

Biocontrol Potential of *Trichoderma Harzianum* and *Trichoderma Viride* Against Soil-Borne Plant Pathogens

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Abstract: Soil-borne fungi and oomycetes like *Fusarium*, *Rhizoctonia*, *Pythium*, and *Sclerotium* annually steal a significant proportion of yield from vegetables, pulses, and field crops. Their ability to persist and persist in an effective form in the soil matrix makes them difficult targets for control using either conventional chemical control (or for many oomycetes, biological control using predatory chemicals) alone, and the reliance by large crop and horticultural enterprises upon chemical fumigants and fungicides (at least over time) has recently had to be questioned due to the effects of this practice upon the soil fauna flora environment and human health. Biological control by fungi (most particularly the filamentous fungi *Trichoderma harzianum* and *T. viride*), has because of this received much attention in the last few decades, and the two species are now some of the largest selling and most researched formulations used in the direct biological control of plant pathogens. This review gives a summary of the current knowledge of the mode of action of these two *Trichoderma* species. The group discusses their taxonomy and ecology, the main modes of their activity (mycoparasitism antibiotics competition, induced resistance induction and plant growth stimulation), the laboratory procedures used to test antagonism, a summary of their performance against the major pathogens and practical points on formulation and deployment in the field, taking Indian situations into account. They examine why biocontrol results are so inconsistent by considering strain identification, soil factors and product quality. Overall the data give the impression that *T. harzianum* and *T. viride* can be an important component of integrated disease management, mostly when used preventively, but do not offer a replacement for crop rotation, field hygiene and resistant varieties.

Keywords: *Trichoderma harzianum*; *Trichoderma viride*; biological control; soil borne pathogens; mycoparasitism; induced systemic resistance; integrated disease management

1. INTRODUCTION

Ask any grower who has had a nursery bed wiped out by damping-off or a tomato crop killed from the bottom up and you will find the story is generally the same. The problem was under the surface, it was hidden until it was too late, and the applications made to the leaves did nothing. Soil-borne pathogenic diseases are one of the most difficult to manage, partly as the pathogen species are capable of being longevity in the soil for years, laying dormant until a host is found (Papavizas, 1985; Punja and Utkhede, 2003).

The chemical option: soil fumigation, seed dressings, drenches has been the response for decades. There are circumstances where they have not and do not have a suitable alternative.

They are costly, they may attack and kill the involved pathogen and the pathogenic organisms may evolve tolerance. Residue issues and worker health and safety regulations have compounded the pressure. Biological control (the suppression of the pathogen by the products of the biological organism) has progressed from being a laboratory term to one that is viable in that environment (Chet and Inbar, 1994; Punja and Utkhede, 2003).

Trichoderma is not new to the biocontrol world. Woo et al. traces the lines of research that began with Weindling (1932) who demonstrated that a Trichoderma isolate could directly antagonise other soil fungi. Since then the genus has been touted as protective of crops, and today products based on the fungus are available on every inhabited continent and comprise a significant proportion of registered commercial biofungicides (Woo et al., 2014). Trichoderma formulations are amongst the best known biocontrols in India, largely due to the low cost of mass cultivation of the fungus and the interest of farmers towards low-input options (Sharma et al., 2014).

This story boils down to two species. Trichoderma harzianum and Trichoderma viride are found in soils all over the world, grow rapidly on simple media, and have been applied to a broad spectrum of pathogens. Even so, literature on them and other Trichoderma species is often confusing. One finds reports of both perfect control and no activity, and it is not always clear whether the reason is the biological differences amongst factors or differences in manufacturing or application of the formulations (Howell, 2003; Lorito et al., 2010).

The point of this review is to tie these threads together. After a brief glance at the pathogens themselves, we describe the two species and where they fit in the broader genus. We then look at the mechanisms of disease suppression, the host assay methods for measuring that suppression, and the evidence for control of the most important pathogens; finally formulation field use, limitations of the approach, and emerging directions for the research.

2. SOIL-BORNE PATHOGENS: WHY THEY ARE SO HARD TO MANAGE

Soil-borne pathogens share a few features that make them hard to beat. Many produce hard yellowish survival structures² such as chlamydospores, sclerotia, microsclerotia, and oospores that can survive dry summers, cold winters or the drought of a crop rotation. Many have broad host ranges and so switching to another crop is not a viable break to inoculum pressure. And they attack the root system, where by the time symptoms appear above ground it is usually too

late (Papavizas, 1985). Table 1 shows the pathogens that most frequently appear in Trichoderma studies, and the diseases they cause and how they survive.

