Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil microbial communities and soil enzyme activities

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SUMMARY

Five strains of *Trichoderma* with known biocontrol activities were assessed for their effect upon pea growth and their antagonistic activity against large *Pythium ultimum* inocula. The effect of *Trichoderma* inocula upon the indigenous soil microflora and soil enzyme activities in the presence and absence of *Pythium* is assessed. In the absence of *Pythium*, *Trichoderma* strain N47 significantly increased the wet shoot weight by 15% but did not significantly affect the dry weight, whilst strains T4 and N47 significantly increased the root weights by 22% and 8% respectively. Strains TH1 and N47 resulted in significantly greater root lengths. *Pythium* inoculation significantly reduced the root length, the number of lateral roots and nodules and significantly increased the root and rhizosphere soil fungal populations. *Pythium* inoculation significantly reduced the plant wet and dry shoot weights and significantly increased the wet and the dry shoot/root ratio. All the *Trichoderma* strains reduced the number of lesions caused by *Pythium* and increased the number of lateral roots. The effect of the *Pythium* on emergence and shoot growth was significantly reduced by all the *Trichoderma* strains except strain To10. Inoculation with *Trichoderma* strains TH1 and T4 resulted in significantly greater wet root weights (62% and 57% respectively) in the presence of *Pythium* compared to the *Pythium* control. Strain N47 significantly increased the shoot/root ratio compared to the *Pythium* control. Inoculation with *Trichoderma* strains T4, T12 and N47 significantly reduced *Pythium* populations. *Pythium* increased the activity of C, N and P cycle enzymes, whilst four *Trichoderma* strains reduced this effect indicating reduced plant damage and C leakage. Overall, strains T4 and N47 had the greatest beneficial characteristics as both these strains improved plant growth in the absence of *Pythium* and reduced plant damage in the presence of *Pythium*. The dual properties of these strains improve the commercial application giving them an advantage over single action inocula especially in the absence of plant pathogens.

INTRODUCTION

Modern agriculture is highly dependent on chemical pesticides, in order to control plant pathogens. Fungicides and fumigants commonly have drastic effects on the soil biota, as they are intentionally applied at much higher rates than herbicides and insecticides (Fraser, 1994). These methods are time-consuming and uneconomical, pollute the atmosphere, and are environmentally harmful as the chemicals build up in the soil (Nannipieri, 1994). Furthermore, the repeated use of such chemicals has encouraged the development of resistance among the target organisms (Goldman *et al.,* 1994). This has resulted in the use of ever-increasing amounts of pesticides and has prompted the search for new strategies of pest control to reduce or eliminate the use of pesticides (Cook & Granados, 1991; Lorito *et al.,* 1994). For instance, integration of biocontrol agents with reduced doses of chemical agents has a potential for controlling plant pathogens with minimal impact on the environment (Chet and Inbar, 1994).

Trichoderma spp. have been extensively studied as potential biocontrol agents (e.g. Lynch, 1990; Papavizas, 1992). However, some studies have also shown that *Trichoderma* spp can stimulate the growth of a number of vegetable and bedding plant crops (e.g. Baker, 1988; Lynch, *et al* 1991a,b). Lynch *et al.* (1991a,b) investigated the effect of *Trichoderma* on the growth of lettuce, and its ability to control damping off diseases caused by *Rhizoctonia solani* and *Pythium ultimum*. They investigated whether a number of *Trichoderma* strains had a direct effect on lettuce establishment and growth in the absence of pathogens. It was found that the fungal treatments reduced the emergence time of seedlings compared to the controls. From the results and those of Ousley *et al.,* (1994) they concluded that specific *Trichoderma* strains have the potential to consistently increase plant growth (Lynch *et al.,* 1991a). The prospects for control of *Pythium* damping-off of lettuce with *Trichoderma*, *Gliocladium*, and *Enterobacter* spp (*E. cloacae*) were also studied. The bacterial antagonists did not perform as consistently as the fungi.

Furthermore, the view of synergism between fungal and bacterial antagonist as shown by Kwok *et al.* (1987) was not shared by Lynch *et al.* (1991b). However, they demonstrated that to thoroughly eliminate the adverse effect of the damping-off pathogen, a threshold level of *T. harzianum* antagonist was needed.

Ousley *et al*. (1993) investigated the effect of autoclaving plant growth promoting *Trichoderma* isolates on the germination and growth of lettuce. Some strains turned out to be good plant growth promoters even after autoclaving. It was inferred that seedling growth promotion by *Trichoderma* is a balance between growth inhibiting and growth promoting properties and autoclaving some strains alters this balance.

