Application of the Ultimate Arbuscular Mycorrhizal Inoculant MYCOGEL(R) in Japan: Results and Prospects

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Application of the Ultimate Arbuscular Mycorrhizal Inoculant MYCOGEL® in Japan: Results and Prospects

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Abstract

Arbuscular mycorrhizas (AM) are mutualistic symbioses occurring between the vast majority of land plant roots and a reduced group of soilborne fungi, the arbuscular mycorrhizal fungi (AMF). The fungus provides the plant of water and mineral and organic nutrients acquired very efficiently from the soil via fungal hyphae, which enhances plant nutritional status and physiological equilibrium, and results in higher yield and a healthier and more sustainable crop production. However, the obligate biotrophic status of AMF has hampered up to recently the large-scale production and application of AMF as inoculants. Conventional AMF inoculants consist of solid grain or powder substrates mainly containing dormant fungal spores, usually in a too-low percentage and often difficult to detect and verify for their vitality. These inoculants are difficult to apply homogeneously via watering systems, slow to establish symbiosis and, what is worst, usually contain non-desired microorganisms, due to their non-*in vitro* production and formulation. This situation gave a U-turn ten years ago with the presentation of the first ultrapure, gel-type mycorrhizal inoculant in the world, MYCOGEL®, produced and commercialized *in vitro* to preserve all its quality and traceability from the lab to the field. MYCOGEL® promotes a very quick and specific AM response to the plant, thus exerting all AM benefits from the beginning of its lifespan, to finally enhance fruit production in terms of amount and quality. In this paper we present the first results obtained in Japan on MYCOGEL® application to different crops in agronomic conditions. Rice, green onion, lettuce, tomato, onion, green pepper, celery and grape were the crops tested, with important increases in crop productivity, quality and yield value. The relative importance of the percentage of mycorrhizal colonization of the roots *vs*. the agronomic effects observed is also discussed.

Introduction

For many years, arbuscular mycorrhizas (AM) have been referred to as one of the most promising solutions to the increasing use (and abuse) of chemical fertilizers, phytochemicals and pesticides in plant production. The ability of AM fungi (AMF) for nutrient (especially phosphorous) and water uptake, transport and release to the host root via their extraradical hyphae has been early described and largely documented (Smith and Read, 2010). More recently, an important role of AMF in alleviating both biotic and abiotic stresses in plants has also been reported, making certainly this group of symbiotic fungi a target for research and technology transfer. All this considering, it could appear surprising that, after more than five decades of AM research, AM inoculation is not yet a 'must' in all plant growers' notebooks and protocols.

The main reason for this is one of the most intriguing and complex characteristics of these groups of soilborne fungi: their obligated biotrophic nature, i.e., the fact that they are unable to complete their life cycle in the absence of a host root to establish symbiosis with. Although some advances have been made recently on the clues of this obligated biotrophy (Bago and Bécard, 2002; Jiang *et al*., 2017; Keymer *et al*.,

2017; Luginbuehl *et al*., 2017), AMF axenic culture and mass production seems to be far from reality nowadays. In fact, the closest we are to this possibility is the so-called monoxenic culture of AM (*in vitro* co-culture of AMF and root organ cultures, Declerck *et al*. 2005).

Until 1988, the sole way to culture AMF was to prepare solid substrates, to grow plants (either from seeds or seedlings) on it, and to add a "starter" (usually consisting of AMF dormant spores or mycorrhizospheric soil) containing propagules of one or more species/isolates of the fungi. After 6 to 12 months these would have colonized the plant and their extraradical mycelium would have extended within the substrate, producing new spores and thus propagating. It is easy to understand that such conventional technique was not suitable to carry out fine studies on AMF biology (such as biochemical or genetic studies), and absolutely inadequate to produce traceable, pure inoculum with minimal standards of quality. Although some researchers tried to establish *in vitro* cultures of AMF, firstly axenically (Mosse, 1962), then using root organ cultures (Mosse and Hepper, 1975), it was not until Bécard and Fortin (1988) adjusted the culture medium to make it compatible for both root and fungus, that the first complete successful monoxenic culture of AMF was established. Eight years later, St Arnaud *et al*. (1996) reported successful growth of extraradical mycelium and extensive sporulation in an independent compartment from the root. These two papers opened wide the door towards progress in mycorrhizal research, and consequently towards mass production of AMF propagules under *in vitro* conditions.

In 2005, Cano and Bago went a step forward, and designed a protocol for mass production of AMF by using, as a basis, monoxenic cultures of special characteristics. These cultures, after being processed, render a semisolid, gel-type inoculant containing not only dormant spores, but also infective hyphae and active mycorrhizal root pieces whose vitality and infectivity is preserved by the gel formulation. Initially (2005) presenting a concentration of 2 x 10^3 propagules/ml, it has reached nowadays a total concentration of 5 x 10^4 propagules/ml, a rate never seen before in AM technology. After being tested both in lab and agronomic conditions, this brand new mycorrhizal inoculant, named MYCOGEL®, was released to the market in 2007, firstly in Spain, then internationally.

