Do people really know how *Trichoderma* species work?

Why *Armillaria* is a problem.

Armillaria is a soil borne pathogen that damages a range of plant species. *Armillaria* root rot remains a major limitation to establishment of crops on land where forest trees were planted, then removed and are not totally removed thoroughly during land preparation. The root and stump remnants are essential food bases for ensuring the longevity of *Armillaria*. Symptoms of *Armillaria* infection in kiwifruit include poor growth, small leaves, yellowing of the foliage, premature leaf drop, cane die-back, and ultimate vine death. Further to the above, vines affected by *Armillaria* exhibit sudden wilt and collapse during the summer as crop loads, nutrient deficiencies and water stress increase.

Chemical control and how it failed.

Armillaria root rot is notoriously difficult to control. Traditional orchard management of *Armillaria* through the removal of tree / vine stumps is virtually impossible, therefore chemical controls were tested for the eradication of this pathogen. Systemic and non-systemic fungicides were trialed for the control of *Armillaria* and subsequent field experiments showed that the chemicals failed to fully eradicate the rhizomorphs (shoe-string growth of *Armillaria* around the roots by which the pathogen can spread) in soil even with as high as 10,000mg/L (West). Because the currently available chemicals for controlling *Armillaria* were either ineffective or phytotoxic, there was a need for an alternative approach to the problem (Baker and Snyder).

Biological control using *Trichoderma* species

Perhaps the most thoroughly studied antagonist of *Armillaria* are *Trichoderma* species. There is a lot of research being undertaken in New Zealand at present about the biological control methods for *Armillaria* using *Trichoderma* species. Research has been undertaken on an intensive level overseas since 1914 using *Trichoderma* as an antagonist to *Armillaria*, however significant findings were made by Dumas and Boyonoski in 1992 with regards to the mode of action of *Trichoderma* species.

They found using electron microscopes that certain *Trichoderma* species attacked, penetrated and destroyed outer tissue of the *Armillaria* rhizomorphs and, once inside, they killed *Armillaria* hyphae by coiling and direct penetration. After one week, the rhizomorphs infected with each of these *Trichoderma* species were devoid of hyphae.

How *Trichoderma* species work.

Some species of *Trichoderma* have been shown to parasitize other fungi, compete aggressively for nutrients and produce antibiotics (Klein and Everleigh). These attributes facilitate the species capacity to colonize particular habitats in which they may hamper development of various fungal species including some plant pathogens. *Armillaria* is found in the soil as mycelia or rhizomorphs (shoestring- like growth) associated with dead roots and stumps. To be effective against *Armillaria*, *Trichoderma* spp must be capable of colonizing and antagonizing the pathogen in infected plant tissues.

Hyperparasitic strains of *Trichoderma* coil around hyphae of the host fungi and degrade their cell walls enzymatically as has been demonstrated by electron microscopy of hyphal interactions between certain species of *Trichoderma* and *Armillaria* (Dumas and Boyonoski). In addition, some of them produce diverse antibiotics that also enable them to limit growth and activity of other fungi. These attributes make some strains of *Trichoderma* useful as biocontrol agents (Elad et al.).

Systemically Acquired Resistance (SAR) is another mode of action which has been documented, however as a stand alone method of activity against *Armillaria*, it can not be warranted as *Armillaria* is ultimately a soil borne pathogen and spreads through the soil over time, and therefore the source of the inoculum needs to be addressed **via** the soil. The merits of SAR need to be investigated further and the costs weighted out.

Factors that affect *Trichoderma* spp and best timing for applications

Factors that determine the success of *Trichoderma* in controlling soil borne pathogens include population densities or antagonistic strains in the soil and the type of preparation of the antagonist applied. At low population densities, antagonistic strains of *Trichoderma* would be limited in their efficacy against *Armillaria*. Therefore, the introduction of *Trichoderma* spp annually into the soil is quite critical and *Trichoderma* populations should be maintained on a regular basis for healthy soils which will be inhibitive to *Armillaria*. The relationship between incidences of *Armillaria* with that of *Trichoderma* indicates higher incidence of *Armillaria* at low incidence of *Trichoderma* (Jeger and Termorshuizen). Choose a product which is easy to handle, has a guaranteed analysis and an expiry date these factors can influence the amount of colony forming units (cfu) or concentration of viable *Trichoderma* spores. The application of *Trichoderma* needs to be budgeted into orchard management annually in order for New Zealand growers to best overcome this detrimental pathogen.

Companies have advocated the use of soil injections of *Trichoderma* spp in August as a means to control *Armillaria*. August is not suitable for application of *Trichoderma* species. The temperature optima for *T. harzianum* ranges between 30-38^{o}C, whereas *T. koningii* optima ranges between 32-35 ^{o}C (Danielson and Davey). These organisms can grow and proliferate at temperatures below these optima, however for aggressive antagonism of *Armillaria* which grows successfully in the months of May to July (cooler temperatures) in New Zealand, *Trichoderma* spp need to be applied in early Autumn and Spring to reduce the inoculum of *Armillaria* before it begins to proliferate, while soil temperatures are still above 13^{o}C. Most importantly, for most soils, significantly fewer colony forming units of *Trichoderma* spp developed at 8^{o}C compared to 16^{o}C or 24^{o}C (Roiger et al). What are soil temperatures in August in any part of New Zealand? Therefore, will suggested *Trichoderma* treatments be effective in the cooler temperatures found in August?