Few commercial preparations of *Trichoderma* are available for controlling plant diseases (Coley-Smith *et al*., 1991), possibly because the non-biological methods of control used at present are more reliable. For *Trichoderma* preparations to provide realistic alternatives to chemical control, their efficiency and reliability need to be improved and knowledge of their ecology and effects on the soil ecosystem needs to be enhanced. The integration of the use of biocontrol and plant growth promoting strains of *Trichoderma* may be a useful asset in the commercialisation and marketing of such *Trichoderma* isolates. Therefore, the assessment of strains for both of these assets *in vivo* is essential for the fulfilment of this. Besnard and Davet (1993) found a number of *Trichoderma* strains that were simultaneously plant growth promoters (tomato and cucumber) and biocontrol agents (*Pythium*), however, they used a sterile potting medium and did not use fresh soil in their experiments.

Wild type isolates of *Trichoderma* with known biocontrol activities are assessed here for their effect upon pea growth and their antagonistic activity against large *Pythium ultimum* inocula, the

effects of the *Trichoderma* inocula upon the indigenous soil microflora and soil enzyme activities in the presence and absence of *Pythium* were also assessed.

MATERIALS AND METHODS

Soil description

The soil used was sandy loam of the Holiday Hills series, taken from Merrist Wood Agricultural College (Surrey), and had been under permanent pasture for at least 15 years. The analysis of the soil, conducted at the University of Surrey, was pH 5.4, particle ratio 10:9:81 clay: silt: sand respectively, and organic matter content 1.6 % by weight.

Fungal strains

Trichoderma strains were collected from various sources and tested for biocontrol activity in plate assays against *Pythium.* The experiment described below was conducted twice, the first with ten *Trichoderma* strains chosen from plate assays, replicated three times (results not shown) and the second with five strains chosen from the first experiment, replicated five times (data presented here). The five strains selected were: *Trichoderma harzianum* strains TH1 (IMI 275950), T4 (IMI 298372), T12 (IMI 298373) and N47 (IMI 288110), and *Trichoderma pseudokoningii* strain To10 which was obtained from M. Welland of Abies seeds and is available in the University of Surrey culture collection.

Material from stock cultures were grown on potato dextrose agar (PDA) at 25^0C for 3 days (primary plates). Mycelial discs (5mm diameter) from primary plates were then used to inoculate secondary PDA plates from which spore suspensions were prepared after 7 days' growth at 25^0C. Suspensions were prepared by detaching the spores from the surface of colonies into sterile distilled water using a glass spreader. The concentration of each *Trichoderma* strain was adjusted to 10^6 spores/ml^{-1} by dilution and direct counting using a haemocytometer. The spores of each

Trichoderma strain were suspended in a 0.75% guar gum solution in which the pea seeds were imbibed. Control seeds were imbibed in sterile guar gum.

Pythium ultimum (IMI 308273) was obtained from CABI Biosceince. Material from stock cultures was grown on plates of PDA at 25^0C for 3 days (primary plates). Four 5mm disks were cut and placed in a flask containing: 95 g of sand, 5 g of organically grown porridge oats and 20 ml of distilled water, all previously autoclaved twice. The flasks were incubated at 25^0C for 3 weeks before being homogenised in a blender and mixed with coarsely sieved soil at a concentration of 3%.

Experimental design

Pythium inoculated or uninocuated soil (150 g) was placed in experimental microcosms consisting of 210 mm high acetate cylinders, slotted between the top and base of plastic 90 mm diameter Petri dishes creating semi-enclosed microcosms (Naseby and Lynch, 1998). Each *Trichoderma* treatment and controls were replicated five times in the presence and absence of *Pythium*. Each microcosm consisted of eight imbibed seeds, planted at a depth of approximately 1 cm below the soil surface. Twenty-five ml of water was added to each microcosm before they were placed in a random design into a growth chamber (Vindon Scientific) set at a 16 hour photoperiod with a day/night temperature regime of 21^oC/15^oC respectively. The relative humidity was maintained at 70%. The experiment was conducted twice the first with 10 *Trichoderma* strains, replicated three times (results not shown) and the second with five strains chosen from the first experiment, replicated five times (data presented here)

Sampling and analysis

After 21 days growth the microcosms were harvested, the following plant measurements were made; the plant shoot and root (wet and dry) weight, number of nodules, length of each root, the number of lateral roots and the number of lesions. Rhizosphere soil (closely associated with the plant roots) samples were collected and stored at 4^0C. The samples were subsequently assayed for soil acid and alkaline phosphatase, urease, β-glucosidase, N-acetyl glucosaminidase, cellobiosidase and chitobiosidase by the methods of Naseby and Lynch (1997).

