Agriculture, Ecosystems and Environment, 29 (1989) 153-161
Elsevier Science Publishers B.V., Amsterdam
Printed in Czechoslovakia

CONCEPTUAL APPROACHES FOR THE RATIONAL USE OF VA ENDOMYCORRHIZAE IN AGRICULTURE : POSSIBILITIES AND LIMITATIONS

S. GIANINAZZI, A. TROUVELOT and V. GIANINAZZI-PEARSON
Laboratoire de Phytoparasitologie, Station d'Amélioration des Plantes, INRA, BV 1540, 21034 DIJON CEDEX, France

ABSTRACT

A strategy is proposed of plant inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi based on appropriate biological tests (soil endomycorrhizal potential and fungal effectiveness/soil receptivity test) that provide information on whether it is necessary to inoculate and which fungi to use. It is suggested that the inoculum production methods should be chosen in function of the techniques used for plant production and of the economic criterion. Examples of successful inoculation of micropropagated apple rootstocks and ornamental plants sown in commercial nurseries are presented.

INTRODUCTION

At present, agricultural techniques do not generally take into consideration the existence of VA endomycorrhizae. However, since the pioneer experiments of J. MENGE on Citrus (MENGE et al. 1977) the importance of VAM for plant production has become more and more widely accepted, and several private enterprises are now interested in producing VAM inoculum. As for other soil microorganisms, the successful use of VAM fungi can only be achieved under certain conditions and therefore the conceptual approaches for their rational use need to be defined. These are based on the use of appropriate biological tests that provide information on whether it is necessary to inoculate and which fungi to use. The biotests are outlined in this paper and examples are given of how we are using them in our programme on the application of VAM to increase plant production. Some new approaches for better exploitation of effective indigenous and introduced fungal populations under field conditions are also discussed.

BIOLOGICAL TESTS

Soil endomycorrhizal potential. The variation between soils in the number of propagules that generate VAM can be very important and their estimation can vary

with the biotest used to quantify them. These biotests are generally based on the Most Probable Number (MPN) estimations calculated after diluting out propagules in a soil and baiting them with a test plant. As suggested by GIANINAZZI-PEARSON et al. (1985) in order to obtain values that most closely reflect the ecological reality existing in a soil, environmental conditions should be changed to a minimum and dilutions should therefore be done using the same, but disinfected, test soil. Environmental conditions, host plant (rapidly rooting, highly mycotrophic) and growth period should be constant for comparative tests of different soils ; growth period should not exceed 5 to 6 weeks at 20°C in order to evaluate the more aggressive fungi able to infect plants during their early stages of development, when VAM have the greatest effects on plant growth.

Fungal effectiveness. Fungal populations vary not only quantitatively, but also qualitatively, that is in their ability to improve plant growth. It is therefore necessary, parallel to an MPN test, to analyse fungal effectiveness. This is based on isolating all propagules from a given volume of soil and reintroducing them into the same volume of disinfected soil. Growth of a test plant (highly endomycorrhiza-dependent) is then compared with that in disinfected soil without fungal propagules and in non disinfected soil containing all the indigenous microflora. The first comparison (disinfected vs reinoculated soil) gives an indication of the potential effectiveness of a VA fungal population in a given soil, whilst from the second (disinfected vs non disinfected soil) we can deduce the real effectiveness of the VA propagules, i.e. whether these are modulated by the presence of other competitive organisms in the soil. VAM fungi in a given soil are not necessarily adapted for maximum ability to enhance plant growth. In this case inoculation of more efficient fungi can be envisaged. It is necessary for this to have a collection of VAM fungi caracterised for their effectiveness under given physical and chemical soil conditions such as pH, level of available P, intended fertilizer treatments and possibly soil texture. Comparison under controlled conditions in disinfected and non disinfected soil of the effectiveness of the chosen VAM fungi with that of the indigenous populations will unable us to predict the outcome of controlled inoculation, and therefore decide whether it is worthwhile to inoculate or not in the field and eventually which VAM fungi to use. The lack of appropriate fungal markers, apart from a very few exceptional situations (ABBOTT and ROBSON 1982), for distinguishing and following the development of an introduced VAM fungus among the indigenous population in a non disinfected soil, should not be considered a major obstacle, since this will be reflected in the improved plant growth and/or level of infection in effectiveness tests. As for the endomycorrhizal potential test, this

biotest should be limited in time, to 6 to 8 weeks in this case, in order to consider only VAM fungi that are highly effective at the early and critical stages of plant establishment.

