Tecnologías Naturales Internacional

Frequently Asked Questions (FAQ)

Microorganisms for the treatment of roots:

Mycorrhizal fungi, *Trichoderma* and beneficial bacteria (PGPR)

This section gives answers to frequent questions about the application, efficacy, compatibility, durability, quality and safety of our microorganisms for the treatment of roots.

Application

How often should the microorganisms be applied and in which quantities?

There is a general rule of thumb: Better small amounts often than one big amount once. The aim is to maintain stable populations in the soil that protect the roots over longer periods of time.

Free living microorganisms, such as rhizobacteria and *Trichoderma*, frequently establish big populations initially that can collapse again in the course of the growing cycle. In this case the inoculum should be applied several times.

Reapplying small amounts periodically over extended periods of time also helps to maintain a divers array of beneficial strains. After several species of microorganisms have colonized the root surface successfully, their distribution patterns start to shift. A species that is very dominant at the beginning may fall out completely later. Awkwardly, it may be precisely this species which renders especially valuable services to the crop at a more advanced stage of its development, for example throughout the highly demanding moment of fruit growth.

The situation is slightly different in microorganisms that are closely associated with the tissue of plant roots, such as mycorrhizal fungi or rhizobia inside root nodules of legumes. Plants that were inoculated well and entirely after germination retain these symbiotic partners until the end of there lives. Reinoculating does generally make less sense.

Reapplication of Endospor

Positive surprises are not uncommon when reapplying endomycorrhizal fungi. Vineyards and orchards that are several years old often profit from a fresh mycorrhization by Endospor. The benign effect of this “refresh” seems to be linked to the competition that occurs between different mycorrhizal species. After the novel application high performing strains replace species that are less efficient in parts of the root system.

The reapplication should take place at a time of the year when roots tend to grow. In temperate climes this happens in spring. The best moment in tropical and subtropical climes is at the end of the rainy season or – in irrigated crops – at the end of winter.

Should the microorganisms be multiplied before the application?

Some users propagate the organisms prior to application. Typically, a commercially available spore product undergoes a reproductive process under non-sterile conditions for several days. The aim of this “enrichment” is to generate more infective material for the treatment of plants. Also, once the microorganisms have advanced from spores to colonies or mycelia, they are able to colonize the root surface more immediately.

We agree that this reproduction can be advantageous at times. Nevertheless, we think that it is not justified in the face of readily available inocula that are technically mature and conveniently priced. Considering the long list of possible disadvantages, we do not recommend to multiply the organisms prior to application.

The growth medium consists of an energy source (molasses, dextrose…), nutrients (N, P…) and other sources of organic material (compost, fibers…). Sometimes these components may not be digested completely by the microorganisms, for example when the fermentation is being interrupted. Applying these components to the plants can have unforeseen consequences.

The additional investment in time and work can only be justified by the substantially improved efficacy of the microorganisms. This criterion is rarely being fulfilled in the context of a modern, economically run plant production.

Possible disadvantages of multiplying prior to application

Undigested residues of sugar can serve as a readily available energy source for pathogens that are already present in the crop, such as *Xanthomonas* or *Phytophthora*. In contrast to the new-comers, these established organisms are well adapted to the environment and thus able to use the sugar immediately for an explosive growth before they can be suppressed by the beneficial microorganisms. This danger is especially real for leftovers on the leaves after spraying.

The additional input of nitrates and phosphates can cause imbalances in a highly fine-tuned plant production. Above all care should be taken with hydroponic and semi hydroponic production systems. Their computer controlled, highly optimized nutrient inputs can become imbalanced by the unintended import of an additional nutrient source. There is anecdotal evidence that microorganisms that are multiplied in this way can enter into competition for nutrients with the plants. This cannot happen when applying our high-tech products.

When crude plant material is used as the source for carbohydrates it can become the avenue for the introduction of pathogens because they are multiplied just as all other microorganisms in the mixture. These materials should be exposed to the high temperatures of a composting process first, in order to kill pathogenic fungi and bacteria. In contrast, the propagation of beneficial rhizobacteria and *Trichoderma* does not allow for high temperatures.

This last point leads to the following general consideration: Good commercial inocula consist of blends of pure strains that are produced separately under sterile conditions in the highly controlled environment of industrial bioreactors. This kind of incubation precludes the co-production of undesired microorganisms. All conditions that are important for the growth are strictly controlled, so that the strains do not loose their desired properties. Leftovers of growth media are limited to a minimum. All these factors lead to consistently high quality that is being monitored constantly according to scientifically objective criteria. This is crucial because it is the only way to assure that good results obtained from applications in the field in the first year can be achieved again in the following years.