A 1g fresh root sample was taken from each replicate and macerated in 9 ml of sterile quarter strength Ringers solution using a pestle and mortar. One gram of rhizosphere soil from each replicate was also suspended in 9ml of sterile quarter strength Ringers solution. Filamentous fungal populations were quantified by plating a ten fold dilution series of each root macerate or soil suspension onto 10% malt extract agar containing 100 ppm streptomycin and 50 ppm rose bengal. Plates were incubated at 20^oC for 5 days before enumeration. P1 medium (Katoh and Itoh, 1983) was used for the enumeration of root indigenous, fluorescent *Pseudomonas*, plates were incubated at 25^oC and enumerated after 5 days growth. Tryptone soya agar (10%) was used for the enumeration of total culturable bacteria.

VP agar (Lumsden *et al*., 1990), based on potato dextrose agar, was used to enumerate *Pythium* and contained the following supplements: Vancomycin (200mg l^{-1}), Pimaricin (10mg l^{-1}), Penthanodichlorobenzene (100mg l^{-1}), Streptomycin (50mg l^{-1}) and Rose bengal (2.5mg l^{-1}). The media of Papavizas and Lumsden (1982) was used to enumerate *Trichoderma* spp and consisted of 500ml of V8 (vegetable juice) centrifuged with 7.5 g Calcium carbonate, from which 200ml of the supernatant was made up to 1 l^{-1} with distilled water and 15 g of agar was added. After autoclaving the following antibiotics were added to the media: 100 ppm of Neomycin sulphate, Bacitracin, Penicillin, and Chrononeb, 25 ppm of Chlorotetracycline

hydrochloride, 20 ppm of Nystatin, 500 ppm of Sodium Propionate and Alkylaryl Poliether alcohol (2ml l^{-1}).

Statistical analysis

Data were analysed using SPSS for Windows (SPSS inc.) by means of a one way ANOVA and subsequently differences between treatments (multiple comparisons) were determined using least significant differences (LSD).

RESULTS

Biocontrol and plant growth

Pythium inoculation reduced the emergence of pea seedlings (Table 1) and this effect was significantly suppressed by all the *Trichoderma* strains except strain To10, with strains T4 and N47 providing the greatest control.

Trichoderma strains TH1, T4, N47 and T12 significantly increased the wet shoot weights by 51%, 41%, 45% and 13% respectively compared with the *Pythium* treated control (Table 1). *Trichoderma* strains TH1 and T4 resulted in a significantly greater wet root weight in the presence of *Pythium* compared with the *Pythium* control (Table 1). Similarly, inoculation with *Trichoderma* strains TH1 and T4 as well as strain T12 resulted in significantly greater dry root weights (Table 2). *Pythium* inoculation significantly increased the wet and the dry shoot/root ratio (Tables 1 and 2) indicating a dramatic effect on root growth. Strain N47 significantly increased the wet shoot/root ratio compared with the *Pythium* control.

Pythium inoculation significantly reduced the root length, the number of lateral roots and nodules (Table 3). Inoculation with all the *Trichoderma* strains significantly reduced the number of lesions caused by *Pythium* and all but strain To10 increased the root length. All the *Trichoderma* inocula significantly increased the number of lateral roots in comparison with the *Pythium* control but did not significantly affect the number of nodules per root system.

Plant growth promotion

In the absence of *Pythium,* strains TH1 and T12 significantly increased the emergence of pea seedlings compared with the control, which is also consistent with the increased emergence with *Pythium* present (Table 1). Strain N47 resulted in similar pea emergence both in the presence and absence of *Pythium* and significantly increased the wet shoot weight by 15% in the absence of *Pythium*. However, none of the *Trichoderma* strains significantly affected the dry weight (Table 2). Strain T4 and N47 resulted in significantly greater wet root weights than the control, by 21% and 8% respectively. *Trichoderma* strain T4 had a significantly lower wet shoot/root ratio than strains N47, TH1 and T12.

Trichoderma strains TH1 and N47 significantly increased the root length, the number of nodules per root system and the number of lateral roots per root system in the absence of *Pythium* (Table 3), whilst strain To10 significantly reduced the number of lateral roots.

Microbial populations

Pythium inoculation significantly increased the root and rhizosphere soil bacterial populations and the root fluorescent *Pseudomonas* population (Table 4). All the *Trichoderma* inocula reduced the soil total bacterial populations in the absence of *Pythium,* however, only strain TH1 reduced the soil bacterial population in the presence of *Pythium.*There were no significant differences in the fluorescent *Pseudomonas* populations among the *Trichoderma* treatments in the absence of *Pythium.* The only effect of *Trichoderma* in the presence of *Pythium* was with the inoculation of strain To10 which resulted in a significant increase in the *Pseudomonas* population.

