Managing Pathogenic Fungi with Other Fungi — *Trichoderma*©

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INTRODUCTION

Horticultural practices can be very disruptive to the natural biological balances that exist in nature, particularly insect and soil microbe balances. When the balance is disrupted, only the most robust and highly adaptive organisms tend to dominate. In most cases the new inhabitants are the unwanted pests and diseases. Due to the increasing environmental and social pressures being placed on horticulturalist to control pests and diseases more sustainably, chemical control options are becoming very unpopular. This has opened the door to the use of integrated pest management (IPM) practices and other biological control agent (BCA) options.

Today there is a wide range of commercially available predatory and parasitising insects that can be introduced into farming systems to control insects. There is also a range of bacteria and entomopathogenic fungi that have been developed for the purpose of insect control including *Bacillus thuringiensis* strains, *B. subtilis* strains, *Metarhizium* sp., *Beauvaria* sp., *Verticillium* sp., and commercial formulations of Actinomycetes and *Strobilurus* sp. metabolites. The commercialisation of fungi that control other fungi has generally been focused on two groups of microbes namely *Bacillus* sp. and *Trichoderma* sp. This paper focuses on the use of *Trichoderma* sp. as a viable BCA.

TRICHODERMA AS A BIOLOGICAL CONTROL AGENT

In recent years there has been a rapid increase in commercially available BCAs for soil-borne pathogens. One of the most common of the fungi-based BCAs is *Trichoderma. Trichoderma* has been widely researched because of its ubiquitous nature and its capacity to be easily cultured out of soils. The most widely researched species that have been studied for their BCA properties are *T. viride, T. harzianum, T. koningii, T. hamatum,* and *T. virens.*

Trichoderma sp. are favoured by the presence of high levels of plant roots, which they colonize readily. Some strains are highly rhizosphere competent, i.e., able to colonize and grow on roots as they develop. The most strongly rhizosphere competent strains can be added to soil or seeds by any method. Once in contact with the roots, depending on the strain, they will colonize either the root surface or the cortex. Thus, if added as a seed treatment, the best strains will colonize root surfaces even when roots are a metre or more below the soil surface and they can persist at useful numbers up to 18 months after application. However, most strains lack this ability.

Foliar Diseases. The first successful commercialisation of *Trichoderma* in Australia was a product called Trichodex® (Registered trademark of Abbott Laboratories). Trichodex (*T. hazianum*, strain T39) was fully registered in 1997 for the control of *Botrytis cinerea* in grapes. This use pattern for *Trichoderma* is unusual when we consider that *Trichoderma* is a soil-borne organism. However, this is a typical example of how adaptable *Trichoderma* can be if the environmental conditions are favourable (presence of adequate moisture).

Other formulations of *Trichoderma* have registrations in U.S.A. for foliar diseases. For example PlantShield HC™ (T-22 Strain) (PlantShield HC 22G is a registered trademark of Bioworks, Inc. Geneva, U.S.A.) which was developed by Professor Gary Harman (Lecturer, Plant Pathology, Cornell University) is registered with the EPA to control powdery mildew on New Guinea impatiens (*Impatiens hawkeri*) and pumpkins, as well as downy mildew in snapdragons (*Antirrhinum majus*), and *Botrytis* in geraniums (*Pelargonium*) and strawberries (*Fragaria ×ananassa*) (Harman, 2000). We have screened Trich-A-Soil, a formulation of *T. harzianum* and *T. viride* (Dr. Percy Wong NSW Dept. Agr. isolate) against *Colletotrichum graminicola* (anthracnose) and found it to have considerable inhibitory effects against this pathogen of foliage and fruit.

Trials conducted in strawberries on the Sunshine Coast, Queensland have shown Trich-A-Soil also has potential for controlling Powdery mildew.

Soil Diseases. Being a soil-borne fungi *Trichoderma* performs at its best in soil. There are many scientific papers that demonstrate the effectiveness of *Trichoderma* for soil-borne disease. Some of the more common diseases controlled by *Trichoderma* are *Pythium* sp., *Fusarium* sp., *Rhizoctonia* sp. (Chet, 1987), *Sclerotinia* sp. (Lo et al., 1996), and *Sclerotium* sp. (Metcalf, 2001)

Dr. Wong (pers. Comm.) has demonstrated the effectiveness of Trich-A-Soil for the suppression of *F. oxysporum* and *R. solani*. The *T. viride* in Trich-A-Soil was isolated from soils in the central west of NSW for the control of Take-All Patch (*Gaeumannomyces graminis*) in wheat. Mycoparasitic activity has been detected indicating that it actively seeks out pathogenic fungi.

