NURSERY PAPERS
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Mushroom for improvement: inoculation of nursery stock with microbes

INTRODUCTION

Just as most insects are no threat to nursery plants, most of the microbial life in the environment poses no danger to production nurseries or to the greenlife growing in them. However, the organisms that do cause damage to plants are generally very destructive.

To mitigate the risk of harmful pathogens, most nurseries focus on hygiene, particularly during the propagation phase of production – for example, using new or appropriately treated containers, and sterilising media, tools and surfaces. Some extend their hygiene practices to hand washing, foot baths and wheel washes, for example.

However, no system is perfect and bacteria and fungal spores can be carried by wind and water so even with the best precautions there is always an infestation risk.

At worst, sometimes the very practices designed to safeguard a nursery can render it more vulnerable by creating a vacuum that is ripe for pathogens to inhabit.

IT'S NOT ALL BAD

In the same way that there are beneficial insects, there are beneficial microbes. In some circumstances, deliberately introducing species can with positively influence plant development, growth and establishment, as well as providing a barrier to less friendly organisms moving into a sterile environment.

Before you consider introducing beneficial organisms, as you would with beneficial insect introductions, make sure you accurately identify both existing and introduced species. It's also important to be aware of the effect pathogen control activities like sprays and drenches may have on the 'good guys' in your nursery.

What are the good microbes?

BACTERIA

The best-known beneficial microbes in plant production are bacteria – specifically Rhizobia, which form a symbiotic relationship with many plants, particularly legumes, peas and beans, as well as plants in the Mimosaceae family, including Acacias.

Rhizobia can be found in nodules that naturally form on the roots of host species in response to the presence of the bacteria in the soil. The bacteria form colonies inside the nodules, take nitrogen gas from the atmosphere and convert it into water-soluble ammonia which plants can directly absorb.

Producers of these plants commonly introduce appropriate rhizobium species to host plants under nursery conditions, as they are unlikely to be present in the artificial growing media in containers.

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Roots of a Phaseolus seedling showing rhizobium nodulation.

Inoculated plants grow and develop better than un-inoculated plants thanks to the extra nitrogen. The bacteria may also break down organic components of the potting media, releasing other plant nutrients and providing an additional boost to plant health.

Rhizobium inoculum is well established and you can purchase it commercially to use in plant production. Research continues into other bacteria that may help plant growth. Of particular interest are so-called 'probiotics' for plants.

Some bacteria, such as Pseudomonas fluorescens, have been shown to help plants resist fungal pathogens such as damping off diseases. Other bacteria produce hormone mimics which encourage plant growth (or reduce it under adverse conditions), or help breakdown chemicals produced by plants under stress (such as drought or waterlogging), which can improve their recovery when conditions improve.

Most of this work revolves around plants grown in soil, and evidence for applications in nursery conditions with constructed organic media (e.g. pine bark-based mixes) is so far limited.

However, there is the potential to commercialise products like these, and some suppliers are already selling products which claim numerous benefits for inground growers. Treat these products with caution – it's hard to guard against the possibility that they contain organisms other than the beneficials they advertise.

FUNGI

When most nursery growers think of fungi, they automatically think of pathogens.

Many fungal pathogens thrive in the same warm temperatures and high humidity conditions as the host plant species they attack.

But most terrestrial plants rely on fungi for survival under natural conditions. Well over 90% of land plants can form what we call mycorrhizal relationships. This is a particular type of symbiosis between a fungus (myco) and a plant root (rhiza). Mycorrhizal relationships benefit both the plant and the fungus. They take several forms that are found in ecosystems all over the world.

The four recognised types of mycorrhizas are:

- Arbuscular mycorrhiza
- ectomycorrhiza
- Orchid mycorrhiza
- Ericoid mycorrhiza.

All four provide nutrients to their host plants by breaking down organic matter and releasing soluble nutrients the plant can absorb. The fungus often effectively increases the physical size of a plant's root system so it can extend further into the soil. In the case of some terrestrial orchids, the fungus largely takes the place of the plant's own root system, and in some species seeds will not even germinate without the presence of the appropriate fungal species

Arbuscular mycorrhizas

Arbuscular mycorrhizas are the most common mycorrhizal type. Over 200,000 plant species are known to form arbuscular mycorrhizal relationships. These include trees, non-woody plants and grasses as well as primitive bryophytes like hornworts and liverworts (the bane of many nurseries). The name comes from the formation of tiny tree shaped (arbuscular) structures of fungal hyphae (branching filaments) inside plant root cells. This is something plant growers would generally try to avoid but, in this case, they are a beneficial invasion. The fungi also grow out around the roots into the growing medium.

