INVESTIGATIONS ON NATURAL ENEMIES AND BIOLOGICAL CONTROL POSSIBILITIES OF *BEMBECIA SCOPIGERA* (Scopoli) (LEPIDOPTERA : SESIIDAE)

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ABSTRACT

In field studies , two larval parasitoids *Bembecia scopigera* were found . The proportion of parasitised larvae in 1, 2 and 3 year - old infested sainfoin fields between 1984- 1987 varied from 9.7 to 42.4 %. Between years , the contribution of *Bracon crocatus* which is a new species for Turkey and *Chelonella nitens* to the rate of parasitism were about 7.5 % - 41.1 % and 0.08 % - 4.7 % , respectively. *B. crocatus* , a gregarious parasitoid of the pest , was found to have potential for biological control in the future.

Further, *Beauveria* spp. , *Fusarium* spp., *Penicillium* spp. and *Bacillus* spp. were isolated from larvae contaminated with diseases. *Bacillus thurigiensis* (16000 IU / mg a.i) was used in the field against the larvae of *B. scopigera* as biological control agent.

INTRODUCTION

Bembecia scopigera is the most important pest of sainfoin (*Onobrychis viciifolia*) fields in the Central Anatolia Region. The pest infests plants during the first year and shortens the life of the crop and results in early dislodging of the plants.Consequently control of the pest is required , by various measures including biological control, etc.

One of the oldest and most successful methods of controlling insect and related pests is by using their natural enemies , including parasitoids which attack and destroy them. Biological control agents traditionally have included predators, parasitoids and mic-

robial pathogens (van Emden, 1989).

To find potential candidates to use as biocontrol agents, survey of possible natural enemies is often carried out in the area of the world assumed to be the centre of evolution of the pest species.The aim of the present study was to determine the natural enemies of *B. scopigera* and their effectiveness . In addition, microbial preparates were tested against the larvae .

MATERIALS & METHODS

The parasitised larvae separated from healthy larvae were counted at 7- 10 day intervals in 1, 2 and 3 year - old infested sainfoin fields between 1984- 1987. Larvae which were parasitised by gregarious and solitary parasitoids were evaluated and the percentage of them parasitised obtained . Disease larvae which have characteristics such as melting, lengthening and hardening of the body or a covering by fungus, or darkening of colour were seperated from healthy larvae.

Furthermore, a preparate including *Bacillus thurigiensis* (Bt)was used against the larvae of *B . scopigera* .

RESULTS & DISCUSSION

It was found that the preparate including Bt (16000 IU / mg a.i) had low effectiveness - 40 .7 % of the larvae killed.

In this study , two larval parasitoids were obtained from cultures as follows ; (i) *Bracon crocatus* (Hymenoptera : Braconidae) , a gregarious parasitoid . Some 4- 53 parasitoid eggs were counted in the host larvae, (ii) *Chelonella nitens* (Hymenoptera : Braconidae) , a solitary parasitoid. Diseases identified included *Beauveria* spp. , *Fusarium* spp., *Penicillium* spp. and *Bacillus* spp.

It was found that parasitism occurred between 21 May - 13 August 1984 ; 28 March- 25 September 1985 ; 16 April - 7 August 1986 and 7 May - 12 August 1987. The percentage of parasitism by the two species of parasitoid for field of various ages is shown

in Table 1 .

Rhinotachina modesta , *Ipobracon triangularis* , *Exerites roborator* and *Leskia aurea* were a larval parasitoids of *B. scopigera* , but were ineffective (Bourniel & Khial , 1965 ; 1968) . Doğanlar (1982) found that the percentage of parasitism by *C . nitens* was less than 5 % on *B. scopigera* . Aubert (1978) reported that *Lissonata pimplator* was a larval parasitoid of *B. scopigera* . It was reported that parasitoids of *B. scopigera* were rare (Vuola & Korpela 1981).

It is concluded that *B. scopigera* , a gregarious parasitoid of the pest, was found to have potential for biological control in the future. Attempts are being made to rear and release this parasitoid.

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TABLE 1 . The effectiveness of parasitoids of *Bembecia scopigera* (Scopoli.)

