

MYCOGRO AG[®] AND MYCOGRO HORT[®]

- MYCORRHIZA PRODUCTS MADE IN NEW ZEALAND

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Abstract

Changes in environmental policies are forcing plant producers worldwide to reduce the input of agrochemicals and demand alternative solutions for sustainable plant production. Accordingly, there is much interest in the use of arbuscular mycorrhizal fungi (AMF) as biofertilisers across both agriculture and horticultural systems. However, the adoption of AMF-based biofertilisers has been hampered due to the prevalence of poor quality commercial products on the market. Many claim to have high propagule numbers but laboratory analysis has often found low numbers or no viable AMF. Chemical fertiliser compounds are also often present, and these may support short-term plant growth. New Zealand farmers should not miss out on potential gains in fertiliser use efficiency and plant health that can be achieved by the use of AMF fungi. With financial support of the New Zealand Royal Society, MBIE, Grasslanz Technology Ltd, AgResearch Ltd, and German industry and research partners, a mass production system for high quality, NZ-sourced mycorrhizal fungi has been developed. The products MycoGro Ag[®] and MycoGro Hort[®] are currently under evaluation in various commercial field trials. The application of MycoGro Ag[®] in lettuce, plantain and chicory and MycoGro Hort[®] in *Pittosporum* resulted in higher plant biomass compared to control treatments. Quality assurance of commercial AMF products is a key issue. To ensure MycoGro Ag[®] and MycoGro Hort[®] meet or exceed customer's expectations, these products are extensively tested for the presence of the correct fungal strains, propagule numbers and viability.

Introduction

Arbuscular mycorrhizal fungi (AMF) are present in most soil ecosystems. They are obligate root symbionts that need a host plant to complete their life cycle. Figure 1 shows the typical structures of AMF. For the majority of plant species, including agricultural and horticultural crops, uptake of water and minerals, particularly phosphorus, is mediated by AMF. Other beneficial effects include the stabilisation of soil aggregates, alleviation of plant stress, and protection against plant diseases, which collectively give improved plant growth. Some agricultural practices, particularly the application of agrochemicals, can reduce the abundance and diversity of mycorrhizal populations in soils (Plenchette et al. 2005, Bunemann et al. 2006, Vosátka and Albrechtová 2008). High phosphate levels in particular have a negative effect on mycorrhiza development and usually eliminate the effect of applied AMF products (Janos 2007; Douds et al. 2012). Sustainable management of agricultural ecosystems should include the efficient management of soil microorganisms (Jeffries et al. 2003, Selosse et al. 2004, Bunemann et al. 2006, Vosátka and Albrechtová 2009, Gianinazzi et al. 2010).

Horticultural practice often uses soil-free or fumigated substrates in which mycorrhizal fungi are absent so that application of a viable AMF strain could be useful in these situations.

Overseas there are several mycorrhiza products on the market. However, Tarbell and Koske (2007) found that few products contained viable fungi able to colonise their host plants. These products are often labelled with a list of AMF species, ectomycorrhiza species, and other fungi and bacteria said to be included in the product. However, the actual package content is frequently different to the label and the presence of viable microorganisms can be very low or none. Vosátka et al. (2012) tested five different commercial inocula on maize and found that some inocula were able to enhance maize biomass when grown in autoclaved zeolite and fertilized weekly by Hoagland solution. However, mycorrhizal colonization of the host occurred only in two of the five products. Their conclusion was that nutrients provided with the inoculum caused a short boost in plant growth. Similar results have been reported by Rowe et al. (2007). AgResearch has tested four mycorrhiza products imported into New Zealand and when applied as recommended there was no effect on plant growth and no root colonization (unpublished results). Without specialised laboratory equipment it is not possible for AMF product users to test the microbial composition of these products. Additionally, all four products tested were formulated as fine powders, which makes application in agricultural practice difficult. A research programme was therefore instigated to develop a mycorrhiza inoculum based on New Zealand-sourced species. With the support of two German industry partners (Cuxin, INOQ), one German research organisation (ZALF) and financial support from Grasslanz Technology, two mycorrhiza products have been developed providing both the horticultural and agricultural industry with a quality controlled product in a form readily used in existing agricultural machinery. MycoGro Ag® is a drillable granule with a particle size of 1–4 mm. It can be either cultivated into the soil prior to sowing or direct drilled with seed and fertiliser. MycoGro Hort® will be available as 1–2 mm or 1–4 mm granules. This study presents some initial experiments to evaluate the two NZ made products.