MYCOGEL® does not contain any other microorganism besides AMF, nor any additive other than certain compounds issued naturally during the plant-fungal symbiotic association, which are retained by the gel and which may act as signals to the plant roots, thus preparing them for the imminent colonization while activating plant metabolism even prior to AMF contact (Cano *et al*., unpublished). MYCOGEL® is presented in a ready-to-use format, easily soluble in tap water to be applied either via irrigation systems, root immersion, injection to soil or spray-application to the substrate of seedlings boxes. This ensures an easy application, compatible with all usual agricultural practices, thus avoiding special care or restriction imposed by conventional mycorrhizal products.

Materials and methods

The experiments here described were carried out at different agronomic sites in Japan, by using as main testing product MYCOGEL® (Agrocode Bioscience™, Roquetas de Mar, Almería, Spain), an ultrapure AM-inoculant produced *in vitro* by means of patented technology and protocols (Cano and Bago, 2005). Conversely to conventional AM inoculants, which consist of solid pellets or thin, unsoluble powder, MYCOGEL® is a semi-solid gel containing at least 5 x 10^4 AMF propagules/ml.

In some of the experiments, two additional products besides MYCOGEL® were added: Rhyzo® (Kimitec Group™, Roquetas de Mar, Almería, Spain) is a root enhancing additive and a bio-nutrient whose power is based on its specific aminogram, B-vitamins and high phosphorous concentration. It is particularly indicated for encouraging rooting, and activating the root system, thus increasing growth potential for all type of crops during the initial stages of the vegetative cycle. Dosis used for this product was 1g/L when applied to seedling boxes, and 3 x 0,3g/L when applied directly to the soil. The second product used in some of the experiments was Bombardier® (Kimitec Group™, Roquetas de Mar, Almería, Spain), a liquid fertilizer obtained from the concentration of selected vegetables, with high concentration of nitrogen, amino acids, organic matter, polysaccharides, and fulvic acid. Dosis used for this product was 2cc/L every 15 days.

Detailed protocols for the different experiments carried out are summarized in **Table 1**. Protocols vary depending on the crop studied, since MYCOGEL® doses should be adjusted to the type of root (morphology and growth speed), to be applied as close as the growing roots and as early as possible after planting, in order to obtain the effects as soon as possible. Usually, one application of MYCOGEL® is enough to obtain the desired effects in annual crops; sometimes reinforce doses are recommended in particular situations, such as pluriannual crops, however this was not the case of the experiments described here.

Table 1 also describes the type of chemical fertilization used in each experiment, which, in general, should never be applied together with MYCOGEL®, but at least two weeks before or after, not to interfere with AMF germination and/or root colonization. The duration of each experiment was correlated to the lifespan of each crop, and it is also shown for each experiment in **Table 1**. Finally, parameters measured for each experiment are specified in the last column of the Table.

All numerical results presented were statistically analyzed by Tukey's test at a significance level of p=0.05. Where appropriated, different letters show statistical significant differences between treatments.

To test mycorrhizal colonization of MYCOGEL®-treated plants, trypan blue staining was carried out on samples of the different roots for each experiment, according to standard protocols (Phillips and Hayman, 1970). Percentage of colonization was measured according to Giovanetti and Mosse (1980).

Results and Discussion

Results obtained for each of the experiments described in this paper are summarized in **Table 2**. Detailed aspects for each of the experiment are shown in **Figs. 1 to 8**.

Table 1. Detailed protocols for the different experiments carried out in Japan with MYCOGEL® as AMF inoculant.

Exp. No.	Plant (variety)/ site of culture / trial surface/ plant density	Treatments	MYCOGEL® application and doses	Fertilization used	Experiment duration	Parameters measured
1	Rice (Yukiwakamaru) Yamagata 2000m^2 150 plants/m^2	- Rhyzo - MYCOGEL® +Rhyzo	- To seedlings in seedling box - 1 week before planting - 1ml/L	Basal 150Kg NPK/Ha; Top dressed with fertilizer (10-10-10)	4 months	- Total productivity (Kg/Ha) - Yield value (€/Ha)
2	Green onion (Morinokanade) Yamagata 300m^2 30 plants/m^2	- Untreated - MYCOGEL® +Rhyzo	- 1 ½ month after planting 1L/Ha	Basal 40Kg NPK/Ha; Top dressed with fertilizer (10-10-10)	4 months	- Root development - Total productivity (product size)
3	Lettuce (Raptor) Ibaraki 500m^2 7,5 plants/m^2	- Untreated - MYCOGEL®	- 1 day before plug seedling planting - 1ml/L	100Kg N/Ha	40-50 days	- Total productivity - Yield value -Resistance
4	4.1 Tomato (Rinka) Ibaraki 300m^2	- Untreated - MYCOGEL®	- 2 weeks after planting - 1L/Ha	Chemical fertilizer as appropriated	5 months	Root development
	4.2 Tomato (Rinka) Ibaraki 300m^2	- Untreated - MYCOGEL® +Rhyzo	- 2 weeks after planting - 1L/Ha	Chemical fertilizer as appropriated	5 months	- Average yield/plant -Root weight/plant - Average stem diameter/ plant
	4.3 Tomato (Rinka) Ibaraki 300m^2	- Untreated - MYCOGEL® +Rhyzo +Bombardier	- 2 weeks after planting - 1L/Ha	Chemical fertilizer as appropriated	5 months	- No. of damaged fruits - Average yield/plant
5	Onion (Lucky) Tokyo 300m^2 30 plants/m^2	- Untreated - MYCOGEL® at planting - MYCOGEL® 2 weeks after planting	- 1 or 2 week after planting - 1L/Ha	Basal 20Kg NPK/Ha	4 months	- Total productivity - Yield value
6	Green pepper (Ace) Ibaraki 300m^2 13 plants/m^2	- Untreated - MYCOGEL® + Bombardier	- 2 weeks after planting - 1L/Ha	Basal 300Kg N/Ha, chemical fertilizer as required	8 months	- Total productivity - Yield value
7	Celery Nagano 300m^2	- Untreated - MYCOGEL®	- To seedlings in seedling box - 0.5ml/L - 2 ~ 3 days before transplanting	Coated fertilizer	2 ½ months	- Plant vigour - Stem uniformity - Root volume - Size / plant
8	Grape (Mascot Zipangu) Okayama 300m^2	- Untreated - MYCOGEL® + Bombardier	- 1L/Ha	Basal, with compost	4 months after product application	- Plant vigour - Post-yield