The artisanal reproductive process harbors a whole range of uncertainties. The duration of the fermentation and the ambient temperature under which it takes place tend to vary. As a consequence, dramatic changes may occur in the resulting species composition, the remains of the growth medium and the application properties. Since many microorganisms grow together, some species might be suppressed by competition and fall out all together. Mycorrhizal fungi, for once, cannot be reproduced in this way and most likely do not survive this process. The efficacy of the species that do survive may suffer at least partially. Thus, the reproduction process can result in highly variable end products which perform differently when applied to plants. This great number of unknowns has the potential for surprises which can distort planning schedules and time tables and in the worst case cause loss.

Is there an overdose for the application of microorganisms?

The fertilization with an overdose of mineral salts can cause the “burning” of the plants. This is not the case for the application of microorganisms. In other words, they either do their job or die. An overdose does not exist in a purely technical sense. Rather, the art of finding the right application rate consists in achieving effects through a sufficient amount of product and within an adequate budget.

What is the right amount of water for the application of the microorganisms?

Our products are fine powders or granulates. Some applications require them to be blended into potting mixes or to be mixed with the seeds. Often though they are mixed with water and sprayed or injected through the irrigation system.

For example, the product Bactiva is often applied at a rate of 30g per 10L of water. It is important that the spores of the microorganisms are drenched into the root zone. On the other hand, the water absorption capacity of the substrate should not be surpassed, in order to avoid flushing the spores out (for example by dripping through the bottom of irrigation trays).

From a biological perspective it is not important how much water is being applied, as long as there is enough water around for spore germination. In other words, the main function of the water is to serve as a vehicle. The aim is to introduce the spores as completely as possible into the root zone. The amount of water depends primarily on the application method and the equipment. It should allow for an even distribution of the product in a given production unit (germination tray, pot, raised bed, field or orchard).

Differences between foliar / soil applications

The amount of water should be well calibrated when applying biological products, such as *Bacillus thuringensis*, *Beauveria bassiana* or *Metarrhizium anisopliae* to the leaves of the plant. The efficacy of these products depends on the degree of surface covered. At the same time, a high concentration of spores is crucial to a good "hit score" when trying to reach all pathogens on the leaves.

The success of applications in the root system does not depend so much on the concentration of the spores in the immediate proximity of the root and its coverage of the surface. These bacteria and fungi can spread by forming colonies and mycelia. They are saprophytes and can make use of organic matter in the soil to grow towards the pathogens that they control. Conventional wisdom has it that this is virtually impossible for the spores of many microorganisms that live in the nutrient deficient cuticle of the leaves.

Is it necessary to adjust the pH of the water prior to mixing it with microorganisms?

All microorganisms thrive at a specific pH range that boosts their growth and efficacy. This is why some suppliers recommend adjusting the pH of the water before introducing the microorganisms. We do not support this view because the microorganisms chosen by us can cope with irrigation water that is somewhat acid or alkaline as long as this pH lies within the limits tolerated by plants. Even if the range is not in the optimum, they do not suffer permanent damage and for this reason there is no need to adjust the pH of the water when mixing.

Ultimately, it is the pH that the microorganisms encounter in the rhizosphere and the soil that affect their performance. Our strains tolerate more extreme pH-values than the plant. This explains why inoculated plants frequently outperform untreated plants on extremely acid or alkaline soils. The prime example is the successful reforestation of abandoned US mines with the help of the ectomycorrhizal fungus *Pisolithus tinctorius* after years of futile attempts without the fungus.

When does it make the most sense to apply microorganisms?

Applying beneficial microorganisms at regular intervals can improve production success particularly when negative soil properties harm the development of plants and soil organisms. They stimulate the growth of roots that have been damaged by transplant, fight with disease or suffer from soil compaction by boosting the development of the healthy parts while suppressing the pathogens.

Experience shows that it is uniquely beneficial to introduce microorganisms in soils that have been cultivated with conventional methods. Usually, the plants respond with an improved nutrient uptake, enhanced growth and higher yields.

Applying microorganisms makes a lot of sense when it is not conceivable that sufficient amounts of beneficial strains and species reach the rhizosphere by natural processes. This is especially true for endomycorrhizal fungi which have spores with large diameters. Normally they are not carried by air and thus absent from germination trays.