Year and infested sainfoin fields	Percentage of parasitoid larvae		
	Average (and range) of parasitised larvae	*B. crocatus*	*C. nitens*
1984-1 year-old	41.2 (2.7 - 63.6)	41.1(2. 7 - 63.6)	0.9 (0.0 - 0. 9)
1985-2 year-old	35.8 (10.7 - 68 .0)	33.4 (33. 4 - 71.6)	2.5 (0.0 - 6.8)
1986-3 year-old	14.2 (1.7 - 30.7)	9.7 (1.7- 19.8)	4.5 (0.0 - 19.9)
1984-2 year-old	26.7 (2.5 - 42. 2)	25.3 (2.5 - 37.9)	1.4 (0.0 - 5. 9)
1985-3 year-old	21.8 (2.0 - 47.1)	20.9 (2.0 - 45.1)	0.9 (0.0 - 5.7)
1984-3 year-old	21.4 (0.8 - 41.4)	19.7 (0.8 - 41.4)	1.7 (0.0 - 7.4)
1985-1 year-old	37.7 (13.6 - 66.3)	34.2 (13.6 - 62.3)	3.4 (0.0 - 11.4)
1986-1 year-old	12.2 (1.1 - 26.5)	7.5 (1.1 - 18.8)	4.7 (0.0 - 22.9)
1986-2 year-old	13.7 (0.0 - 31.3)	10.7 (0.0 - 23.3)	2.9 (0.0 - 9.8)
1987-2 year-old	9.7 (5.0 - 22.1)	8.9 (5.0 - 19.1)	0.7 (0.0 - 6.5)
1987-1 year-old	14.9 (1.4 - 52.5)	10.2 (1.4 - 28.8)	4.7 (0.0 - 23.8)

POTENTIAL FOR BIOLOGICAL CONTROL OF *SCLEROTINIA SCLEROTIORUM* IN WINTER OILSEED RAPE WITH *CONIOTHYRIUM MINITANS*

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ABSTRACT

A field trial, with winter oilseed rape, was conducted to determine the effect of soil incorporations of a maizemeal-perlite preparation of *Coniothyrium minitans* on sclerotial survival and apothecial production of *Sclerotinia sclerotiorum*. The mycoparasite infected sclerotia and decreased sclerotial survival, carpogenic germination and production of apothecia. Effects were greatest when inoculum of *C. minitans* was applied in autumn, at the time of sowing, rather than when applied in spring. *C. minitans* survived in soil for two years and spread considerable distances to infect sclerotia in control plots. Despite the inoculum potential of *S. sclerotiorum* being reduced by the *C. minitans* treatment, no disease control was obtained. The reasons for this failure of *C. minitans* to control Sclerotinia stem rot in oilseed rape, and possible strategies to improve its efficacy in the field are discussed.

INTRODUCTION

Stem rot of winter oilseed rape caused by *Sclerotinia sclerotiorum* was first recorded in England in 1973 on a crop grown in Berkshire (Anonymous, 1973, 1975) and, since then, it has occurred irregularly over a gradually increasing area as the crop has been more widely grown (Jellis *et al.*, 1984). Incidence of stem rot has generally been low in the UK and consequently crop losses have been small except

in disease 'hot spots', confined mainly to the southern counties of Kent and West Sussex (Jellis *et al.*, 1984; Davies 1986). However, in more recent years, severe infections have been widespread throughout the UK, with yield losses in excess of 20% (Fitt *et al.*, 1992).

Once the pathogen is established, it is extremely difficult to control. Even though crop rotation and different cultivation methods can reduce the build-up of the pathogen in soil (Archer *et al.*, 1992), they cannot be relied upon as effective means of control because of extrinsically produced ascospores initiating infections (Williams & Stelfox, 1979). Foliar-applied fungicides, including the dicarboximides and MBCs, have been shown to give effective control provided that they are applied at the correct time just before petal fall (Bowerman & Gladders, 1993). However, no reliable disease forecasting system is currently available in the UK, and routine applications of fungicides for disease control are expensive. These problems and the environmental concerns over the use of pesticides have led to the search for biological control of stem rot.

Coniothyrium minitans has already shown potential for biological control of *S. sclerotiorum*. Solid-substrate soil incorporations of the antagonist have been shown to control *S. sclerotiorum* in sunflower (Huang, 1980), celery and lettuce (Whipps & Budge, 1992; McQuilken & Whipps, 1995), but have yet to be tested in oilseed rape. This paper reports a small-scale field trial conducted to determine the effect of solid-substrate soil incorporations of *C. minitans* on sclerotial survival and apothecial production of *S. sclerotiorum*, and Sclerotinia stem rot in oilseed rape.

