavior of these two species in soil improvement compared to other species evaluated was in correspondence with the high volume of green and dry biomass incorporated by these species, as well as the quality of this material (high C: N rate). These high volumes caused significant soil organic matter variation, which also improved water-holding capacity and physical soil properties related to the most agronomically favorable aggregate percentage (21).

Furthermore, the use of green manures/cover crops prevents the direct impact of raindrops on the soil, avoiding its breakdown and surface sealing formation, increasing infiltration and preventing erosion; besides reducing water runoff speed and decreasing soil particle drag[F].

CHEMICAL EFFECTS

Green manures/cover crops while growing and their shoot/root residues promote important effects on chemical soil fertility. The main chemical effects expected when using green manures/cover crops are[F]:

- Increase soil nutrient content and availability due to a high organic acid formation that favors weathering process of soil minerals and improves less-available nutrient solubilization, mainly phosphorus. They also increase nitrogen content by biological fixation of legumes used as green manures as well as the recycling of leaching elements (nitrogen, calcium, magnesium, potassium, etc.) through biomass of species with deep roots.
- Neutralize toxic elements such as aluminum by forming organic complexes.
- Improves nutrient absorption capacity and storage by accumulating organic matter.
- Improves nutrient distribution in soil profile, favoring calcium and magnesium transport and nutrient release, mainly phosphorus, during root system decomposition.
- Raise soil pH by mineralizing organic anions to CO_2 and H_2O.

The influence of green manures on soil N content is attributed to the supply of N derived from BNF, if legumes are used, which increase its availability to subsequent crops and make nutrient balance positive in the soil-plant system[G].

With the rapid legume mineralization in the soil, significant quantities of N and other elements are released that favor plant growth and the non-used fraction stays as a residual effect on subsequent crops (22).

In Philippines, eight legume species were evaluated as nitrogen fertilization substitutes and, in two years, *Sesbania rostrata* and *Crotalaria juncea* accumulated excessive N compared to rice requirements; also, they increased soil organic carbon and total nitrogen (23).

In addition, green manures reduce P adsorption effect associated with increased soil organic matter content, leading to complex formation that block adsorption sites on Fe and Al oxide and hydroxide surface (24).

[G] Pozzi, C. Estudo de sistemas de uso do solo en rotações de culturas en sistemas agricolas brasileiros: dinâmica de nitrogênio e carbono no sistema solo – planta – atmosfera. Tesis de Doutorado, Universidad Federal Rural do Rio de Janeiro, Rio de Janeiro, Brasil, 2005, 120 p.

It was proved that legume root system easily extracts poorly-soluble nutrient elements, especially phosphorus. Among the most efficient plants on this concern are *Lupinus albus*, *Fagopyrun esculentun*, *Eucalyptus gummifera*, *Brassica napus*, *Cajanus cajan* and *Arachis hypogaea* (25).

Regarding this subject, the interest in using available P-mobilizing plants as green manure is renewed, so as to increase its availability in the main crop. These plant species, as *Tithonia diversifolia*, are capable of obtaining less-labile soil phosphates to be accumulated in its biomass, even at higher levels than their own needs or P requirements (26).

Some of these species, under deficit P conditions of soils with neutral or slightly alkaline pH, can be self-supplied by the element and leave a fraction available to other crops, due to its root exudates that decrease pH in rhizosphere and mobilize P present in this soil zone (27).

A beneficial effect of green manures is nutrient recycling if using plants that expand their root systems to deep soil horizons and absorb nutrients from these layers. After cutting plants and their mineralization, there is a gradual release of available nutrients for topsoil crops (24).

In Cuba, studies performed about the influence of green manures on some chemical soil properties have found a positive effect of these plants on exchangeable cations and profitable P contents in the soil soil[H].

Regarding soil K content, species with the greatest increases were *Mucuna aterrimum*, *Canavalia ensiformis, Dolichos lablab* and *Crotalaria juncea*. Green manures also increased soil profitable contents of Ca, Mg, and P (Table II).

Taking this into account, it is stated that P coming from green manure/cover crop decomposition or from soil organic constitutes a significant amount that gradually feeds soil solution phosphorus, increasing plant absorbing efficiency. In addition, green manures/cover crops recycle lot of potassium through their biomass, like calcium and magnesium, so that they help maintain satisfactory levels of these available nutrients in plants[F].

