

Mycorrhizal fungi to control replant disease: existing diversity in apple orchards

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Abstract

A major problem of fruit growing in Flanders is a shortage of uncultivated soils available for new plantings. Fruit growers must therefore plant new trees on fields already used for the cultivation of fruit. This can lead to replant disease and might cause poor growth and consequently delay the first harvest with one or more years. The use of fertilizers proved to be insufficient to enhance the growth of trees experiencing replant disease. Although both biotic and abiotic factors can result in replant disease, it is known that the major cause is a complex of nematodes and fungi. Arbuscular Mycorrhizal Fungi (AMF) can prove their usefulness with regard to these problems, as it is known that these symbiotic organisms can improve nutrient and water uptake and pathogen resistance in their host. To which extend these AMF can contribute to the preservation and recovery of healthy soil ecosystems in the fruit growth will be examined in a recently started project. The final goal is to develop an efficient and in practice usable AMF inoculum. A first important aim is to determine the existing AMF diversity in apple orchards based on modern molecular techniques. Preliminary results already gave some insights in the AMF composition of the examined apple trees. Additionally, major differences in composition of the AMF community were observed among different orchards with a multivariate analysis.

Keywords: Apple, replant disease, arbuscular mycorrhizal fungi, diversity

Introduction

AMF are obligate root symbionts of a wide variety of host plants. They are specialized members of the vast population of microorganisms that naturally occur in the rhizosphere (Smith and Read, 2008). Consequently, AMF may provide a sustainable solution to control replant disease. Since 2001, AMF have been classified into a separate fungal phylum: the Glomeromycota (Schüßler *et al.*, 2001). Nowadays, 241 species are described which are classified in 4 orders and more than 10 families (Schüßler and Walker, 2010; Redecker *et al.*, 2013). The majority of species belong to the Acaulosporaceae, the Gigasporaceae, the Claroideoglomeraceae and the Glomeraceae. AMF are multifunctional within an ecosystem. In general, they provide an extensive development of mycelium around the root epidermis and within the cortical cells. They enlarge the contact surface and increase the storage capacity of water and nutrients by means of the presence of million small vesicles and ramifications (arbuscules). The vigour of trees will increase due to the additional uptake of water and nutrients from the soil. As a result, less fertilizer has to be used. Moreover, the young trees can reach their maximum production capacity one year earlier. Trees are also less susceptible to abiotic stress conditions such as for example

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drought stress (Bothe, 2009). The beneficial effect can vary from influencing the uptake of nutrients to achieving a major effect in reducing root colonisation by soil pathogens, depending on the involved group of AMF (Maherali and Klironomos, 2007). AMF can also be divided in 2 specific categories, i.e. generalists and the specialists. Generalist are common AMF which are globally widespread. The specialists, on the other hand, are occurring only locally (Öpik *et al.*, 2010). How these AMF can contribute to the preservation and recovery of healthy soil ecosystems in pome fruit orchards will be examined in a recently started project. A first important aim was to determine the existing AMF diversity in apple orchards.

Material and Methods

Samples of roots and soil were collected from approximately 30 selected orchards, aimed at sufficiently covering natural variation regarding orchard age, soil structure, location and type of cultivation. Additionally, samples were collected from 3 nurseries and 2 standard orchards. A standard soil analysis was performed on the soil samples to determine the soil texture, pH, %C and the content of P, K, Mg, Ca and Na. Moreover, the actual nitrate reserve (NO₃-N) and the phosphate capacity of the soil (P Olsen, Olsen *et al.*, 1954) were measured. An innovative molecular technique, i.e. 454 pyrosequencing, was chosen to identify the AMF community in these orchards. First, the performance of a series of genetic markers, specific for AMF, was tested. These markers allow visualising the genetic variation in a part of the genome of the AMF in order to distinguish different families, and even species. Subsequently, the best markers were selected to investigate the whole dataset (i.e. AMV4.5NF-AMDGR).

Results and Discussion

Analysis of a subsample of the root samples already gave some first insights in the AMF composition. An overview of the different detected AMF families is shown in figure 1. The majority of the identified AMF belong to the Glomeraceae family.

