Biological Plant Protection – Mechanisms and Systems for Nurseries Using Beneficial *Trichoderma* Fungi©

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INTRODUCTION

Nursery plants are vulnerable to fungal infection, which may occur at any stage of their life span on foliage or roots. The use of bark- or peat-based growing media has many advantages over soil in the attempt to produce a near perfect environment to establish and maintain a healthy root structure. However, the often initially sterile nature of this type of medium creates a microbiological vacuum ideally suited to becoming occupied by opportunistic disease-causing fungi. Augmentation of media with beneficial microorganisms offers a sustainable management tool for growers of commercial plants, which affords protection against disease and has the potential to minimize reliance on chemical pesticides.

Biological control agents have been the subject of extensive research for many years and have shown good potential for the control of some plant diseases, especially those caused by soil-borne fungal pathogens (Harman and Bjorkman, 1998). However, few are yet available as proven commercial formulations. During the past decade public pressure placed on food producers to decrease their use of chemical pesticides has increased considerably and resulted in new opportunities for integrated pest management (IPM) involving biological control agents in many areas of commercial horticulture.

The bio-control properties of *Trichoderma* species have been extensively investigated for many years and it has been shown to be effective in the control of many soil-borne pathogens causing root rots, wilting, and damping off diseases (Papavizas, 1985; Harman and Bjorkman, 1998; Hjerljord and Tronsmo, 1998). Since the term "immunising commensal" was first coined to describe activity of *Trichoderma* (Ricard, 1977) this organism has shown considerable potential to have an impact on protective management of some common fungal diseases in plants (Ricard and Highley, 1988; Hunt and Gale, 1998a,b; Bailey and Lumsden, 1998). It is within this context that some aspects of recent experimental work with commercial formulations of *Trichoderma* species to develop Trichoprotection® (Agrimm Technologies Ltd. Christchurch) programmes are discussed below. Trichoprotection® involves the introduction and maintenance of well-defined strains of *Trichoderma* species to occupy the biological niche for the benefits of improved plant growth and health.

SOIL DISEASES

During the development of Agrimm's commercial product formulations over the past decade, laboratory studies (Hunt, 1999) have shown considerable inhibitory and mycoparasitic activity of various *T. harzianum* and *T. viride* strains towards a number of soil-borne fungal pathogens (Table 1). A number of these pathogens including *Phytophthora, Fusarium, Pythium,* and *Rhizoctonia* species have relevance in the nursery industry.

Table 1. In vitro biofungicidal activity of Agrimm *Trichoderma* strains.

Pathogen	Host	Disease	Inhibition
Armillaria mellea	Kiwi fruit vines	Root and trunk rot	78
Fusarium oxysporum f. *canariensis*	Palm trees	Root rot, wilt	80
Fusarium oxysporum f. *pisi*	Peas	Wilt	43
Phytophthora cactorum	Apple trees, ornamental shrubs	Trunk canker	64
Pythium irregulare	Vegetables, ornamental shrubs	Damping off	44
Pythium ultimum	Vegetables, ornamental shrubs	Damping off	75
Rhizoctonia solani	Ornamental shrubs	Damping off	34
Sclerotium cepivorum	Onions, garlic	Onion white rot	80
Sclerotinia minor	Lettuce	Stem rots	31

Inhibition represents mean values (%) from duplicate cultures obtained from a number of experiments conducted between 1993 and 1999 (see Hunt, 1999 for experimental details).

THE TRICHODERMA FAMILY

In its natural environment *Trichoderma* is a resident of the litter and woody plant debris in humus or associated with plant matter in the soil. It acts as a mycoparasite or saprophyte to establish a niche for itself often at the expense of other fungi, which it may use as an alternative source of nutrients. It has been clearly demonstrated actively parasitising a number of basidiomycetes including *Rhizoctonia solani* and *Armillaria mellea* (Lewis and Papavizas, 1980). Taxonomically there are a number of species of *Trichoderma* but *T. harzianum, T. viride, T. kongeii,* and *T. hamatum* are the species most commonly referred to as having biocontrol activity. These species differ microscopically in both mycelium and spore morphology. Being saprophytes, *Trichoderma* species have developed effective mechanisms of competing with other fungi in the same ecological niche. Some or all of these mechanisms of action enable various *Trichoderma* species and strains to be effective as bio-control agents.

MECHANISMS OF ACTION

There have been a number of mechanisms by which *Trichoderma* species have been shown to aggressively occupy and maintain a presence in a defined biological niche. Any one or combination of these mechanisms may confer bio-control properties on a particular *Trichoderma* strain. The three latter mechanisms are all being actively researched by Agrimm Technologies Ltd in their attempts to improve the activity and efficacy of their commercial product formulations.