EVALUATING THE USE OF GREEN MANURE NITROGEN

The efficient use of nutrients has gained more attention with greater fertilization costs and the continuous worry about environmental impact (28). Several methodologies have been developed to quantify nutrient profitability. Among them, the method of differences is based on the assumption that soil nutrient amount absorbed by the plant is independent of the nutrient amount applied.

The method of differences is performed by means of assessing N absorption in two plots: one of them without N fertilizer (or organic nitrogen source) to estimate N amount provided by the soil, and another plot, where organic manure or fertilizer is added, to estimate N amount absorbed by the plant from the soil and the added source, and by calculating the difference of N absorbed by plants in the plot with added nutrients minus the plot without this application, then estimating N amount from the applied source absorbed by the crop (28).

It is a very representative method, easily applied under experimental plots or production areas conditions to quickly calculate the effects of applying nutrients. However, its disadvantage is that in experiments with fertilizer response, plants from fertilized plots take more soil N than unfertilized ones (primming effect), which is attributed to a source effect of N applied on its soil availability (29).

Fertilized plants grow more vigorous, develop better roots and explore a larger soil volume (36). Green manures seem to cause primming effect, releasing soil N resulting from its incorporation (31).

The efficiency of incorporated N with green manures also depends on the volume and quality of incorporated material, allelopathic effects, weed and pathogen control, known as a whole as rotation effect (32).

[H] García, M. Contribución al estudio y utilización de los abonos verdes en cultivos económicos desarrollados sobre un suelo Ferralítico Rojo de la Habana. Tesis de Doctorado, INCA, La Habana, Cuba, 1997, 100 p.

Table II. Effect of different green manure species on the chemical characteristics of a Red Ferralitic soil

Treatment	Exchangeable cations (cmol 100 g^{-1})			P (mg kg^{-1})	pH	MO (%)
	K	Ca	Mg			
Control	0,42	11,8	2,55	255	6,77	2,31
Crotalaria juncea	0,56	12,9	3,55	297	6,87	2,25
Sesbania rostrata	0,45	12,25	3,37	269	6,95	2,15
Mucuna aterrimum	0,64	12,3	3,9	279	6,6	2,31
Canavalia ensiformis	0,67	12,1	2,92	272	6,45	2,17
Sorghum vulgare	0,49	12	3,5	269	6,56	2,32

Adapted to García (1997)[D]

USING ^{15}N ISOTOPE IN GREEN MANURE STUDIES

^{15}N is used in agricultural research studies for BNF quantification, efficiency and profitability of N sources and its dynamics in the soil, water and plants (22).

In studies about N dynamics, it is difficult to distinguish its origin. The use of ^{15}N can accurately estimate its routes in the system (33). It is a very precise method; however, its use is expensive and requires special equipment and conditions, so that it is generally used under controlled environments, which are not representative of production conditions.

The isotopic dilution method is based on isotopic composition differences of various available N sources for plant growth. Thus, it is possible to study and quantify plant absorption irrespective of the nutrient source employed (29).

To determine N fertilization efficiency, two closely related parameters are employed: percentage of N derived from fertilizer (% NddF), which is an isotopic quantification independent of yield and sensitive to detect differences between fertilization treatments. The second one is N recovery efficiency of the fertilizer (ERNF), which depends on yield and is based on mass balance, obtained by chain calculation and, therefore, subjected to a greater experimental error (22).

The results from applying the method of differences are more similar to those obtained by the isotopic method, to the extent that the soil has lower contents of organic matter and total N[I].

In some investigations made to evaluate green manure N profitability, either by the isotopic method or that of differences, it has been shown that in terms of nutrient supply, *Crotalaria juncea* and *Mucuna pruriens* provided between 25 and 38 % of total N absorbed to rice crop (*Oryza sativa*) (31).

Meanwhile, other authors have found that from 5 to 17 % N is recovered by subsequent wheat crop, from 7 to 12 % N is lost by leaching or volatilization and from 50 to 78 % is retained in the soil (34).