Mycorrhization

Seedlings that are germinated in peat moss or similar substrates usually cannot form mycorrhizae without the application of mycorrhizal spores. However, treating the plant with the fungus at an early stage gives the fungus the opportunity to invade the whole root area. In the process the fungus undergoes a maturing process just like the plant. Finally, at the moment of transplant, a well developed mycelium supports the plant to overcome the transplant stress. Nonmycorrhizal transplants normally come into contact with mycorrhizal fungi in the field sooner or later. Nevertheless, a late mycorrhization means that they loose out on an important head start at a crucial early moment of the growth cycle.

There is a huge diversity of crops that benefit from mycorrhization. However, those crops that give high yields over many years, such as vineyards and orchards, are especially attractive. The cost-benefit analysis is unambiguous and they should be treated early on and possibly several times. The investment in Endospor is minute as compared to other costs of crop production and the possible gains. The calculation is different for short lived crops, such as lettuce where the costs of mycorrhization can easily outweigh the benefits. In this case a field trial should give a clearer picture.

Why do not all growers and agronomists already apply microorganisms?

The worldwide use of microorganisms in plant production is steadily climbing. Their potential to complement or substitute for chemical fertilizers and plant protection products has been proven scientifically. However, their use of microorganisms is still in its infancy when compared to the predominance of chemicals. The reasons are manifold and sometimes subject to controversial discussions.

Many suppliers of biological plant protection products argue that users and lawmakers are being influenced by the intense lobbying of the financially powerful chemical industry. Manufacturers of biological alternatives too have increased their lobbying activities over the past few years. However, these outfits are for the most part small, local and service orientated. Their lobbying efforts tend to pale in the face of the ubiquitous presence of multinational chemical companies.

Small manufacturers of biological products cannot pay for the costly registration processes of plant protection products that are set by the high standards applicable to the chemical industry. At any rate this hold true for the present initial stage with the absence of big and lucrative markets that would justify these investments.

As a result, suppliers of "soil amendments" or "plant fortifiers" have to limit their claims to vague statements about the mode of action and advertise any aimed for pesticidal effects as "side effects". Once corned into this category, effective products are put together with dubious "snake oils" that do not pass a thorough scientific efficacy test.

The buyer cannot rely on commonly accepted standards for this nascent industry when choosing between different microbial products. Even the concentration of the stated ingredients can only be verified by costly analysis. This results in a buyers beware marketplace. By this count some few "lemons" of doubtful quality can harm the reputation of the whole industry, especially if fraudulent suppliers exaggerate claims for a quick buck.

Over time, the increasing interest and turnover unleashes a growth in high quality products and the consumer's brand awareness. We look with confidence into the future and believe that new technologies that create real value will prevail eventually.

"Conservative" users

The often quoted assertion that many growers and farmers are "conservative" and thus are not easily convinced by new developments does not explain anything. The sector has been shaped by all those technological trends that have transformed other branches of the economy, such as industrial rationalization, computerization and globalization. Neither is there a shortfall of regular crisis moments that spur change, like the increase of energy and fertilizer costs or the ever more limited portfolio of plant protection products due to more stringent legal restrictions and the emergence of pest resistance.

Is it possible to use soil-borne microorganisms for the control of leaf disease or for foliar fertilization?

Mycorrhizal fungi cannot grow on leaves. They have to be applied to the roots. This is different for many free living bacteria and fungi of the rhizosphere. Most can grow in the aerial parts of the plant if they find a nutrient supply. Still, they are exposed to a number of adverse conditions that hamper the conquest of this habitat.

In contrast to the abundance of organic matter in the soil and the rhizosphere, there are hardly any nutrients on the cuticle of the leaves (phyllosphere). *Bacillus* and *Trichoderma* cannot move around actively. Instead, they extend through a growth medium by forming colonies and mycelia. Motionless, void of an energy source, exposed to the dangers of drought and ultraviolet radiation, they can hardly contribute to the protection and fertilization of the plant.

Exceptions

There are isolated cases of successful applications of soil-borne microorganisms in the aerial parts of the plant. For example, bees are made to tread on an area soaked with *Trichoderma* spores when leaving the hive. The bees deposit the spores on strawberry flowers and thereby help to prevent *Botrytis* after fruit formation. Antagonistic soil microorganisms can also partially protect the root crown which is disproportionally at risk of rot.

