Use of Beneficial Microorganisms for Improvement in Sustainable Monoculture of Plants

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INTRODUCTION

The introduction of bark- or peat-based soilless media in the commercial nursery industry has resulted in a more consistent medium for establishment, growth, and sale of most species of plants. Provided the medium has been supplemented with adequate nutrients and appropriate chemical pesticides are readily available, plant growth can be initiated and maintained economically. However, the microflora of the medium will seldom approach that of "healthy" soil in either magnitude or diversity of soil microorganisms. In many cases soilless media may be severely depleted of beneficial microorganisms extenuating the need for regular application of pesticides to control potentially harmful soil pathogens. In the past decade an increasing awareness has occurred of the important role beneficial microorganisms can play in commercial horticulture, provided sympathetic management practices are implemented for their establishment and maintenance. During this time the number of commercially available products based on soil-derived microbes has increased considerably. The identity, source, and use of some examples of these products will be summarised below together with selected case studies of their application in specific situations.

Table 1. Beneficial soil microorganisms.

Class	Activity			
	Symb.*	Bact.	Fung.	Insect.
Bacteria				
Rhizobium spp.	+			
Bacillus subtilis		+/-	+	
Pseudomonas spp.		+	+	
Streptomyces griseoviridis			+	
Agrobacterium radiobacter		+		
Serratia entomophila				+
Fungi				
Trichoderma harzianum	+/-		+	
Fusarium oxysporum			+	
Coniothyrium minitans			+	
Pythium oligandrum			+	
Candida oleophila			+	
Glomus spp.	+		+/-	
Bavaria bassiana				+
Metarhizium anisopilae				+
Nematodes				
Heterorhabditis spp.				+
Steinernema feltiae				+
Steinernema carpocapsae				+

* Symbiotic, bactericidal, fungicidal, insecticidal

TYPES OF BENEFICIAL MICROORGANISMS

Soil-derived microorganisms with well documented beneficial properties towards plants can be simply classified into three groups with individuals belonging to bacteria, fungi, and nematodes. Some examples within these groups are shown in Table 1.

All the examples depicted in the table have been used as bioactive ingredients in various commercial product formulations (Fravel, 1997). Most organisms have a single beneficial activity although *Pseudomonas* spp. show both bactericidal and fungicidal activity while *Trichoderma harzianum* and *Bacillus subtilis*, which are primarily fungicidal, and *Glomus* vesicular arbuscular (VA) mycorrhiza, which are primarily symbiotic, have been reported to have other activities (Samuels, 1996; Linderman, 1994).

COMMERCIAL PRODUCTS

An excellent summary of commercially available products for biocontrol of plant pathogens has been assembled by Fravel (1997) and is accessible on the Internet. Some examples of these products, their activity and details of the manufacturer are outlined in Table 2.

Most products are available as wettable powders to be watered or sprayed on to the soil, or as pellets or prills to be mixed with soil or media. The exception is Trichoject which is a liquid suspension of *Trichoderma* for injection into trees and vines.

Table 2. Commercial biocontrol products.

Product[1]	Manufacturer[2]	Agent	Activity
Epic	Gustafson	*B. subtilis*	*Alternaria, Rhizoctonia, Fusarium, Aspergillus* spp.
Intercept	Soil Technologies	*P. cepacia*	*Rhizoctonia, Fusarium, Pythium* spp.
Mycostop	Kemira	*S. griseoviridis*	*Alternaria, Rhizoctonia, Fusarium, Aspergillus* spp.
Polygandron	Plant Production Institute	*P. oligandrum*	*Pythium ultimum*
Soilgard	Thermo Trilogy	*G. virens*	*Rhizoctonia solani, Pythium* spp.
T-22G	Bioworks Inc.	*T. harzianum*	*Rhizoctonia solani, Fusarium, Pythium, Sclerotinia* spp.
Trichopel	Agrimm Technologies	*T. harzianum, T. viride*	*Phytophthora, Rhizoctonia Pythium, Fusarium, Sclerotinia* spp.
Vaminoc	MicroBio	*Glomus* spp.	*Fusarium, Pythium, Phytophthora* spp.
Trichoject	Agrimm Technologies	*T. harzianum, T. viride*	*Armillaria mellea, Eutypa lata, Chondrostereum purpureum, Phytophthora*

[1] Manufacturer's trademaks. [2] For addresses see Fravel, 1997.