In some studies conducted on rice crop in Cuba using isotopic techniques, through ^{15}N analysis, it was obtained that green manure *Sesbania rostrata* did not contribute directly to the total N content of rice plants, at least within its first cycle, despite having encouraged significantly N accumulation by the crop, which always presented similar excess ^{15}N proportion (similar isotopic dilution) with green manure incorporated and without it. The low availability of N derived from green manures could be associated with the low rate of organic N mineralization, due to the reducing conditions existing as a result of a rapid soil flooding after plant incorporation; in addition, green manure effect on these conditions suggests that this material stimulated microbial activity, causing a proportional priming effect to native N and fertilizer availability in the soil (35).

Several authors have studied green manure effects on maize (*Zea mays*) nutrition and found profitability coefficients ranging between 30 and 72 %. The causes of low efficiency could be leaching, denitrification, volatilization and N immobilization by soil microorganisms (22, 36).

Results obtained in Cuba have reported profitability coefficients of N derived from green manures ranging from 20 to 50 %, depending on green manure species evaluated, or its combination with supplemental

[I] Álvarez, M. Los abonos verdes: una alternativa para la producción sostenible de maíz en las condiciones de los suelos Ferralíticos Rojos de la Habana. Tesis de Maestría, INCA-UNAH, La Habana, Cuba, 2000, 69 p.

doses of mineral fertilizer, which can accelerate mineralization process of incorporated green manures and increase N profitability coefficient (37).

REPLACING OTHER N SOURCES BY GREEN MANURES IN AGRICULTURAL SYSTEMS

The replacement of mineral fertilizers by organic ones has been limited by the need to be produced in the same place where they are going to be used (38). In this sense, green manures have certain advantages over other organic fertilizers.

In India, it has been indicated that by adding *Sesbania rostrata* to rice (*Oryza sativa*), 50 % N fertilizer can be replaced (39, 40). Crop yields after incorporating 20 t ha^{-1} *Vigna unguiculata* as green manure were higher than the control without N. The combined treatment of vigna plus 60 kg N ha^{-1} mineral fertilizer yielded twice rice than the control and 24 % higher than the treatment with the optimum dose of 120 kg ha^{-1} N (47).

Some studies performed on rice crop in Cuba has found that by applying 50 % of the optimal mineral fertilizer dose together with *Sesbania rostrata*, agricultural yields increased more than 1 t ha^{-1} of paddy rice for two consecutive harvests.

When studying N profitability coefficient of green manures, determined by the isotopic method, 42 % N-*Mucuna pruriens* for rice and 20 % N-*Crotalaria juncea* for corn were recorded. By combining N fertilizers and green manures, the effect was additive with increased yields surpassing the treatment of the highest mineral fertilizer dose (31).

The contribution of green manures to corn yield was equivalent to the application of 97, 91, 89 and 78 kg N ha^{-1} for *Mucuna pruriens*, *Crotalaria juncea*, *Cajanus cajan* and *Arachys hipogaea* respectively (42). Corn had a better response to increase yields and leaf N and P contents in subsequent crops with legumes used as green manures, compared to subsequent crops with grasses (43).

With regard to corn, the combined use of *Canavalia ensiformis* with mycorrhizal inoculation of the main crop allowed the crop to reach similar dry matter yields to those obtained by the production control, under microplot conditions, with 50 % reduction of mineral fertilizer dose applied (44).

Concerning potato crop (*Solanum tuberosum)* in Cuba, Canavalia replaced up to 50 % its N fertilizer needs. The highest yields were obtained by combining canavalia and 40 kg ha^{-1} N to replace up to 75 % the recommended dose of mineral fertilizer (38).

The combined use of green manures and mineral fertilizers promoted higher nutrient quantities with reduced soil potential acidity and increased Ca and Mg contents in the aerial part of sugarcane plants (*Saccharum* spp.) (33).

In China, with an intensive cultural system, it was determined that employing cover crops as corn (*Zea mays*), chrysanthemum (*Chrysanthemum seguetum*) and edible amaranth (*Amaranthus mangostanus*) in rotation with cucumber (*Cucumis sativus*), the risk of losing N is reduced whereas P and K availability is enhanced. In addition, both soil microbial biomass population and diversity increase, which helps obtain higher yields in the main crop (45).