Competition/Antagonism. *Trichoderma* is a fast-growing fungus which, under favourable conditions of suitable temperature and moisture will grow quickly and aggressively to occupy an ecological niche, very often at the expense of other micro-organisms. In this situation the *Trichoderma* out-competes other microorganisms for nutrients and may literally smother and/or antagonise any competitive growth by overgrowing other fungi.

Abiosis (Metabolite Production). Metabolites produced by *Trichoderma* include proteolytic enzymes such as cellulases, xylanases, and chitinases which all have strong cell wall degrading activity allowing *Trichoderma* to digest cellulose-based plant material in its saprophytic role. Some *Trichoderma* strains also produce antibiotics, which have potent antifungal activity. Both types of metabolites allow *Trichoderma* to have an effective array of chemical agents with which to deter the growth of other competing microorganisms.

Mycoparasitism. *Trichoderma* has the capacity to act as a parasite of other fungi to not only kill these other microorganisms but also use them as a food source. Not all strains of *Trichoderma* have mycoparasitic properties towards other fungi and many soil-borne pathogens may be resistant to this activity. However, if demonstrated, mycoparasitism is a powerful biocontrol property of various strains from a number of species of the *Trichoderma* family. (Fig. 1)

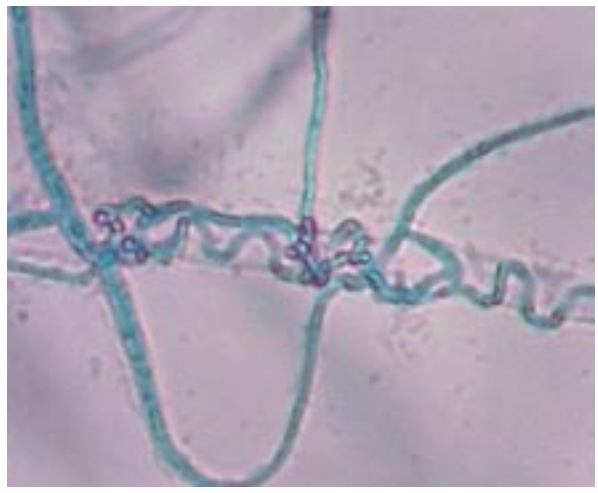

Figure 1. Photomycrograph showing myco-parasitism of *Pythium irregulare* by *Trichoderma harzianum* strain AGSS28. Thin strands of *Trichoderma* mycelium coil around the central strand of *Pythium*. Note the lack of staining of the *Pythium* strand with lactophenol blue indicating loss of cytoplasm.

Elicitor Response. Plants respond physiologically in a number of ways in their attempts to eliminate or contain the threat from a foreign invading pathogen. Chemicals produced by the plant as a consequence of some physiological processes can be transported to other areas of the plant where they are able to stimulate further protective responses (i.e. they act as elicitors). *Trichoderma* has been shown to act as an elicitor in stimulating the plant's natural defence mechanisms by producing phytoalexins, which are chemicals with antifungal activity.

TRIALS ON GROWING MEDIA

Over the past five seasons trials have been conducted in a number of countries on growing media to evaluate the efficacy of three of Agrimm's commercial formulations containing selected strains of *T. harzianum* for plant establishment and disease protection.

PATHOGEN CHALLENGE TRIALS

***Pythium* Challenge.** Replicated pot trials were established in August 1999 by Dr. Paul Simmonds, Bord na Mona, Dublin, Ireland, to assess a *Trichoderma* formulation [(Trichodry™ (Agrimm Technologies Ltd. Christchurch), 1 kg • m^3] use at seeding time with and without challenge from *Pythium* species. Pea seeds were sown into 4-inch pots, (10 seeds per pot, 15 replicates per treatment), containing peat-based growing media, plus or minus Trichodry™, which had been added 1 week prior to planting. One half of the pots were challenged with a spore suspension of *Pythium* applied during the watering stage at planting. All pots were watered and germination recorded at 7 and 14 days with fresh weight of the plants recorded at 28 days.

Table 2. Germination and fresh weight of pea seedlings challenged with *Pythium* spore suspension.

Treatment	Germination (%)		Fresh wt. (g)
	7 days	14 days	
Control	87.3	90.7	119.2
Control + *Pythium*	13.3	25.3	19.3
Trichodry™	84.0	90.0	114.1
Trichodry™ + *Pythium*	43.3	52.0	53.3

Results (Table 2) showed that both germination and total fresh weight of seedlings challenged with *Pythium* and treated with *Trichoderma* was significantly increased ($p < 0.05$, Duncan's F test) compared with seedlings challenged without *Trichoderma* treatment. This treatment affected a three-fold increase in germination over the control treatment in the *Pythium*-challenged seedlings.