Trich-A-Soil also has inhibitory effects against *Phytophthora cinnamomi. Phytophthora* sp. is not a common pathogen known to be suppressed by *Trichoderma* however some evidence exists in the literature that *Trichoderma* has potential to control *Phytophthora* sp. in apples (Smith, et al., 1990). Avocado growers in the Atherton Tablelands (for North Queensland) who have problems with *P. cinnamomi* have seen positive responses from the regular use of Trich-A-Soil. It has been suggested by some researchers that *Trichoderma* has potential to be very effective against *Phytophthora* sp. because its cells walls are mainly chitin based and *Trichoderma* is a major producer of chitinase, a chitin-denaturing enzyme.

Mode of Action of *Trichoderma*. Biological systems involve highly complex physical and biochemical interactions. The most detailed research on the mode of action of *Trichoderma* has been Harman's (2000) work with *T. harzianum*. Harman has concluded that this species has the following modes of action: mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced plant resistance, solubilization and sequestration of inorganic nutrients, and inactivation of the pathogen's enzymes.

It is highly likely that this list is incomplete for *T. harzianum*. Therefore, if all other *Trichoderma* sp. were included, the list would be much longer.

Mycoparasitism is one of the most potent BCA mechanisms of *Trichoderma*. This mechanism is highly complex in its own right with up to 20 separate genes and gene products (enzymes) involved in the process. Some of the most active enzymes in this range are the chitinolytic enzymes. In 1998, Lorito (1998) listed 10 separate chitinolytic enzymes involved in mycoparasitism. In addition, diversity exists within another range of enzymes called glucanase (Benítez et al., 1998; Lora et al.,

1995) and proteinase (Geremia et al., 1993). These "sugar-based" enzymes are also believed to be involved in the process of systemic acquired resistance (SAR) in the host plant (Yedidia et al., 1999; De Meyer et al., 1998). This is outside the scope of this paper, however, it is believed that induced SAR effects from *Trichoderma* may explain much of the foliar disease suppression observed in plants that have been inoculated with *Trichoderma* either by foliar application or soil inoculation.

Recent research conducted by Metcalf (2001) has demonstrated the effectiveness of an indigenous Tasmanian strain of *T. koningii* against white onion rot (*Sclerotium cepivorum*). Metcalf also documented the chitinolytic enzymes involved in the mycoparasitism.

Rhizosphere Competence. The colonizing ability of *Trichoderma* at the rhizosphere is critical to its performance as a BCA. Much of the selection criteria for soil-based BCAs involve determining the rhizosphere competence of the organism. If it has weak colonizing effects, it has little future as a BCA. Trich-A-Soil is very rhizosphere competent as can be seen from Fig. 1, showing good colonization along the length and growing tip of a wheat root.

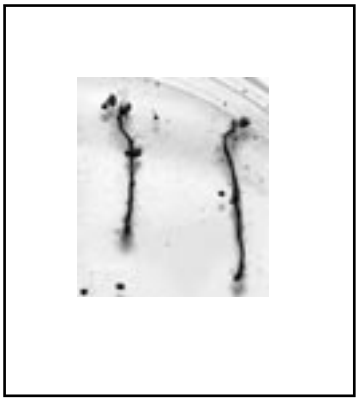

Figure 1. Nodules along the root is *Trichoderma viride* colonization.

Trichoderma will colonise plants at varying levels. Trials conducted by the University of Sydney at two different sites indicated that Trich-A-Soil colonizes very well. The lower levels of colonization in the Penrith soil can be attributed to the fact that this green did not receive regular irrigation.