In Chile, using cover crop mixture of *Trifolium subterraneum*, *Medicago polymorpha* and *T. michelianum* in grape crop (*Vitis vinifera* L.) cv. Cabernet Sauvignon, with low fertility soils, there were clear advantages compared to traditional fertilization, in terms of improving crop N nutrition. The study showed that green manures have a positive impact on dry matter and N content increases, due to the high N contribution of legumes, like that of N fertilization. Through isotopic techniques, it was proved that about 20 % total N accumulated by the crop came from legumes (46).

In Brazil, when studying different green manures in subsequent crops with horticultural crops, it was found that this culture system allowed to increase lettuce (*Lactuca sativa*) and carrot (*Daucus carota*) productivity, with fresh and dry mass increases of both horticultural species, yielding more than 200 g fresh mass per plant, highlighting rotations with *Canavalia ensiformis* and the mixture of *Mucuna aterrimum* and *Zea mays*, which surpassed the production obtained in presence of *Cajanus cajan*, cow dung and organic manure type Bokashi (de Almeida, 2009)[J].

[J] de Almeida, K. Adubos verdes na producao de alface e cenoura, sob sitema organico. Tese de Doutorado, Universidade Estadual Paulista, Faculdade de Ciencias Agroomicas, Brasil, 2009. 114 p.

ARBUSCULAR MYCORRHIZAL FUNGI (AMF)

Mycorrhizae are mutual symbiotic associations existing between certain soil fungi and higher plant roots. Fungi are benefited by carbonated sources derived from the plant and, in turn, it is benefited by a deeper soil exploration, so increasing water absorption capacity, mineral nutrients, plant growth and its development[A].

FEATURES AND BENEFITS

Among the features and benefits that mycorrhizae provide to plants are a higher water and nutrient absorption capacity, which allows the host to better support adverse soil and climate conditions, to improve biomass and crop production as well as enable to form stable soil aggregates (6, 47, 48).

Three types of mycorrhizal associations have been defined considering their morphoanatomical and ultrastructural characteristics: Ectomycorrhizae, Ectendomycorrhizae and Endomycorrhizae. The latter are not visibly detected; they form an outer hypha network and penetrate inside cortical cells without colonizing the endoderm. It is the most widespread group in the planet and is divided into several subtypes, arbuscular being the most representative and the most important group in tropical ecosystems.

The extensive extra-radical hypha network plays an important role in soil conservation, improving soil aggregation. Organic compound production by AMF hyphae and other microorganisms in myco-rhizosphere can help join micro-aggregates and macro-aggregates together (49).

AMF are spread through spores, mycelia and colonized root fragments, which constitute propagules as a whole and colonize host plant roots to develop and give rise to new propagules (50). Most propagules in the soil are located at the first 15 to 20 cm deep, directly related to aeration and organic matter content (51).

Mycorrhizal symbiosis in short-cycle crops develops sequentially, passing through different growth stages, either microbial (latency, exponential, stabilization or plateau and death or full sporulation) or vegetable, according to plant host phenological phases. At the onset of plant fruiting, there are always higher mycelium and colonization percentage values whereas lower spore values than at harvest end, which makes evident a close relationship with carbon supplied towards roots (52).

AMF ROLE IN PLANT NUTRITION

Plants inoculated with effective AMF species causes a marked increase in nutrient absorption and translocation processes, either by interception, diffusion or mass flow.

This benefit is complex and can result from various mechanisms, such as a greater soil exploring surface, higher root absorbing capacity, non-accessible nutrients taken by non-mycorrhized roots, benefits of other microorganisms in the rhizosphere, amortization of adverse soil pH effects, aluminum, manganese, other heavy metals, salinity, water stress and pathogen attack (53, 54, 55).

The main N absorption mechanism is by mass flow, which transfers soil solution through water potential gradient regulated by transpiration (56).

A greater N absorption as NH_4^+ and NO_3^- is provided by AMF in effective symbiosis with the host and causes the uptake of necessary nutrients by plant growth stimulation. Under low level conditions, AMF permits less-available nutrient absorption (57).

AMF play a vital role in taking phosphorus from the soil, mainly in tropical areas where available P amounts are low. The mechanism to improve absorption through AMF takes place from the ability to explore a larger volume of substrate and by increasing root absorbing capacity (interception) or diffusion, which is nutrient transport throughout a concentration gradient (57).