The fungi involved in this type of symbiosis (a kind of living arrangement between members of two different species) are completely dependent on their relationship with plants for their energy source – sugar, which comes from photosynthesis. Because Arbuscular mycorrhizas are difficult to grow on their own, they are also difficult to classify, so estimates of the number of fungal genera involved are anywhere between 300 and 1,600. They are all microscopic and don't form any fruiting bodies (like toadstools or mushrooms) that are visible to the naked eye.

Ectomycorrhizas

This group of mycorrhizas effectively form a sheath around the outside of the plant roots, with fungal hyphae extending out into surrounding soil. A much smaller number of plant species form ectomycorrhizas, but that includes many temperate gymnosperms, particularly the Pinaceae family, and hundreds of tree and shrub species.

Puff balls of the ectomycorrhizal fungus Rhizopogon luteolus (left) and an inoculated Pinus seedling (right) photos courtesy of HVP forestry nursery Victoria

While the number of plant species involved is relatively small, it's estimated that over 20,000 different fungi can form ectomycorrhizal connections to plants, which shows that many different fungi can form mycorrhizas with the same plant species, and the same individual fungus may form mycorrhizas with multiple different plants.

These fungi often produce large fruiting bodies visible at particular times of year as toadstools, mushrooms, puffballs and bracket fungi, for example. These have been successfully used in forestry in many parts of the world, introduced either in the nursery or at planting to improve plant establishment for timber plantations.

Orchid mycorrhizas

These are specific to the Orchidaceae family and, as with arbuscular mycorrhizas, the fungal hyphae are found within the plant cells of the orchids. Many orchid species, particularly terrestrial native orchids in Australia, can't germinate from seed unless there's an appropriate fungal partner in the soil. This has presented huge problems for revegetation and conservation of native orchid species.

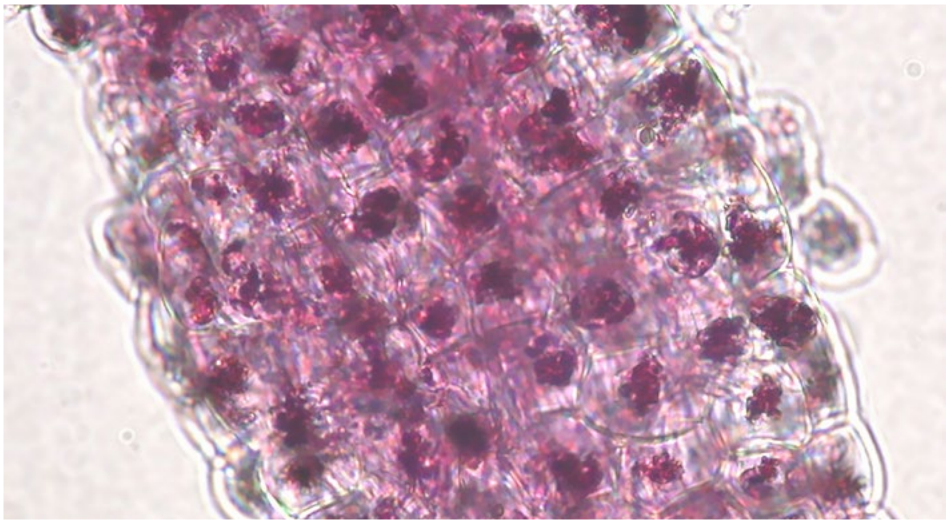
There are somewhere between 20,000 and 35,000 orchid species in the world and, so far, over 25,000 fungi have been found to form orchid mycorrhizas – and pairings seem to be very specific.

The fungi involved are also macrofungi, often producing visible fruiting bodies. Additionally, some of the fungal species involved can form ectomycorrhizas with other species of plant, possibly even transferring nutrients between plants under natural conditions.

Ericoid mycorrhizas

The Ericacae family, consisting of around 4,000 species globally, including many acid loving plant species including Rhododendrons and Vaccinium spp. The family was expanded to include the former plant family Epacridaceae of around 30 genera of Australian natives including Epacris and Astroloma. These plants are unique in that their roots systems generally do not feature root hairs like most other terrestrial plants. Perhaps as an adaptation to this, they have evolved very fine roots which are colonised by at least 150 known species of fungi to form mycorrhizas. The family often grows in low pH (acid) soils and relies on mycorrhizas to break down organic matter in the absence of other microbes that can tolerate the acidic conditions.

The fungi grow into the root cells of the plants, and individual plants may have mycorrhizas with multiple different fungi at any given time. As with other mycorrhizal relationships, these fungi may also form relationships with liverwort species, and the liverworts possibly act as a reservoir of inoculum for newly germinated seedlings in the Ericaceae. Some research shows enhanced germination of Ericaceae seeds when known mycorrhizal fungus species, are introduced however results have been variable.



Hair root of Vaccinium seedling with established ericoid mycorrhizal fungus inside root cells.

