Trial Protocol & Analysis
Greenhouse & Nursery Growers

Every grower that decides to trial MycoApply® mycorrhizal inoculants comes from a different perspective and is looking for different benefits. It is important to remember mycorrhizae provide plants one key advantage, they increase the root absorptive area of the plant, allowing the plant to better capture the nutrients and water. From these plant improvements, the full array of benefits emerge. These benefits are discussed in more detail during the analysis section of this document. This Trial Protocol and Analysis provides a general overview of mycorrhizal fungi, outlines the basics of trialling mycorrhizal products, and provides compatibility and cultural protocols, in order to optimize your ability to best utilize these products, observe and document the benefits, and ultimately experience the return on investment (ROI) that these symbiotic organisms provide professional growers.

General Information

- Mycorrhizal fungi have evolved with plants for over 450 million years to develop symbiotic relationships benefiting both the fungus and the plant. Nearly 95% of all major crop species have mycorrhizal relations that provide carbon, sugars, and lipids to the fungi, and in return provide nutrients and water to the plant.
- Endomycorrhizae are the most common and have a mutualistic association with approximately 80-85% of plant species. They colonize plant roots intracellularly (inside the root cell). They are considered generalists, meaning any endomycorrhizae can develop a relationship with any endomycorrhizal plant.
- Ectomycorrhizae form with about 5-10% of the plant species. They colonize plant roots extracellularly (outside the root cell). Conifers (cone bearing) and many hardwood trees have ectomycorrhizal relationships. Ectomycorrhizae are often specific to certain plant species. If you are running your trial on ectomycorrhizal plants, please contact a Mycorrhizal Applications representative to determine which product is best for the plants you are growing.
- Two common horticultural families, Ericaceae and Orchidaceae, form mycorrhizal relationships with other types of mycorrhizae, which are not commercially available. Additionally, approximately 5% of plant species are non-mycorrhizal. Common non-mycorrhizal families include: Brassicaceae, and Caryophyllaceae.
- Prior to commencing your trial, review the *"Mycorrhizal Status of Plant Families and Genera"* list or interactive database and determine whether your crops are endomycorrhizal (Endo) or ectomycorrhizal (Ecto) to identify which MycoApply product you should trial. Endo-only and Ecto-only products are available. If you are growing both endo and ectomycorrhizal plants, then choose an Endo/Ecto blend which will cover both.
- The next task is to decide what form of product that you plan to use, a dry granular/powder/ injectable powder formulation or a non-aqueous liquid. Use the dry granular/powder to incorporate into potting soils or when mixing with landscape soils. The injectable powder and non-aqueous liquid products can be added to water to form a mycorrhizal suspension, and then used as a dip, drench, etc. The suspension will require initial agitation, and the particles will pass a 50-mesh screen. We do not recommend using a horticulture injector dosing system with the MycoApply powder products.

Mycorrhizal Trialing Basics

- MycoApply products use the term "propagules" instead of "spores" to determine how much product to apply per pot or per plant. However, different products have different counts of propagules per pound/gram and it is the quantity of propagules that is important to the success of inoculation for the plant. For drenching, the desired propagule count per container varies depending on the size of the container, the maturity of the plant, and the crop time. Refer to the various *"Drench and Boom Spray Recommendation Charts"* for more information. If you plan to incorporate the mycorrhizae into your soil, refer to the *"Soil Incorporation Chart"* for more detailed information. Mycorrhizal propagules may survive for several years in the soil before germinating and colonizing the host plant. Thus, it is important to place the propagules in the root zone, near actively growing roots. Ideally this is within ½ inch (13 millimeters) of the growing root. These root tips produce exudates that signal the fungi for colonization.

- If the crop is going to be sub-irrigated or drip-irrigated treated at low volumes, then it is better to either incorporate the propagules into the container substrate or apply a heavy drench prior to placing the potted plants in these irrigation systems for the best distribution of the propagules in the growing substrate. The recommended drench rate, for optimal distribution of the propagules, represents about 8-10% of the container volume for peat or bark-based substrates, assuming that the mix is initially moist. Dry soils will require a greater quantity of water, but not more propagules. Saturated soils should be avoided because they will experience very little downward movement of propagules into the root zone. A third clear water drench may be needed to move the propagules down to the root zone.