Moreover, mycorrhizae can accelerate unavailable P absorption by the interaction between root exudates of some plant species that are capable of solubilizing P (25).

There are high concentrations of K and Mg in both mycorrhized and non-mycorrhized plants. They move easier than P in soil solution. In some cases, a higher nutrient absorption coincides with an indirect effect to remove P deficiencies (52), although some experimental works have suggested that AMF role to obtain

K represents an important way for plants to get it[G].

FACTORS AFFECTING AMF EFFICIENCY

Several biotic and abiotic factors influence the establishment and functioning of mycorrhizal symbiosis.

Light: in areas with high solar radiation, colonization levels are generally high; however, if it weakens, plant response to this symbiosis decreases. High colonization depends, among other factors, on a higher photosynthetic rate, which implies a greater production and metabolite exchange (52).

Temperature: AMF are formed under temperature ranges of 18 to 40 °C, the optimum being for most species, close to 30 °C. The influence of temperature is variable on AMF-mycorrhized plants and it is related with the right combination of fungal species-host and plant-developing phase (54).

pH: AMF response to pH has been studied due to its effect on plant productivity, the direct effect on endophyte and host plant physiology as well as the indirect effect through changes in base-changing capacity (BCC). Different AMF species tolerate diverse pH ranges (58).

On the other hand, pH effect must be considered in selection studies of AMF species with high symbiotic efficiency, either on association productivity or on fungal reproduction mechanisms (52). Some AMF species are not adapted to different pH conditions from those of the native soil where they were isolated; thus, pH is essential in establishing AMF species per soil type (54).

Seasonality: in geographical regions where it is possible to contrast at least two climatic seasons, such as rainy and dry weather, diverse native AMF species and its functionality may vary (51). External factors as seasonality and management influence AMF spreading and may affect symbiosis under field conditions (59).

Different studies have demonstrated the influence of soil type on mycorrhizal functioning and seasonality, because in poorly-drained soils with high moisture retention, the highest colonization percentages are recorded during the dry season (60) while in well-drained soils, the highest colonization percentages belong to the rainy season (61, 62, 63).

Soil cover: as AMF are obligatory symbionts, their distribution in cultivated soils is strongly influenced by vegetation (6).

In fallow land, natural plant mycorrhization is poorly developed and there is no correlation between spore population and fallow duration (64); however, AMF spore number increased significantly with higher number of weed species (diversity) present in fallow land (65).

If the soil is kept naked during its preparation, hypha viability sharply decreases by the absence of host plants or if non-mycotrophic plants are introduced at crop rotation scheme (49).

When analyzing the frequency of appearance of native AMF spores per cover type, it was found that agroforestry systems, followed by natural forest, showed the highest mean root colonization, over 30 %. By contrast, plot covers, monocultures and grasslands recorded the lowest root colonization percentage values (51).

In other studies, these authors demonstrated that native arbuscular mycorrhizal symbiosis was more effective in ecosystems with highly heterogeneous covers; thus, it is very important to promote agro-bio-diverse agricultural production systems (66).

Mycorrhizal plant dependence (MD): it comes from the degree of relationship existing between the plant and the fungus to obtain maximum productivity at a given soil fertility level and it is an intrinsic plant property (4, 53).

MD calculation can not be extrapolated to natural conditions where native mycorrhization develops (52). Nutrient requirements and a low absorption capacity, if there is no mycorrhiza, are correlated with high MD, which is not equal to a high crop response to inoculation (47).

Plants can be grouped according to MD degree as follows: obligatory mycorrhized plants present a much reduced growth in the absence of AMF symbiosis and colonization rates are above 60 %; facultative mycorrhized plants have a more profuse and developed root system, although under adverse soil conditions they respond to mycorrhization. Colonization rates are below 50 %; non-mycorrhized plants do not form any association (4).

Mycorrhizal effectiveness: it is the ability of an endophyte to positively influence plant growth, increase propagule number or improve nutrient transfer. It results from the physiological interaction between symbionts (4).

It is determined by the type of mycorrhizogenous fungus, host plant, symbiotic interface and specific type of soil or substrate, its degree of fertility and water availability (39). The main form to quantify mycorrhizal effectiveness is by evaluating host plant response during its growth (4).