Soil receptivity. Soils are known to differ in their receptivity to microorganisms (ALABOUVETTE 1986) ; this will be a key factor for the successful introduction of non indigenous VAM fungi into a soil. However, because VAM fungi are obligate biotrophs, soil receptivity to them can only be tested in the presence of the host plant ; this is a prerequisite for, and therefore already evaluated in, effectiveness tests.

STRATEGY OF INOCULATION

The two biotests for endomycorrhizal potential and fungal effectiveness, together with current knowledge about the endomycorrhizal dependency of different plant genera and species, will give us the essential tools for a rational use of VA fungi (Table 1).

INOCULUM PRODUCTION

VA fungi must be multiplied on living roots and this obliges us to use techniques for inoculum production that are different from those generally employed for other biotechnically interesting fungi. This, usually considered as a major disadvantage, appears . our experience to be an advantage, since the risk of maintaining in a culture collection VAM fungi that have lost their symbiotic properties is very low because these are easily detectable by considering the growth of the stock culture host plants. On the other hand, difficulties of producing VAM fungi on living plants arise from the precautions that have to be taken in order to obtain 'clean' (pathogen-free) inoculum. Inoculum is presently being produced in large quantities on plants growing in either soil or artificial substrates and prepared for use as sieved soil (GIANINAZZI 1982, MENGE 1983), bound to inorganic carriers (DEHNE and BACKHAUS 1986) or as extracted spores that have been artificially bound to an inorganic carrier (J. WOOD personal communication). Each method has its own advantages and limitations ; the way of producing inoculum needs to be decided in function of the techniques used in plant production and of the economic criterion. For example, this year in France, we have prepared a "soil type inoculum" in agricultural waste soil produced by a sugarbeet company, as a means of commercially utilising this side product. Surface disinfected VAM can provide an excellent form of inoculum for certain special crops where it is essential to conserve a minimum degree of microbial contamination at the moment of outplanting from axenic conditions (e.g. micropropagated plants).

Table 1. Potential and strategy for micro-symbiont inoculation (S. GIANINAZZI et al. 1986)

I- SOIL :

Indigenous micro-symbiont populations (propagule number)	Micro-symbiont effectiveness	Potential for inoculation	Strategy
Absent	0	+++	Inoculate
Low	-	++	Inoculate
	+	+	Increase the indigenous population
High	-	++	Inoculate
	+	0	Maintain indigenous level

II- PLANT : Establish the dependence of cultures on the microsymbiont for plant growth.

Table 2. Effects of VAM fungi on growth of micropropagated apple root-stock (M26) one year after outplanting into field plots on the 'Domaine d'Epoisses' INRA, Dijon, F.

Measurements per plant	Non disinfected soil			Disinfected soil		
	Control	G.m.	G.i.	Control	G.m.	G.i.
Fresh weight(g)	82bc	107b	116b	40c	181a	194a
Diameter(mm)	12.2b	13.7ab	13.9ab	8.8c	15.9a	15.8a
Number of branches	8.2b	17.5a	17.5a	9b	18.5a	17.2a

Means of 4 replicates. Results in each line followed by the same letter do not differ significantly from each other (P= 0.05).
G.m. = *Glomus mosseae* ; G.i. = *Glomus intraradices*.

CONTROLLED INOCULATION TRIALS

As indicated in Table 1, several situations exist in which controlled inoculation of VAM fungi could be advantageous for production and management of the success of the controlled inoculation under these circumstances.

Low level of indigenous VAM fungi. The non disinfected, fertilised arable soil from 'Domaine d'Epoisses' has a very effective VAM population (Table 2 and 3) and three separate tests of its endomycorrhizal potential gave high values of 980 (test plant : onion), 1480 (test plant : onion) and 2600 (test plant : clover) propagules/kg of soil (Table 3 and Table 1 in GIANINAZZI-PEARSON et al. 1985). These observations led us to investigate the effect of decreasing propagule numbers of effective VAM fungi on plant yield under field conditions. Results presented in Table 5 show that plant yield could be significantly increased even with only 50 propagules/kg of soil, although optimum results were obtained with 167 and 500 propagules/kg of soil. When this soil is disinfected, therefore, 200 propagules/kg of soil is probably the minimum inoculum density required. Experiments in non disinfected soil have not yet been done, but it seems likely that in the case of effective indigenous VAM fungal populations, the density of propagules required would be higher than 200 because of the competition with other existing soil microorganisms. This competition is well illustrated by comparing the development after harvest of VAM fungi in non disinfected soil (Table 1 in GIANINAZZI-PEARSON et al. 1985) with that in disinfected soil (Table 5) : in the latter case propagules increased 2 to 5 times. In spite of this we should be able to predict a successful inoculation using the fungal effectiveness /soil receptivity test. The same must be true when the indigenous VAM fungi are not effective, but in this case the possibility of finding appropriate fungi may be lower because the existing competition with other soil microorganisms will be reinforced by that with non effective indigenous VAM fungi.