Is it possible to boost the growth of beneficial microorganisms specifically

Our free-living microorganisms derive their energy from the breakdown of organic compounds. For this they need a range of supplements that also form part of the growth media used to cultivate microorganisms in Petri dishes. Adding these materials to the soil benefits all species of microorganisms no matter their effect on the plant. So it is not possible to exclusively target the desired microorganisms in this way

Still, generally speaking, it is commendable to add compounds that stimulate the growth of soil-borne microorganisms sustainably. An active and ecologically stable soil community bestows a certain resistance on plants against the attack of pathogens. However, one should avoid readily digestible sources of energy, such as sugars that can be broken down easily. Materials which exhibit microscopically small structures that increase their surface area have also turned out as favorable environments for microorganisms to grow.

New approaches

Apart from these general considerations, there are new attempts to boost exclusively the growth of beneficial microorganisms. It seems that some polyphenols act as messengers that stimulate the establishment of endomycorrhizal fungi in the root system. Also, the addition of selective growth media on the leaves can boost the development of a desired group of microorganisms on the cuticle. However, at present these new ideas have not yet been established successfully on a commercial scale.

Efficacy

Is it possible to guarantee the efficacy

Maintaining and strengthening the plant's health is the main contribution of our microorganisms. A biofungicide reduces the amount of a soil-borne pathogenic fungus without eradicating it completely. However, the remaining infective units are prevented from causing disease. We do not aim for the total destruction of the pathogenic fungus at the expense of the "patient" the plant. Instead our goal is to avoid damage *and* achieve maximum yield through healthy plants.

The application of conventional chemical substances follows a monocausal linear thinking. For example, if leaves loose their color as a symptom of a lack of nitrogen, adding nitrogen will result in a darker green.

Microorganisms tend to display an abundance of different modes of action and interactions. Dealing with complex biological systems requires comprehensive reasoning instead of a linear mindset that is restricted to simple cause-effect chains. In practice it is often impossible to predict efficacy with quantifiable accuracy. Instead, we ask ourselves about the likelihood of several effects to occur all at once, often reinforcing each other. These effects then often outperform the solutions offered by an approach purely based on chemicals.

Do biological products perform as well as chemical products?

Our biological products are of the highest quality and, when applied correctly, frequently get as good results as conventional chemical products.

Biological crop protection values prevention more than cure, often in marked contrast to the chemical plant protection approach.
The antagonistic microorganisms in the product Bactiva can improve problem soils substantially, particularly if they have accumulated great quantities of pathogenic fungi that are almost uncontrollable by conventional fungicides. When a soil has been exposed for many years to a glut of chemical fungicides, their replacement with antagonistic microorganisms represents a sudden and profound shift in the protection strategy. This can result in a dramatic improvement of plant health and growth.

The treatment with biological agents does not require the plant to rid itself of toxic chemicals by means of energy consuming biochemical processes. This is part of the reason why using biological control agents often increases yield.

Fertilization

Biofertilizers cannot completely replace chemical fertilizers in high performance crop production systems.

Nitrogen fixing bacteria only bind a limited amount of nitrogen (up to a maximum of 70kg/Ha), but on the other hand, they are capable of channeling this nitrogen efficiently to the plant without loosing the major part as in the case of chemical fertilization.

Microorganisms give the plant access to otherwise locked up phosphorus and potassium. However, they cannot fix theses elements from the air or simply “create” them out of nowhere. Hence, they can only help replacing chemical fertilizers when those elements are locked up in sufficient quantities in the soil where they are unavailable to the plants. This means that microorganisms cannot contribute phosphorus and potassium in an artificial substrate void of any mineral fertilizer.

An initial needs assessment should shed light on whether a chemical product can be replaced by a biological product. After looking at the results, these recommendations should then be improved further with the help of our technical support.

If the microorganisms really work that well, why is it that big companies do not offer them?

The use of microorganisms usually requires more technical support by trained specialists than the use of chemicals. For example, after a treatment with a chemical insecticide all insects fall dead from the leaves. In contrast, the success of bioinsecticides may rely on knowledge of the pest’s life cycle in order to determine the right moment for the application.

A holistic reasoning and a clear focus on technical services is required for dealing with biological systems. This is mostly incompatible with the sales structures and organizational culture of big multinational chemical companies. This opens opportunities for niche firms that dedicate all their effort on the special services related to biological crop management.

If microorganisms are capable of preventing disease, why doesn't it always say so on the package?

The product has to be submitted to a costly registration process so that pesticidal claims can be included on the label. Fortunes may be invested without any guarantee for a successful registration or subsequent commercial breakthrough. In the face of the present low level of sales in biological crop protection, the huge investments bear unpalatable economical risks for most companies.