***Rhizoctonia* Challenge.** A similar set of replicated pot trials was also established by Dr. Simmonds to assess another *Trichoderma* treatment on pea seedlings after challenge with *R. solani*. Pea seeds were sown into pots [(3 seeds per pot, 5 replicates per treatment), containing 400 ml, peat-based growing media plus and minus Trichoflow™ (Agrimm Technologies Ltd. Christchurch) (1 g • $litre^{-1}$)] that had been added 1 week prior to planting. One-half of the pots were challenged with a spore suspension of *R. solani* in water added to the media immediately prior to planting. All pots were watered, grown in a glasshouse without supplementary heat or light, and germination was recorded at 7 and 14 days with fresh weight of the plants recorded at 29 days.

Table 3. Germination and fresh weight of pea seedlings challenged with *Rhizoctonia solani* spore suspension.

Treatment	Germination (% @ 14 days)	Fresh wt. (% control)
Control	100	100
Control + *Rizoctonia solani*	14	0
Trichoflow™ + *Rizoctonia solani*	55	35

Results (Table 3) showed that both germination and total fresh weight of seedlings challenged with *Rhizoctonia* and treated with *Trichoderma* were significantly increased ($p < 0.05$, Duncan's F test) compared with seedlings challenged without *Trichoderma* treatment. This treatment effected a four-fold increase in germination over the control treatment in the *Rhizoctonia*-challenged seedlings.

Growth Enhancement Trial. Replicated pot trials were established by Dr. Ian Harvey, Plantwise, Lincoln, to assess the effect of *Trichoderma* treatments on the root growth of cucumber plants glasshouse-grown on sawdust media under fertigation. Cucumber seedlings (variety 'Telegraph') were raised from seed in a glasshouse and planted into black polythene planter bags (22 cm diameter) full of sawdust plus and minus *Trichoderma* treatment (Trichodry™ – 1 $kg \cdot m^{-3}$) which had been added prior to planting. There were four replicates per treatment. Each bag was arranged in a random order in the glasshouse, watered, and supplied daily with 200 ml liquid nutrient solution. The bags treated with *Trichoderma* received further treatment (Trichoflow™ – 2.5 $g \cdot litre^{-1}$ water) at 3 weekly intervals. Plants were inspected regularly, stem diameters measured 35 and 56 days after planting and all plants destructively assessed for root fresh weight after 4 months.

Table 4. Stem diameter and root fresh weight of glasshouse cucumber plants.

Treatment	Stem diam. (mm)		Root Wt.
	35 days	56 days	(g)
Control	9.91	10.84	82.2
Trichodry™ + Trichoflow™	10.40	10.94	178.2

Although mean stem diameter was marginally increased at both 35 and 56 days with the *Trichoderma*-treated plants this was not significant compared with control after assessment by ANOVA. Mean root weight from the *Trichoderma*-treated plants was significantly different ($p < 0.05$) and was increased by 216% over control plants.

CONCLUSIONS

Although *Trichoderma* has shown clear potential in scientific-institute-based experiments as a biocontrol organism for more that four decades consistent commercial success has been elusive. It is likely that there are many reasons for this; for example, lack of suitable strain(s) selection, consistent active ingredient manufacture, product formulation, product packaging and storage, and finally technical product sales support. Any one, or combination of these factors has the potential to compromise a *Trichoderma*-based commercial product. The experiments reported in this paper form a very small part of an extensive database that has been developed during the past decade with Agrimm's Trichoprotection® product formulations. Consistent commercial results are being achieved with these formulations in all aspects of horticulture including nursery applications. The use of multiple strains of *Trichoderma*, unique to Trichoprotection® formulations allows greater spread of protective activity in the product. These factors together with the attention to detail that is achieved in the manufacturing and quality control of the active *Trichoderma* ingredient and the follow up support for the use of Trichoprotection® products of-

fer the best chance for continued success by the end user. Furthermore, key to the consistent success is an active research programme in many areas of horticulture, which allows results from new research to be applied in product development. An example of this is the recent availability of specific strains of *Trichoderma* with defined bio-activity towards *Sclerotinia minor*, licensed from Lincoln University, to be incorporated into a new formulation, Trichodry™ 6S. When lettuce seedlings grown on this formulation have been transplanted into fields with a known history of *Sclerotinia* lettuce drop, excellent protection of this disease has been demonstrated in commercial plantings (Stewart and Rabeendran, 2000).

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