Sometimes native strains do not cause the highest effectiveness, which may be related to a low concentration of native propagules, or because they have a higher adaptability and possible microbial functionality; however, it does not always mean a greater mycorrhizal efficiency (52, 67).

Soil-strain specificity: the positive response to AMF inoculation depends on three factors: inoculated species, amount of mycorrhizal propagules, soil type and its fertility. The latter defines the efficient species for an edaphic-climatic condition, although the effectiveness reached by inoculation depends on the management given to the plant and soil (67).

One of the most important results found in Cuba was that, irrespective of all crops studied, there was a highly efficient AMF strain for each soil, obtaining the largest responses with such inoculation. Therefore, there was a high soil-efficient strain specificity, whose effect was completely reproducible along different years of experimental repetitions[A].

Undoubtedly, this is a positive element of high impact to manage mycorrhizal associations in crops, since it enables to select efficient strains for a given condition.

Most AMF do not have a high specificity with the host, which is one of the bases for managing crop sequence inoculation. Selectivity usually occurs between AMF population species and the plant as influenced by edaphic conditions (50, 68).

Glomus species have a wide range of functional distribution predominating in high and mid-fertility ecosystems, which are extremely efficient and competitive. Results obtained in Cuba allowed extending that range to low fertility conditions and establish that *Glomus cubense* species has the best results in Red Ferralitic soils, when studying strain effectiveness to inoculate different crops (67, 69, 70).

By analyzing data collected out of more than 100 experiments conducted with different crops in soils of high and low input agriculture, it was observed that plant response to different AMF strain inoculation in soils with low input agriculture was related to properties associated with soil types. *Glomus fasciculatum-like* and *G. etunicatum-like* strains had a high efficiency in relatively rich soils in nutrients and organic matter; *Paraglomus occultum* and *G. mosseae-like* behaved better in relatively poor soils in nutrients whereas *G. mosseae* and *G. manihotis* in mid-fertility soils (71).

They did not find any significant relationship between plant response to AMF strain inoculation and soil properties where high-input agriculture was implemented, probably due to changes induced by different host plant species and to soil properties modified by the history of intensive production. They concluded that knowing the behavior of AMF strains under certain environments could be the key to the efficient management of mycorrhizal symbiosis, through inoculation, in agro-ecosystems.

Nutrient availability: it depends on soil type and nutrient supply needed to supplement crop requirements. The lowest inoculation effects are obtained with efficient strains and very high nutrient availability whereas the greatest effectiveness is achieved with average availability. If this is low or zero, symbiosis does not work properly, so that plants show less growth and lower inoculation effectiveness (47, 72, 73).

It is estimated that the association between AMF and the host consumes between 5 and 10 % of photosynthetic products; such cost will be compensated if the plant is under suboptimal nutrient supply conditions (53).

For an efficient symbiosis, nutrient availability in the system should be lower than the commonly used for non-mycorrhized plants. Obtaining plants with optimal growth in the presence of low nutrient amounts is due to their higher absorption efficiency by mycorrhized plants, thus, to a greater nutrient profitability coefficient (67).

High nutrient availability decreases the presence of mycorrhizal structures inside roots, indicating that mycorrhizal effectiveness reduction is the result from symbiotic inhibition or malfunctioning. A greater fungal structure number is necessary to ensure the proper symbiotic functioning in low fertility soils (57).

MYCORRHIZATION EFFECT FOR AN OPTIMAL NUTRIENT SUPPLY

Joint application of inoculation with low mineral fertilizer doses increase symbiotic effectiveness, which is expressed by a higher mycorrhizal colonization and yield, obtaining an optimal fertilizer dose that is lower than the recommended one to obtain similar production volumes in the absence of inoculation (48, 67).

Applying higher doses than the optimal ones to mycorrhized plants reduce mycorrhizal symbiosis to almost inhibit it; however, yields do not decrease, indicating that plants ensure their nutritional requirements but not through mycorrhization. The optimal fertilizer dose for mycorrhized plants depend on crops in question and soil fertility (53, 74).

Decreasing nutrient doses by using AMF range between 25 and 50 % mineral fertilizer dose recommended for each crop (75), which is achieved at the expense of nutrient absorption increases from the soil and fertilizers that leads to their profitability coefficient increases and to a critical index decrease of soil elements (67).