CASE STUDIES

Three case studies will be considered, two based on *Trichoderma* products as a biopesticide and a growth enhancer and one based on a *Glomus* VA mycorrhiza as a root inoculant. *Trichoderma* spp. are ubiquitous in origin and while generally saprophytic in activity, they occupy an ecological niche within the layer of decaying plant matter in both forest and agricultural soils where they assist in the formation of humus. They also demonstrate antagonistic and/or mycoparasitic activity towards a wide range of other soil-resident and wood-invading fungi from which they may derive an alternative source of nutrients. It is this property which has led to extensive investigation for many decades into their potential use as biological control agents (Samuels, 1996; Papavizas, 1985; Chet, 1993). *Trichoderma* was first registered for use as a biopesticide in the early 1980s and is now approved for commercial use in many countries including, Israel, USA, France, Belgium, Sweden, U.K., Australia, and New Zealand (Samuels, 1996).

Mycorrhizal fungal associations are also very common in nature with some 90% of higher plants interacting in a beneficial way with this group of fungi (Hunter, 1998). The term mycorrhiza is derived from the Greek for fungus root and describes the mutually beneficial relationship between fungus and plant where the plant's root system is extended by intimate interaction with the fungal mycelium. *Glomus* spp. are a group of endomycorrhizal fungi found naturally associated with many species of plants grown commercially where they assist with phosphorus uptake while also conveying improved resistance to water and disease stress (Linderman, 1994).

1) *Trichoderma* Biopesticide. Trichoject is described as a high-dose liquid injectable formulation of *T. harzianum* and *T. viride* which is registered in New Zealand for control of *C. purpureum* causing silver leaf of orchard fruit trees and protection against *A. mellea* root rot of kiwifruit vines. Trichoject has been administered to mature kiwifruit vines by injection of 20-ml dose into a 6-mm-diameter hole drilled in the trunk at ground level using a hydraulic injector for application. Results from field trials performed in orchards showing high disease pressure and deaths from *Armillaria* when followed for a number of seasons (Hunt, 1998) have shown significant improvement in the survival of treated vines compared with untreated controls (Fig. 1). Survival of untreated control vines show a death rate of up to eight times that of treated vines ($p < 0.005$). Contract injection of vines at risk in orchards throughout the Bay of Plenty region of the North Island of New Zealand has resulted in excess of 20,000 vines being treated since product registration in 1996.

Trichoderma has been isolated from vines 5 years after injection suggesting protective effects could last for extended periods (Hunt, 1998).

2) *Trichoderma* Soil Conditioner. Trichopel is described as a nutritive kernel pellet formulation with multiple strains of *T. harzianum* which is sold in New Zealand and Australia as a soil and media inoculant and conditioner. It has been evaluated as a plant growth enhancer (McPherson and Hunt, 1995) and in trials with commercially grown glasshouse tomatoes planted in sawdust-filled bags supplied with nutrients by liquid fertigation. Treated plants received 5 g per bag Trichopel G well mixed into the surface layer to a depth of 100 mm.