Table 1: Major soil-borne pathogens targeted in *Trichoderma* biocontrol studies

Pathogen	Typical diseases	Example hosts	Main survival structures
<i>Fusarium oxysporum</i>	Vascular wilts, root rots	Tomato, chickpea, cotton, banana	Chlamydospores in soil and plant debris
<i>Rhizoctonia solani</i>	Damping-off, root and stem rots, sheath blight	Vegetables, cotton, potato, rice	Sclerotia and mycelium in debris
<i>Pythium</i> spp.	Seed rot, seedling damping-off, root rot	Almost all seedling crops	Oospores
<i>Sclerotium rolfsii</i>	Collar rot, southern blight	Groundnut, tomato, chilli, many others	Sclerotia
<i>Macrophomina phaseolina</i>	Charcoal rot, root rot	Soybean, sorghum, sesame, cotton	Microsclerotia
<i>Sclerotinia sclerotiorum</i>	White mould, stem rot	Brassicas, legumes, sunflower	Sclerotia
<i>Verticillium dahliae</i>	Verticillium wilt	Cotton, potato, tomato, olive	Microsclerotia

Control methods tend to be hierarchical, with resistant varieties used where they are available, but there are not available for all host-pathogen pairings and resistance can fail (break down). Rotation and sanitation may reduce the amount of inoculum, but do not normally eradicate it; soil solarisation can work in semi-tropical climates; fungicides and fumigants are the supplements. Biological control can be placed into this hierarchy as a further stratum, but the special feature is that a living agent can, in principle, occupy the root zone and exploit it for some period of time, rather than rapidly breaking down as a chemical would (Chet and Inbar, 1994):

3. THE GENUS TRICHODERMA

3.1 Taxonomy and a note on naming

Trichoderma is a large and very chequered ascomycete genus. Although Rifai (1969) outlined some semblance of order to the chaos with what he called nine 'species aggregates'; Bissett (1991) further subdivided the genus into sections; 'based on the rapid growth of branches of the conidiophores, and various conidial characters'. *T. viride*, *T. harzianum* is assigned to Trichoderma and *Pachybasium* respectively. These are the teleomorphs of *Hypocrea*, the latter sex that couples *T. viride* is *Hypocrea rufa* (Samuels, 2006).

The effect of which cannot be underestimated, as the name *T. harzianum* has been used so widely that the whole complex has gained the name. (An extensive reclassification of the group revealed many of the familiar commercial biocontrol isolates, marketed for so long as *T. harzianum*, actually belong to other species in this complex. (Chaverri et al., 2015). This does not mean that anything written previously was incorrect, but that an experiment or observation expressed for *T. harzianum* may not necessarily be applicable to different isolates with a different name. We have used the usual names through this review, in agreement with the papers cited and refer to this problem again in section 8.

3.2 Ecology

Trichoderma spp. Are among the most common of fungi in soil, they can found in forest litter, rotting logs and on plant roots, and are speedy colonists and fairly tolerant of pH; actually nearly all isolates produce copious spores and are easily dispersed. Most *Trichoderma* spp. Grow either at room temperature or at fairly warm temperatures, approximately mid twenties to around thirty degrees Celsius, but this can depend upon the particular Strain (Papavizas, 1985) which is one of the reasons they have been successful in tropical and subtropical farming systems.

They are easily isolated. Elad et al. (1981) devised a selective medium, which greatly simplified the task of enumeration of *Trichoderma* propagulae in soils, and similar media are still employed in many laboratories. Once isolated in culture, they are very simple to identify to genus level by their rapidly growing green colonies and branched conidiophores, which bear clusters of spores. Species identification is trickier, and in the present day it generally calls for DNA sequence data (Samuels, 2006; Druzhinina et al., 2011).

What is interesting about *Trichoderma* for agriculture is that it is not just a passive inhabiter of soil. Many strains have been described as opportunistic, avirulent plant symbionts: they colonise the outer tissues of roots, they parasitise other fungi, and they induce alterations in the plant that promotes its defences and in many cases, growth (Harman et al., 2004). Genome comparisons indicate predation of other fungi is the primitive lifestyle of the genus, with the plant associated state evolving after that (Kubicek et al., 2011).

