Organic apricot production: Towards an ecologically intensive orchard self-sufficent in inputs: Focus on use of AMF for cultivation of rootstocks

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Abstract

In a context of lower crop productivity in organic farming compared to conventional farming, fruit production has to become ecologically intensive, but at the same time, limit dependence on off-farm inputs, in particular pesticides used in organic farming, organic fertilizers and fossil energies. An organic apricot orchard was set up in the aim of promoting natural processes in the soil as well as the tree, by acting in both time and space. In this context, experimentation on stimulation of root mycorrhization for better assimilation of mineral elements has been carried out. Analyses of soil showed native abundance and a diversity of endomycorrhizal fungi species, not justifying the use of artificial mycorrhization of trees. In order to verify this hypothesis, pot tests were performed on rootstocks (peach tree GF305) in four modalities: soil sterilized or not at 110 °C, inoculated or not with a commercial product containing a mix of mycorrhizal fungi (Glomus sp.). The plants were grown in cold shelters without fertilizer for 10 weeks. The results showed a better growth of young peach trees, as well as a higher N-tester index in the modalities with the commercial arbuscular mycorrhizal fungi (AMF) product and with non-sterilized soil in comparison with plants growing only in sterilized soil. Furthermore, the combination "non-sterilized soil and commercial AMF product" showed an increase of 40%, 45% and 32% of height, dry shoot weight and dry root weight, respectively compared to plants growing in non-sterilized soil without commercial AMF product. In conclusion, supply of commercial AMF product could improve the efficiency for growth promotion of plants even though the soil is naturally rich in AMF. The mechanisms explaining this observation must be investigated with further experimentation.

Keywords: Mycorrhiza, rootstock, apricot, peach tree.

Introduction

Organic agriculture is a good model for moving agriculture towards agroecology. However, a number of failures in this cropping system could eventually weaken it, particularly for the fruit sector. The yields of organic fruit production are still lower than those obtained in conventional crops. The need to meet the growing market demand for organic fruit and to ensure the economic sustainability of organic farms requires increasing the productivity of orchards. But organic farming contributes to the depletion of non-renewable energies and greenhouse gas emissions because of the high mechanization.

It is therefore essential to implement innovative practices in order to reconcile competitiveness and respect of the environment. In a production system without livestock, an apricot orchard has been set up in compliance with the specifications of organic farming, in which several levers of agroecology have been implemented.

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The aim was to favor natural processes with a varied agro-ecosystem, as well at soil level for a better availability of water and mineral elements, as the tree to improve its tolerance to pests. Thus, before planting, it was necessary to mobilize nutrient cycles to feed the soil; according to the needs of trees. In the area, rank management has been geared with appropriate techniques to avoid competition and inter-rank management promoted biodiversity and the ecosystem services. The different levers used during the experiment are the following techniques: (i) choice of a rustic and autofertile variety plant material, and an adapted rootstock; (ii) management of the size tree (for an early fruit set); (iii) use of green compost produced on the farm and charcoal (biochar); (iv) use of mulching and organic amendments; (v) recycling the biomass of nitrogen-fixing legumes used as green manure; (vi) maintenance of the presence of auxiliaries for a biological control to limit the use of pesticides (notably sulfur and copper); (vii) cover crop management for limiting rank competition; (viii) stimulation of root mycorrhization. In this work, we will present results obtained with the experimentation realized on root mycorrhization of rootstocks (peach trees).

Material and Methods

In order to know if the soil used for the cultivation of the rootstocks was naturally rich (or not) in AMF, a soil sampling was carried out on October 2012. This soil has been previously (autumn 2011) sown with 6 species of leguminous: *Medicago sativa, Medicago lupulina, Melilotus officinalis, Onobrychis sativa, Trifolium repens, Vicia sativa.* For analyse, the soil was diluted (6 dilutions tested and 5 replicates per dilution) in a sterile substrate. Each dilution was the growing medium of a barfiola clover seedling. After 5 weeks of cultivation, the roots were stained according to the technique described by Phillips and Hayman (1970). The most probable number of mycorrhizal fungus propagules was estimated using Cochran Tables 100-1 and 100-2 (1950). In parallel, molecular analyzes have been realized: total DNA was extracted from three soil sub-samples (3 x 250 mg) with a commercial kit (NucleoSpin Plant II, Macherey-Nagel). The DNA amplifications were then carried out with, on the one hand, specific primers of the families *Glomeraceae*, *Acaulosporaceae* and *Gigasporaceae*; and, on the other hand, specific primers of *Rhizophagus intraradices*, *Funneliformis mosseae*, *Claroideoglomus etunicatum*, *Funneliformis geosporus* and *Claroideoglomus claroideum*.