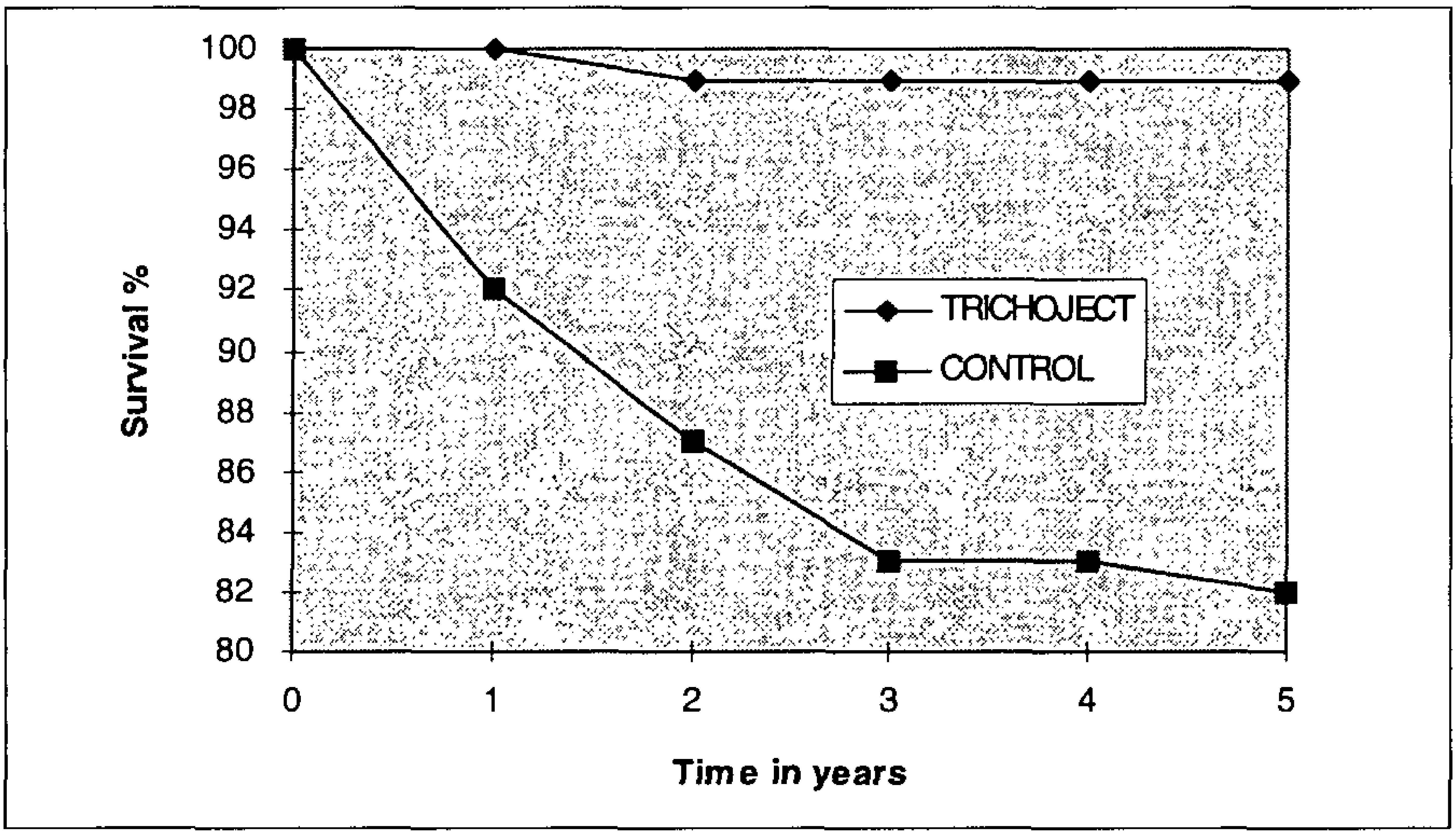

Figure 1. Survival of kiwifruit vines treated with Trichoject.

Results showed a significant increase in stem diameter measured at first truss on two varieties of tomatoes treated with Trichopel (Table 3). Although the mean crop weight for one of the varieties measured was not significant, total crop weight increased by 6.3% with treatment.