3.3 *Trichoderma harzianum* and *T. viride* compared

The two species are similar enough to be interchangeable but distinct enough to justify separating them out. Their main characteristics are covered by these table 2. They are all taken from arable soils, they all used as parasites to fungi and they all can produce multiple antifungal compounds. Though in practice *T. harzianum* is shown to be the more rhizosphere-compatible organism, but *T. viride* is one of the most common species of *Trichoderma* that can be bought in India (Sharma et al., 2014). They are merely tendencies, not accurate, and the differences between strains within a species will often be greater than between species.

Table 2: Comparison of *Trichoderma harzianum* and *Trichoderma viride*

Feature	<i>T. harzianum</i>	<i>T. viride</i>
Section (Bissett, 1991)	<i>Pachybasium</i>	<i>Trichoderma</i>
Sexual state	<i>Hypocrea lixii</i> (species complex)	<i>Hypocrea rufa</i>
Conidiophore branching	Densely branched, often in pairs or whorls	Less regular branching, more open
Conidia	Smooth-walled, globose to subglobose, green	Subglobose, with rough or warted walls, dark green
Notable metabolites	Harzianic acid, harzianolides, peptaibols, 6-pentyl- α -pyrone	Viridin, peptaibols, 6-pentyl- α -pyrone (coconut-like odour)
Main modes of action	Mycoparasitism, antibiosis, competition, induced resistance, growth promotion	Mycoparasitism, antibiosis, competition, some growth promotion

Feature	<i>T. harzianum</i>	<i>T. viride</i>
Taxonomic caution	Now recognised as a species complex; identity of commercial strains often revised	Species boundaries also refined by molecular work

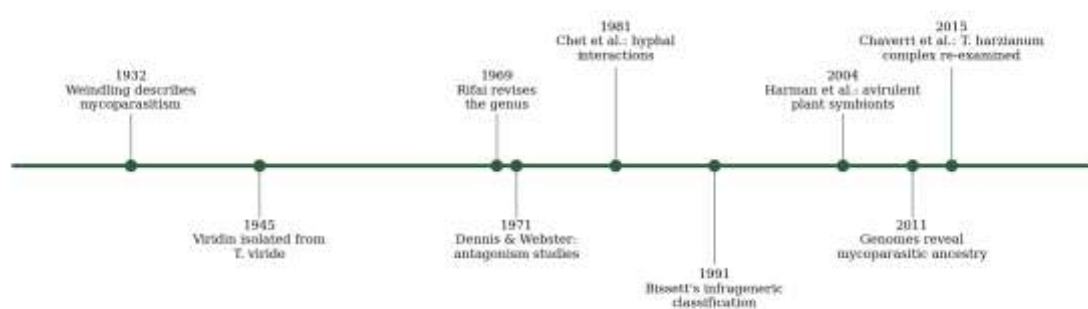


Figure 1. Selected milestones in *Trichoderma* biocontrol research, from the first description of mycoparasitism to modern genomic and taxonomic studies.

4. MECHANISMS OF BIOCONTROL

There is no all-encompassing 'trick' that *Trichoderma* uses to give disease control. Instead, given strain, pathogen, and environment, multiple mechanisms operate simultaneously, each at a different level of importance (Howell, 2003; Bentez et al., 2004). Understanding can be improved by classifying them as whether they directly affect the pathogen or through the plant, such as in Figure 2 and summarised in Table 3:

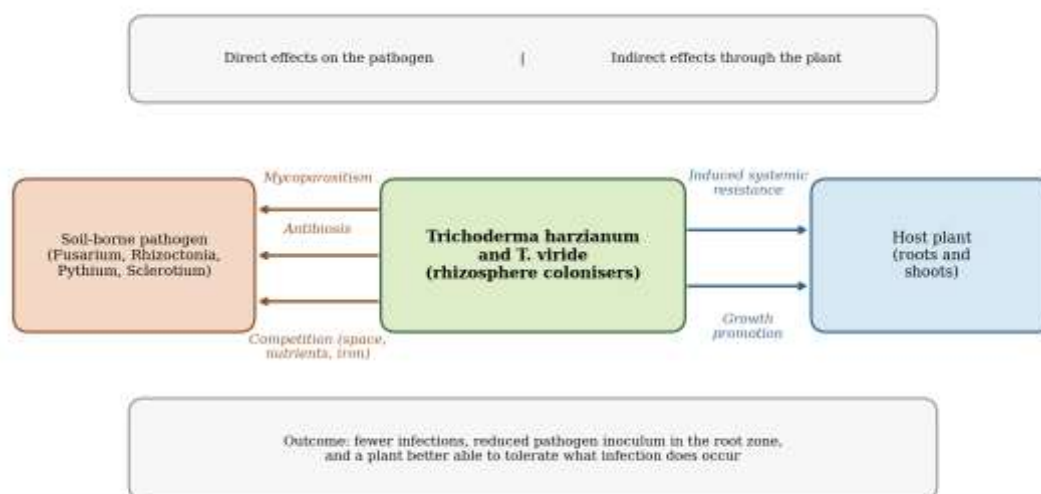


Figure 2. Conceptual summary of how *T. harzianum* and *T. viride* suppress soil-borne disease, through direct action on the pathogen and indirect action through the host plant.

4.1 Mycoparasitism

Mycoparasitism, that is the direct parasitism of one fungus on another, is perhaps the mode of actions that has most been connected to Trichoderma. The first description of the occurrence was by Weindling (1932), followed by the first microscopy work. Trichoderma hyphae grow in the direction of the susceptible fungus, occasionally coiling around in the same way as a nematode is coiled in preparation for parasitism, followed by formation of a hook or subglobose appressorium that grows against the infection court (Dennis and Webster, 1971c; Chet et al., 1981). Scanning electron microscopy of the interaction between *T. harzianum* and *Rhizoctonia solani* and *Sclerotium rolfsii* showed hyphal coiling, penetration, and subsequent collapsing of the Phytopathogenic cells (Elad et al., 1983).

The attack also has a chemical component. Trichoderma produces a range of cell-wall degrading enzymes, like chitinases, β -1,3-glucanases and proteases that attack the cell wall. Viterbo et al. (2002) summarized the evidence that these are important in biocontrol and pointed out the tendency of multiple enzymes to act synergistically, with combinations of enzymes being more inhibitory than single isolates. Other aspects of the fungus have a key role to play: signaling within the fungus, for example G-protein and MAP kinase pathways, help it detect the host and trigger the attack (Zeilinger and Omann, 2007). This is confirmed by

genome comparisons: the mycoparasite gene families are expanded in the sequenced species (Kubicek et al., 2011).

Mycoparasitism has particular importance with sclerotial pathogens as *Trichoderma* is able to colonise and break down sclerotia, reducing future inoculum in the soil (Elad et al., 1983; Papavizas, 1985).

4.2 Antibiosis

The second direct mechanism involves 'chemical warfare at a distance.' It has been known for some time now that *Trichoderma* spp. Excrete volatile and non-volatile substances capable of inhibiting the growth of other fungi. As early as 1945 Brian and McGowan extracted viridin from *T. viride* but failed to identify it, though they did describe it as a 'highly fungistatic substance' (1945). Dennis and Webster's (1971a, 1971b) then identified a variation between the genera in the production of the non-volatile and volatile antibiotics, because of this shedding light on the 'intrusiveness' of *T. viride* on mycellia.

The overall number of compounds has been brought much more up-to-date now, and literature reviews have included several classes of secondary metabolites in this genus: peptaibols (short, membrane-active peptides), pyrones (for example 6-pentyl--pyrone: a compound responsible for the coconut-like aroma common to many cultures), and different polyketides and terpenoids (Vey et al., 2001; Reino et al., 2008). Vinale et al (2006) identified several main metabolites from 2 commercial strains and attributed them to the activity against specific pathogens. Harzianic acid from *T. harzianum* can have both antifungal activity as well as plant-growth promoting activity (Vinale et al., 2009). This list emphasizes the chemistry involved, but does not describe how much each metabolite, or other, is actually present in native soils, where contamination levels are difficult to determine.