- Mycorrhizae begin to colonize newly emerging plant roots within a couple of days after inoculation but can be applied before the seed germinates or a cutting starts to root. The benefits of inoculation start to become more prominent to the grower approximately 8 weeks after inoculation. Typically, the plant starts to benefit after 3-4 weeks. For mycorrhizal evaluation trials, it is best to test trial crops with a total crop time longer than 8 weeks. We suggest applying mycorrhizae during propagation phase or after you receive plugs or liners, so you can start the process sooner and see positive results earlier. A grower of young plants (plugs & liners) needs to ensure homogeneity of the mycorrhizal application(soil incorporation or drench) due to the small soil volume in the plant cell. An additional benefit of applying the mycorrhizae earlier is reduced costs, since you are applying the mycorrhizae to a smaller soil volume. Once treated during propagation, the mycorrhizae stay with the plant throughout its entire life and moves with the plant when it gets bumped up into a larger container. The benefits of mycorrhizae will become more apparent when the plant is moved from the plug/propagation tray into the finished container. Typically, you only need to treat plants once with mycorrhizae during production of the plant.

- The simplest trial is to grow plants with and without the mycorrhizae treatment under similar growing conditions. Avoid adding additional variables, so you can clearly see the difference. Mycorrhizae should make your plant more effective and efficient in the uptake of nutrients and water. Wait to reduce fertilizer and water inputs until you have demonstrated the benefit of adding the mycorrhizae on its own. Under optimal growing conditions found in most commercial greenhouse and nursery operations, mycorrhizal benefits may not be obvious, hence the reason for this protocol. To avoid biological and environmental variables, use large numbers of plants (100 or more if possible) for each treatment. Be sure to label each treatment so you can track the performance of the treated plants. It is also a good idea to physically separate the two treatments. Mycorrhizae can transfer from one plant to another via a shared water source or physically if the application splashes during treatment.

- Plant selection is a critical step in trialling the use of mycorrhizae. Often growers want to trial "problem" plants. This often is a poor choice. Since the plant is probably a "problem" plant due to several factors that may not be positively impacted by the mycorrhizae. We suggest you trial a plant that is part of the core of your plant offering. Mycorrhizae can benefit specialty portions of your product offering, but the best return on investment will come from when the mycorrhizae positively impact the main plants in our operation.

- Using fewer propagules than recommended will slow colonization and delay a desired response. A non-uniform application of the mycorrhizae (soil incorporation or drench) will cause variability in response, and lack of uniformity of the crop. Applying more propagules than recommended will have no adverse effect on plant performance. Applying the wrong type of mycorrhizae or applying mycorrhizae to a non-mycorrhizal plant will not negatively impact your crop, but should also not show any benefit.

Cultural Protocols

Irrigation	• Growers are often categorized as either growing wet or dry. If you are a wet grower, where access to available water by the plant is never an issue, you have the potential to negate one of the primary benefits of mycorrhizae. Dryer growers will more reliably see more visible benefits from the application of mycorrhizae. Often growers choose to grow wetter to reduce risks, especially if you or your team are less experienced. We suggest you research some greenhouse and nursery water management best practice, so that you can avail yourself and your plants to this key benefit of mycorrhizae usage. At some stage in a plant's life, it will be faced with lack of available water. In these cases, the mycorrhizal inoculated plant will turn on the ability to access mycorrhizae available water in the rhizosphere and mycorrhizal stored water in the hyphae. Plants choose the path of least resistance and choose the readily available water first before it uses the mycorrhizae to access additional water or pull from mycorrhizal water reserves.
Fertilizers	• Most greenhouse and nursery crops are over-fertilized, and this results in soft, vegetative growth with high nitrogen levels, reduced carbohydrates, reduced root development and greater disease susceptibility of roots, stems, leaves and flowers. High levels of water-soluble nitrogen and phosphorus suppresses most mycorrhizal activity (both connections and utilization of the benefit) because it reduces the need of the hosts for the mycorrhizae benefits. At some stage in a plant's life, it will be faced will experience low access to nutrients. In these cases, the mycorrhizal inoculated plant will turn on the ability to access mycorrhizae available nutrients in the rhizosphere and mycorrhizal stored nutrients in vesicles and exchanged in the plant by the arbuscule. Plants choose the path of least resistance and choose the readily available nutrients supplied by overfertilization first before it uses the mycorrhizae to access additional nutrients or pull from mycorrhizal nutrient reserves. • We recommend liquid fertilizer growers use a fertilizer that is low in phosphorus and has a high percentage of nitrate-nitrogen. For example, instead of using a 20-20-20 fertilizer, consider using a 20-10-20 fertilizer, or even better a 15-5-15 or 15-2-15 fertilizer. If you can evaluate other variables, consider reducing the 200-400 ppm N to 100-150 ppm N for a comparison. Consider using a clear water application between fertilizer applications to reduce readily available nutrients. If you must apply a high phosphorus analysis fertilizer, then apply it three or more weeks after inoculation to avoid inhibiting the mycorrhizal colonization. Controlled release and organic forms of fertilizers release their nutrients very slowly and do not increase the water-soluble nutrients in the substrate excessively, therefore they work very well with mycorrhizae.

