BRASSICA CROPS AND A *STREPTOMYCES* SP. AS POTENTIAL BIOCONTROL FOR CLUBROOT OF BRASSICAS

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ABSTRACT

Two glasshouse experiments and a field trial were carried out to evaluate the potential of brassica crops which contain high levels of glucosinolates for control of clubroot of brassicas. *Brassica rapa* crops were grown for about 70 days in a field which was infested with *Plasmodiophora brassicae*. In the first glasshouse experiment, the leaf and stem of the plants were harvested, chopped into small pieces and mixed with clubroot-infested soil in punnets. Chinese cabbage seedlings were then transplanted into the punnets. In the second glasshouse experiment, soil samples were taken in punnets from plots where the *B. rapa* crops had been rotary hoed and left to decompose for about three weeks. Chinese cabbage seedlings were transplanted into the punnets. In both experiments, *B. rapa*-treated soil reduced ($P<0.001$) the clubroot severity on the root systems of the seedlings compared to the untreated control. A field trial was carried out on clubroot-infested soil to evaluate *B. rapa*, *B. napus* and a *Streptomyces* sp. for clubroot control. The two brassica crops and *Streptomyces* sp. reduced ($P=0.05$) clubroot severity on root systems of Chinese cabbage plants, except where *B. napus* was planted at a lower rate (8 plants/m²).

Keywords: *Brassica* spp., biocontrol, biofumigant, *Streptomyces* sp., clubroot.

INTRODUCTION

Clubroot of brassicas, caused by *Plasmodiophora brassicae* (Woronin), 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.

Good progress has been made towards controlling clubroot through the use of chemicals (Cheah 1995; Cheah et al. 1999), disease-resistant cultivars (Falloon et al. 1997) and biological control (Cheah et al. 2000a). We have also identified *Brassica* spp. having high levels of glucosinolates that could be used as a component of an integrated disease management strategy for clubroot. The negative impact of *Brassica* tissue on harmful soil micro-organisms is well documented (Chan & Close 1987; Kirkegaard et al. 1994) and has been reviewed by Brown & Morra (1997).

This paper reports the results of a glasshouse test and field trial to evaluate the potential of two species of *Brassica* as biofumigants and a *Streptomyces* sp. as a biocontrol agent for the control of clubroot. These two *Brassica* species were screened and selected for their high levels of glucosinolates in plant tissues by Crop & Food Research at Lincoln.

MATERIALS AND METHODS

Glasshouse experiment 1

Brassica rapa L. (turnip) plants were grown for about 70 days in a commercial field plot which was severely infested with the clubroot pathogen. It was found that the root

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systems of these plants were not infected by the disease. Leaves and stems of these plants were taken and chopped into small (1-5 mm) pieces. Eighty grams (fresh weight) of this plant material was mixed with 250 g clubroot infested soil in a punnet. Eight Chinese cabbage seedlings cv. Wong Bok (14 days old) were transplanted into a punnet and kept under intermittent mist in a glasshouse (25°C) for 5 weeks until disease assessment. Control seedlings were grown in unamended field soil. The treatments were replicated five times.

Disease was assessed by removing the seedlings from each punnet, washing the root systems and visually scoring them for clubroot severity (0 = healthy root systems to 5 = completely clubbed).

Glasshouse experiment 2

Brassica rapa plants were grown (16 plants/m²) for about 70 days in a commercial field plot which were severely infested with clubroot pathogen. The plants were then ploughed and rotary-hoed (12 cm deep) into the soil. The plant materials were left to decompose for 3 weeks. The treated soil was taken and placed in punnets. Eight Chinese cabbage seedlings (14 days old) were transplanted into a punnet and kept under intermittent mist in a glasshouse (25°C) for 5 weeks until disease assessment. Control seedlings grew in untreated soil. The treatments were replicated five times. Disease was assessed as in experiment 1.

Field trial

A field trial was carried out at a commercial grower's property on clubroot-infested soil (pH 6.5) in Palmerston North. Seedlings of *B. rapa* and *B. napus* L. (rape) were transplanted into field plots and grown for about 70 days. Two planting densities (8 and 16 plants/m²) were used for each species (Table 1). The plants were then ploughed and rotary hoed into the soil to a depth of 12 cm. The plant materials were left to decompose for 3-4 weeks.