Experiment 1. Effect of MYCOGEL® on rice (var. Yukiwakamaru)

The application of MYCOGEL® + Rhizo® on rice (var. Yukiwakamaru) rendered better, healthier-looking plants compared to the just Rhizo®-applied treatment (**Fig.1**). This was translated into a higher productivity (+10.6%) and higher yield economic value (+179.80 €/Ha) at the end of the experiment (**Fig. 1, Table 2**). It is important to note here that in a previous experiment, the application of Rhizo®-only to the plants rendered a 19,3% extra productivity when compared to an untreated plot; this suggests that the MYCOGEL® + Rhizo® combination could induce up to a 30% increase in crop productivity, and over 500€/Ha extra earnings for the grower when compared to untreated plants.

It was known that arbuscular mycorrhizal inoculation has a positive effect on rice when applied prior to flooding conditions, resulting in greater grain production and a better nutritional status of rice plants (Solaiman and Hirata, 1995, 1998). These experiments were conducted under greenhouse conditions, but, could they be translated to agronomic conditions? The results shown here confirm the utility of rice inoculation with arbuscular mycorrhizas in the "real world" when using the appropriated product, and open new prospect to extensive use of MYCOGEL® on this crop, of key importance for social economy in different Countries around the world.

Experiment 2. Effect of MYCOGEL® on green onion (var. Morinokanade)

The application of MYCOGEL® on green onion (var.

Table 2. Results obtained in the different experiments carried out in Japan with MYCOGEL® as AMF inoculant.

Exp. No.	Crop	Product	Parameter measured	Control	Treated	Difference
1	Rice (var. Yukiwakamaru)	MYCOGEL® +Rhyzo®	Total productivity (Kg/Ha)	5450	6030	+10.6% (+580 Kg/Ha)
			Yield value (€/Ha) (0.31€/Kg)	1689.50	1869.30	+179.80€
2	Green onion, (var. Morinokanade)	MYCOGEL® +Rhyzo®	Total productivity (2L category)	51%	60%	+9%
3	Lettuce (var. Raptor)	MYCOGEL®	Total productivity (Kg/Ha)	22200	24000	+8.10% (+1800 Kg/Ha)
			Yield value (€/Ha) (0,18€/Kg)	3996	4320	+324€
			Increased resistance to Spot Bacterial Disease	--	Yes	--
4	Tomato (var. Rinka)	MYCOGEL® (4.1)	Root development	--	Higher	+10% to 30%
		MYCOGEL® +Rhyzo® (4.2)	Average yield per plant (Kg)	2.4	3.0	+24.5%
			Root weight per plant (g)	175.1	176.9	+1%
			Average stem diameter per plant (cm)	1.859	1,954	+5.1%
		MYCOGEL® +Rhyzo® +Bombardier® (4.3)	Number of damaged fruits	21	13	-40%
			Average yield per plant (Kg)	1.46	1.99	+36%
5	Onion (var. Lucky)	MYCOGEL®	Total productivity (Kg/Ha)	54000	59000	+9.26% (+5000 Kg/ha)
			Yield value (€/Ha) (0,25€/Kg)	13500	14750	+1250€
6	Green pepper (var, Ace)	MYCOGEL® +Bombardier®	Total productivity (Kg/Ha)	70000	87500	+25% (+17500 Kg/Ha)
			Yield value (€/Ha) (0,63€/Kg)	44100	55125	+11025€
7	Celery	MYCOGEL®	Plant vigour, stem uniformity, root volume, size per plant	--	Higher	--
8	Grape (var. Mascot Zipangu)	MYCOGEL® +Bombardier®	Plant vigour, post-yield	--	Higher	--