Fungicides	• Mycorrhizal fungi are soil-borne fungi that may be susceptible to certain older chemistry fungicide products. The "MycoApply Interactions with Turf and Horticulture Fungicides" table is a list of our present knowledge of the compatibility of mycorrhizal fungi and fungicides. A US and Canadian version of the document is available. Many fungicides, including all biological fungicides, are compatible with mycorrhizal inoculation and development. The inoculation/symbiosis stage is the most susceptible period.
	• Most foliar applied fungicides (except systemics, such as Bayleton or Strike) have little effect on the mycorrhizal fungi.
	• Seeds treated with fungicides typically do not impair mycorrhizal development since the germinating roots carry the mycorrhizae away from the treated seed.
	• Terms and interpretations used in the *"MycoApply Interactions with Turf and Horticulture Fungicides"* table are as follows:
	  • "OK" – These fungicides can be safely used and should maximize the impact of the mycorrhizal inoculation.
	  • "Avoid Use" – If these fungicides are used as a part of the planned production:
	  1. Apply the fungicide and wait a month.
	  2. Apply the MycoApply mycorrhizal inoculant.
	  3. Allow the mycorrhizal fungi time to colonize, approximately 6 weeks, before applying an "avoid use" fungicide after inoculation.
	  • "Insufficient Data" – These products have not been tested to determine their impact on mycorrhizae. We suggest you trial with and without the fungicide on a small scale before you proceed with use of any of these products.

Other Chemical Treatments	• Mycorrhizal fungi are generally not harmed by herbicides, nematicides, or insecticides. For trial purposes, we suggest not applying mycorrhizae with one of these products to avoid an additional variable
Applying with Fertilizers	• If you want to apply your fertilizers and mycorrhizae at the same time, we suggest using MycoApply EndoThrive. It can be added to a fertilizer stock tank and applied with a liquid fertilizer. The EndoThrive concentrate can be stored for a period of time within the fertilizer stock tank. For trial purposes, we suggest not applying the fertilizer and mycorrhizae together to avoid an additional variable.

Mycorrhizal Applications®

Trial Analysis
and Conclusions

Evaluating Your Mycorrhizal Trial

- At the conclusion of your trial, analyze the plants and make comparisons between treatments that are important to you, your crop, and your bottom line. Visual comparisons are always good because those are important to both you and the buyer. If you have 100 plants of each treatment, then choose the best 20 plants and the worst 20 plants of each treatment and compare them side-by-side. Compare the top growth and compare the root systems of the treatments. Commonly observed differences include increased roots, shorter crop time, better branching, darker foliage, and more flowers. Typically, if the trial plants have gone under some sort of stress (heat, nutrient, or water) the comparison between the mycorrhizae-treated plant and the non-treated plants will be stronger, and the performance differences will be more pronounced.
- Another evaluation option includes all plants from all treatments. This time you need to go through all the plants and pick which plants are #1 grade and ready for sale now, then #2 grade and ready to sell within a few days and #3 grade for plants that probably will not be saleable anytime soon. Often mycorrhizae-treated plants can be sold bench run since the crop is more uniform. The potential labor saving can be enormous when growers move to a more uniform higher grade of plants and transition to a bench run picking system.
- Another important consideration is shelf-life of the treated and untreated plants. Allow some of the plants to wilt after applying a uniform amount of water, simulating stress after leaving the greenhouse. Determine how many days transpire before wilting, and then determine how fast the plants recover after irrigation. Are there favorable differences between the treatments? Can shrinkage be reduced by the treatments? Plants treated with mycorrhizae typically last 24-48 hours longer between waterings than untreated plants. Just think of all the production costs you will save if you increase the number of plants you sell at full price.
- In future trials, you may choose to reduce some input costs, such as water, fertilizer and/or pesticide applications. These reductions in production costs can also add up, both for your business's bottom line, and for the environment due to reduced water and fertilizer runoff. The presence or absence of mycorrhizal propagules will not cause harm to greenhouse-grown or nursery-grown plants, so weak or dead plants in either treatment would indicate problems in initial plant quality and/or growing conditions, rather than a treatment effect.
- There are many variables that go into a successful trial. If the growing conditions of the trial were not typical of your production practices, then you may wish to repeat the trial under a different environment or for other crops before drawing a conclusion. It is important to remember that mycorrhizae are biological organisms, and their response varies depending on the environment, plant genetics, and production protocols. The plant's responses that you are evaluating were driven by the plant's needs and the mycorrhizae's ability to satisfy those needs.