A. Streptomyces sp. (S99) which has been shown to give significant control of clubroot (Cheah et al. 2000a) was also included in this trial. This isolate was cultured and grown in autoclaved crush maize in plastic bags for 4 weeks at 22°C. The concentration of the inoculum was about 20 x 10⁸ colony forming units/cm³. The crush-maize inoculum was spread on the soil surface of each plot (about 500 g/m²) and incorporated by rotary hoeing to a depth of about 12 cm.

Chinese cabbage cv. Wong Bok seedlings were transplanted into trial plots. Control plots had not contained *Brassica* plants. The trial design was a randomized complete block with six replicates, each consisting of six treatments. Each treatment plot consisted of a single row of 10 plants spaced at 0.3 m. Crop management followed routine commercial practices.

Plants were harvested from each plot at maturity (14 weeks). All root systems were lifted and scored for clubroot on a 0-5 scale, where 0 = healthy root system and 5 = complete clubbing on the whole tap root. Data were analysed using ANOVA procedures of the Genstat5 statistical package. Field data were logarithmically transformed to perform analysis of variance and back-transformed.

RESULTS

Glasshouse experiment 1

Chinese cabbage seedlings grown in soil with chopped *B. rapa* tissues had a mean root score of 0.6 (SEM=0.1). This was a lower ($P<0.001$) clubroot severity than the untreated control seedlings (mean = 3.4, SEM=0.2).

Glasshouse experiment 2

The clubroot disease severity on root systems was generally low. Seedlings of Chinese cabbage grown in soil that was treated with *B. rapa* had a mean clubroot score of 0.2 (SEM=0.1), which was less ($P<0.001$) than the untreated control seedlings (mean = 1.5, SEM=0.5).

Field trial

The disease level on root systems of Chinese cabbage plants was low. All treatments (except treatment 3) reduced ($P=0.05$) clubroot severity on root systems compared to

the untreated control (Table 1). *Brassica napus* at the lower rate (8 plants/m²) was not significantly different from the untreated control. No phytotoxicity was observed on the treated plants.

TABLE 1: Mean clubroot score on root systems of Chinese cabbage after soil treatment with *Brassica* spp. and *Streptomyces* sp. in field trial.

Treatment	Rate (plant/m ²)	Mean root score ¹	Logarithm of mean root score ²
1. <i>B. rapa</i>	8	0.6	-0.55
2. <i>B. rapa</i>	16	0.6	-0.47
3. <i>B. napus</i>	8	0.8	-0.24
4. <i>B. napus</i>	16	0.6	-0.45
5. S99		0.6	-0.50
6. Untreated		1.4	0.32
LSD (P=0.05; df=25)			0.66

¹Clubroot score: 0 = healthy root system, 5 = complete clubbing on the tap root. These figures are the back-transformed means of root score rounded to 1 decimal place.

²Data were logarithmically transformed to perform analysis of variance.

DISCUSSION

Both glasshouse experiments and the field trial showed that all treatments reduced the clubroot severity on root systems of Chinese cabbage plants, indicating that these *Brassica* spp. are potentially good candidates for biofumigants for the control of clubroot.

Our results support past findings that *Brassica* tissues actively suppress soilborne diseases (Brown & Morra 1997). Chan & Close (1987) reported that in glasshouse tests, *B. napus* and four species of *Brassica* reduced the root rot severity and *Aphanomyces euteiches* Drechsler oospore numbers on peas. In a field trial they also found that these *Brassica* spp. reduced the *Aphanomyces* root rot.

The mechanism of biofumigant action against clubroot involved is not fully understood. However, glucosinolates, a group of thioglycosides that are present within the tissue of *Brassica*, are thought to be involved (Fenwick et al. 1994). After tissue damage, glucosinolate molecules are cleaved by a thioglucosidase, producing many products including isothiocyanates, nitriles and thiocyanates. Isothiocyanates are biologically active, disrupting cellular components by denaturing protein structure (Kawakishi et al. 1983). However, there may be other factors involved in reducing clubroot severity.

In this trial, *Streptomyces* sp. (S99) was shown to reduce the clubroot severity, confirming our previous findings (Cheah et al. 2000a).

Our results indicate that the *B. rapa* and *B. napus* tested, and a *Streptomyces* sp. (S99), can provide an additional control strategy for integrated disease management for clubroot. Other methods including cultural practices, liming, resistant cultivars and chemicals (Cheah et al. 2000b) should also be used for control of clubroot.

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