Commercial Considerations for Selection of *Trichoderma* as a BCA. The success of microbiological BCAs is dependent on numerous environmental variables. Temperature, pH of soil, moisture content, EC levels, organic matter content, and soil porosity are critical. However, *Trichoderma* needs to be delivered into the environment at a sufficiently high concentration to colonise effectively. The difficulty is knowing what levels one must deliver the BCA into the environment for effective biological control. Work conducted by Lo et al. (1996) demonstrated that effective biological control with the T-22 strain of *Trichoderma* in turf situations was 1 million colony-forming units (viable spores) per gram of soil. This inoculation rate appears to be standard based on work they have conducted in other crops (Lo et al., 1996).

A commercial formulation of *Trichoderma* with low colony-forming unit (cfu) values is not going to be practical or economically feasible due to the quantities needed for effective biological control. Table 1 shows four commercially available *Trichoderma* products available in Australia and their cfu levels.

Importantly, *Trichoderma* is a living organism. The survival of such an organism under commercial distribution can be a challenge. Suppliers must ensure product does not encounter extremes in temperature and prolonged periods in storage. Shelf life is generally limited to a few months if cfu levels are to be maintained at sufficient numbers to be of any use as a BCA. The fresher the material, the better the cfu levels and the greater the chance of successful colonization and efficacy.

Table 1. Commercial *Trichoderma* products.

Product	Trichoderma sp.	cfu/g
Amnite[a] T 100 (Enviro Gold A-100 + Trichoderma) (Nutri Life Tricho-shield)	*T. harzianum*	1×10^8
Promot™ TRI-D 25™	*T. koningii* *T. harzianum*	5×10^8
Trichoprotection Trichoflow©	T. viride *T. harzianum*	1×10^8
Trich - A- Soil	T. viride *T. harzianum*	1×10^9

™ TRI-D 25 and Promot are registered trademarks of JH Biotech, Inc. Ventura, California.
© Trichoflow is a registered trademark of Agrimm Technologies Ltd, Christchurch NZ.
[a] Amnite T 100 is a registered trademark of Cleveland Bio, Stockton on Tees, England

CONCLUSION

The use of BCAs in crop protection is increasing. This is due to the environmental benefits but also the commercial necessity. Pathogens are rapidly building resistance to current fungicides almost as fast as they are being released. New chemistry, which meets Occupational Health, Safety and Environmental standards, takes years to develop and once one does reach the market it is often cost prohibitive.

Biological Control Agents like *Trichoderma* offer safe and effective adjuncts to synthetic chemistry. Biological control agents are also favourable because of the low risk of chemical resistance due to their innately complex mode of action. I believe BCAs like *Trichoderma* will eventually become common place in closed production systems like plant propagation and soil-based hydroponic crop production. This is because the BCAs can easily be introduced into the growing system and the growing conditions can be controlled to favour the BCA.

The only major restriction to the proliferation of microbial-based BCAs is their commercialisation limitations. Naturally occurring organisms cannot be patented unless they can be modified genetically which would limit their use from an environmental and regulatory standpoint. Therefore, funding for BCAs research will remain limited. It is up to companies like Organic Crop Protectants to make certain that local microbial BCA research and commercialisation continues. This can only be achieved through the industry supporting products like Trich-A-Soil.

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A Fast and Reliable Method of Plant Propagation©

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HOW I DISCOVERED THIS METHOD

I often trim the trays of plants in 2-inch tubes and I noticed that some of the trimmings fell on top of the potting mix in the tubes and if I left them there they took root quite quickly. So I decided to duplicate the process in the igloo environment and I was amazed to see how quickly the clippings took root in the controlled environment. So I started trialling various sorts of plants and was very pleased to see how well this worked. About a year after I had started trialling this I was very interested to read Ian Gordon's article (2001) in the Australian Horticulture magazine and while his methods were similar, his method required a much more sophisticated setup than I had available. The beauty of this method is that it requires very little cost and labour to set it up and means that large numbers of plants can be propagated very quickly and cheaply. It is not applicable for all plants but I think I can demonstrate that it certainly works very well with a great number of plants.

HISTORICAL USE OF A SIMILAR METHOD BUT WITH A DIFFERENT APPLICATION

See Australian Horticulture June 2001 article *Using Ultra Soft Tips For Cutting Propagation* by Ian Gordon. I haven't found any other references similar so if you have been using this for 50 years — don't hassle me!

WHY THIS METHOD WORKS

- Plant hormones (auxins) are concentrated in area of the plant where active cell division is happening.