In the meantime, the products are simply labeled as "soil amendments" or "biostimulants" and do not contain any pesticidal claims.

At which temperature do our microorganisms work best? At which temperature don't they work?

The development of our microorganisms reaches an optimum at warm temperatures (between 25°C and 35°C). Even higher temperatures can suppress their growth, but real losses occur only above 42°C when coagulation can take place, i.e., when proteins may be damaged irreversibly.

The microorganisms contained in our products must not be exposed to temperatures above 40°C during storage and handling. Beware that this can easily be reached in a car or in the tubes of an irrigation system in summer

Trichoderma doses not grow at temperatures below 10°C. Nevertheless, we routinely observe good results in cultures of temperate climes, probably because at low temperatures the activity of pathogenic fungi is also limited.

Most of our microorganisms should not be stored below freezing point because ice crystals might form in the cells and pierce through their membranes

Generally speaking, the conditions that are suitable for plant growth are also benign for the microorganisms that are contained in our products. This includes the temperature range. Microorganisms that live in close association with the roots and that tolerate hot and cold temperatures help the plant to withstand extreme temperatures.

How to judge whether the application of microorganisms was successful?

After a root has been treated with Bactiva it tends to grow more lushly. Its color is lighter and it is equipped with more root hairs. The root ball retains more earth when shaken. The root gains weight in comparison to the aerial parts of the plant.

Plants that were treated with Bactiva and Endospor withstand transplant, adverse conditions and lack of nutrients better. They are also more resistant to soil-borne pathogens and less prone to disease in all parts of the plant. They have higher yields, live longer and produce over longer periods of time.

Apart from these results that are easy to appreciate, the microorganisms can also be detected by laboratory methods.

Not all roots that are inoculated with mycorrhizal fungi show more vigorous growth. As opposed to ectomycorrhizae, the endomycorrhizal association cannot usually be seen with the naked eye. The successful performance of the symbiosis depends on the fungal mycelium to be well established in the soil. Contrary to common believe a good performance is not always correlated with a strong presence of the fungus in the roots.

Notwithstanding, this percentage of colonization is often measured by applying costly methods.

High concentrations of our strains of *Trichoderma* can be found in the proximity of the root with a light microscope for weeks after the application of Bactiva. The higher their concentration the more comprehensive they protect against fungal root rot.

Experience shows that soils and substrates that are treated with antagonistic microorganisms can still feature rather high concentrations of pathogenic fungi without the plants showing any symptoms of disease.

How much fertilizer can be saved when using nitrogen fixers and phosphate solubilizers?

The crop's demand for nutrients depends on many things, such as, the soil's natural content of macronutrients and micronutrients available to the plants, the special needs of the crop, its stage of development, the production system, and the desired yield. A fertilizer recommendation should take all of these considerations into account and be issued on the basis of a proper chemical analysis.

Hence, there are no blanket recommendations for savings on fertilizers through the application of mycorrhizal fungi in combination with rhizobacteria (such as the products Endospor 33, Fosfonat, and Endospor Dry Mix). Nonetheless, many empirical data show that the nitrogen and phosphate input for agricultural crops can be lowered by 20% in the first year. If yields do not decrease a further reduction to 30% and afterwards 40% can be achieved.

Beware of overambitious promises by those who advocate replacing the entire chemical and organic fertilizer program through the use of microorganisms. Please let our technical support assist you for the duration of several growth cycles when planning for the savings on fertilizer programs.

How much additional yield can be expected from the application of microorganisms?

There are frequent reports of yield increases in the range of 5 to 10% in agricultural crops after the use of Endospor 33, Fosfonat or Endospor Dry Mix. But similar to the discussion on fertilizer savings, it is impossible to give an across-the-board answer to this question.

Experience shows that treatment results in rather large yield increases when the base line yield lingers well below the potential for the crop. The production of maize (corn) can be expected to increase by 10% when it usually only attains 6ton/Ha, whereas maize that routinely yields 12ton/Ha should not be expected to gain more than 5%.

Compatibility

Are our microorganisms compatible with chemical products?

The recommendations for the use of biological products should contain a compatibility list that informs about the interactions with chemical plant protection products and fertilizers. This is especially true when employed as a tool of integrated crop management and in the gradual process of conversion from chemical to biological management.