MYCORRHIZAL INOCULATION AND ROTATION WITH GREEN MANURES

Through crop rotation, efficient plants in AMF multiplication, such as *Helianthus annuus*, *Crotalaria juncea*, *Canavalia ensiformis*, *Cajanus cajan* and *Stizolobium aterrimum*, increase inoculum amount in the soil, favoring subsequent crop colonization and improving their nutrition and production (6, 76).

Inoculating with efficient AMF strains increases spore number in any sequence but it depends on the number of inoculations, the crop in question and the preceding crop (67).

Thus, studies made under micro-plot conditions recorded an initial AMF propagule multiplication, from 83 spores in 50 g soil at the beginning of sequence, up to values between 731 and 1594 spores in 50 g soil, depending on the values found, on the species involved within evaluated sequences and on their mycorrhizal or non-mycorrhizal inoculation (Table III)[C].

Mycotrophic green manure growth, in the absence of mycorrhizal inoculation, increases native colonization percentage in the subsequent crop (6).

This effect is directly related to a higher native propagule number produced by such plant growth (49), but sometimes this development does not achieve an adequate soil AMF reproduction or a positive plant response, since propagules are found in very low amounts or species present are not effective (67).

If green manures are inoculated with an effective AMF strain, soil mycorrhizal inoculum content increases as well as the subsequent crop growth (77).

The use of green manures should not be circumscribed to the joint application with AMF strains when planted, but its greater importance must be given to the initial inoculation of green manure species with efficient AMF strains. In this way, soil mycorrhizal reproduction, a more vigorous green manure growth and a substrate shape with very high concentration of efficient propagules are achieved, enabling an effective and more economic plant mycorrhization (67, 78).

Table III. Variation on AMF spore number in two crop sequences and in the presence of mycorrhizal inoculation

Starting the sequence	First crop of the sequence		Second crop of the sequence	
83	Fallow	454 b	Canavalia	731 c
			Canavalia - HMA	1077 b
	Brachiaria	832 a	Canavalia	854 bc
			Canavalia - HMA	1594 a
Es χ		0,14 *		0,08 *

Adapted from Martín, 2009

** Means with different letters in the same column differ, according to Duncan test (p<0,05)

Fallow: land resting for three months

Green manures employed in certain types of soil and agro-productive systems do not fully guarantee nutritional requirements for an effective mycorrhization; in these cases, it is necessary to supplement with some amounts of mineral or organic manure, which are much lower than those applied to intensive productive systems or in presence of non-mycorrhized crops (67).

In this regard, it has been proved that by canavalia-corn rotation and in the presence of mycorrhizal inoculation, N rates required to achieve a maximum stable yield up to 75 % of the recommended dose for that type of soil are reduced, and such decrease is directly related to N that green manure was able to provide, derived from N biological fixation process (11).

Another AMF effect on legumes is the tripartite symbiosis with *Rhizobium* bacteria. Symbiosis with bacteria provides biologically fixed N while AMF increase P absorption, favoring BNF. Legumes provide photosynthates to bacteria and AMF (73).

Canavalia ensiformis is a high potential plant for AMF colonization, which is able to promote even the subsequent crop colonization (56).

In Cuba, it has been found that canavalia multiplies native mycorrhizal propagules into large quantities in the soil (7); therefore, it is interesting to study the introduction and multiplication of efficient strains through inoculation.

Thus, recent studies have shown that canavalia inoculated with efficient strains according to soil type, is able to multiply mycorrhizal propagules and enhance effective mycorrhizal colonization of the subsequent crop (12).

EFFICIENTLY MYCORRHIZED AGRICULTURAL SYSTEMS

Efficiently mycorrhized agricultural systems are those where, by inoculating efficient AMF strains, plants achieve a better mycorrhizal symbiotic functioning, giving rise to higher growth, nutrient uptake and yield, compared to non-inoculated plants (7).

Therefore, the use of these soil microorganisms in agriculture is an alternative to mineral fertilizers. From the ecological point of view, their use may reduce energy application, agricultural ecosystem degradation and nutrient losses from agricultural soils. In addition, the productive capacity of the system is maintained, preserving biodiversity and contributing to a more stable and long-term sustained production in balance with the environment.