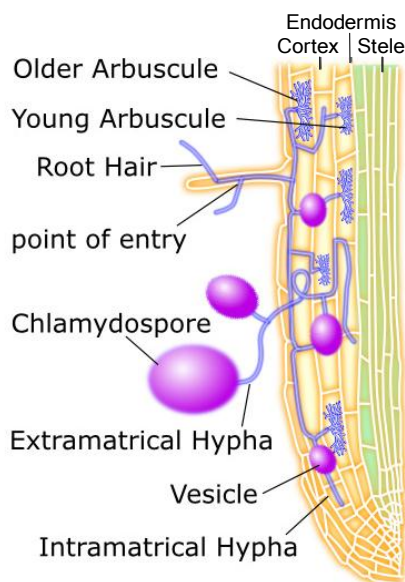


Fig. 1 Characteristic structures of arbuscular mycorrhizal fungi inside and outside of plant roots. Modified figure by Mark Kelly, Heartspring.net© 2012

Propagation of AMF for commercial application

The propagation of MycoGro Ag® and MycoGro Hort® was carried out in a plant based system using NZ Atiamuri pumice (1–4 mm, Industrial Processors Ltd) as carrier material. At the end of the five month propagation process, the pumice was sieved to remove bulk root material that could affect the handling of the final product (e.g. during drilling). The final

product contains three fungal species: *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler 2010, *Claroideoglossum lamellosum* (Dalpé, Koske & Tews) C. Walker & A. Schüßler 2010 and *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders. These fungi were isolated from dryland farm soil in Canterbury. The minimum propagule number in the product is 100 infectious units per gram. The product label contains further information such as pH, mineral content, application rates and recommended storage conditions. Grasslanz Technology Ltd has commenced the process of protecting MycoGro Ag® and MycoGro Hort® under the Plant Variety Right Act.

Quality control

Root staining, spore counts and spore viability

During the five month propagation period root colonization is monitored by staining fresh roots to determine colonisation progress. Once the inoculum is harvested spore counts are determined. Spore viability is usually high after harvest and can be monitored in stored inoculum by using the viability stain Iodonitrotetrazolium chloride (INT). Viable spores appear red after INT staining, whereas dead spores remain a pale to yellow colour.

Most probable number assay

The assay used to estimate AMF propagule numbers is the *most probable number* assay (MPN). This assay measures the presence or absence of mycorrhizal propagules (spores, colonised root fragments and fungal hyphae) in a host plant in a dilution series. Even though the assay is indirect (absolute numbers of propagules are not measured), it has the advantage of providing a single value that can be compared directly with other tests in the same assay. The disadvantage is that environmental factors, type of inoculum, and manipulation of the inoculum can affect results (Wilson and Trinick, 1982). The assay is carried out in 60-cell propagation trays with *Plantago lanceolata* as host plant. Three 10-fold dilutions are tested (1:10; 1:100; 1:1,000). Each dilution treatment has 6 replicates. Six plants without mycorrhiza serve as the control. After 35 days roots from each individual plant (n = 24) are stained and examined for presence or absence of colonization using a dissecting microscope. In dilutions with low inoculum, roots must be scanned carefully for signs of colonisation. Neither detached hyphae nor germinated spores are counted as propagules. The number of colonised plants in each of the three dilutions is applied to a table of most probable numbers.

Identification by DNA sequencing

At the end of each propagation season, colonised roots as well as spores were used to identify the species present in MycoGro Ag® and MycoGro Hort®. DNA was extracted from roots using a Plant DNA isolation kit (MoBio Laboratories Inc.) and DNA was PCR amplified using the method described by Lee et al. (2008). PCR products were then purified and sequenced by MacroGen Inc., Seoul, South Korea. The fungal DNA sequences were matched to reference strains on an international database (<http://maarjam.botany.ut.ee/>).

Product evaluation

Experiments were carried out to validate MycoGro Ag® and MycoGro Hort® under field conditions and in pot trials.

Lettuce. Pot trials using lettuce provides a fast way to test inoculum and optimise rates. Seedlings from a commercial nursery in Christchurch were used to set up this pot trial. The substrate was a low fertility seed raising mix and 10% of MycoGro Ag® was blended into the substrate. The pot size was 1.5 L and each pot was filled to 1.2 L. For the MycoGro Ag® treatment 4.5 L seed raising mix was blended with 0.5 L MycoGro Ag® and then aliquoted

between pots. For control pots the seed raising mix was blended with 0.5 L sterile pumice. The recommended application rate for the imported product (A) was 1.5–2 kg/ha which equates to 0.15–0.20 g/m². This presented a too small weight of product to be reproducibly weighed for pot trials so that for practical reasons a much greater weight of product was applied: 2 g powder (product A) and 10 % sterile pumice per 1.2 L seed raising mix (equivalent to 151kg/ha). All treatments were replicated four times. The pots were placed in a tunnel house in August 2013 and fertilised weekly using Peter’s Excel CalMag Grower (Scotts®) at 0.8 g/L). After 8 weeks the lettuce fresh weight was measured from destructively harvested plants. The MycoGro Ag® treatment produced 54.8 % greater fresh weight than the control ($p = 0.02$) and 49% greater than the imported product ($p = 0.013$) (Fig. 2). There was no statistically significant difference between the plant fresh weight for the imported product (A) and the untreated control.