MATERIALS AND METHODS

Inoculum production of fungi

Sclerotia of *S. sclerotiorum* were produced on sterilised wheat grain, cv. Armada, following inoculation and incubation at 20^{0}C for 3 weeks (Mylchreest & Wheeler, 1987). Batches of twenty sclerotia (*c.* 3-6 mm diameter) washed clean of adhering wheat were placed in Terylene net bags (*c.* 5 x 5 cm, mesh < 2 mm) for immediate use in the field trial. Maizemeal-perlite inocula of *C. minitans* were prepared using a method described previously (McQuilken *et al.*, 1995).

Field trial

The field trial was conducted at Imperial College's field station, Silwood Park, near Ascot, Berks., UK. Soil from the trial site was free of sclerotia of *S. sclerotiorum*. The trial was arranged in a randomised block design with three replicate plots for each treatment. Plot sizes were 1 m^2 separated by 2 m of the same crop. Five days after sowing, maizemeal-perlite inocula of *C. minitans* were evenly

applied to plots (0.8 l m^{-2}) and raked into the soil surface to a depth of *c.* 3 cm. Immediately after incorporation, ten Terylene net bags of sclerotia were buried (1-2 cm deep) individually at random positions within each 1 m^2 plot and labelled. Inocula were also incorporated into plots in the following spring just before the end of stem extension (26 March). Controls consisted of untreated plots and plots treated with inocula killed by autoclaving. To control annual grass and broad-leaved weeds, Kerb 50 W (500 g kg^{-1} propyzamide) and Dow Shield (200 g l^{-1}) were applied at recommended rates in late October and early February, respectively. A spring top dressing of nitrogen (200 kg N ha^{-1}) was applied in March. No other pesticides or fertilisers were applied.

A bag of sclerotia was removed from each replicate plot in October and then at monthly intervals thereafter. Numbers of sclerotia recovered from each bag, their viability and infection by *C. minitans* were assessed (Whipps & Budge, 1990). Survival of *C. minitans* in the soil was also monitored by soil dilution plating on Oxoid potato dextrose agar (PDA) containing Triton X-100 and Aureomycin (Whipps *et al.*, 1989). Numbers of apothecia in each 1 m^2 plot were counted at regular intervals during April, May and June. Apothecia were not removed after counting. Just before petal fall, samples of petals (30-50) were removed from each plot and plated onto PDA. Plates were incubated for 14 days at 18-20°C and scored for the presence of *S. sclerotiorum*. Disease was assessed at pod senescence (mid-July).

RESULTS

Survival and infection of sclerotia

In comparison with the control plots, consistently fewer sclerotia were recovered from plots treated with autumn soil incorporations of *C. minitans* at all monthly samplings except the first in October (Table 1). There was also a general decline with time in the numbers of sclerotia recovered from plots treated with the antagonist in autumn. Spring soil incorporations of the antagonist in March had no effect on subsequent sclerotial recovery in comparison with the corresponding controls.

The highest levels of sclerotial infection (74-100%) by *C. minitans* always occurred in plots treated with autumn soil incorporations of the antagonist (Table 2). Even by the first sampling (October; 7 weeks after soil incorporation), up to 98% of the sclerotia recovered were infected by *C. minitans*. The antagonist also spread to control plots by November (11 weeks after soil incorporation), infecting low numbers of recovered sclerotia (3-12%) throughout the trial period. Between April and August, sclerotial infection by *C. minitans* was higher in plots treated with spring soil incorporations of the antagonist than in the corresponding controls.

However, the levels of infection were considerably lower than in those sclerotia recovered from plots treated with autumn soil incorporations of the antagonist.

TABLE 1. Effect of autumn and spring soil incorporations of *Coniothyrium minitans* on percentage recovery of sclerotia of *Sclerotinia sclerotiorum* by the antagonist from plots of oilseed rape.

Sampling month	Treatment		
	Untreated control	Autumn *C. minitans*	Spring *C. minitans*
Oct	100	100	-
Nov	99±0.2[a]	75±6.6	-
Dec	97±1.4	50±6.1	-
Jan	99±0.4	35±7.8	-
Feb	99±0.6	22±7.1	-
Mar	95±2.7	17±6.6	-
Apr	97±2.1	15±6.4	85±8.3
May	98±1.3	9±3.4	98±1.7
Jun	88±2.6	18±8.9	88±6.8
Aug	65±5.1	4±1.9	78±3.6

[a]Values are means ±SE

TABLE 2. Effect of autumn and spring soil incorporations of *Coniothyrium minitans* on percentage infection of sclerotia of *Sclerotinia sclerotiorum* by the antagonist from plots of oilseed rape.