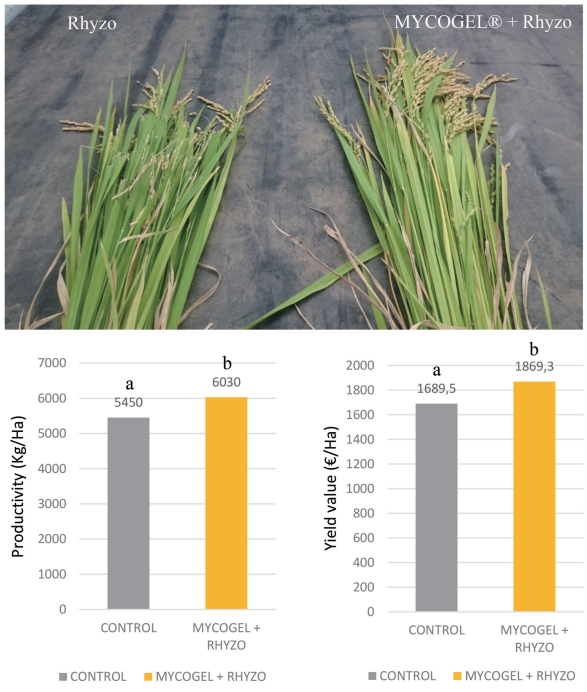

Fig. 1. Results obtained after application of MYCOGEL® + Rhyzo® on rice (var. Yukiwakamaru). Different letters show significant differences (p=0.05)

Morinokanade) resulted in a very important increase in product size (**Fig. 2**): a 9% increase in plants belonging to the "2L category" was noted when treated with MYCOGEL®, compared to untreated control plants (**Fig. 2, Table 2**). Since the fruit of the biggest size gets the highest value at the market, this translates into important extra earnings for the grower. Besides this, an important increase in root volume of MYCOGEL®-amended plants compared to control plants was obtained, which indicates a better nutritional status and physiological development of inoculated plants.

Experiment 3. Effect of MYCOGEL® on lettuce (var. Raptor)

In this experiment a Spot Bacterial Disease Resistant lettuce variety (Raptor) was used. This disease is a newly-described one, caused by the bacteria *Xanthomonas campestris pv. vitians*, which affects mainly lettuce and causes devastating losses which have been reported to be up to 80-100%. Therefore, it was quite important to assess the compatibility of MYCOGEL® to resistant varieties and test its potential to even ameliorate crop production under these conditions.

Although at a first glance control lettuce produced in the experiment could appear bigger in size (**Fig. 3**), total productivity, measured as Kg/Ha, showed otherwise (**Fig. 3, Table 2**): an increase of 8.1% in production of MYCOGEL®-

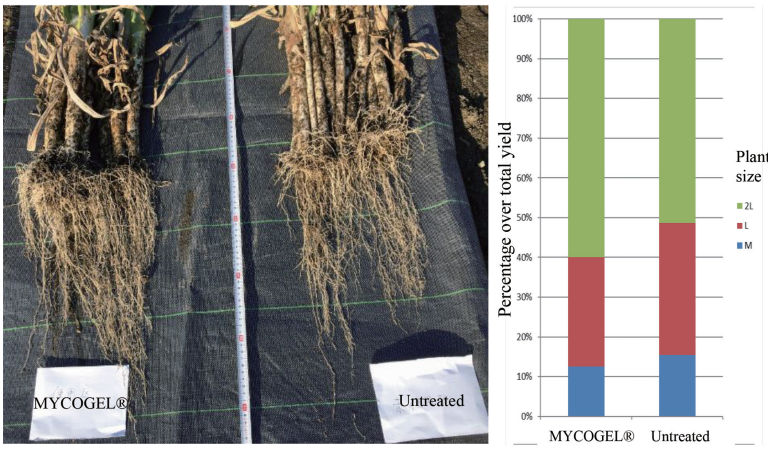

Fig. 2. Results obtained after application of MYCOGEL® on green onion (var. Morinokanade).

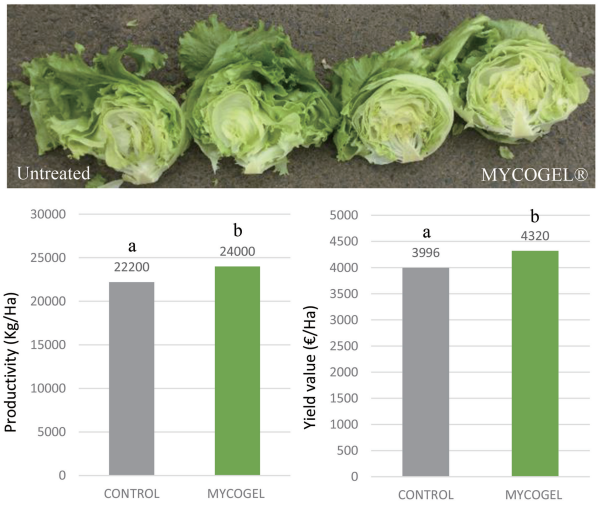

Fig. 3. Results obtained after application of MYCOGEL® on lettuce (var. Raptor). Different letters show significant differences (p=0.05)

amended plants was obtained. This would translate into +324€/Ha extra earnings for the grower. MYCOGEL®-treated lettuces wrapped firmly and beautifully, and no signs of the disease could be seen on them.