4.3 Competition

Competition, because it is not immediately obvious, is also easily missed. *Trichoderma* is fast growing, can utilise a broad menu of substrates and rapidly takes up nutrients from the root surface, this way depriving pathogen spores of the exogenous nutrients they need to germinate. Competition for space at inoculation sites, or competition for iron through siderophores, also apply (Chet and Inbar, 1994; Bentez et al., 2004). Even on aerial plant surfaces, work with a strain of *T. harzianum* used against *Botrytis cinerea* found that competition for nutrients and

interference with the enzymes produced by the pathogen are both part of its mode of action (Elad, 2000). An ability to get there first may be a great asset for fast pathogens such as *Pythium* that infect at seed and seedling stage.

4.4 Induced resistance and plant growth promotion

The picture changed again in the 1990s when we found out that it was not only the plant that was talking to *Trichoderma*, but the other way round too. Yedidia et al (1999) demonstrated that when *T. harzianum* was colonising cucumber roots, activity of enzymes tied to the defence response increased in the roots and leaves and there was strengthening of the cell wall at points of attempted pathogen entry. This was followed by work that showed that *Trichoderma* spp. Could induce systemic resistance, a state of improved defence throughout the whole plant, through elicitor activity involving jasmonic acid and ethylene signal transduction pathways (Shoresh et al (Shoresh et al., 2010; Hermosa et al., 2012). 2010; Hermosa et al. 2012).

This is often accompanied by growth promotion. Colonised plants will often have larger root systems, increased nutrient uptake and better stress tolerance, including higher resistance to drought and salinity (Harman et al., 2004; Harman, 2006; Vinale et al., 2008). As a larger, more vigorous root system can also show increased resistance to infection, distinguishing between disease effects and growth effects in the field can be difficult. Several mechanisms have now been reviewed at the genomic level (Mukherjee et al., 2013) and that work suggests that 'Trichoderma should also be appreciated as a plant partner and not just as a fungal pathogen'.

Table 3: Main biocontrol mechanisms of *T. harzianum* and *T. viride* and key references

Mechanism	What happens	Main agents	Key references
Mycoparasitism	Hyphae recognise, coil around and penetrate pathogen hyphae or sclerotia	Chitinases, glucanases, proteases; signalling pathways	Chet et al., 1981; Elad et al., 1983; Viterbo et al., 2002
Antibiosis	Diffusible and volatile compounds inhibit spore germination and hyphal growth	Peptaibols, viridin, pyrones, harzianic acid	Brian and McGowan, 1945; Vinale et al., 2006; Reino et al., 2008

Mechanism	What happens	Main agents	Key references
Competition	Faster uptake of nutrients and colonisation of space and infection sites	Rapid growth, siderophores, broad substrate use	Chet and Inbar, 1994; Benítez et al., 2004
Induced resistance	Root colonisation primes defence pathways throughout the plant	Elicitors, jasmonate and ethylene signalling	Yedidia et al., 1999; Shores et al., 2010
Growth promotion	Larger roots, better nutrient uptake, stress tolerance	Hormone-like effects, metabolites such as harzianic acid	Harman et al., 2004; Hermosa et al., 2012

Another final note. Howell (2003), reflecting on the history of these ideas, argued that the dominant mechanism can vary not only among species but among strains of a species, and even among pathogens being attacked by the same strain. A useful caution. Demonstrating that *T. harzianum* works predominantly via antibiosis against a particular target pathogen cannot be used to generalize the biocontrol activity of that strain to *T. harzianum* overall.

5. MEASURING ANTAGONISM IN THE LABORATORY

Agar plates are used for most screening. The most common test is dual culture: a disc of the pathogen is placed in one side of a Petri dish, another of *Trichoderma* in the other and the Petri dish incubated until the pathogen in a control plate without *Trichoderma* is at the edge of the dish. The effects on the two colonies are noted (Dennis and Webster, 1971c; Skidmore and Dickinson, 1976).

Results are expressed in either of two ways. The first is the percent inhibition of radial growth, calculated by Vincent (1947), $PI = [(C - T) / C] 100$, in which C is the radial growth of the pathogen on the control plate and T is its radial growth when *Trichoderma* is present. For purely speculative purposes, if the control colony grew out to 90 mm and the treated to 27 mm, then the inhibition would be 70 percent. An alternative is a classification system. Bell et al. (1982) suggested a five class system to describe the relative extent to which each fungus had overgrown the other (Figure 3), and it has continued to be popular because growth measurements are unable to record such interactions adequately.