High level of indigenous VAM fungi. When high levels of indigenous, effective VAM fungi are present in a given soil, inoculation is not necessary (Table 3). However, use of a metalaxyl or aluminium ethyl phosphonate-based fungicide (J.A. MENGE, personal communication) that can eliminate or considerably reduce the level of competitive soil fungi like Pythium sp. or Phythophthora sp. without affecting VAM fungi (see MORANDI, in this volume), could be an interesting way of increasing the endomycorrhizal effect.

In soils with high populations of ineffective VAM fungi, inoculation may not increase plant production unless the soil is disinfected, because of microbial (symbiotic or not) competition. We know very little about competition between

different VAM fungi, or between them and other soil microorganisms and more research is needed. We have, however, recently discovered pea mutants which do not form VAM in pot tests (DUC, TROUVELOT, GIANINAZZI-PEARSON, GIANINAZZI, unpublished results). If some VAM fungal strains could selectively overcome this resistance, as is the case for certain nodulation mutants with different endomycorrhiza-dependent plant species.

Table 3. Comparison of the effectiveness of the indigenous VAM fungi of the 'Domaine d'Epoisses', Dijon, F. with *G. intraradices* using *Allium cepa* cv. Augusta as a test plant.

Treatments	Propagule numbers/kg of soil	Infection(%)	Dry weight g/plant
Disinfected soil	10b	4c	0.2b
Disinfected soil + *G. intraradices*	1890a	90a	9.6a
Non disinfected soil	1480a	60ab	11.4a
Non disinfected soil + *G. intraradices*	980a	48b	13.2a

Means of 4 replicates. Results in each column followed by the same letter do not differ significantly from each other (P= 0.05).

Table 4. Increased growth (%) of several woody species one year after inoculation in a nursery (Levavasseur & Fils, Ussy, 14420 Potigny, F.) as compared to uninoculated controls.

Plant species	Heights G.i.	Heights G.m.	Diameter G.i.	Diameter G.m.
Ampelopsis veitchii	+ 73**	+ 45**	+ 13	+26**
Berberis thunbergii	+ 30**	+ 3	+ 16	-2
Cercis siliquastrum	+ 1	+ 20	+ 7	+26**
Cornus sanguineum	+ 17	+ 39**	+ 2	+14
Fraxinus excelsior	+ 12	+ 91**	+ 8	+50**
Hibiscus syriacus	+ 26*	+ 70**	+ 31**	+14
Ligustrum japonicum	+ 15	+ 21	+ 25**	+26**
Liquidambar styraciflua	+244**	+106**	+107**	+50**

Significantly increased : P= 0.01 (**) and P= 0.05 (*)
G.m. = *Glomus mosseae* ; G.i. = *Glomus intraradices*.

Table 5. Soil inoculum density of *G. intraradices* in disinfected field plots : effect on leek yield, endomycorrhizal infection and soil endomycorrhizal potential after harvest.

	Propagule numbers before crop/kg of arable soil			
	500	167	50	0
Yield (g/plots)	1021ab	1273a	789b	466c
Infection level *				
F%	93a	83a	87a	18b
M%	69a	60a	61a	6b
A%	61a	54a	52a	3b
Propagule numbers after harvest	29333a	8600b	18400ab	173c

* TROUVELOT *et al.* 1985. Results in each line followed by the same letter do not differ significantly from each other (P= 0.05).

Absence of indigenous VAM fungi. This situation is typical in nurseries where artificial (inert) potting mixtures or substrates are used, or where soil is disinfected before planting. In these cases, only the fungal effectiveness/soil receptivity test will be necessary. Among 14 different substrates tested for their relative ability to ensure suitable fungal and plant development, we have chosen a peat/soil/gravel mixture (25:50:25) as a standard substrate for our programme on the introduction of VAM fungi into the production cycle of micropropagated woody fruit crops. Results obtained with the latter will be limited in the present paper to apple tree root-stock ; others concerning vines (RAVOLANIRINA *et al.*) and oil palms (BLAL and GIANINAZZI- PEARSON) are presented elsewhere in this volume. Plants were inoculated in May with *Glomus mosseae* (LPA5) and *G. intraradices* (LPA8) during the *post in vitro* acclimatisation period, transferred to a glasshouse and then outplanted in October into disinfected or non disinfected soil in a field nursery. The introduced fungal strains were chosen for their compatibility with the clay loam nursery soil (pH 7.0, 30 to 40ppm Olsen P). As could be predicted, the VAM fungi developed very well and stimulated plant growth in pots ; this initial advantage was amplified after outplanting in the field plots with disinfected soil (Table 2). The advantage of the controlled inoculation was reduced in non disinfected soil. This can be explained by the fact that : 1- the uninoculated plants grew better because they were infected by indigenous VAM fungi ; 2- the inoculated plants' growth was less than in disinfected soil probably due to a negative effect of other soil microorganisms eliminated by soil disinfection. Comparison of the effectiveness of the indigenous VAM fungi with that of the introduced fungi showed that the former were in fact very effective (Table 3). As indicated in