Table 3. Glasshouse tomatoes (*Lycopersicon*) treated with Trichopel.

Measurement	Trichopel mean ± s.d.	Control mean ± s.d.	Statistics p value
Stem diameter [1]			
'Taupo'	12.69 ± 1.56	11.69 ± 0.91	0.002 s.
'Evita'	9.33 ± 1.07	8.59 ± 0.66	0.014 s.
Crop weight [2]			
'Taupo'	34.30 ± 6.36	32.26 ± 5.16	0.189 n.s.
Total Crop Weight [3]	445.94	419.43	6.32%⇧

[1] Mean stem diameter (mm) from 10 replicates measured at the first truss.
[2] Mean weight (kg) per week harvested Monday, Wednesday, and Friday over a 4-week period.
[3] Total crop (kg) harvested from 240 plants sampled in each treatment.
Abrev: s.d. = Standard deviation; s. or n.s. = significant difference of the two means by Students t test; ⇧= increase in Trichopel treatment weight over control.

3) Glomus Root Inoculant. Vaminoc is described as VA mycorrhizae stabilised on an inert clay matrix which is sold in U.K., New Zealand, and Australia as a root inoculant to improve plant growth. It has been evaluated as a root inoculant to enhance plant growth in trials with vegetables, fine turf grasses, and forestry tree species. Six species of trees grown commercially in forest nurseries were evaluated for VAM infection after 5 to 6 weeks growth from germination in pots containing Vaminoc inoculant and compared with sorghum as a positive control. Microscopic examination of seedling roots showed five of six species to contain either mycelial infection or inclusion bodies (vesicles) after 6 weeks (Table 4).

The sorghum-positive controls all showed clear evidence of mycelial infection as well as the presence of numerous inclusion bodies. *Acacia melanoxylon* was the only species not to show any evidence of infection.

Table 4. Reaction of tree seedling roots to growth in Vaminoc.

Plant	Mycelial infection[1]	Inclusion bodies[2]
Sorghum bicolor	+	+++
Pseudotsuga menziesii	-	+
Eucalyptus nitens	-	+++
Pinus radiata	-	+++
Cupressus macrocarpa	+	+++
C. lusitanica	+	++
Acacia melanoxylon	-	-

[1] + Glomus mycelium present, - no mycelium.
[2] +++ Numerous, ++ moderate, + scattered inclusion bodies, - no inclusion bodies.

CONCLUSIONS

There is a considerable range of commercial products currently available which are based on beneficial microorganisms derived from both bacteria and fungi. Many of these products offer the commercial nursery opportunities to introduce these organisms into new management techniques for sustainable improvement in plant growth and disease protection.

REFERENCES

Chet, I. (ed.). 1993. Biotechnology in plant disease control. Wiley-Liss, New York.

Fravel, D. 1997. Commercial biocontrol products for use against soilborne crop diseases. Internet web page: http://www.barc.usda.gov/psi/bpdl/bioprod.html.

Hunt, J.S. 1998. *Armillaria* in kiwifruit—A long term management problem. N.Z. Kiwifruit J. 126:32-34.

Hunter, M.N. 1998. The relevance of mycorrhizas to the nursery industries. Nursery Industry Association of Australia, Annual Conference, Hobart (in press).

Linderman, R.G. 1994. Role of VAM fungi in biocontrol. pp. 1-25. In: F.L. Pfleger and R.G. Linderman (eds.). Mycorrhizae and Plant health. Amer. Phytopathol. Soc. St. Paul, Minnesota.

McPherson, D. and **J.S. Hunt**. 1995. The commercial application of *Trichoderma* (beneficial fungi) in New Zealand horticulture. Comb. Proc. Intl. Plant Prop. Soc. 45:348-353.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. Annu. Rev. Phytopathol. 23:23-54.

Samuels, G.J. 1996. *Trichoderma*: A review of biology and systematics of the genus. Mycol. Res. 100(8):923-935.