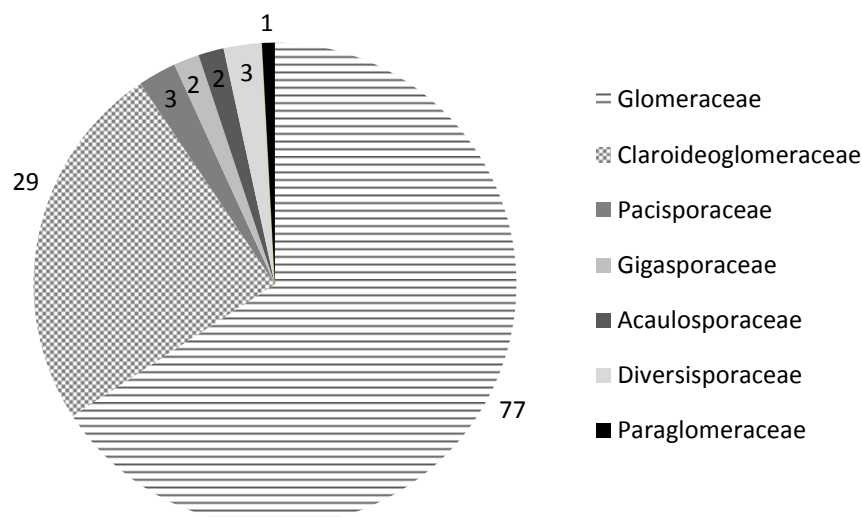


Figure 1: Proportion of the different AMF families detected during a first preliminary 454-screening of 30 apple trees, divided over 5 orchards.

Additionally, major differences in composition of the AMF community were observed when different orchards were compared based on a multivariate analysis (Figure 2). Each point in the figure represents an apple tree. Trees which are situated close to each other have a similar AMF composition. The AMF diversity in roots of organic cultivated apple trees is generally higher than in roots of conventional cultivated apple trees. In a next step, the correlation between AMF diversity and environmental characteristics will be investigated.

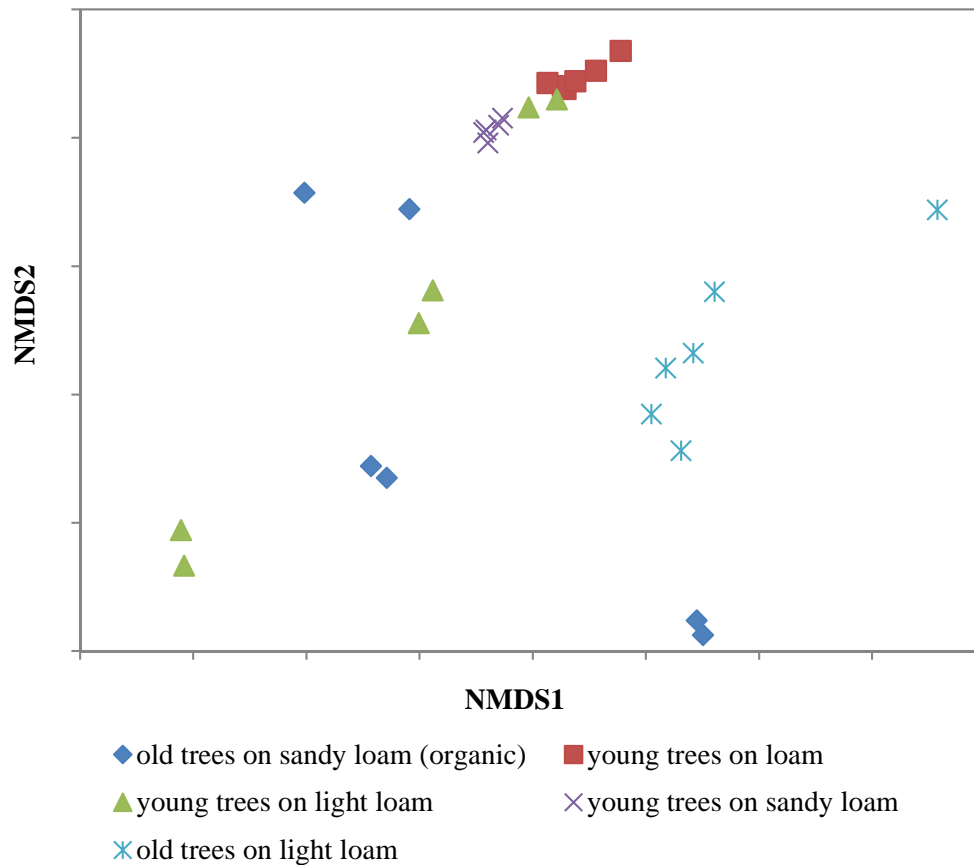


Figure 2: Presentation of the AMF community of 30 different apple trees divided over 5 different orchards. The graphic is based on a multivariate analysis of the preliminary 454-sequencing results.

Conclusion

Analysis of root samples of 2-19 years old trees revealed that AMF belonging to different families are present in Flemish apple orchards. Moreover, major differences in composition of the AMF community were observed between different orchards.

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