Sampling month	Treatment		
	Untreated control	Autumn *C. minitans*	Spring *C. minitans*
Oct	0	98±1.1	-
Nov	3±1.7[a]	74±10.9	-
Dec	12±4.7	85±8.9	-
Jan	7±3.1	100	-
Feb	6±2.0	97±2.9	-
Mar	8±2.9	95±2.5	-
Apr	9±2.6	97±2.2	28±8.4
May	6±2.8	94±2.5	28±6.5
Jun	7±2.8	93±4.2	38±6.1
Aug	3±1.8	98±1.4	38±9.9

[a]Values are means ±SE

Apothecial production and disease incidence

Apothecia were first observed in treatment plots on 16 April (flowering) and were present until late June (leaf senescence) (Table 3). Most apothecia were produced from late April until mid-May. No new apothecia were found after the end of May and numbers declined thereafter. During peak apothecial production (23 April - 21 May), very low numbers were produced from sclerotia in plots treated with autumn soil incorporations of *C. minitans* compared with control plots, or those treated with spring soil incorporations of the antagonist. However, spring soil incorporations of the antagonist had some effect on apothecial production later on in the growing season. On 11 June (seed development (most pods green)), fewer apothecia were present in spring *C. minitans*-treated plots compared with controls.

TABLE 3. Effect of autumn and spring soil incorporations of *Coniothyrium minitans* on numbers of apothecia produced by sclerotia of *Sclerotinia sclerotiorum* in plots of oilseed rape.

Sampling date	Treatment		
	Untreated control	Autumn *C. minitans*	Spring *C. minitans*
16 Apr[a]	15±3.7[b]	1±0.8	15±5.7
30	47±7.2	2±1.6	40±13.8
7 May	30±6.1	0	26±13.0
14	60±6.4	2±1.0	52±14.7
28	24±3.1	1±0.5	15±5.1
4 Jun	24±3.1	1±0.5	15±5.1
11	14±3.2	0	1±0.5
18	2±0.9	4±3.8	0

[a]16 Apr was 31 weeks after burying sclerotia/autumn soil incorporation of *C. minitans*

[b]Values are means ±SE

Samples of petals removed from all treatment plots just before petal fall and plated onto PDA produced typical colonies of *S. sclerotiorum*. In general, numbers of petals from which the pathogen was isolated were lower in plots treated with autumn soil incorporations of *C. minitans* compared with either the controls, or plots treated with spring soil incorporations of the antagonist. At pod senescence, *S. sclerotiorum*-diseased plants were present in virtually all treatments, but not in all plots. Overall, only a low level of disease (0-20% of stems affected) was present

throughout the trial and there were no significant differences between the *C. minitans*-treated plots and the control (data not shown).

Survival of *C. minitans* in soil

C. minitans exhibited a general decline in colony forming units (CFUs) cm^{-3} of soil with time in all plots treated with inocula of the antagonist. However, the antagonist could still be detected at 10^4 CFUs cm^{-3} of soil for up to two years after the last soil incorporation.

DISCUSSION

Autumn treatment with *C. minitans* reduced the inoculum potential of *S. sclerotiorum* in soil. Sclerotial recovery was reduced, and carpogenic germination and apothecial production were inhibited. These effects of *C. minitans* have been reported before in field trials against *Sclerotinia trifoliorum* in the absence of plants (Turner & Tribe, 1976), and in glasshouse trials with *S. sclerotiorum* in lettuce and celery (Whipps & Budge, 1992). However, this is the first report of such effects of *C. minitans* on *S. sclerotiorum* in the UK under field-grown oilseed rape. Spring treatments were not as effective at reducing the inoculum potential as autumn treatments. It is possible that spring treatments with *C. minitans* may not provide sufficient time for the antagonist to infect sclerotia and prevent carpogenic germination of *S. sclerotiorum*. Alternatively, it may be related to the environmental conditions prevailing following soil incorporation. Tribe (1957) correlated poor infection of sclerotia of *S. trifoliorum* by *C. minitans* in spring with low soil temperatures and greater infection in the autumn with higher soil moisture. Similar conditions may have prevailed during this trial.