Pythium inoculation significantly increased the root and rhizosphere soil fungal populations (Table 5). The root fungal populations in the absence of *Pythium* were significantly greater than

the control with the inoculation of all the *Trichoderma* strains except strain TH1, whilst strains TH1, T4 and T12 significantly increased the soil fungal population in the absence of *Pythium*. The rhizosphere soil and root *Trichoderma* populations were greater in the *Pythium* treated soil than in non-infested soil (Table 5). The *Trichoderma* populations of the two controls (without *Trichoderma* inoculation) were below the level of detection of around 10^3. The root and rhizosphere soil *Trichoderma* populations did not significantly differ with the different inocula in the absence of *Pythium*. However, strain To10 resulted in the greatest root *Trichoderma* population and strain T4 the greatest rhizosphere soil population in the presence of *Pythium*. Inoculation with *Trichoderma* strains T12, N47 and T4 resulted in significantly lower *Pythium* populations than the control and strain TH1 (Table 5).

Soil enzyme activities

There were significantly higher enzyme activities in soil infected with *Pythium* (Table 6), in most cases by a factor of 3 or more. Inoculation with a number of *Trichoderma* strains significantly reduced the effect of *Pythium.* Strains TH1, T4, N47 and T12 significantly reduced β-glucosidase, NAGase and chitobiosidase activities relative to the *Pythium* control. Strains TH1, T4 and T12 significantly reduced the alkaline phosphatase activity, whilst strains TH1, T4 and N47 significantly reduced the urease activities relative to the *Pythium* control. However, strain To10 did not affect any of the enzyme activities relative to the *Pythium* control and none of the strains affected the acid phosphatase and cellobiosidase activities relative to the *Pythium* control. The only significant effects with respect to the control in the absence of *Pythium* was an increase in NAGase and β glucosidase activities with the inoculation of strain To10.

DISCUSSION

Plant growth measurements were used to assess the potential impact of the different inocula on crop production. *Pythium ultimum* had a significant effect on all the plant measurements, which is in accordance with the fact that it is a major pathogen of pea and is most destructive at the seedling stage (Kommedahl *et al*., 1981). *Pythium* inoculation reduced pea emergence and this effect was suppressed by all the *Trichoderma* strains except strain To10, with strains T4 and N47 providing the greatest control. These results are in concordance with the work of Cook (1994) who suggested that the ability of *Pythium* to rapidly colonise the host plant before other fungi is an essential part of their pathogenicity. Therefore, seed inoculation with *Trichoderma* can dramatically reduce in the effect of *Pythium*.

All the *Trichoderma* strains, except strain To10, significantly improved the growth of plants in the presence of *Pythium* and significantly reduced the damaging effect of the pathogen. *Trichoderma* strains TH1, T4 and N47 had the greatest overall effects, resulting in increased plant weights and root lengths, and reduced root lesions. This is corroborated by previous work where strain TH1 was shown to control *Pythium* damping-off of lettuce (Lumsden *et al.,* 1990), and increase both plant stand and plant fresh weight (Migheli *et al.,* 1994).

The fact that *Trichoderma* strains T4, N47 and T12 significantly reduced the *Pythium* population demonstrates that these *Trichoderma* strains have antagonistic activity towards the pathogen, which is related to an improvement in plant production. Baker (1989) suggested that the time-course of mycoparasitism indicates that mycoparasitism of *Pythium* cannot be the mechanism of antagonism when the agent is applied to seed, and the production of an antibiotic (‘routing factor’) by *Trichoderma* spp. was the causal factor in biological control of *Pythium* spp. However, three (TH1, N47 and T12) of the five strains used retarded the growth of *Pythium* by

the production of soluble diffusable metabolites and subsequently totally overgrew and mycoparasitised the *Pythium* in plate assays (data not shown). The other two strains (To10 and T4) did not totally overgrow the *Pythium* but retarded the growth of *Pythium* by the production of soluble diffusable metabolites. This suggests that the reduction in the *Pythium* population is related to the mycoparasitic activity of the *Trichoderma* rather than the production of metabolites.

Plant growth stimulation (in the absence of *Pythium*) has been shown with a number of *Trichoderma* strains (Ousley *et al.*, 1994) on other plants such as lettuce, petunia and marigold. In this study, the *Trichoderma* strains were preliminarily selected for biocontrol properties before plant growth stimulation. It is evident from these results that inoculation with strains TH1, T4, N47 and T12 resulted in varying degrees of plant growth promotion, whilst strain To10 overall had a slight detrimental effect on plant growth. Strain N47 was the most consistent plant growth promoter resulting in significant increases in most of the plant growth measurements reported and was the only strain to increase the plant shoot weight.

