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Endomycorrhizal Fungus Inoculum Production Techniques

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Introduction

The beneficial role of the VAM symbiosis on plant growth has been thoroughly documented, especially in the last decade.

One of the main factors which prevents controlled experimental comparisons between mycorrhizal and non-mycorrhizal conditions is the lack of "high grade" VAM inoculum. Included in the above category are: inoculum free of pathogens; high concentration of inoculum per given unit of volume; high efficacy of the species and/or biotypes of the inoculum and the need for the inoculum to be readily storable and deliverable in nature.

If the above could be achieved, inoculum would be available for use in the following:

1. Field experimentation to identify the most beneficial crop-soil situations as to where directed inoculations should be used.
2. Establishment of a basis for commercial production of inoculum for use under agricultural conditions in both annual and perennial crops.

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Work Plan and Accomplishments To-Date

With the above as the aim, experiments were undertaken to ascertain the following:

1. Which crop species are most suitable for inoculum production.
2. How best to control possible biotic agents which can interfere in inoculum production.
3. Which substrates are most suitable for growing the mother plants.
4. What nutritional regimes are most efficient with regard to: production, ease of application and cost.

1. Crop species to be used for inoculum production

During the time frame covering this report the following crop species were investigated or are at present under investigation. Maize, Sudan grass, melon, wheat and safflower.

Some of the above were chosen on the basis of previous results obtained by us and because of the ease of growing them under greenhouse conditions during different seasons: corn and melon in spring, summer and fall; Sudan grass, year round; wheat and safflower, winter.

On the basis of the preliminary results it seems that corn is best during the seasons used as it grows rapidly and produces an extensive root system. If the mycorrhizal infection is very high, corn roots turn yellow. In small scale comparisons made as to cv. differences, we found that a cv. obtained from West Germany had a very high percent of infection.

We experienced some problems, especialy in summer, with Sudan grass in terms of chlorosis, induced by poor N nutrition. Melon, although readily infected, seems to be a poor choice for inoculum production due to its comparatively small root system. We have found

melon to be very useful in estimating soil populations of VAM fungi by using the most probable number (MPN) technique.

Wheat and safflower are at present under investigation.

2. Controlling biotic agents which can interfere in inoculum production

Assuming that the primary VAM fungal inoculum is pathogen free, that the seed used to grow mother plants has been checked and found to be pathogen free as well, and that the substrates are free of biotic agents or treated to kill same, secondary sources of biotic agents which can greatly effect efficient VAM fungal production can be introduced via the water applied to pots, be wind deposited or arrive on six legs.

We have found that several biotic agents can greatly interfere with inoculum production, the main two being the obligately parasitic fungus Olpidium brassicae and a species of collembola (springtails). Based on studies conducted by us to eliminate or prevent Olpidium, we have come to the conclusion that chemical means are not sufficiently effective. On the assumption that the spores were water borne, we installed a water sterilizing unit (by U.V. radiation). Since this installation we have observed that all our roots are Olpidium free. An experiment, in conjunction with the commercial company producing the sterilizing units, will be conducted in the near future to quantify our preliminary results. We should point out that in our studies we could find from very little to no infection of VAM in root portions infected with Olpidium. Since the introduction of the system, VAM infection in corn has been 90%.

Although there is some information with regard to the use of chemicals to control collembola, we prefer to use them only as a last resort. What seems to work very well is the application of strips of paper with a long-lasting glue around the pots. This traps the colemba, and prevents their access to the substrates in the treated pots.

3. Substrates to be used for inoculum production

Numerous plant growing substrates have been used to obtain VAM inoculum. Two main categories are available: inert and non-inert. In the inert category we can include sand; Turface, an expanded montmorillonite clay; LECA, lightweight expanded clay aggregate, and tuff, volcanic rock. Non-inert can be soil; peat, soil and peat mixtures, and mixtures of sawdust and soil, among others. In substrates of the first category the nutrient status of the mother plants can be controlled better.

From our experience, both in sand and Turface, root production is better than in non-inert mixes. Better root production is obtained in Turface than in sand, especially in corn. We have obtained excellent infection of corn with the VAM fungus *Glomus intraradicis*, in Turface.