Even though *C. minitans* was able to reduce the inoculum potential of *S. sclerotiorum*, no disease control was obtained. The small plot size and the low level of disease (< 20%), caused by dry weather conditions during flowering, made detection of statistically significant effects difficult. Since ascopores of *S. sclerotiorum* require water for germination, colonisation of petals and subsequent infection of oilseed rape stems, periodic irrigation of the crop during continued dry conditions at flowering and petal fall is likely to encourage disease development in future trials. In view of the possibility of long distance spread of ascospores between plots (Williams & Stelfox, 1979; Archer *et al.*, 1992), it is recommended that bigger plots separated by large guard areas of oilseed rape are also used. This will help to prevent ascosporic inocula produced in control plots from infecting oilseed rape in plots treated with *C. minitans*.

C. minitans survived in soil in this trial for up to two years and spread to infect sclerotia at a considerable distance from soil incorporation. This confirms the potential of *C. minitans* to survive and infect sclerotia of *S. sclerotiorum* in the long term (Whipps & Budge, 1992).

The reduction in inoculum potential of *S. sclerotiorum* resulting from soil incorporation of *C. minitans* indicates the potential that may exist for this biocontrol agent to control Sclerotinia disease in oilseed rape. However, further field trials are required at different sites to evaluate *C. mintans* under a wide range of soil types and conditions. It is also necessary to optimise the timing of application of the biocontrol agent to achieve practical field use. Another treatment before stem extension may also be required to attack those sclerotia which develop on plants in the rosette stage following autumn-winter infection (McQuilken *et al.*, 1994). Foliar spore sprays during flowering may also be required to prevent *S. sclerotiorum* using petals as a food base to infect plants.

ACKNOWLEDGEMENTS

We wish to thank Dr. B. E. J. Wheeler for constructive discussion throughout this work and the gardening staff at Silwood Park for field assistance. We are also grateful to Dr G. E. Jackson for assistance with data collection. Financial support for this work, provided by the Ministry of Agriculture Fisheries and Food (MAFF) and EEC, is gratefully acknowledged.

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THE POTENTIAL FOR RESISTANCE TO CYST NEMATODES IN TRANSGENIC PLANTS WHICH EXPRESS ANTIBODIES.

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ABSTRACT

Potato cyst nematodes (PCN) are important pests of potato crops worldwide. Monoclonal antibodies (MAbs) are valuable tools for the identification of nematodes through their ability to recognise species-specific antigens and they also have potential for use in novel control strategies. Specific antibodies have been expressed *in planta* (plantibodies) that bind to plant viruses and provide some protection against viral attack. A panel of MAbs to PCN has been produced at Rothamsted and is currently being screened by indirect immunofluorescence for antibodies that recognise targets important in nematode development. The proteins recognised by the selected MAbs will be characterised. MAbs with potential to interfere in nematode development will be cloned as short-chain antibody fragments for eventual expression in plants. Protocols will be devised to analyse the efficacy of these MAbs in reducing nematode infection and development. Preliminary results on the identification and characterisation of nematode antigens recognised by Mabs with the potential to be used as 'plantibodies' are presented.

INTRODUCTION

Potato cyst nematodes (PCN) are agricultural pests of great economic importance. In the UK they are estimated to cause loss of about 10% of the value of the annual potato crop (Evans & Stone, 1977). They are root endoparasites not exceeding 1mm in length at any developmental stage. The two PCN species *Globodera rostochiensis* (Wollenweber, 1923) and *Globodera pallida* (Stone, 1973) probably originated in the Andean region of South America and were taken, sometime in the 19th century, to Europe which then became a secondary centre of distribution (Brodie, 1984). PCN have now spread throughout the world to at least 50 countries (Baldwin & Mundo-Ocampo, 1991).

PCN decrease yield directly by decreasing the size of the host root system, which in turn affects water and nutrient uptake and leaf duration (Brodie *et al.*, 1993). Further, indirect losses are incurred by the necessity to grow crops of lesser value in crop rotations and from the costs of maintaining quarantine schemes, advisory services and resistance breeding programmes. Resistant cultivars, however, cannot be used continuously because they promote the selection of virulent pathotypes. There is no commercially available cultivar with full resistance to *G.*

pallida and nematicides and crop rotation are also less effective at controlling this species of PCN.