The increased growth response of plants caused by *T. harzianum*, depends on the ability of the fungus to survive and develop in the rhizosphere (Kleifield and Chet, 1992). A possible mechanism for increased plant growth is an increase in nutrient transfer from soil to root, which is supported by the fact that *Trichoderma* can colonise the interior of roots (Kleifield and Chet, 1992). Many microbial treatments of plants have in the past been shown to have positive or negative influence on plant growth (Lynch *et al*., 1991). However, Ousley *et al*. (1993) found that autoclaving *Trichoderma* inocula did not remove growth-promoting properties and suggested that seedling growth promotion by *Trichoderma* could be a balance between growth inhibition and growth promotion properties, with the balance altered in some strains by autoclaving. The slight inhibitory effect of *Trichoderma* strain To10 may be related to the

production of volatile pentyl and pentenyl-pyrones by *T. harzianum* (Lumsden *et al*., 1990) which, besides being fungistatic and effecting biocontrol action (Tables 1 and 2) can have phytotoxic side effects at high doses.

Inoculation with strain T4 resulted in a large increase in root weight, but did not effect the shoot weight, which resulted in a large decrease in the shoot/root ratio. The only other strain to increase the root weight was strain N47, which also increased the shoot weight and therefore did not affect the shoot/root ratio. The conversion into shoot/root ratio has been used extensively in the past (Clark and Reinhard, 1991) and has been suggested to be an indicator of plant stress, whereby the lower the shoot/root ratio (or the higher the root/shoot ratio) the more stressed the plant. Causes of plant stress include nutrient limitations (including oxygen, Drew and Lynch, 1980) and therefore, a decrease in shoot/root ratio may indicate such stress, although pathogenic effects do not necessarily comply with this rule. It should be recognised however, that such stressed plants may be more effective in acquiring water and nutrients as a result of the expanded root system. Thus this is a positive adaptive response to such stresses and this could be a useful trait in low nutrient or dry soils.

The increased root and rhizosphere soil bacterial populations and the root fluorescent *Pseudomonas* population in the presence of *Pythium* is due to the pathogenic effect of the pathogen causing nutrient leakage from the root. Only strain TH1 reduced this effect, which is due to this strain causing the greatest reduction in the effect of the pathogen on plant growth, indicating that the pathogen caused less root damage in the presence of strain TH1. All the *Trichoderma* inocula reduced the soil total bacterial populations in the absence of *Pythium*, however, this effect may be due to the *Trichoderma* inocula and fungal population taking up an increased proportion of the soil niche, indicated by increased soil fungal populations. Therefore, there would be increased competition for limiting nutrients, leading to a decrease in the bacterial

population in comparison with the control and an increase in fungi such as *Trichoderma*, which are strong soil colonisers.

Fluorescent Pseudomonads are natural biocontrol agents found in the soil, and these results indicate that the introduction of the *Trichoderma* strains (without *Pythium*) did not adversely affect these useful populations. The only effect of *Trichoderma* in the presence of *Pythium* was with the inoculation of strain To10, which resulted in a significant increase in the *Pseudomonas* population. This is likely to be a result of the slightly deleterious effect of this strain causing increased root leakage/damage, which allows a greater population of aggressive rhizosphere/root colonisers such as fluorescent pseudomonads.

The increase in root and rhizosphere soil fungal populations in the presence of *Pythium* again is due to the root damage caused by the pathogen and the resulting nutrient leakage. The increases in root and soil fungal populations with most *Trichoderma* inocula in the absence of *Pythium* are in part due to the addition of the inocula. This is indicated by the *Trichoderma* populations being undetectable in the controls (below 10^3) and therefore, the *Trichoderma* inocula had an additive effect on the total fungal populations. The lack of an increase in the fungal population with the TH1 inocula in the absence of *Pythium*, and an actual decrease in the presence of *Pythium* may be related to the potent antagonistic properties of this strain against a wide range of fungi (Lynch, 1987).

The fact that the *Trichoderma* populations of the two controls (without *Trichoderma* inoculation) were below the level of detection allowed direct quantification of the different inocula without the aid of genetic markers. The greater rhizosphere soil and root *Trichoderma* populations in *Pythium* treated soil again is due to the increase in available carbon sources from the damaged roots. All the strains were recovered from the soil and root at similar levels in the

absence of *Pythium* indicating similar colonisation abilities in this soil. However, strain To10 resulted in the greatest root *Trichoderma* population in the presence of *Pythium*, which may be related to this treatment having the lowest root mass and therefore the greatest damage and carbon leakage.

The microbial population results revealed that the soil infected with *Pythium* had significantly greater bacterial and fungal populations than uninfected soil. This is likely to be due to increased leakage of carbon compounds from the diseased roots. The results also show that microbial population measurements can be indicative of the extent of damage caused to the plant by the pathogen.