The beneficial fungi contained in our products are usually compatible with insecticides, herbicides, antibiotics, and even a great number of fungicides. Sometimes fungicides cause a slight growth inhibition. For example, a fungicide might reduce the success of the colonization by our strain of the endomycorrhizal fungus *Glomus intraradices*. We define the fungicide as compatible as long as it does not cause the mycorrhization of the roots to fall below 80% of its normal value.

High phosphate concentrations (>40ppm in a nutrient solution) can slow down the colonization process by the mycorrhizal fungi to the extent that there is no colonization within the narrow time frame of plant production. Some crops, such as weed, respond to mycorrhization with higher than normal yields only after halving the phosphate input.

The beneficial bacteria contained in our products usually tolerate insecticides, herbicides, and fungicides but must not be combined with antibiotics.

As a general recommendation, bacterial and fungal spores should not be mixed with plant protection products and fertilizers in the same tank. Special caution must be taken when using broad spectrum antimicrobial biocides, such as chlorine and hydrogen peroxide.

Which cultural practices have to be adjusted when using microorganisms?

We pursue the aim of an integrated crop management with an ever more complete use of new microbiological elements within the solid framework of conventional cultural practices. Our products can be applied alongside the vast majority of chemical products, with the exception of antimicrobials and some few chemical fungicides. This allows for their step-by-step introduction without the total abolishment of other products or cultural practices.

Our long term goal is to replace chemical products as completely as possible by biological alternatives. We are delighted by every client who becomes so convinced by the protective properties of the microorganisms in the product Bactiva as to suspend the use of chemical fungicides against root rot altogether.

Our advisers assist in this incremental buildup of trust through a respectful relationship. We know that the shift from conventional practices to biological crop management always includes a shared learning curve. In the process we value years of experience and mistrust radical “solutions”.

Recommended practices

Cultural practices that improve soil ecology may not be a must but they are still advisable because they contribute to the formation of humus and suppress the proliferation of pathogens. This is especially true for the use of organic material and biostimulants that boost soil live (e.g.: compost, worm compost, fish extract, humic acids and fulvic acids, sea kelp extract). It also includes less tilling or even zero tillage, the use of suitable planting densities and crop rotation in summer and winter. The fertilizer program should be adapted to the results of up-to-date chemical analysis that also includes microelements and continuous measurements of pH values and electric conductivity. Our technical assistance encompasses all of these biological, chemical and organic components.

Which plants profit from the use of our microorganisms?

The roots of all plants are associated with beneficial microorganisms. Our product Bactiva can be employed successfully with all crops. Even the roots of epiphytes, such as ornamental orchids, are widely treated with Bactiva. The only exceptions are aquatic plants for which our microorganisms are not suited.

The products Bactiva, Endospor and Ectospor have been applied primarily and on a commercial scale to the following crops:

- Vegetables and fruits: tomato, chili, potato, lettuce, cucumber, strawberry, blackberry, raspberry, water melon…
- Legumes: bean, pea, soya, broad bean, chickpea, peanut...
- Grain and corn: corn, wheat, barley, millet...
- Ornamentals: poinsettia, rose, cut flowers…
- Forest / fruit trees: pine, oak, peach, avocado, walnut…
- Cash crops: sugar cane, cotton, oil palm...
- Green areas: golf courses, sports facilities, urban parks…

Mycorrhiza

Some plants do not associate with mycorrhizae. These include mainly members of the cabbage family (*Brassicaceae*), knotweed family (*Polygonaceae*), pink family (*Caryophyllaceae*), fat-leafed plants (*Crassulaceae*) and *Chenopodiaceae*, as well as many aquatic plants or plants that are associated with water, such as the sedges (*Cyperaceae*) and rushes (*Juncaceae*), and specific plant families, including carnivorous plants and parasites.

These plants cannot be treated with Endospor. However, they can be successfully inoculated with Bactiva (with the exception of aquatic plants).

In addition, different types of plants require distinct mycorrhizal fungi. They are commonly divided into mycorrhizal types, each of which is being formed by different fungal genera (ectomycorrhizas, ectendo-mycorhizas, arbutoid mycorrhizas, ericoid mycorrhizas, VA mycorrhizas, orchid mycorrhizas, and monotropoid mycorrhizas).

By far the most common form is the VA mycorrhiza (VA = vesicular-abuscular). It is formed mainly by fungi of the genus *Glomus* which is the main ingredient of the product Endospor.

The most commonly used product in forestry is Ektospor. It contains the ectomycorrhizal fungi *Pisolithus* and *Rhizopogon*. These fungi colonize the roots of all conifers and deciduous trees of the birch (*Betulaceae*), beech (*Fagaceae*), and willow family (*Salicaceae*).