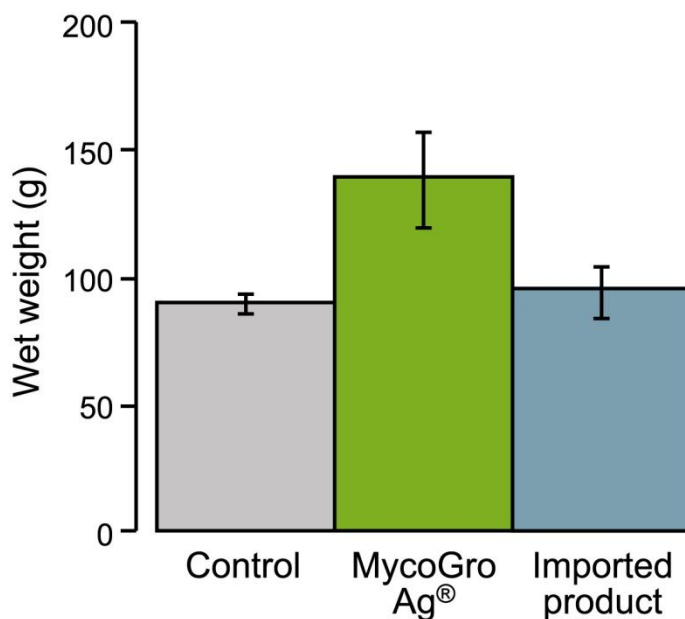


Fig. 2 Plant fresh weight of lettuce 8 weeks after transplanting seedlings into 1.5 L pots containing two mycorrhiza products and a no mycorrhiza control. Error bars are standard deviations.

Plantain. In autumn 2013 three different application rates of MycoGro Ag® were applied in a plantain field trial located in Springston (Canterbury) alongside two imported mycorrhiza products applied at label rates. MycoGro Ag® was cultivated into the soil at 50, 100, and 200 kg/ha prior to planting. All plots, including controls, received 380 kg/ha dolomite. The two imported products were applied according to the recommendations on the package at 2kg/ha (product 1) and 3 kg/ha (product 2) by mixing the powder with seed. Treated seed together with 200 kg/ha ammonium sulphate was then drilled into 9.6 m² plots with 4 replicates per treatment in a randomized block design. Shoot dry matter was measured in October 2013 by cutting 1 m² quadrates per plot. Data was analysed by a paired t-test. At a rate of 50 kg/ha MycoGro Ag® plantain shoot dry matter was 118% greater than the fertiliser control ($p < 0.05$). Higher rates of MycoGro Ag® did not increase dry matter above the 50 kg/ha rate but all three MycoGro Ag® resulted in significantly greater DM compared to the control ($p < 0.05$). Neither imported product had a significant effect on shoot dry matter. Note that the product labelled as “Imported 1” in Figure 3 is identical to product “A” used in the lettuce trial.

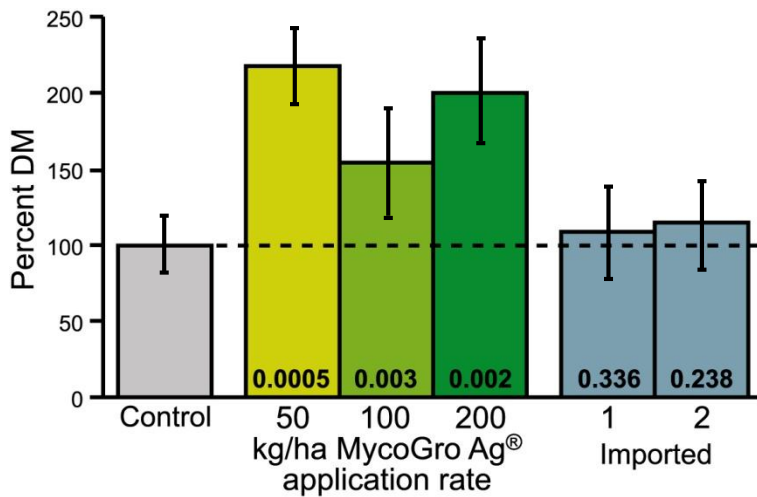


Fig. 3 Percent shoot dry matter of field grown plantain (*Plantago lanceolata* cv. Tonic) compared to control. Three different application rates of MycoGro Ag® were applied. Two imported products were applied according to the manufacturer’s recommendation. Error bars are standard deviations. The numbers within bars represent *p*-values.