Therefore, we can conclude that the application of MYCOGEL® for lettuce production resulted not only in better yield and increased earnings, but also in healthier plants and a decrease losses caused by Spot Bacterial Disease.

Experiment 4. Effect of MYCOGEL® on tomato (var. Rinka)

Three different experiments were carried out with tomatoes as a testing crop. On the first of them, in which application of MYCOGEL®-only was compared to untreated control plants, inoculated plants showed a higher root development (**Table 2**), ranging between +10 up to +30%, as well as a larger root hair volume, which indicated a better nutritional status of the plant. It was not possible to finish this experiment up to fruit production.

In the second experiment with tomatoes, inoculated plants were amended with a combination of MYCOGEL® +Rhyzo®. In this case crop production analysis rendered an increase of 1% in root weight per plant and a 5.1% increase in average stem diameter per plant, which finally brought to an increase of +24.5% in average yield per plant (**Table 2, Fig. 4**). This should translate on important extra earnings for the grower. Finally, on the third experiment carried out with tomatoes, untreated plants were tested against MYCOGEL® + Rhyzo®, and MYCOGEL® + Rhyzo® + Bombardier® -inoculated plants respectively. Results rendered an extra crop production (measured as average yield/plant) of +18% in the case of MYCOGEL® +Rhyzo® plants), which raised to a +36% in the case of MYCOGEL®+Rhyzo®+Bombardier® -treated plants (**Table 2, Fig. 4**). As shown in **Fig. 4**, treating plants with the combination MYCOGEL®+Rhyzo® +Bombardier® also resulted in a lower number of damaged roots at the end of the experiment compared to untreated plants; namely a 40% decrease in negative symptoms was noted.

Treated tomatoes appeared healthier-looking and more reddish compared to untreated ones, which is in agreement with certain results in which lycopene content was increased by MYCOGEL® inoculation in different tomato varieties (Reva *et al*., 2018). Lycopene is a recognized anti-oxidant and anti-cancer agent (Ono *et al*., 2018), therefore inoculation of tomato plants with the combination of MYCOGEL® + Rhyzo® + Bombardier® may not only be an economic question, but also a healthy question, in agreement with social demand and government directives.

Experiment 5. Effect of MYCOGEL® on onion (var. Lucky)

In this experiment two inoculation timing were tested: first, applying MYCOGEL® at planting; and second, applying it 2 weeks after planting. The results obtained show (**Fig. 5, Table 2**) a 10% increase in average weight per plant compared to untreated plants in the first case (at planting) and a 5% increase in average weight per plant over untreated plants in the second case (2 weeks after planting). It is well-known that application of arbuscular mycorrhizas to plants should be carried out as early as possible along the plant lifespan, since young plants are more receptive to AM symbiosis, but also because of the interest of obtaining the mycorrhizal-induced beneficial effects as soon as possible. Our results confirm