Is it possible to apply *Trichoderma* and bacteria such as *Bacillus subtilis* mixed together?

The topic is subject to extensive debates by experts. Our experience so far has shown that *Trichoderma* and *Bacillus subtilis* can indeed occasionally harm each other through competition, antagonism, or even parasitism. However, these rare and generally weak negative interactions are more than compensated by the numerous advantages that come with a complex mixture. The combination of several highly active species in our products increases the probabilities of success and their potential for applications in different crops and a wide range of environmental conditions and production systems.

Is it possible to combine different products that contain microorganisms?

The ecological balance of a natural species community becomes more stable with an increasing number of participating species and strains. This rule also applies to the living community of the soil and the immediate sphere of influence of the roots, the rhizosphere. Here it is an ecologically stable, species-rich community of microorganisms that helps the plant to thrive even in poor conditions.

However, the microorganisms of two or more commercial products might occasionally interact with each other antagonistically when combined. It is conceivable, for example, that a strongly dominant but not very effective nitrogen fixer displaces the less dominant but more productive nitrogen fixer of another product altogether.

To avoid this risk, only products should be combined that complement each other. Each product should also have shown its efficacy as a stand-alone product. In this case a combination of several products should yield superior results than a single product.

Can microorganisms be combined with all substrates?

Each species can only live, grow and reproduce within a certain range of environmental conditions. This so-called ecological amplitude is usually wider for our microorganisms that colonize the roots than for their host plants, i.e., they can tolerate a larger variation in environmental conditions, such as temperature, altitude, acidity and availability of water. Crucially, the plants ability to withstand extreme pH values and temperatures or a lack of water also increases through under the influence of the microorganisms. Thus microorganisms widen a plant’s ecological amplitude by its buffering effect on extreme environments.

This finding answers a series of questions regarding the demands of microorganisms. Good growing conditions for plants are also beneficial for the microorganisms contained in our products. Consequently, microorganisms can be combined with all plant substrates.

However, the suitability of a substrate or a solution as a carrier for microorganisms that are stored for some time before being applied to crops, has to be confirmed on a case-by-case basis through laboratory tests.

Mixing our endomycorrhizal fungi with compost and storing the blend for a few weeks before use results in unsatisfactory levels of colonization on plant roots in three-quarters of the cases. However, this observation masks large and hard to explain differences between different types of compost.

At a sufficient level of freely available water saprophytic microorganisms, such as *Bacillus* and *Trichoderma*¸ can germinate and colonize the entire potting mix or soil. This results in a positive head start. However, such a population can collapse again when stored for too much time.

Durability

What is the shelf life of our microorganisms?

The durability of different species and strains of microorganisms differs widely. Bacteria such as *Bacillus* that form real spores (endospores) are the most enduring creatures known to science.

The situation is quite different for bacterial species that do not form true spores, such as *Pseudomonas*, *Azospirillum* and *Azotobacter*. They lose viability after a few months unless they are dried thoroughly and stored at temperatures below freezing point.

Our strain of *Trichoderma harzianum* and the endomycorrhizal fungus *Glomus intraradices* remain viable for at least two years when stored correctly. Spores of ectomycorrhizal fungi usually have longer shelf lives than spores of endomycorrhizal fungi.

The resistance of *Bacillus* spores

The spores of *Bacillus subtilis* survive experiments which mimic the conditions that prevail throughout the ejection from the atmosphere by asteroid impact, the following travel through the solar system and the impact on another planet. It has also been reported that *Bacillus* spores germinated successfully after having spent 250 million years in salt domes. It seems plausible that such resistant spores travel on debris between planets and thus perhaps even might have given origin to life on Earth (panspermia).

The durability of our products

Determining the expiration date is not easy when organisms with different life spans are mixed into a single product. We have decided to include short-lived high-performance strains, such as *Pseudomonas fluorescens* in the blend. All the same we also make sure that long-lived species of *Bacillus* accomplish the task even after storage for prolonged periods of time.

Our experience has shown that many products give excellent results under experimental conditions in the laboratory, but they disappoint when exposed to the harsh conditions of a commercial distribution system. That is why we pay special attention to durability and resistance when selecting our strains and production methods. In general, our products are designed to assure that 98% of the spores still germinate after 18 months of storage at 25°C.

For how long do our microorganisms remain viable when mixed with water or substrate?

There is usually no easy answer to this question. Rather, experimental evidence has to shed light on the duration on a case-by-case basis. There is no problem with the temporary storage for a day or so. However, if the period extends to several days or weeks, professional advice should be sought.