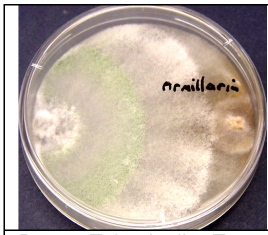
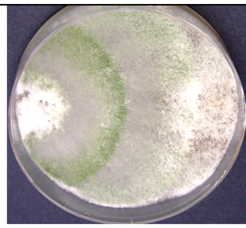
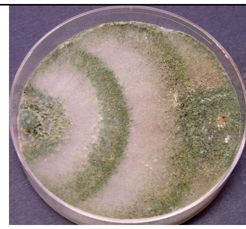
Once soil temperatures exceed 15^{o}C, then this is the best timing for *Trichoderma* spp to be introduced into the soil. After all, *Armillaria* is a **soil borne** pathogen. There is no necessity for the application of *Trichoderma* to be made through air blasters into the soil as competent *Trichoderma* species can grow through the soil profile and can be applied as ground applications (through boom sprays or through fertigation) and adequate rainfall is sufficient to aide *Trichoderma* to proliferate through the soil profile.

In vitro trial work done with *T. koningii* and *T. harzianum*

There is significant data supporting the use of *Trichoderma* species *in-vitro* and this has recently been document in New Zealand after the evaluation of these two strains was made under lab conditions. Both strains have merits as antagonists to *Armillaria* and the type of antagonism is evident in the photographs.

Dual culture of *Armillaria mellea* and *T. koningii & T. harzianum* at 20 C, over a period of 9 days. Armillaria had been growing 7 days before *T. koningii & T. harzianum* was started on the left side of plate. Note the relatively rapid growth of *T. koningii & T. harzianum* which enables it to effectively overtake and overgrow Armillaria in nature.

		
Day 1 after seeding *T. koningii* and *T. harzianum*. Cover kept in place until colonies cover agar surface.	Day 2. Note rapid 2-day growth of *T. koningii* & *T. harzianum* cf 9 days for Armillaria.	Day 3. *T. koningii* & *T. harzianum* has reached the edge of the Armillaria colony.

		
Day 4. *T. koningii* & *T. harzianum* has almost overgrown Armillaria. *T. koningii* & *T. harzianum* sporulation evident.	Day 5. Cover removed for clarity. Overgrowth complete. Zones of *T. koningii* & *T. harzianum* sporulation.	Day 9. Note heavy *T. koningii* & *T. harzianum* sporulation where Armillaria colony was.

Armellaria is very slow growing at 20 C and made only 10 mm of radial growth in 7 days, at which time *T. koningii & T. harzianum* were started. *T. koningii & T. harzianum* rapidly covered the plate, reaching the *Armillaria* colony in 3 days, overgrowing it by day 5 and obliterating it from view by day 6. *T. koningii & T. harzianum* are effective antagonists of *Armillaria*.

Field work done with *T. koningii* and *T. harzianum*

This has been trialed and noted in the Te Puke region whereby several growers have trialed commercially available strains of *T. koningii* and *T. harzianum* in order to measure the antagonism caused by these two strains of *Trichoderma* in field applications via the soil. Two applications were made into the soil, the first in October where a positive change was noticed in the health of vines at flowering. In February, a second application was made and white mycelial growth was noticed throughout the orchard (growth of *Trichoderma* species) the following day. No more of the vines have been lost since and these vines have yielded a full crop and new callusing of the bark and new cane growth was quite evident. Trial work began in 2001 and continues in this region.

Further trial work is currently being conducted in California on two products, the first being Enzone® (Entek Corp., Brea, CA) a standard fungicide containing thiocarbamate and Promot® (J.H Biotech, Inc., Ventura, CA) a biological product containing *T. koningii* and *T. harzianum* (Elkins et al).

Summary

The merits of *Trichoderma* species as a potential biocontrol agent for *Armillaria* have been promising for many years now. The true merits of this organism lie in the management approach adopted by growers with regards to this organism. To best utilize *Trichoderma* species, the following must always be remembered;

1) at low population densities, antagonistic strains of *Trichoderma* would be limited in their action against *Armillaria*, therefore bi-annual applications need to be made early in April/ May and in October whereby *Trichoderma* populations can actively grow in the soil and antagonise *Armillaria* in suitable soil temperatures,
2) budget for *Trichoderma* applications as a routine management tool in kiwifruit orchards,
3) be aware of the soil temperatures when you apply *Trichoderma* products as soil temperatures below 13^{o}C will not actively promote the growth of this organism,
4) do not be drawn in by fancy application methods of *Trichoderma* species as these are an added cost which do not increase the efficacy of *Trichoderma* products,
5) ensure that vines do not suffer from water stress or nutrient deficiencies as vines which are stressed in any way have an increased susceptibility to *Armillaria*. Therefore, take fertilizing vines and water management seriously, conduct soil and leaf analyses to best ascertain the nutrient status of kiwifruit vines. A healthy balanced soil system and vine are less susceptible to pathogens and promote conditions which are favourable for *Trichoderma* species to proliferate,
6) ensure the *Trichoderma* product chosen is easy to handle and can guarantee viable spore counts,
7) investigate the use of fungicides and the effects these can have on the *Trichoderma* product you have chosen to use,
8) Finally, take *Trichoderma* species seriously. The merits of this organism are more than meets the eye.

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Excerpts from A Review on Trichoderma species as Biological control agents.

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