Measurement of soil enzyme activities may be useful for gaining a greater understanding of the nature of perturbations caused to ecosystem function and has been used as an indicator of the effect of microbial inoculation (Naseby and Lynch, 1998). Soil enzyme activities have also been used as an indicator of Carbon leakage from roots (Naseby and Lynch, 1998; Naseby *et al.*, 1999). The large increase enzyme activities found in the presence of *Pythium* therefore indicate a dramatic increase in C and nutrient leakage from roots due to root damage. The C cycle enzyme activities such as β-glucosidase, NAGase and chitobiosidase are directly related to carbon availability, whereas P cycle enzyme activities are inversely related to P availability (Tabatabai, 1982; Tadano *et al.*, 1993). In conditions of high C availability, such as root leakage, P is a more limiting nutrient and demand increases resulting in an increase in phosphatase activity (Naseby and Lynch, 1998). Therefore, the *Pythium* must have caused a decrease in the available phosphate, thus causing an overall increase in activity. The decrease in available P took the form of an increase in the available carbon in the rhizosphere (by root leakage). Urease activity (N cycle) was also increased by *Pythium* infection, which again indicates increased C availability as shown by Naseby *et al.*, (1999).

The trend found in the alkaline phosphatase activity was not repeated with the acid phosphatase activity. However, the majority of the acid phosphatase activity may be of a different origin to the alkaline phosphatase. Acid phosphatase is mostly of plant and associated fungal origin (Tarafdar and Marschner, 1994), whereas the alkaline phosphatase is more likely to be of microbial origin. If this is the case, then the effects of the inocula upon acid and alkaline phosphatase, in some circumstances, can be independent. This is supported by the work of Naseby and Lynch (1997) where rhizosphere acid phosphatase did not show significant differences with the inoculation of bacteria, addition of substrates and did not show a trend with soil depth. The acid phosphatase activity would be more dependent upon the nutritional status of the plant, therefore, the damage caused by *Pythium* resulting in the loss of C from the roots would reduce the P requirement of the plant.

Strains TH1, T4, N47 and T12 reduced the effect of the *Pythium* on soil enzyme activities by varying degrees. All four strains reduced the β glucosidase, chitobiosidase and NAGase activities indicating a reduction in C leakage from the root. Furthermore, the reduction in alkaline phosphatase activity with strains TH1, T4 and T12 also indicates a reduction in available C as P is less limiting with these treatments than with the *Pythium* control. Urease activity (N cycle) was also reduced by strains TH1, T4 and N47 in relation to the *Pythium* control which again indicates a comparative reduction in available C. The reduction in a number of enzyme activities by strains TH1, T4, N47 and T12 therefore indicates a reduction in plant damage and subsequent C leakage caused by *Pythium*, and is also related to increases plant growth described earlier with these strains. This is supported by the fact that inoculation with strain To10 resulted in similar enzyme activities to the *Pythium* control and this strain did offer any protection to the plant as shown by the plant growth measurements.

As few of the enzyme activities were affected by most of the *Trichoderma* strains in the absence of *Pythium* it follows that these inocula did not have a large effect on the nutrient status of the rhizosphere or nutrient exudation /leakage from the root (Naseby and Lynch, 1999). The only strain to increase the β-glucosidase activity was strain To10, which also increased the NAGase activity, this effect may be due to a slight detrimental property of this strain as shown by the decrease in the number of lateral roots and a small insignificant decrease in the root weight. Therefore, strain To10 may have damaged the root system causing a slight increase in C leakage, which increased C cycle enzyme activities. The enzyme activity results demonstrate that such measurements are sensitive indicators of the effect of *Pythium* on plant roots and of the protective effect of the *Trichoderma.*

Overall, strain N47 had the greatest beneficial characteristics as it consistently improved the pea growth measurements in the absence plant pathogens and also had an antagonistic effect against *Pythium ultimum* which reduced the damage caused to the pea plants by the pathogen. Strain T4 also improved several of the plant growth measurements and had good biocontrol properties, whilst strain TH1 was the best biocontrol agent. The dual properties of these strains improve the commercial application giving them an advantage over single action inocula especially in the absence of plant pathogens.

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Table 1 Emergence, wet shoot and root weights (g) of pea plants as affected by *Trichoderma* inocula and *Pythium ultimum*.

	Soil without *Pythium*						Soil treated with *Pythium*					
	Control	To10	TH1	T4	N47	T12	Control	To10	TH1	T4	N47	T12
Emergence	0.93^{c}	0.95^{cd}	1.00^{d}	0.93^{c}	0.95^{cd}	1.00^{d}	0.68^{a}	0.73^{a}	0.85^{b}	0.95^{cd}	0.88^{bc}	0.81^{b}
Shoot	4.84^{c}	4.54^{c}	4.87^{c}	4.95^{cd}	5.57^{d}	5.02^{cd}	2.88^{a}	2.48^{a}	4.35^{bc}	4.07^{b}	4.18^{bc}	3.25^{b}
Root	6.27^{c}	5.81^{c}	6.17^{c}	7.62^{e}	6.76^{d}	6.07^{c}	2.18^{a}	1.90^{a}	3.53^{b}	3.43^{b}	2.62^{a}	2.23^{a}
s/rRatio	0.77^{ab}	0.78^{ab}	0.82^{b}	0.66^{a}	0.83^{b}	0.83^{b}	1.33^{c}	1.32^{c}	1.44^{cd}	1.40^{c}	1.66^{d}	1.48^{cd}

To10, TH1, T4, N47, T12: *Trichoderma* strains inoculated.