Table 1 there is little likely benefit in inoculating plants before transplanting into non disinfected field soils containing effective VAM fungi.

We have also studied the interest of introducing VAM fungi in commercial nurseries where soil disinfection is a current practice. Soils from different nurseries in northern France were tested for their compatibility to our strains using the fungal effectiveness/soil receptivity test. Selected nursery beds were then inoculated with G. intraradices or G. mosseae before seeding ; usual nursery practices were otherwise used for plant production. Growth enhancement was observed with all the tested plant species but not always with both fungi, underlining the importance of plant-fungus interactions (Table 4). It is therefore reasonable to think that inoculation with both VAM fungi would have ensured better growth of all the plants. We are now experimenting with a 'cocktail' inoculum containing more than one fungus which we think will guarantee Rhizobium populations (GRESSHOFF et al. 1986), this could allow the development of plant cultivars that will form endomycorrhiza only with certain, specifically selected (GIANINAZZI and GIANINAZZI-PEARSON 1986) VAM fungi. This would overcome problems of high levels of non effective indigenous inoculum as well as the possibility of introducing inoculum, which is of high quality and has a precise target.

In conclusion, we can say that knowledge accumulated about VAM in the past years has now created the conditions to begin rationally using them for plant production.

REFERENCES

ABBOTT, L.K., ROBSON, A.D.: Infectivity of vesicular-arbuscular mycorrhizal fungi in agricultural soils.-**Aust. J. Agric. Res. 33**: 1049-1059, 1982.

ALABOUVETTE, C.: Fusarium-wilt suppressive soils from the Château-Renard region : review of a 10-years study.-**Agronomie 6**: 273-284, 1986.

DEHNE, H.W., BACKHAUS, G.F.: The use of vesicular-arbuscular mycorrhizal fungi in plant production.I. Inoculum production.-**J. of Plant Diseases and Protection 93**: 415-424, 1986.

GIANINAZZI, S., GIANINAZZI-PEARSON, V.: Progress and headaches in endomycorrhize biotechnology.-**Symbiosis**: 139-149, 1986.

GIANINAZZI, S., GIANINAZZI-PEARSON V., TROUVELOT, A.: Que peut-on attendre des mycorhizes dans la production des arbres fruitiers ?-**Fruits 41**: 553-556, 1986.

GIANINAZZI-PEARSON, V., GIANINAZZI, S., TROUVELOT, A.: Evaluation of the infectivity and effectiveness of indigenous vesicular-arbuscular fungal

populations in some agricultural soils in Burgundy.-**Can. J. Bot. 63**: 1521-1524, 1985.

GRESSHOFF, P.H., DAY, D.A., DELVES, A.C., MATHEWS, A., OLSON, I.A. et al.: Plant host genetics of nodulation and symbiotic nitrogen fixation in pea and soybean.-In: Evans, M.J. et al. /Ed./: **Nitrogen Fixation Research Progress.** Pp. 19-25. Martinus Nijhoff Publisher, Amsterdam 1986.

MENGE, J.A.: Utilization of vesicular-arbuscular mycorrhizal fungi in agriculture.-**Can J. Bot. 61**: 1015-1024, 1983.

MENGE, J.A., LEMBRIGHT, A.H., JOHNSON, E.L.V.: Utilization of Mycorrhizal Fungi in Citrus Nurseries.-**Proc. Int. Soc. Citriculture 1**: 129-132, 1977.

TROUVELOT, A., KOUGH, J.L., GIANINAZZI-PEARSON, V.: Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle.-In: GIANINAZZI-PEARSON, V., GIANINAZZI, S. /ed./: **Physiological and genetical aspects of mycorrhizae.** Pp. 217-221. INRA, Paris 1986.

Gianinazzi, S., Trouvelot, A. and Gianinazzi-Pearson, V., 1989: Conceptual approaches for the rational use of VA endomycorrhizae in agriculture: possibilities and limitations. Agric. Ecosystems Environ., 29: 153-161.