Efficacy Analysis

Colonization	Typically a mycorrhizal root colonization assay is not needed. Plant appearance and performance are often sufficient to evaluate the trial. However, mycorrhizal root colonization assays are available at Mycorrhizal Applications. A root assay confirms whether the roots have been colonized or not. The results are reported as a percentage of colonized roots in the sample and do not determine the adequacy of colonization for plant performance. Colonization percentages vary by plant type and environment. Please contact your Mycorrhizal Applications Representative for instructions for sampling, shipping requirements, and pricing.
Plant Analysis	**There are many growth parameters that can be measured or evaluated for each treatment.** **Visual:** 1. Visually, determine plant color of each treatment. Is one treatment darker green than the other or more variegated? 2. Is one treatment more compact or taller than the other? 3. Look at the root ball of each plant outside of the pot. You may wish to wash the roots in running water to make comparisons in root system development. 4. Is one treatment more advanced, and would be ready to sell sooner? **Measurements:** 1. Measure plant heights and obtain an average. 2. Measure plant diameters and obtain an average. 3. Count the number of branches and obtain an average. 4. Count the number of flowers and/or fruits and obtain an average. 5. Determine the difference in production costs (nutrients, water, labor, crop time) between the treatments. 6. Place dollar values on the plants to determine the difference in crop income. **Pay-By-Scan Measurements:** 1. Percent sell through. 2. Time at retail before sell. 3. Dollar value of plant returns.
Possible Return on Investment (ROI)	**Cost Savings:** 1. Reduction in fertilizer costs per unit. Compare tissue analyses of treated and untreated plants if you have reduced fertility levels for your trial. 2. Reduction in pesticide (fungicides, insecticides, nematicides, herbicides, PGRs) applications. Mycorrhizae use and the better stronger root system and improve plan vigor are a strong foundation to build an IPM program around. 3. Reduced water usage per unit, resulting in less run-off issues for the facility. 4. Fewer plant losses due to less transplant shock. This has become more important as the product mix has moved to more royalty costing cuttings and less seed produced plants. 5. More uniform growth resulting in shorter crop time and more "bench-run" harvesting. Enabling growers to reduce labor and become more mechanized. 6. Enhanced ability to handle stress during shipment to customer. Plants come off the truck looking better. 7. Improved plant shelf-life, reducing plant maintenance costs and reducing plant losses at retail. 8. Increased survival rates in the landscape for flowers, trees, and shrubs. This can lead to less plant returns by customers, which in turn increases profitability for the retailer and the grower. **Plant Benefits:** 1. Drought tolerance reduces plant wilt and severity. 2. Reduced plant mortality. 3. Quicker root and plant establishment. 4. Increased flowering and fruiting. 5. Adaptability tolerance. Plants better able to grow outside of ideal pH and EC range. 6. Expanded root network for water and nutrient absorption and their utilization. 7. Promotes healthy root systems and suppresses root pathogens and disease development. 8. Provides a measure of "crop insurance" that can withstand stress and optimize plant performance under less-than-optimal growing conditions.

We look forward to hearing about the results of your trial!

Please contact your Mycorrhizal Applications Representative when you have completed your trial and assessed your return on investment.