BIOLOGICAL CONTROL OF CLOVER CYST NEMATODE (*HETERODERA TRIFOLII*) WITH FUNGI PARASITIC TO NEMATODES OR THEIR EGGS

F.S. HAY and L. BATESON

AgResearch Grasslands Research Centre,
Private Bag 11008, Palmerston North, New Zealand

ABSTRACT

The ability of four fungi to suppress nematode reproduction and ameliorate yield loss of white clover (*Trifolium repens*) in soil infested with clover cyst nematode (*Heterodera trifolii*) was examined in a pot experiment. Three fungal parasites of nematode eggs (two sterile fungi and *Scolecobasidium constrictum*) and a parasite of nematodes (*Hirsutella rhossiliensis*) were added to soil in the form of alginate beads. At eight weeks after sowing, the mean number of cysts which developed per pot was reduced by *Scolecobasidium constrictum*, one of the sterile fungi and *H. rhossiliensis*. *H. rhossiliensis* also reduced the number of cysts per gram of plant dry matter and maintained dry matter yield per pot and per plant at a level not significantly different from that of control pots (without nematodes) or pots treated with *H. rhossiliensis* alone. The number of eggs which developed within each cyst was increased in the presence of *H. rhossiliensis*. Results suggest that fungi such as *H. rhossiliensis* may have the potential to enhance the establishment of white clover.

Keywords: Nematodes, egg-parasitic fungi, nematophagous fungi, biological control

INTRODUCTION

Clover cyst nematode (*Heterodera trifolii* Goffart) is considered one of the most damaging of plant-parasitic nematode species to white clover (*Trifolium repens* L.) in New Zealand, causing reductions in yield, nitrogen fixation and persistence of white clover in pasture (Mercer 1994). The lifecycle of *H. trifolii* takes approximately four weeks under ideal conditions. Nematodes hatch from eggs as second stage juveniles which burrow into root tissue and form specialised feeding sites (syncytia). Females remain in this sedentary position and pass through two more stages to become adults which lay scores of eggs internally. Following egg production, females die and their bodies harden to form protective structures around the eggs (cysts). The use of chemicals for the control of this pest is uneconomic and environmentally undesirable, therefore alternative strategies for control are being investigated. These strategies include breeding plants which are resistant or tolerant to nematode feeding, development of pasture management practices which reduce the impact of nematodes or biological control (Mercer 1994). This paper describes some preliminary trials in the selection of fungal biological control agents of *H. trifolii*.

MATERIALS AND METHODS

Fungi

Three fungal parasites of *H. trifolii* eggs (a sterile red fungus, a sterile dark fungus and *Scolecobasidium constrictum* Abbot) and a fungus which parasitises second stage juvenile nematodes by means of adhesive conidia (*Hirsutella rhossiliensis* Minter & Brady) were used in this experiment. *H. rhossiliensis* was isolated from soil at AgResearch Flock House, Bulls, New Zealand using the method of Duddington

(1955). The sterile dark fungus was isolated from diseased eggs of *H. trifolii* obtained from cysts on roots of white clover in pots in a glasshouse, while other fungi were isolated from diseased eggs obtained during a survey of clover cyst nematodes in New Zealand pastures (Hay and Skipp 1993). Fungi were grown on potato dextrose agar (PDA) in Petri dishes for one to two months. For each fungus, three 1cm² blocks were taken from the edge of colonies on PDA and each macerated in 15ml of sterile water with an Ultraturrx homogeniser for 15 seconds. Macerate was mixed with 55 ml of a 18g/l solution of sodium alginate. This suspension was added dropwise into 500 ml of a 43 g/l solution of calcium gluconate. After 20 minutes, the resulting beads were collected by filtration and dried at 25°C for 45 hours. Beads had a mean weight of 0.015g and were refrigerated until required.

Nematodes

Cysts of *H. trifolii* from white clover in the glasshouse were picked from roots and placed on Whatman number 1 filter paper suspended over water by a coarse mesh in a Petri dish. Several such hatching dishes were refrigerated until required, at which time dishes were placed in an incubator (20°C) to allow nematodes to hatch. Second stage juveniles (J2) which had migrated through the filter paper into the water beneath were collected after four days, concentrated by filtration through a millipore apparatus and counted in a Doncaster dish.

Pot experiment

The experiment was a completely randomised design with the following treatments, with and without nematodes: a) control, b) *S. constrictum*, c) sterile dark fungus, d) sterile red fungus, e) *H. rhossiliensis*. Pasture soil was obtained from the research farm at AgResearch, Palmerston North, air-dried, sieved and pasteurised by microwaving 1 kg quantities on high power for 3 minutes in a 800 watt microwave oven. For each treatment, 8 pots (Lily Polarcup 225ml containers, Carter Holt Harvey distributors) were half-filled with 100g pasteurised soil and covered with 50g pasteurised soil containing alginate beads (2.5g beads/100g soil). Twenty seeds of *T. repens* cv. 'Huia' were planted in each pot and covered with another 25g pasteurised soil containing beads to give approximately 125 beads per pot. At 7 days after sowing, seedlings were thinned to 15 per pot. At 15 d after sowing, a suspension of *H. trifolii* was pipetted onto the surface of 4 pots of each treatment (approximately 7000 J2/pot). Pots were placed in temperature controlled tanks (20°C) and watered to weight at regular intervals to maintain a consistent moisture regime between pots. At four weeks after sowing, pots were treated with 10 ml of modified Hoagland and Knop's solution (Chen *et al.* 1961). At eight weeks, cysts were extracted from pots by elutriation of soil and collected from the overflow on a 180µm sieve in approximately 30ml water. Cysts remaining on the roots were picked off manually under a binocular microscope. Suspensions of cysts were made up to 50 ml with water and a 10 ml aliquot counted in a Doncaster dish. Fifty cysts were taken from the suspension and ground in an eppendorf tube containing 300 µl water to release eggs. A 100 µl aliquot was made up to 10 ml and the number of eggs counted in a Doncaster dish. Plants were dried overnight at 100°C and weighed. Data were analysed by ANOVA using Microsoft Excel and treatment means separated by LSD.