Experimentation has been carried out in April 2013 with pre-germinated GF 305 peach cores placed in 1.2-liter pots in a substrate composed of soil from the apricot plot, previously sown with leguminous (see above). Four modalities have been tested: sterilized soil or not at 110 °C, inoculated or not with a commercial product containing six species of AMF (Glomus sp.). The support, based on clay, is amended with bio-additives (natural minerals, marine algae extracts, keratin, humus, chitin). The plants were grown in cold shelters without fertilizer for 10 weeks. The shoot length of seedlings was measured regularly. Measurements of the chlorophyll content index were made on three dates with the N-tester. After 10 weeks of cultivation, the trees were taken off, the aerial part and the roots weighed separately and put in an oven to evaluate their dry mass. Colonization of young peach roots was evaluated. The analysis is based on the examination of 30 small fragments of the root system (Phillips and Hayman, 1970). These fragments, after staining, were examined under a microscope and scored according to a class scale (Trouvelot et al. 1986).

Results

Analyzes of soil gave an estimate of 340 000 mycorrhizal propagules per kg of soil (min 102 000, max 1 122 000). The molecular analyzes showed the presence of the families

Glomeraceae and *Acaulosporaceae.* Among *Glomeraceae*, *Rhizophagus intraradices*, *Funneliformis mosseae*, *Claroideoglomus etunicatum*, *Funneliformis geosporus* were detected.

Analyses on plants showed a better growth of young peaches (Table 1), as well as a higher N-tester index (Figure 1) in the modalities with the commercial AMF product. The estimation of the mycorrhization rate reveals a high colonization in both modalities with non-sterilized soil, with very high levels, both in frequency (number of colonized roots) and intensity of colonization per root (Figure 2). On the other hand, with the sterile soil modalities, the contribution of the commercial AMF product slightly improved the colonization of the roots by the endomycorrhizas, in frequency but not in intensity, which remains very weak.

Table 1: Peach growth measurements after 10 weeks of pot cultivation. Significantly different measures are followed by a different letter in brackets (Newman-Keuls test at the 5% threshold).

	Sterlized soil	Sterilized soil + commercial AMF product	Non-sterilized soil	Non-sterilized soil + commercial AMF product
Height (mm)	557	583	628	878
Dry shoot weight (g)	2.32 (b)	3.26 (ab)	3.05 (b)	4.43 (a)
Dry root weight(g)	1.08 (c)	1.43 (bc)	1.82 (ab)	2.40 (a)

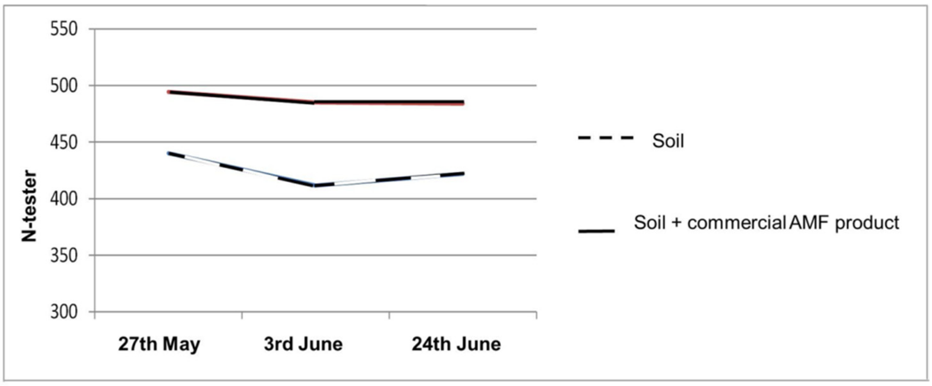

Figure 1: Evolution of the chlorophyll content index of the N-tester. Significant differences for the 3 dates (Newman-Keuls test at the 5% threshold).