These systems are valid for low input conditions and a more technical agriculture, allowing high yields and enhancing soil life. At the same time, contamination caused by excessive fertilization and the negative effects of drought (67) are reduced.

Those soils dominated by native fungi with high effectiveness are not suitable for inoculation. In this case, AMF need to be managed to maintain their high population levels, which will be used in inoculation programs (53).

The greatest number of AMF species and inocula is in low-input systems (80). Many agricultural practices employed in high-input systems have relatively small native AMF populations, while organic systems increase their population in preventing the use of agrochemicals and promoting biodiversity (81).

The use of mycorrhizae as biofertilizers does not mean to stop fertilizing; otherwise, fertilization becomes more efficient and the dose to be applied may be reduced to ranges of 50-80 %.

At present, the compatibility between mycorrhizal symbiosis and the proper fertilization management as well as the need to redefine fertilizer requirements and critical nutrient levels in the soil for mycorrhized crops with efficient strains, should be consciously introduced as a basis for agricultural exploitation.

CORN ROTATION WITH GREEN MANURES AND MYCORRHIZAL INOCULATION

By introducing legume-corn rotations, farmers' dependence on external inputs decrease, system profitability increases, nutrient balance becomes positive and soil quality improves (82).

By using canavalia in subsequent crop with corn, yields increase up to 6,3 t ha^{-1} and have between 40 and 60 % profitability from green manure N. Corn yields are equivalent to those obtained with 200 kg ha^{-1} mineral fertilizer N (83).

Green manures incorporated promote NPK absorption by corn crop, due to the greater element availability stimulated through green manure (84). The use of low mineral fertilizer doses increases the beneficial impact of green manures on the crop[C].

Increased yields of corn planted after incorporating legumes were 1,1-3,2 t ha^{-1} and 1,4-3,8 t ha^{-1} in areas of low and high rainfall respectively, advising that lower doses of mineral fertilizers must be applied together with legumes to increase N profitability coefficient (85).

Under the conditions of our country, green manures have a positive influence on corn yield. This planted in subsequent crop with canavalia had similar yields to the variant with mineral fertilization and higher yields than the absolute control by 1 t ha^{-1}. This type of response has very good results regardless of the time of year in which it is applied (38).

In studies conducted to evaluate corn response to mycorrhizal inoculation, it has been reported that AMF-inoculated plants accumulated 40 % more dry matter and N than non-inoculated ones, growing in a soil with alfalfa (*Medicago sativa*) residues (86). *G. fasciculatum* strain along with corn allowed increasing yields between 21 and 77 % according to soil type (87).

Moreover, corn showed high colonization with native AMF strains when planted in subsequent crop with mycotrophic plants in grassland or fallow, which permitted native mycorrhizal endophyte propagation (88).

The preceding crop effect on corn growth is partly due to the increased AMF propagule number caused by the previous crop. In this regard, it is reported that corn in subsequent crop with sunflower (*Helianthus annuus*) increased 49 % dry mass compared to non-inoculated treatments and improved crop root colonization (76).

AMF management through green manures can be a useful practice in sustainable agriculture. Winter green manures multiplied native AMF inocula in the soil, with high colonization of the subsequent corn and increased extra-radical hypha density. The mycorrhization degree of corn (% colonization) was correlated with corn growth and yields (89).

Green manures significantly increase AMF colonization of the subsequent crop and its combined use with mineral fertilizers complements crop N needs, with a significant decrease of the doses to be used due to AMF promotion of absorption (90).

GENERAL CONSIDERATIONS

Green manures, although known for millennia, are not very often used in conventional and highly technical agriculture. Many producers reject their use, mainly because they do not manage the different forms of employment; it does not mean that the producer should be adapted to green manures; otherwise, green manures must be adapted to agro-productive conditions to be used.

If they are going to be employed together with biofertilizers as AMF, it not only will enhance their growth and development but also will increase their benefits to the main crop. Furthermore, by inoculating green manures, plants mostly reproduced by botanical seed will be easily inoculated in established plantations or crops agamically reproduced, which are more difficult to inoculate.

The use of nutritional alternatives in crops, as the two mentioned in this paper, is a need that cannot be delayed, considering the importance of environmental preservation, to obtain healthy food and the rising fertilizer price in the world market.

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