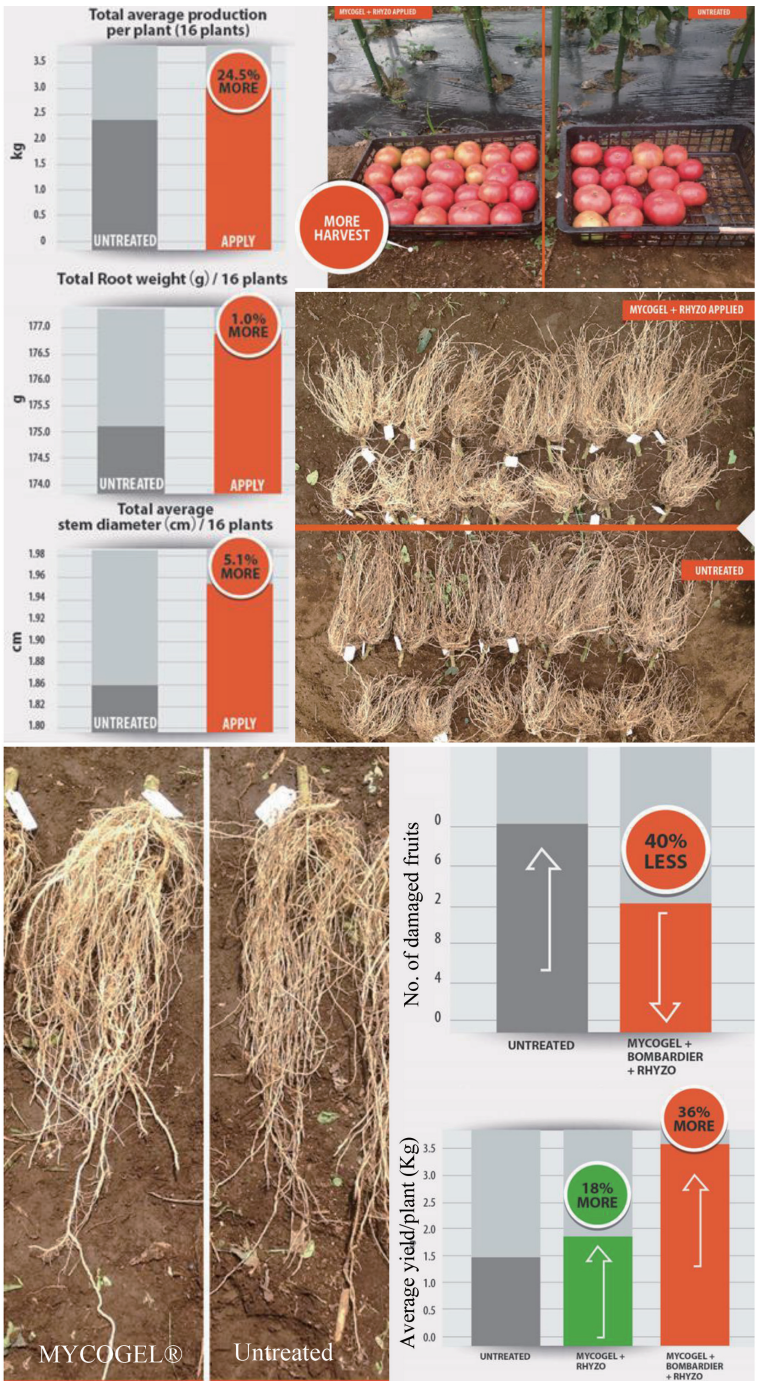

Fig. 4. Results obtained after application of MYCOGEL® + Rhyzo® or MYCOGEL® + Rhyzo® + Bombardier® on tomato (var. Rinka).

these facts and stress the importance of treating plants under agronomical conditions with MYCOGEL® at the earliest convenience, always following the technical instructions provided on each particular case and for each particular crop.

A 9.26% increase in total productivity, measured as Kg/Ha, was obtained when treating plants with MYCOGEL® compared to untreated controls (**Table 2**). Considering an average price in the market of 0.25€/Kg, the economic benefits of applying MYCOGEL® would increase in 1250€/Ha extra income, which is a non-negligible figure. These important economic benefits are even more convincing when considering the extra beneficial properties conferred to the soil by arbuscular mycorrhizas, such as a better aeration, improvement in soil structure, enhancement of soil microbial diversity and C recycling. More than 450 million years of co-evolution between arbuscular mycorrhizas and land plants have certainly result in a natural, environmental-friendly way for agronomic production that humans should take advantage of, moreover now that biotechnological tools such as MYCOGEL® are available.

Experiment 6. Effect of MYCOGEL® on green pepper (var. Ace)

Results for green pepper (var. Ace) with MYCOGEL® combined with Bombardier® are the most spectacular ones among those reported on this paper (**Fig. 6, Table 2**). Up

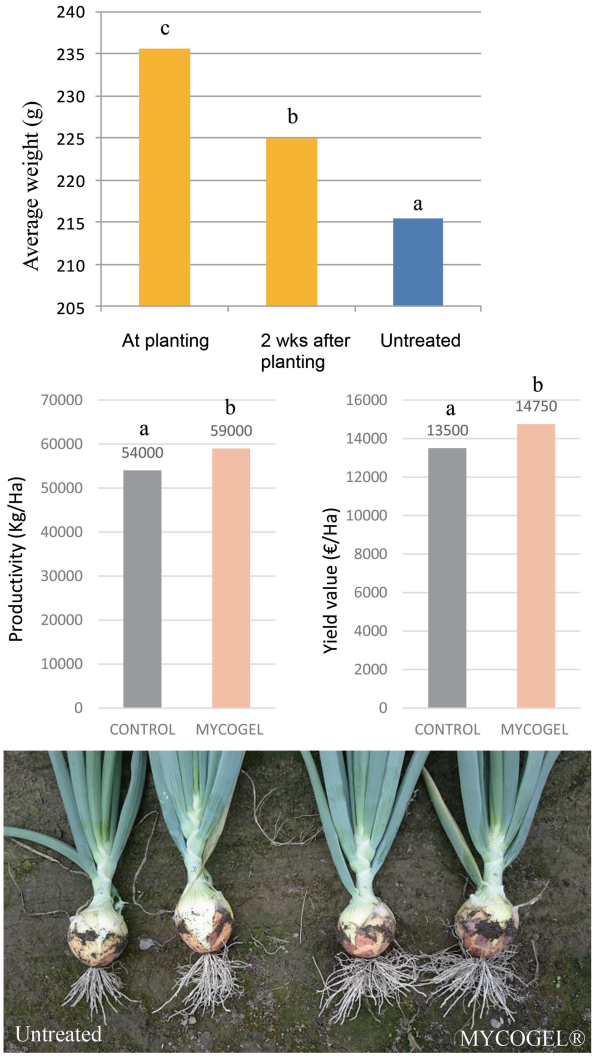

Fig. 5. Results obtained after application of MYCOGEL® on onion (var. Lucky). Different letters show significant differences (p=0.05)

