BIOLOGICAL CONTROL OF CLUBROOT ON CAULIFLOWER WITH *TRICHODERMA* AND *STREPTOMYCES* SPP.

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ABSTRACT

The effectiveness of *Trichoderma* and *Streptomyces* spp. in suppressing clubroot of brassicas, which is caused by *Plasmodiophora brassicae,* was tested on cauliflower seedlings in glasshouse and field crops. The glasshouse experiment showed that of fifteen isolates of *Trichoderma* spp. and one *Streptomyces* sp. tested, six of these reduced ($P<0.05$) disease severity compared to the untreated control. In further testing of these six isolates, three isolates of *Trichoderma* spp. and one isolate of *Streptomyces* sp. significantly reduced disease severity. A field trial was carried out on clubroot-infested land. Crushed maize colonized with *Trichoderma* and *Streptomyces* was spread onto the plots and rotary-hoed into the soil. Results showed that three *Trichoderma* isolates (TC32, TC45 and TC63) and one *Streptomyces* isolate (S99) reduced ($P<0.05$) disease severity on the root systems, but did not increase the top weights of the plants ($P>0.05$).

Keywords: *Plasmodiophora brassicae,* biological control, *Trichoderma* spp., brassicas, *Streptomyces* sp.

INTRODUCTION

Clubroot of brassicas, caused by protozoan *Plasmodiophora brassicae,* is the most serious disease of New Zealand's brassica growing areas, reducing marketable yields and sometimes totally destroying crops. Once the disease has established in the ground, it is very difficult to eradicate and affected areas cannot be used for the cultivation of crucifers until costly soil sterilization has been carried out.

The above-ground symptoms of the disease include wilting of leaves during hot, dry days and stunting of severely affected plants. These symptoms are the direct result of damage to the root systems of infected plants. Roots become severely distorted, forming galls or clubs that characterize the disease. In severe cases, the swollen roots eventually decay as the plant dies.

Good progress has been made towards controlling clubroot through the use of chemicals (Cheah 1995; Falloon *et al.* 1997; Cheah *et al.* 1999), disease-resistant cultivars (Falloon *et al.* 1997) and lime and adjuvant (Cheah and Page 1999). We have also identified biological control as a possible component of an integrated disease management strategy for clubroot. Over the past four years, we have screened and tested the effectiveness of many micro-organisms and natural products for their potential in controlling clubroot. In a field trial, two isolates of *Trichoderma* (TC45 and TC64) and chitosan (crabshell extract) significantly reduced clubroot severity on cauliflower (Cheah and Page 1997).

This paper reports the results of further glasshouse screening of *Trichoderma* and *Streptomyces* spp. and a field evaluation of four isolates for control of clubroot in cauliflower.

MATERIALS AND METHODS

Glasshouse screening

Fifteen isolates of *Trichoderma* spp. and one *Streptomyces* sp. isolate were screened for biocontrol activity against *P. brassicae* using a screening technique developed by Cheah and Marshall (1995). All *Trichoderma* and *Streptomyces* isolates

were collected from infested field soil surrounding the root systems of symptomless plants in commercial growers' blocks at Levin. The assumption was that if no symptoms had developed in the root systems of plants growing in clubroot-infested soil, an antagonist may have prevented infection by *P. brassicae,* as others have found with different pathogens (Linderman *et al.* 1983). These isolates were cultured on potato-dextrose agar plates for 10 days. Individual isolates were then bulked up on sterile crushed maize in 500 ml conical flasks. Flasks were inoculated with agar pieces (about 1 cm^2) cut from the cultures and then incubated at 25°C under light for 10 days before being used. Inoculated flasks were shaken occasionally to loosen mycelial mats and encourage growth.

Chinese cabbage cv. Wong bok (B*rassica chinensis* L.) seedlings were used in all trials because they are susceptible to clubroot infection. Seedlings were grown for 14 days in seed trays in a glasshouse (25°C) then seedlings were transplanted into each plastic pot (18 cm diameter) filled with clubroot-infested field soil collected from Levin. About 200 ml of crushed maize colonized with *Trichoderma* spp. or *Streptomyces* sp. was mixed into the top layer of the soil to about 5 cm deep. The seedlings were drenched with phosphorous acid (Foli-R-FOS 400) at 2 ml/litre after transplanting.

Seedlings were randomised under intermittent mist in a glasshouse (25°C) for three days before being moved to a shade house (about 20°C) for a further four weeks. The experiment was repeated three times. Each treatment consisted of 10 seedlings per pot.

Disease was assessed by removing the seedlings from pots, washing the root systems and then visually rating clubroot severity (0 = healthy roots to 5 = completely clubbed).

In a subsequent experiment six isolates were retested, using the same procedures as described above. The experiment was also repeated three times.