RESULTS

The mean number of plants which had successfully established at eight weeks after sowing was reduced by the presence of nematodes for all treatments except the sterile dark fungus and *H. rhossiliensis* (Table 1). The mean dry matter yield per pot in the absence of nematodes was not significantly different from the control without nematodes for all fungal treatments, indicating that none of the fungi were deleterious to plant growth (Table 1). For all fungal treatments except *H. rhossiliensis*, the mean dry matter yield of plants per pot and per plant was significantly lower in the presence of nematodes than in the absence, indicating that only *H. rhossiliensis* was able to maintain plant growth in the presence of *H. trifolii* (Table 1). Although the number of cysts per pot was reduced by *S. constrictum*, the sterile red fungus and *H. rhossiliensis* in comparison to the control with nematodes, the number of cysts per gram of plant dry matter was

reduced only by the *H. rhossiliensis* treatment (Table 1). The number of eggs per cyst was higher for *H. rhossiliensis*, but not for any of the other fungal treatments.

TABLE 1: The effect of four fungal parasites of nematodes on reproduction of *Heterodera trifolii* and on survival and yield of white clover in the presence or absence of nematodes.

Treatment		Plants/ pot	Plant dry weight (g/pot)	Plant dry weight (g/plant)	Cysts/ pot	Cysts/g plant dry weight	Eggs/ cyst
Control	- ¹	14.8a ²	1.15ab	0.078b	-	-	-
	+	12.0c	0.73c	0.061c	2472a	3399ab	44b
<i>S. constrictum</i>	-	14.5a	1.22ab	0.084ab	-	-	-
	+	12.3bc	0.54d	0.044d	1685cd	3316ab	54b
Sterile dark fungus	-	14.3a	1.14ab	0.080b	-	-	-
	+	14.0a	0.61cd	0.044d	2069abc	3505a	55b
Sterile red fungus	-	13.8ab	1.28a	0.094a	-	-	-
	+	11.8c	0.59cd	0.050cd	1292d	2279bc	30b
<i>H. rhossiliensis</i>	-	14.3a	1.18ab	0.083ab	-	-	-
	+	14.5a	1.11b	0.077b	1750bcd	1586c	102a

¹ With (+) or without (-) *H. trifolii*

² Means within columns followed by the same letter are not significantly different (P<0.05)

DISCUSSION

Treatment of soil with *H. rhossiliensis* was successful in preventing a reduction in dry matter yield of clover in the presence of *H. trifolii*. However, none of the egg-parasitic fungi were able to maintain plant yield at a level comparable to the control (without nematodes), and none were able to increase plant yield in comparison to the treatment with nematodes.

Although some fungal treatments decreased the number of cysts per pot, only *H. rhossiliensis* reduced the number of cysts per gram of plant dry weight. For the egg-parasitic fungi, the reduction in cysts per pot was offset by a more than proportionate reduction in plant yield. The time period of this experiment (eight weeks) and incubation temperature would have been sufficient for two generations of *H. trifolii* to develop. The ineffectiveness of the egg-parasitic fungi may be explained in that they would have had no impact on the invasion and development of the inoculated nematodes leading to reduced yield at this early stage. However, two of the egg-parasitic fungi did reduce the number of cysts at the end of the experiment, probably through parasitism of eggs produced by the first generation leading to fewer J2 invading and developing into cysts in the second generation. *H. rhossiliensis* would have been active against both inoculated J2 and those hatching from eggs of the second generation and was therefore more effective at preventing yield loss in comparison to the egg-parasitic fungi in this experiment.

The increased number of eggs per cyst in the *H. rhossiliensis* treatment may have resulted from an increased food supply available to developing females as a result of there being fewer females per gram of plant material. This indicated that reducing the number of cysts may be at least partly compensated for by an increase in egg production. Further experiments will assess the effectiveness of *H. rhossiliensis* at reduced inoculum density, *H. rhossiliensis* in combination with egg-parasitic fungi and egg-parasitic fungi over a longer time period.

REFERENCES

- Chen, T., Kilpatrick, R.A. and Rich, A.E., 1961. Sterile culture techniques in plant nematology research. *Phytopath.* 51: 799-800.
- Duddington, C.L., 1955. Notes on the techniques of handling predacious fungi. *Trans. British Mycol. Soc.* 38: 97-103.
- Hay, F.S. and Skipp, R.A., 1993. Fungi and actinomycetes associated with cysts of *Heterodera trifolii* Goffart (Nematoda: Tylenchida) in pasture soil in New Zealand. *Nematologica* 39: 376-384.
- Mercer, C.F., 1994. Plant parasitic nematodes in New Zealand. *N.Z. J. Zool.* 21: 57-65.