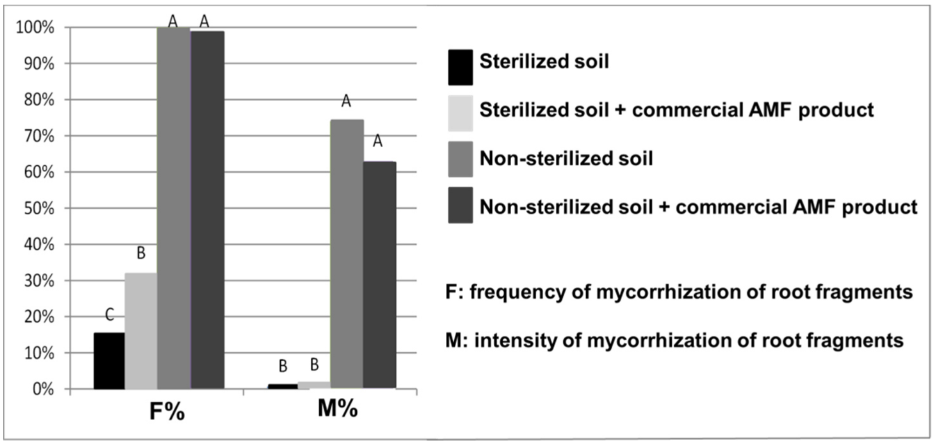

Figure 2: Rate of colonization of peach roots by endomycorrhizal fungi, after 10 weeks of cultivation. Significantly different measures are followed by a different letter (Newman-Keuls test at the 5% threshold).

Discussion

Mycorrhiza is a mutualistic symbiosis between beneficial fungi and a plant. Mycorrhizal fungi facilitate plant development through better access to soil resources and may limit the effects

of biotic and abiotic stresses. Artificial mycorrhization can enhance the potential of natural mycorrhization and its stimulation of root development when the natural mycorrhizal propagules concentration of the soil is insufficient.
Before planting, analyzes have been realized to measure the natural mycorrhizal propagules concentration of the soil serving as a support for the experiment. They showed that the soil was rich in endomycorrhizal fungi. Furthermore, molecular analyzes showed a high diversity of AMF fungi with four species detected. The previous crop cycles (sowing with leguminous, which are known as stimulator of mycorrhization via a good nodulation of roots (Guillotin et al. 2016)) thus induced an abundance and a diversity of endomycorrhizal fungi species. In order to check if an artificial mycorrhization of trees was still needed despite these good results, pot tests were performed on rootstocks peach tree GF305.

The combination “non-sterilized soil and commercial AMF product” showed an increase of 40%, 45% and 32% of height, dry shoot weight and dry root weight, respectively compared to plants growing in non-sterilized soil without commercial AMF product. In conclusion, supply of commercial AMF product could improve the efficiency for growth promotion of plants even though the soil is naturally rich in AMF. The mechanisms explaining this observation must be investigated with further experimentation.

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References

Cochran, W.G., (1950). Estimation of bacterial densities by means of the “most probable number”. Biometrics, **6**: 105-116.

Garcin, A., Millan, ML., Jay, M., Loquet, M., Brachet, B., Villenave, C., Mercier, N., Masquelier, S. (2014). L’abricotier en agriculture biologique : vers un verger écologiquement intensif et autonome en intrants ? *INFOS CTIFL* **301**: 38-49

Guillotin, B., Couzigou, J - M and Combier, J - P (2016) NIN Is Involved in the Regulation of Arbuscular Mycorrhizal Symbiosis. *Frontiers in Plant Sciences,* **7**:1704.

Philipps J-M., and Hayman D-S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, **55**: 158-161.

Trouvelot A., Kough J.L., Gianinazzi-Pearson V. (1986). Mesure du taux de mycorhization VA d’un système radiculaire. Recherche de méthodes d’estimation ayant une signification fonctionnelle. In *Mycorrhizae: physiology and genetics*. INRA, Paris, 1986.