Field trial

A field trial was carried out at a commercial grower's property on clubroot-infested soil (Dannevirke silt loam, mean pH 6.5) in Levin. Three isolates of *Trichoderma* spp. (TC32, TC45 and TC63) and one *Streptomyces* sp. (S99) were compared for the control of clubroot on cauliflower cv. All Year Round. These isolates were chosen because they gave significant reduction of disease severity in a glasshouse screening trial (Table 1).

TABLE 1: Mean clubroot score on root systems of Chinese cabbage seedlings after treatment with biocontrol agents in a glasshouse experiment.

Treatment	Mean root score[1]
TC45	0.6
S99	1.1
TC63	1.5
TC32	1.6
TC66	2.0
Phosphorous acid (plant drench @ 2 ml/litre)	2.6
TC42	3.0
Untreated	3.7
LSD (P=0.05; df=20)	2.0

[1]Clubroot score: 0=healthy root systems, 5=complete clubbing of the tap root.

Trichoderma spp. were cultured and grown in crushed maize as described by Budge and Whipps (1991). To prepare inoculum for the field trial, mixtures comprising 2 litres of flaked maize/perlite (15% v/v) and 200 ml tap water in bags were autoclaved twice for 15 min and then inoculated with 100 ml of a suspension of 10^6 spores/ml in distilled water. The bags were incubated at 22°C for 4 weeks. The bags were shaken periodically to distribute the mycelium evenly. The concentration of inoculum for

TC32, TC45, TC63 and S99 was $3x10^7$, $8x10^7$, $6x10^6$ and $28x10^8$ colony forming units per cm^3 respectively.

Maize colonised with *Trichoderma* and *Streptomyces* spp. was spread on the soil surface of each plot (about 4 kg/m^2). The plots were rotary-hoed to a depth of about 12 cm to incorporate the crushed maize into the soil. Cauliflower cv. All Year Round seedlings (8 weeks old) were then transplanted into the trial plot. Control plots were untreated. The trial design was a randomized complete block with six replications, each consisting of five treatments. Each treatment plot consisted of a single row of 10 plants spaced at 0.3 m.

Plants were harvested from each plot at maturity (14 weeks) and the weights of plant tops were measured individually. All root systems were lifted and scored for clubroot on a 0-5 scale, where 0=healthy root systems and 5=complete clubbing on the whole tap root. Data were analysed using ANOVA procedures of the Genstat statistical package.

RESULTS

In the preliminary glasshouse screening of fifteen isolates of biocontrol agents, six isolates (five of *Trichoderma* and one of *Streptomyces*) reduced the clubroot severity on root systems. In further testing of these six isolates, three isolates of *Trichoderma* spp. (TC32, TC45 and TC63) and one isolate of *Streptomyces* sp. (S99) significantly reduced disease severity (Table 1). The field trial results showed that all treatments significantly ($P<0.05$) reduced clubroot severity on root systems but did not increase the weight of the plant tops compared to the control (Table 2). Two isolates, TC32 and TC45, resulted in a significant ($P<0.05$) decrease in plant top weight.

TABLE 2: Mean clubroot scores and fresh plant top weight (root removed) for cauliflower harvested at Levin after treatment with biocontrol agents.

Treatment	Mean clubroot score[1]	Mean fresh top weight (g)
TC32	1.5	337
S99	2.2	433
TC45	2.6	312
TC63	2.6	454
Untreated	3.6	494
LSD (P=0.05; Df=20)	0.7	83

[1]Clubroot score: 0=healthy root systems, 5=complete clubbing of the tap root.

DISCUSSION

The objective of this trial was to screen and test as many biocontrol agents as possible and select the best candidates for further development. In our previous field trial, we found that TC45 reduced the severity of clubroot and increased the weight of the plant tops (Cheah and Page 1997). The results of this trial also showed that TC45 and three isolates significantly reduced in disease severity, but did not increase top weights. Rather, the TC32 and TC45 treatments decreased plant top weight compared to the control.

This result may be related to the effect of the treatments on the growth of the plants. Soon after the treatment, we observed that all treated plants were stunted compared to untreated control plants. However, all plants had recovered by three weeks after the treatment, except plants from the TC32 and TC45 treatments. These stunting symptoms had not been observed in our previous trial, and may have been caused by the different method of application of biocontrol agents in this trial (Bell *et al*. 2000). In the previous trial, plants were dipped into spore suspensions of *Trichoderma* spp. before planting, whereas in the present trial, plants were transplanted into soil in which cultured maize had been incorporated. The latter method meant the root systems of the plants was in

longer contact with the biocontrol agents, which may have adversely affected plant growth. The actual effect and mechanism involved is not known, but *Trichoderma* spp. are known to produce a range of metabolites that may affect the growth of micro-organisms and plants (Ghisalberti and Sivasithamparam 1991).

Our results indicate that *Trichoderma* and *Streptomyces* spp. may be useful biocontrol agents for control of clubroot. Our future work will test different methods and rates of application in order to minimize stunting effects and improve disease control.

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