Quality

How to recognize a "good" biological inoculum?

A biological product is "good" if it works well at reasonable cost. It is not just "good" because it is cheap or has a high concentration of spores or because the manufacturer makes ambitious promises.

When choosing between products one should first hear the opinions of other users. It is preferable to start with the treatment of a small area, avoid radical changes and take into account that recommendations might have to be adjusted after gathering first-hand experience.

How do products that contain microorganisms differ?

Microbial products vary significantly in their species and strain composition, concentration, manufacturing process, preservation method, and additives. Some products come with large quantities of metabolites that have accumulated during the manufacturing process. They are effective immediately, without the need for the microorganisms to start growing first. In other products, the organisms lose their desirable properties in the course of the production process. In addition, manufacturers differ widely in quality standards and their level of experience with their products under market conditions.

This is only the beginning of a long list of variations that makes it difficult to objectively compare products with each other. The user should ask how long the products can be stored and whether they can be incorporated in a given crop management system. Can he count on decent technical support? What is the reputation of the brand and its manufacturer in the market? How many other users does he know?

When faced with the choice between two chemical products, such as two NPK fertilizers or two products based on metalaxyl, the best product is often simply the one with the highest concentration of the active ingredient. When both suppliers have an equal reputation the consumer only has to perform a back-of-the-envelope calculation on price and content. However, a mere comparison of ingredient lists is not sufficient for biological products. A product based on *Trichoderma harzianum* can be more effective than a competing product that boasts a thousand-fold concentration.

How can you verify that a product actually contains the spore concentration that is specified on the label?

Usually it is impossible for the user to examine the spore concentration. He may turn to a state-approved microbiological research outfit. Unfortunately, the reported concentrations often differ widely when splitting a sample into different parts and sending them to several labs. If the user has finally won confidence by receiving similar results by a number of labs, he still cannot be sure that he will receive the same concentration or an equal degree of purity in the following purchase.

In this situation, the reputation of a brand is crucial. We are concerned about our reputation as a manufacturer and operate a strict quality control system which has assured consistently good results for many years. We invite independent research institutes to apply our quality control procedures, so that our statements are transparent and comprehensible to others.

How does TNI secure the quality of its products?

We retain samples for each production run and subject them to quality control. The aim is to determine the number of spores and their ability to germinate by applying generally accepted microbiological methods.

What is the purpose of the additives that are found in many products that contain microorganisms?

The spores are attached to small particles of additives which provide them with a food source and help them to get started.

Critics claim that products with microorganisms are improved by additives such as amino acids, humic acid and seaweed extract, so that a positive effect occurs regardless of whether or not it stems from the organisms.

In the case of our highly concentrated products only a few hundred grams are usually applied per hectare. However, these small amounts of product contain many spores, almost all of which are able to form a colony and thereby to multiply exponentially. Additives cannot grow and reproduce. Applying a few grams per hectare of sea kelp has a negligible effect on plant growth.

Safety

Can our microorganisms cause harm to human health?

Extensive toxicological data were assembled for the product Bactiva. They are based on EPA guidelines for the impact on skin, skin irritation, eye, ingestion and water-living animals. The results justify a "green label", i.e., the LD50 is above 5000mg/kg.

Despite the overall safety of the product, care has to be taken to avoid inhaling the fine powder because the highly concentrated spores and their metabolites can irritate the mucous membranes and the respiratory tract. We use protective suits and breathing masks during the production of the blend. Our constant handling of the spores might otherwise lead to irritations and immune responses.

We therefore recommend a breathing mask and gloves for the application. People who are prone to allergies should be particularly protected. Although the microorganisms were taken from nature and their spores are found in the air we breathe, we are not normally exposed to such large quantities.

If the microorganisms are sprayed on aerial plant parts, they should be washed as soon as possible into the rhizosphere, where they become active. Minor deposits on the leaves and fruits should be washed before eating. They are safe as long as they come as fine remnants of spray applications and not as the pure, highly concentrated product.

May our microorganisms harm the plants?

Bacteria that are reproduced in an artisanal manner by the user may compete for nutrients with the plants, especially when used in semi-hydroponic cultures. This has not been observed for Bactiva, Endospor and Ectospor, nor have our products shown any other adverse effects on plants.

May the repeated use of our microorganisms cause harm to the soil?

The microorganisms contained in our products stimulate the soil and activate it ecologically. There are no negative effects on the quality of the soil.