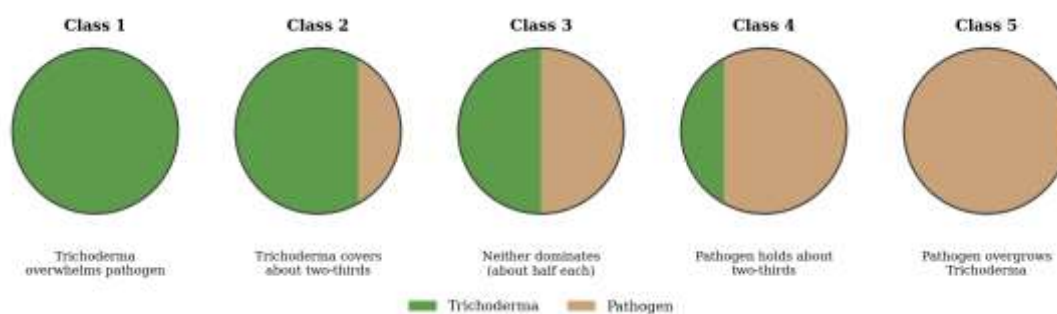


Figure 3. Schematic of the five-class dual-culture rating scale of Bell et al. (1982), from complete overgrowth of the pathogen by *Trichoderma* (class 1) to complete overgrowth of *Trichoderma* by the pathogen (class 5). Plate drawings are illustrative.

The two other plate assays differentiate the effects of different types of antibiotics. The volatile metabolite test involved growing two fungi on separate plates with their lids sealed together, so that only gaseous chemicals could be exchanged. In the non-volatile metabolite test agar that had supported *Trichoderma* was used for pathogen cultivation, or the pathogen was grown in *Trichoderma* culture filtrate. These two formats follow the traditions of Dennis and Webster (1971a, 1971b).

This is a recognized weakness and one that is worth repeating. An isolate that grows rapidly on nutrient agar may fail to establish in a natural soil, where nutrients are limited, other soil microbes are present and the pathogen is working within a host. If nothing else, Papavizas (1985), and Punja and Utkhede (2003) both correctly caution against relying on (only) plate assays. Good screening programmes will so follow up promising isolates with pot studies before they are tested in the field.

6. PERFORMANCE AGAINST MAJOR SOIL-BORNE PATHOGENS

6.1 *Fusarium* wilts

Among the diseases for which biocontrol is most long-standing are fusarium wilts. Sivan and Chet (1986) tested *T. harzianum* against fusarium spp. In cotton, wheat, and muskmelon, noting a reduction in disease in each. This was early indication that the effect was not host-specific and Raper et al. observed the same in peanut. Chickpea wilt caused by *F. oxysporum* f. Sp. Ciceris has received particular attention in India; Dubey et al. (2007) cited several *Trichoderma* spp. In the seed treatment and considered how biocontrol fits into the management of this

disease. The common theme from this work is that biocontrol pathogens are most effective if they can colonize the crop before the pathogen arrives.

6.2 Rhizoctonia, Pythium and damping-off

Seedling diseases are another area where *Trichoderma* has probably been most reliably successful. *Rhizoctonia solani* is a well known target for mycoparasitism the coil around and penetration of its hyphae was recorded both early and in detail (Chet et al (Chet et al., 1981; Elad et al., 1983). 1981; Elad et al. 1983). *Pythium* is a little harder to generalise about; susceptibility to parasitism and antibiotics is easy for hyphae and oospores as they have very thin walls but due to its rapid attack, timing is important, and the mechanism by which it suppresses depends on the strain and situation (Howell, 2003). In both cases, establishing the biocontrol on the seed or seedling medium where it can grow as the seedling root emerges is a popular option.

6.3 Sclerotium-forming pathogens

For pathogens, which over-winter as sclerotia than. *Trichoderma* gives a rare chance in control strategies a way of lowering the over-wintering inoculum. Elad et al (1983) identified attacks on both *R. solani* and *S. rolfsii* by *T. harzianum*, and long-term research has largely supported the idea that *Trichoderma* can colonise and damage sclerotia in soil (Papavizas, 1985). This is in particular significant with *S. rolfsii*, as one sclerotium can cause one new infection, whilst soil populations increase with each cropping.