Chicory. In October 2014 three different application rates of MycoGro Ag® were applied in a chicory field trial at one of Abron Ltd Hawkes Bay on-farm trial sites (dairy). The trial was set up as randomised blocks (7 × 2 m each) with 4 replicates per treatment. MycoGro Ag® was cultivated into the soil at 25, 50, and 100 kg/ha prior planting. All plots received 150 kg/ha coated DAP (diammonium phosphate) at planting and 60 kg/ha SustaiN in December. Dry matter cuts were taken in January by mowing 7 × 0.48 m strips, bulking herbage and drying for ≥24 hours at 80°C until the weight was constant. Data was analysed by “Student’s t-test. Figure 4 shows the dry matter yield which was higher for application rates 25 and 50 kg/ha but results were not statistically significant between treatments and the control (*p* > 0.05).

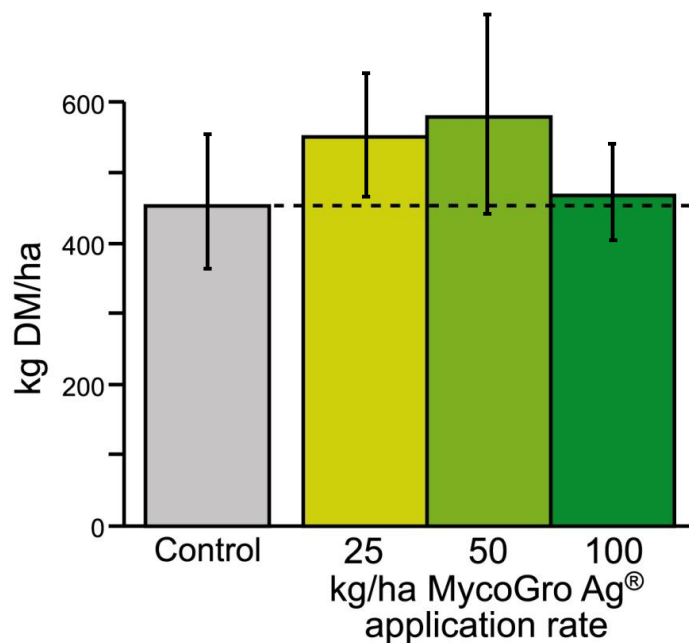


Fig. 4 Chicory dry matter three months after planting. Three different application rates of MycoGro Ag® were applied. All plots, including controls, received 150 kg/ha DAP at planting time. Error bars are standard deviations.

Pittosporum. Two year old *Pittosporum* plants were purchased from a nursery in Christchurch and transplanted into individual pots containing 1.2 L seed raising mix each in July 2014. Three treatments were prepared. Control pots received 0.120 L sterile pumice blended into 1.2 L seed raising mix per pot. MycoGro Hort® was applied at a 10% rate (1.08 L seed raising mix plus 0.12 L inoculum) and pots with the imported product received 0.12 L sterile pumice and 2 g of inoculum. The recommended rate of 2 kg/ha for the imported product would have meant applying 0.0264 g per 1.5 L pot, which was not able to be reliably weighed so a greater amount was substituted, equating to 151kg/ha. Four replicates per treatment were prepared. Plants were maintained in a tunnel house and received regular overhead watering and were fertilised monthly with Peter's Excel CalMag Grower at 1 g/L. After four months plant height was measured. Control plant and plants inoculated with the imported product showed an average plant height of 423mm and 437 mm, respectively. Plants inoculated with MycoGro Hort® showed an average height of 525 mm and were significantly higher than the control ($p = 0.001$) and imported product ($p = 0.005$). Pictures of roots were taken prior field planting and indicated consistently larger root biomass in the MycoGro Hort® treatment (Fig. 5). All plants were then planted into an area with other NZ native plants and will be measured again after 12 months.



Fig. 5 *Pittosporum* roots four months after inoculation with one imported product (A), MycoGro Hort® (B) and without inoculum (C). Control pots contained equal amounts of sterile pumice.

Concluding remarks

There is increasing interest in the use of arbuscular mycorrhizal fungi as biofertilisers across both agriculture and horticultural systems. However, the adoption of AMF-based products has been hampered due to poor quality imported commercial products on the market. Therefore a mycorrhiza product based on New Zealand sourced fungi has been developed. It has been demonstrated in the studies outlined here that MycoGro Ag® and MycoGro Hort® are able to increase plant biomass in agricultural and horticultural plants and field trials are ongoing. A bulk production facility for agriculture AMF has been established and the first commercial quantities will be available in May 2015. MycoGro Ag® adds another tool to farmer's fertiliser toolbox with the purpose of increasing plant performance by unlocking more of the full biological potential of soil and improving environmental outcomes by reducing fertiliser application.

Acknowledgements

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