BENEFITS TO NURSERIES

While it is obvious from over a century of research that mycorrhizas benefit plants in natural ecosystems, their benefits to nursery growers are not so clear. Fungi in nutrient-poor soils can benefit plants by breaking down organic matter and releasing nutrients that would be otherwise unavailable to the plants, but in nurseries, growers are likely to be providing the optimum nutrients for plant growth for the period they are in the nursery environment. This may mean any mycorrhizal fungi are benefiting from plant growth but offering no benefit in return.

The behaviour of mycorrhizal fungi in artificial organic-based growing media is also not as well understood as their behaviour in natural mineral soils, including under regular additional irrigation. Adding fertiliser may also change their behaviour – the fungi may take up nutrients to the detriment of the plants in containers.

These questions aside, there is evidence that plants inoculated with appropriate mycorrhizal species can establish better when planted out, especially in areas where they will receive little follow-up care or maintenance, such as in forestry and revegetation applications.

There are also multiple examples of inoculated tree species being able to rehabilitate contaminated post-industrial sites, such as from mining, and either sequester toxic elements or break down contaminant molecules into less harmful compounds. For these specific purposes inoculation may be a considerable advantage.

Nonmycorrhizal plants

Around 50,000 plant species do not form mycorrhizal symbioses in their roots, for example, many weed species that thrive in unstable environments.

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The Brassicaceae family and the Crassulaceae family don't form fungal relationships. Similarly, the Proteaceae family have their own specialised root structures to cope with their often nutrient-poor soil environments. Many rhizobium hosting plants are also unlikely to form mycorrhizal relationships. Attempting to inoculate nonmycorrhizal species will likely be unsuccessful. For more information about nonmycorrhizal plants visit Mark Brundrett's web resource at *mycorrhizas.info/nmplants.html*

Other microscopic helpers

While fungi and bacteria are the main microbes that benefit plants, there are numerous other lifeforms in soils that benefit plant growth directly and indirectly. A healthy and diverse microbiota in the soil can prevent invasion by pathogens such as phytophthora. This prevention occurs either by competing with the pathogen or directly affecting it (via predation or producing compounds toxic to the species).

Many other microbes feed on organic matter and break it down into soluble forms that plants can absorb, and others still are able to fix atmospheric nitrogen and convert it into ammonium, such as cyanobacteria and actinomycetes.

One genus of actinomycete, the Frankia spp. forms symbiotic relationships with several genera of trees from both the northern and southern hemispheres. Alnus species and Allocasuarinas can have stable Frankia colonies associated with their roots in much the same way as legumes have rhizobium catching nitrogen for them.

Practical considerations for nurseries

Introducing new organisms into a relatively clean environment, such as a nursery, has obvious challenges, even if the potential benefits are clear. Just as with introducing beneficial insects to combat pest insects, introducing beneficial microbes means adjusting nursery systems and/or practices.

Accurate identification of beneficial organisms

Before you Introduce a beneficial microbial agent, you must be absolutely positive about its identity and ensure there is no possibility that it is contaminated with pathogenic organisms. The old practice of 'mixing in a bit of soil' collected from around an established tree is not appropriate for a modern nursery. If you can't obtain uniform cultured microbes from a supplier willing to verify their identity, avoid them.

Mycorrhizal fungi cultured from the roots of a single wild collected plant.

Some forestry nurseries collect fruiting bodies of known mycorrhizal fungi and surface sterilise them. The nurseries then incorporate them into growing media or use them to produce an inoculum that can be applied to established plants later in the production cycle.

Many fungi are able to form mycorrhizal relationships with multiple species of plants, and many plants commonly have multiple mycorrhizas at any time with different fungal partners. *(See pic below)*

It's also important to think about the consequences of introducing of fungal species into the environment, as there have been examples of 'weedy' fungi moving into natural vegetation in Australia. For more information, see Dunk et al's Mycorrhiza article: *pubmed.ncbi.nlm.nih.gov/21573836/*

Pesticide use

In the same way that you would if you were introducing beneficial insects, mites or nematodes into a production environment, you need to make appropriate changes to integrated pest management (IPM) and chemical use. In this way, you can prevent pesticides damaging off-target beneficial species. Fungicides, in particular, are likely to affect both mycorrhizal fungi species and beneficial bacteria and other microbes, which may be present in the growing media. In most cases, managing growing media and plant production using best practice guidelines will prevent fungal contamination.

To check whether you have contaminant fungus is in your media, contact your local plant pest diagnostic service or Grow Help Australia. Don't forget, all production nurseries receive six free samples per year until the end of 2025.

MORE INFORMATION

Download past nursery papers from the Greenlife Industry Australia website at *www.greenlifeindustry.com.au/communications-centre?category=nursery-papers*

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