6.4 Variability in the field

Field results are more variable than plate assays. Several reviews over various decades come to similar generalizations: suppression is usually better in seedling/nursery systems than in long-season field crops, better when the biocontrol is applied before infection occurs, and better when the soil has adequate organic matter to support the fungus. (Papavizas, 1985; Punja and Utkhede, 2003; Lorito et al., 2010). Disease pressure also plays a role. When there is a very high inoculum load, no biological agent alone will probably provide complete control. These generalities fit well with the position that *Trichoderma* is much more likely to succeed within an integrated programme.

7. FORMULATION AND FIELD USE

A good strain that never gets to the field alive but is of little value, and the formulation is often the make or break of many products. Commercial preparations usually consist of spore or chlamydospore products, blended into a carrier to retain viability in storage. The most common form in India is a talc-based powder, but others use grain vermiculite oil and granular carriers, and there are also liquid formulations (Sharma et al (Sharma et al., 2014; Woo et al., 2014). 2014; Woo et al. 2014). The idea of formulation has been around for some time Harman and Bjrkman (1998) expressed it because of this: "formulation should protect the fungus, disperse it, and place it where the insect is; and finally provide the fungus with an environment to flourish in."

Method of application depends on the crop and how the disease is controlled. The method most often recommended is listed in Table 4. The rate of application will depend on the product and crop used and the extension recommendation can vary region by region so growers should always follow the label and local recommendation rather than any one number.

Table 4: Common delivery methods for *Trichoderma* products, with practical considerations

Method	How it is done	Advantages	Cautions
Seed treatment	Seed coated with a spore formulation before sowing	Places the fungus on the emerging root; low product use	Compatibility with any seed fungicide must be checked
Seedling root dip	Roots of transplants dipped in a spore suspension	Protects transplants at a vulnerable stage	Needs fresh, viable product and clean water
Soil application with organic matter	Product mixed with well-decomposed compost or farmyard manure and incorporated	Gives the fungus a food base; suits nurseries and field beds	Manure must be mature; very dry soil limits benefit
Drenching	Spore suspension applied to the root zone	Quick way to treat an established stand	Curative effect limited; works better as prevention

Method	How it is done	Advantages	Cautions
Nursery bed or potting mix	Mixed into the growing medium	Protects seedlings from the outset	Avoid mixing with chemical sterilants at the same time

Certain principles are repeated across the practical literature. Product quality: viability is affected by time and heat, so fresher product from cooler storage is best; application should precede infection if do-able; the fungus takes moisture and a source of energy, so mixing with organic matter generally helps; and the coexistence of other inputs should be checked few fungicides harm *Trichoderma*, but some have significant suppressive effects (Punja and Utkhede, 2003; Woo et al., 2014). Typical practices in integrated programmes then are to place the trickle of fungal mass close by organic amendments or with relatively large, compatible doses of fungicide, and to do so in the expectation that this makes more reliable the antagonist establishment that follows (Lorito et al., 2010).

7.1 Fitting *Trichoderma* into an integrated programme

The natural impulse is to compare a biocontrol agent against a fungicide. That is not the right way to compare them. In the real world, the question for the grower is: will I find that I need less of what I already do, or will I be able to skip some other input, if I add *Trichoderma*? Thinking this way makes these fungi look very natural, and they integrate easily into the common practices of organic and conventional farming. By feeding them, organic residues in good decomposition stage encourage their survival (Papavizas, 1985). By removing fallen leaves, burying infected plant materials and preventing further contamination, several hurdles already have been overcome for them. Varieties that are resistant or tolerant to some of the pathogens help reduce pathogen load even more. Even if fungi colonise the roots of plants that are only tolerant of disease, the biocontrol action may add to the genetic resistance, making a disease suppressive cultivation system (Shoresh et al., 2010).

Perhaps over 90% of the problems with culture and the application of fungi are in the first two stages. But, then one application through the media, by spray or root dip technique, can protect the crop through to harvest in what comes next weeks. This, I think, is where diagnosis and application are most manageable, with the added value of the product far greater than that of

handling a bottomless field. "Greenhouse and nursery systems have most often been the ones in which to use fungal biocontrol agents" and it is easy to see their increasing popularity (2003).