Based on our experiments we have decided to concentrate our efforts on producing inoculum in inert substrates, especially in Turface, LECA and tuff for the following reasons:

1. Turface - this material which is non-porous, provides excellent root development, with very high infection. If high spore production will also be obtained, the entire mass (substrate and roots) can be used as inoculum. If poor spore production is obtained, the roots can be used as starter inoculum in more efficient substrates. Turface can

be sterilized.

2. LECA - This is a material obtained commercially from West Germany. The major advantages are that it contains numerous pores, is very light, and can be sterilized if needed. Roots grown in this substrate also become readily infected. Its main advantage, however, is that the pores of the substrate become filled with mycelium, and of even more importance, with spores of VAM fungi. LECA can thus be readily used as the production and delivery system of VAM inoculum. We especially emphasize the delivery aspect. At present the major constraints in VAM inoculum use are the shelf-life and methodology of delivery. LECA granules containing spores are easily stored; the inoculum, long lived; and the granules used either to infest seedling mixes or delivered to the field by existing agricultural equipment.

In preliminary tests using LECCA carrier inoculum we have obtained excellent infection of corn. Other crops being tested at present using LECA inoculum are citrus and banana seedlings. LECA granules can be ordered in various sizes. We believe that 2-4 mm dia. offers the best choice.

If the substrate becomes infested with pathogens, they can be killed with fungicides that will not affect the mycorrhizal spores.

3. Tuff - this material, which is volcanic ash ground to various sizes, is used in many plant growth mixes. It is highly porous, and for our purposes very cheap and more readily available than the above. Root production, however, in tuff only, is not as good as in the above two. It is also not as light as LECA. However, because of its price and availability we plan to include it in our study, in comparison with LECA.

4. Plant nutrient regimes for optimizing VAM inoculum production

As may be expected, the nutritional status of the mother plants will have the decisive effect on VAM formation, development, host longevity and spore production. Using inert substrates should afford a measure of host nutrient status control and allow development for the best nutritional protocols to optimize root growth as well as inoculum production. Among the factors that need to be controlled:

1. Concentration of the various mutrients in the substrate during the growing period

2. Optimizing N P K ratios to insure good plant growth as well as VAM infection, development and spore production.

In order to accomplish the above, several nutritional methodologies may be utilized. Each has its advantages and disadavantages and it remains to be seen which would be optimal for our purpose. In brief the following may be utilized:

a) Preparing nutrient solutions, which are applied with the irrigation water at given intervals. These are usually prepared using technical grade nutrient salts.

b) Utilizing commercial grade nutrient solutions that are available for use in agriculture. Application as above.

c) Incorporation of individual solid fertilizers in the substrates.

d) Incorporation of slow release full nutrient granules (Osmocote) in the substrates.

The advantage of a and b is that high precision of nutrient application can be achieved. However, these methods are time consuming both with regard to preparation and application. Method a is also relatively costly.

Method c, although time saving and relatively cheap, allows for little nutrient status control. The more soluble nutrients would tend to leach, and others to accumulate, leading to unbalanced nutrient status.

Method d, which allows both the use of granules of differing compositions and of timing of nutrient release, would seem to afford the optimal method of nutrient supply. A mixture of two types of granules should be able to supply optimal nutrient availability during the growing periods of different plants. As only water would then be needed this could be provided via individual emitters, reducing the time needed for plant maintenance.

An experiment which will include various substrates and nutrient applications will shortly be initiated to compare substrate x nutrient interaction effects on the growth of selected hosts and on inoculum production of several VAM fungal species.

The above summarizes greenhouse and laboratory studies related to the project.

As part of ongoing research and within the scope of the project, part of our effort was directed at examining the effect of some of our produced inoculum in a field experiment conducted to evaluate the role of VAM on peanut. Among the factors studied: the role of VAM on peanut growth, nutrient uptake, effect on Bradyrhizobium nodulation and yield. As the results have not yet been fully analyzed, they will be presented in the next report.