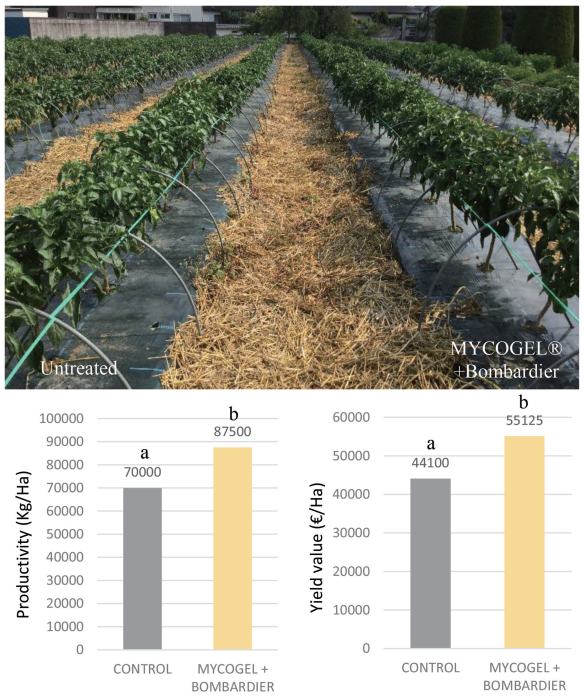

Fig. 6. Results obtained after application of MYCOGEL® + Bombardier® on green pepper (var. Ace). Different letters show significant differences (p=0.05)

to a 25% extra productivity was obtained in terms of Kg/Ha, meaning earnings increase of somewhat 11000€/Ha. As discussed before, this result should encourage growers to make confidence on mycorrhizal technology and on mycorrhizal products which demonstrate high standards of quality. In this sense, it would be desirable that the scientific community, together with governmental rulers and environmental agents would establish clear mycorrhizal product should meet in order to be eligible as market products. Also, it would be interesting that Governments would encourage the growers to use of such products by making them easily available and/or even subsidized. This could promote a cleaner, low-input agronomy while increasing circular economy, which is nowadays one of the main targets across Countries.

Experiments 7 and 8. Qualitative effect of MYCOGEL® on celery and grape (var. Mascot Zipangu)

In these two last experiments just qualitative measurements were carried out, however they pointed once more to the interesting benefits conferred by MYCOGEL® to the crops.

In the case of celery (**Fig. 7**), higher amount of active, white roots, together with higher vigor, uniformity and growth rate was obtained compared to untreated plants. Inoculated plants also presented less steam bend and disease loses, which translated in higher productivity and greater profitability.

In the case of grape, treatment of plants with MYCOGEL® +Bombardier® render an increase in plant vigor, fruit quality and, importantly, fruit longer of post-harvest life (**Fig. 8**): while treated bunches of grapes collected on July, 21st showed little changes after 3weeks (August, 10th), untreated bunches of grapes collected the same day showed rotten units after that time. This effect of extended post-harvest life has been also observed in other fragile cultures, such as strawberries (Huelva, Spain) and tomatoes (Granada, Spain), and probably has to do with the anti-oxidant abilities conferred by mycorrhizal symbioses to the plant. Post-harvest loss is an important issue, especially in export crops which should maintain good appearance and properties as much as possible until arriving to destination. Mycorrhizal extra-endurance properties in this sense would be certainly welcomed by producers.

In these two cultures fertilization was amended by means of coated fertilizer (celery) or basal fertilization just with compost (grape). It is important to stress here that applying the adequate fertilization type and level in crops amended with MYCOGEL® is crucial: in the past (a view is still retained by certain researchers and technical advisors) there existed the idea that applying mycorrhizas was not at all compatible with applying chemical fertilization, and particularly that addition of P was strictly forbidden. This was based on the well-known fact that high P concentrations inhibits AMF spore germination and even root colonization (Hepper, 1980; Same

Fig. 7. Results obtained after application of MYCOGEL® on celery. A, C, E, untreated plants. B, D, F, treated plants.