Emergence-proportion of seedlings emerged five days after sowing, shoot-shoot weight, root–root weight, s/r ratio-ratio of shoot weight to root weight.

Alphabetical letters indicate significant differences at $p<0.05$ level

Table 2 Dry shoot and root weights (g) of pea plants as affected by *Trichoderma* inocula and *Pythium ultimum*.

	Soil without *Pythium*						Soil treated with *Pythium*					
	Control	To10	TH1	T4	N47	T12	Control	To10	TH1	T4	N47	T12
shoot	0.024^{bc}	0.022^{bc}	0.023^{bc}	0.025^{c}	0.024^{bc}	0.024^{bc}	0.015^{a}	0.013^{a}	0.02^{bc}	0.02^{bc}	0.02^{bc}	0.018^{b}
Root	0.44^{c}	0.45^{c}	0.47^{c}	0.51^{c}	0.46^{c}	0.47^{c}	0.17^{a}	0.13^{a}	0.27^{b}	0.27^{b}	0.19^{ab}	0.23^{b}
Ratio	0.054^{ab}	0.049^{a}	0.054^{a}	0.049^{a}	0.052^{a}	0.051^{a}	0.100^{cd}	0.103^{d}	0.080^{c}	0.084^{c}	0.112^{d}	0.072^{c}

To10, TH1, T4, N47, T12: *Trichoderma* strains inoculated.

Shoot-shoot weight, root–root weight, s/r ratio-ratio of shoot weight to root weight.

Alphabetical letters indicate significant differences at $p<0.05$ level

Table 3 Mean root length (cm) and number of lateral roots, nodules and lesions per root system as affected by *Trichoderma* inocula and *Pythium ultimum*.

	Soil without *Pythium*						Soil treated with *Pythium*					
	Control	To10	TH1	T4	N47	T12	Control	To10	TH1	T4	N47	T12
Rlength	11.94^{cde}	14.38^{efg}	16.42^{fg}	12.52^{def}	14.66^{fg}	12.46^{def}	6.54^{a}	8.60^{ab}	11.72^{cde}	10.88^{bcd}	10.10^{bcd}	9.70^{bc}
Lroots	26.40^{d}	22.00^{c}	29.50^{e}	26.40^{d}	29.50^{e}	25.20^{d}	9.00^{a}	19.80^{bc}	17.20^{b}	20.60^{bc}	21.00^{bc}	18.80^{b}
Nodule	6.00^{b}	7.40^{bc}	8.25^{c}	5.60^{b}	8.80^{c}	7.40^{bc}	0.00^{a}	0.60^{a}	1.00^{a}	1.00^{a}	0.20^{a}	0.40^{a}
Lesions	na	na	na	na	na	na	19.00^{c}	5.8^{a}	5.2^{a}	7.60^{a}	11.00^{b}	10.80^{b}

To10, TH1, T4, N47, T12: *Trichoderma* strains inoculated.

Rlength = mean root length, Lroots = mean no.Lateral roots per root system, Nodules-Mean no. of nodules per root system, lesions-mean no. lesions per root system.

Alphabetical letters indicate significant differences at $p<0.05$ level

Table 4 Log total soil and root bacteria and fluorescent Pseudomonad populations as affected by *Trichoderma* inocula and *Pythium ultimum*.

	Soil without *Pythium*						Soil treated with *Pythium*					
	Control	To10	TH1	T4	N47	T12	Control	To10	TH1	T4	N47	T12
RBact	7.24^{a}	7.13^{a}	7.28^{a}	7.29^{a}	7.36^{a}	7.27^{a}	8.01^{bc}	8.07^{bc}	7.99^{bc}	8.39^{d}	7.85^{b}	8.14^{c}
SBact	7.55^{e}	6.91^{cd}	7.02^{d}	6.58^{a}	6.75^{b}	6.84^{bc}	8.28^{g}	8.15^{g}	7.75^{f}	8.30^{g}	8.20^{g}	8.44^{g}
Pseu	5.53^{ab}	5.30^{a}	5.48^{ab}	5.54^{b}	5.48^{ab}	5.26^{a}	5.98^{cd}	6.18^{e}	5.86^{bc}	5.98^{cd}	5.93^{cd}	6.11^{de}

To10, TH1, T4, N47, T12: *Trichoderma* strains inoculated.