Chemicals, if used can be managed with a little care and should not be completely ruled out. Some fungicides are aggressive, and it makes little sense to treat seed with a fungicide that kills any biocontrol present on the seed coat as a seed dressing. Certain formulations are more benign to *Trichoderma*, or certain strains have selected for fungicide tolerance and can be applied together (Woo et al., 2014). A sensible and safe course of action would be to determine compatibility on petri dishes, then pots and finally a collaborative program in a field.

Another justification for the use of biological agents is more generic. A soil that contains a diverse and thriving microbial community, like that which may result from the use of broad spectrum soil chemicals, is less prone to invasion from pathogens (Papavizas, 1985; Chet and Inbar, 1994). The long term effects of *Trichoderma* additions in potentially improving this natural suppression are uncertain, but represent one of the reasons why many agronomists value them as investments in soil quality as well as disease control.

8. CHALLENGES AND LIMITATIONS

If *Trichoderma* was no good, then I would happily write it off, but if its qualities justified it, why have we not replaced chemical control with it? So let me list down the reasons

The first issue is identity. As described above in 3.1, many commercial strains marketed as *T. harzianum*, have Next been identified as strains from other species in the complex (Chaverri et al., 2015). If the label species is wrong then any comparisons made between products or attempts to duplicate a published result are all based on inaccurate information. Accurate strain identification, preferably stored with the culture in a culture collection and identified using molecular markers, would go a long way to alleviating this problem.

Secondly, it is the variability of the environment. The soil pH temperature moisture levels, organic carbon content and the populations of microbes present influence the establishment and activity of the introduced strains (Papavizas, 1985). A strain that was selected in a certain region might be very ineffective elsewhere. This is why local-adapted isolates are generally preferred in a national programme and why tests have been established to select isolates from the native local areas (Sharma et al., 2014).

Quality of the product is the third factor. A biological product is only as effective as the number of living cells present when the product is administered and quality control can be patchy among several small producers in some regions. Shelf life, contamination, and incorrect labelling of spore numbers have all been identified as issues (Woo et al., 2014).

Fourth, the biological effect is tend to be prophylactic and often partial. A grower hoping to see a drench collar knock out a disease within a few days tends to be disappointed. It is expectations and extension advice that count as much as the science itself.

Lastly, the issue of complexity has to be addressed. Is it possible to predict performance with tons of mechanisms working together when each mechanisms varies by strain and pathogen? (Howell, 2003; Lorito et al., 2010). Only field trials in multiple seasons and locations will provide the answer.

9. FUTURE PROSPECTS

Several areas of research have great potential. Genomic and transcriptome studies have already identified the genes that are activated when interacting with pathogens and plants, and this is starting to influence strain selection and improvement (Druzhinina et al (Druzhinina et al., 2011; Mukherjee et al., 2013). 2011; Mukherjee et al. 2013). Lorito et al. (2010) suggested that bridging the gap between these 'omics' results and concrete field research is the next goal, and this is still valid.

Secondly, combinations of biocontrol strains, or combinations of *Trichoderma* with good bacteria, should improve consistency of biocontrol performance, because diverse partners may be adapted to different conditions. Although rather patchy evidence is available, it is a compelling premise. Thirdly, improved formulation, with better carriers, protective additives and delivery formats, should improve shelf life and field persistence (Woo et al., 2014). Fourthly, improving diagnosis and strain specification should tighten quality control.

None of this will matter without farmer understanding. The evidence presented here indicates that the variation in success is largely due to the time, application method and good agronomy. Training and demonstration plots are that means likely to be as valuable as any new variety.

10. CONCLUSION

Trichoderma harzianum and *T. viride* have become perhaps the most intensively researched biocontrol agents. They have multiple modes of action including direct attack to pathogens by parasitism and antibiosis, competition for nutrients and space, induction of host defences and actual or potential growth promotion. The experiences and research studies provided by laboratory and field research from over many decades have firmly established their place against a number of key soil-borne pathogens, mainly in seedling systems and as a pre-inoculation treatment.

They're not silver bullets though. Performance depends on strain soil climate and product quality and timing, and the taxonomy of *T. harzianum* itself calls for careful handling. Sure, they seem better suited to being one component of an integrated disease management programme rather than an alternative to cultural measures such as resistant varieties, rotation, and good nursery practice, or effective use of chemicals where needed. If used as such, and with the right strain and formulation, they provide the farmer with a lower-input option that has been tried and tested for about 100 years.

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