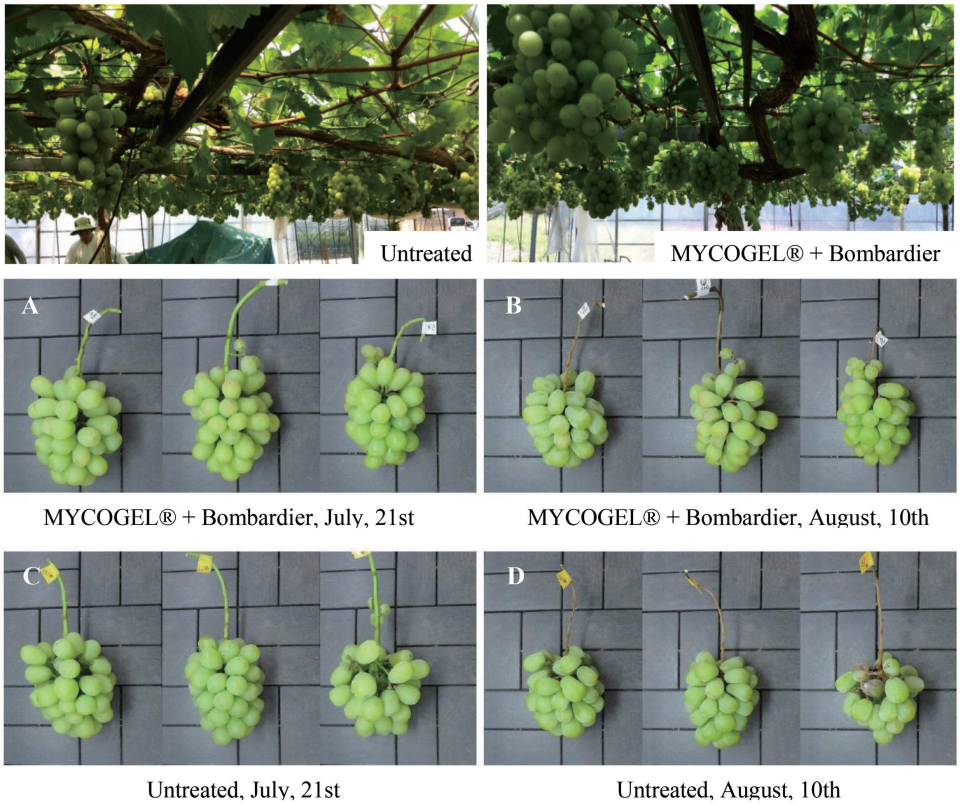

Fig. 8. Results obtained after application of MYCOGEL® + Bombardier® on grape. A, treated plants on July, 21st; B, treated plants on August, 10th; C, untreated plants on July, 21st; B, untreated plants on August, 10th.

et al., 1978). However, P is an essential nutrient for plant grow and crop production and that it should never be avoided, but make it compatible, when inoculating crops with mycorrhiza (Grant *et al*., 2005). To do this, it is important to never apply MYCOGEL® and fertilization at the same time, but waiting for the fungus to have colonized the root (i.e., about 2 weeks), then apply fertilization at convenience. A good alternative to this is to use slow nutrient-release fertilizers, which consist of granules of fertilizer coated with a special resin providing the exact fertilized dosage on each moment of the crop. Also, natural manure as a basal fertilization is a highly compatible practice, exactly the two techniques used in these two experiments.

AMF colonization results

Analyses for mycorrhizal colonization of roots sampled from the different crops tested rendered very low rates of root colonization (<5%). Furthermore, in some of the crops, mycorrhizal colonization could even not be detected. Although these results might be initially surprising, the fact is that low rates of host root colonization are the rule rather than the exception when using *in vitro*-produced AMF inoculum. However, the important effects obtained for treated plants (such as those presented in this paper) are consistent, and have been reported in many trials both under lab or agronomic conditions. How is this possible?

There are three possible explanations for this fact: first, although generally assumed that there is a direct correlation between percentage of root colonization and plant benefits (i.e., the higher the % colonization is, the better the plant nutritional status is), this has been shown not to be true in different situations (Treseder, 2013). For instance, Ávila-Peralta *et al*. (2015) found that, from a given level of colonization on, increase in root colonization by AMF resulted in a decreased symbiotic effectiveness and host benefits, indicating that, in those situations, the AMF behave somehow parasitically rather than symbiotically. Already in 1988 Douds *et al*. shown that there is an optimal level of mycorrhizal colonization above which the plant receives no enhanced nutrient uptake (benefit) yet continues to support mycorrhizal metabolism (cost). This colonization extent depends on many factors, among other: the nutritional status of the plant, water and nutrient availability in soil, light intensity and plant and fungal genotypes (Treseder, 2013). In fact, the final extent of root colonization is a combination of *both*, plant and fungal control of intraradical colonization. It is easy to understand that, from the point of view of the plant, acquisition of the most nutrient and water influx possible with the least cortical fungal "invasion" would be the best situation; on the other hand, from the point of view of the AM fungus, the least energy, resources and C (in terms of fungal structures) it should allocate to its intraradical mycelium to get the most C compounds out from the root, the better. In other words, if just one, very efficient, restricted infection unit would be sufficient to support satisfying bidirectional nutrient transport between both symbionts, that should be good enough to render the observed plant (and fungal) extra growth and benefits. Further research is needed to confirm this hypothesis and to understand why *in vitro*-issued inoculants seem to behave more efficiently at lower colonization rates compared to conventionally-produced inoculants.

A second possible explanation for the reduced AM colonization observed is the difficulty of collecting all root pieces from agricultural soils, in which roots have grown extensively and not confined, as it is the case of pot cultures. It is well known that mycorrhizal colonization is most active at subapical zones of secondary and higher-order roots. These zones are very fragile and are frequently discarded when collecting roots out of the soil. If restricted infection units were established precisely at these zones, the total, real root colonization would be strongly underestimated.

A third possible explanation for the low colonization observed is a more paradigmatic-challenging one: that physical AM colonization of roots is not as necessary as thought for the plant to obtain benefits from the presence of AMF, but the presence of certain signal molecules emitted by the AMF as a response of symbiosis (or pre-symbiosis), which could act as potent biostimulants profoundly affecting plant physiology. This hypothesis is being tested actually with surprising results (Cano *et al*., unpublished) and could make a turning point in our understanding of AM mutualistic symbiosis.

In conclusion, the application of the ultrapure AM inoculant MYCOGEL® to different crops in Japan resulted in important benefits, not only from an economic point of view, but also in terms of healthier fruits and a more sustainable management of agricultural soils. Plant growers can benefit from this new technological tool which copes with the highest standards of quality and efficiency, and is in agreement with the Governmental directives in terms of circular economy.

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