Rbact-root bacteria/g root, sbact soil bacteria/g soil, pseu- fluorescent pseudomonads/g root

Alphabetical letters indicate significant differences at $p<0.05$ level

Table 5 Log fungal populations as affected by *Trichoderma* inocula and *Pythium ultimum*.

	Soil without *Pythium*						Soil treated with *Pythium*					
	Control	To10	TH1	T4	N47	T12	Control	To10	TH1	T4	N47	T12
RFungi	5.15^{a}	5.41^{c}	5.25^{ab}	5.40^{bc}	5.35^{bc}	6.19^{f}	6.10^{e}	6.12^{e}	5.72^{d}	6.21^{f}	5.91^{de}	6.17^{f}
SFungi	5.48^{b}	5.36^{a}	5.95^{c}	6.26^{e}	5.56^{b}	6.25^{e}	6.26^{e}	6.13^{d}	6.47^{f}	6.34^{e}	6.31^{e}	6.14^{d}
STrich	ND	4.41^{ab}	4.00^{a}	4.15^{a}	4.45^{ab}	4.68^{abc}	ND	4.87^{bc}	4.66^{ab}	4.99^{c}	4.87^{bc}	4.56^{ab}
RTrich	ND	4.30^{a}	4.35^{ab}	4.30^{ab}	4.48^{ab}	4.26^{a}	ND	5.18^{e}	4.86^{bc}	4.88^{cd}	4.93^{cd}	5.01^{de}
Pythium	ND	ND	ND	ND	ND	ND	6.10^{c}	5.99^{bc}	6.10^{c}	5.94^{ab}	5.93^{ab}	5.83^{a}

To10, TH1, T4, N47, T12: *Trichoderma* strains inoculated.

RFungi root fungi/g root, SFungi- soil fungi/g soil, STrich- soil *Trichoderma* /g soil, RTrich- root *Trichoderma* / g root, *Pythium- Pythium/g soil,* ND- Not detected

Alphabetical letters indicate significant differences at $p<0.05$ level

Table 6 Soil enzyme activities in the rhizosphere of pea plants inoculated with *Trichoderma* and *Pythium ultimum.*

	Control						*Pythium*					
	Control	To10	TH1	T4	N47	T12	Control	To10	TH1	T4	N47	T12
Acidphos	3.19^{a}	3.91^{a}	4.21^{a}	3.79^{a}	3.37^{a}	3.66^{a}	14.37^{b}	15.19^{b}	15.39^{b}	15.45^{b}	14.47^{b}	15.05^{b}
Alkphos	0.21^{abc}	0.28^{bc}	0.26^{bc}	0.14^{ab}	0.12^{a}	0.16^{ab}	0.82^{e}	0.80^{e}	0.61^{d}	0.63^{d}	0.73^{de}	0.39^{c}
β-gluc	0.64^{a}	0.79^{b}	0.69^{ab}	0.73^{ab}	0.67^{a}	0.71^{ab}	6.52^{d}	6.27^{d}	5.63^{c}	5.63^{c}	5.4^{c}	5.61^{c}
Cello	0.02^{a}	0.04^{a}	0.02^{a}	0.03^{a}	0.02^{a}	0.02^{a}	1.06^{bc}	0.91^{bc}	1.03^{bc}	1.17^{c}	0.77^{b}	0.98^{bc}
Chito	0.03^{a}	0.03^{a}	0.02^{a}	0.004^{a}	0.03^{a}	0.001^{a}	0.21^{c}	0.18^{bc}	0.12^{b}	0.14^{b}	0.13^{b}	0.16^{b}
NAGase	1.01^{a}	1.36^{b}	1.09^{a}	0.94^{a}	0.87^{a}	0.91^{a}	3.02^{d}	2.78^{d}	2.37^{c}	2.29^{c}	2.21^{c}	2.17^{c}
Urease	0.26^{a}	0.25^{a}	0.21^{a}	0.23^{a}	0.26^{a}	0.26^{a}	1.52^{c}	1.33^{bc}	1.2^{b}	1.23^{b}	1.15^{b}	1.33^{bc}

To10, TH1, T4, N47, T12: *Trichoderma* strains inoculated.

Acidphos- acid phosphatase, alkphos- alkaline phosphatase, β-gluc-β-glucosidase, cello-cellobiosidase, chito-chitobiosidase, NAGase- N-acetyl glucosaminitase. Activities expressed as mg PNP released/h/g soil

Urease activity expressed as g ammonia released/h/g soil

Alphabetical letters indicate significant differences at $p<0.05$ level