Cyst nematodes have complex relationships with their host plants. They overwinter as juveniles in eggs contained within a cyst, formed from the female cuticle which tans on death to form a protective capsule for 200-500 eggs (Brodie *et al.*, 1993). The eggs may remain dormant in the soil for up to about 25 years. Hatching is triggered by exudates from host plant roots. Potato root diffusate (PRD) contains up to six active components, and induces hatching by a bimodal action on eggshells and juveniles. Changes brought about by PRD allow juveniles to become sufficiently active to cut through the eggshell and ultimately hatch (Doncaster & Shepherd, 1967).

The juveniles move in the soil searching for a healthy plant and invade young potato roots. Later, young potato tubers may also be subject to invasion by juveniles. Infested tubers and tubers carrying infested soil are the primary vehicles for the dispersal of the nematodes to uninfested regions. The juveniles mature into adults inside the roots or under the surface layer of the tuber. The males are active and migrate in search of young females which, on maturation, emerge through the root surface and spend their entire lives attached to the roots.

Developing juveniles absorb nourishment by the induction of a syncytial feeding site in the host roots (Wyss & Zunke, 1986). These feeding sites act as nutrient sinks and solutes are continually withdrawn by the nematode and replenished by the plant (Hussey, 1989). Nematodes fail to reach maturity if the syncytia are not formed and females often die when there is reduced food availability because of competition from adjacent nematodes. After fertilisation, an embryo develops within each egg to become a second-stage juvenile (J2). The adult female dies after completion of egg development and its cuticle tans. Development from hatching to adult at optimal temperatures takes between 38 and 45 days (Baldwin & Mundo-Ocampo, 1991).

The expression of antibodies in plants has already been demonstrated (Hiatt *et al.*, 1989; Hiatt, 1990). Plant cell culture, transformation and genetic recombination (sexual crossing of regenerated plants) are novel methods for achieving assembly of light and heavy chain pairs and immunoglobulin structures in plants. Plants expressing functional antibodies are found in the progeny of a cross between plants containing the individual heavy or light chains.

Engineering nematode resistance in crops by the production of transgenic plants expressing functional antibody fragments directed to vital nematode proteins has been proposed (Bakker *et al.*, 1993).The engineered single-chain Fv antibody (scFv) is particularly suitable for expression in plants because of its small size and the lack of assembly requirements. The variable domains (V and VL) can be amplified by polymerase chain reaction (PCR) using 'universal primers' and inserted into vectors for *Escherichia coli* expression of scFv antibody, in which the two variable domains are connected by a linker peptide. With this work, (Tavladoraki *et al.*, 1993), first reported a plant phenotype with an attenuation of viral infection derived from a constitutively expressed, virus-specific antibody. The scFv antibodies proved to be functionally stable in the cell cytoplasm. The intracellular scFvs seem particularly

suitable to 'immunomodulate' selected cytoplasmic antigens, unlike whole antibody molecules which need to be targeted to the endoplasmic reticulum for correct assembly/folding and stable accumulation in plants (Tavladoraki *et al.*, 1993).

A panel of monoclonal antibodies (MAbs) to PCN antigens has been produced at Rothamsted, originally for diagnostic purposes, and from these, three MAbs have been shown to react with the nematode amphids, dorsal oesophageal glands and somatic muscles (Curtis & Evans, 1994). The whole antibody panel is now being further analysed in order to identify MAbs reacting with potential targets for antibody inhibition which could disrupt nematode development in the plant. Here we report preliminary results on the identification of MAbs reacting to PCN antigens.

MATERIALS AND METHODS

Antigen preparation

Approximately 50 cysts of *G. pallida* pathotype Pa2/3 and *G. rostochiensis* pathotype Ro1 were soaked in distilled water for 1 d and homogenised using an Eppendorf plastic homogenizer (Biomedix) in 10 µl of PBS on ice (10 cysts/ml). Homogenate of gravid white females of *G. pallida* was prepared as described above. To obtain excretory/secretory products, including stylet secretions, approximately 2000 J2 of *G. pallida* and *G. rostochiensis* were incubated with a solution of 0.2 mg/ml 5-Methoxy DMT oxalate (Research Biochemicals Incorporated) in distilled water for 4 h at room temperature. Two protease inhibitors, 1mM EDTA and 1mM PMSF, were added to the final solution. The protein concentrations were determined using a Bio-Rad assay (Bradford, 1976).

Indirect immunofluorescence

The specific reactivity of the antibodies was localised in cryostat sections of *G. rostochiensis* and G. *pallida* second stage juveniles (J2). To obtain fresh J2, cysts of *G. rostochiensis* and *G. pallida* were soaked in distilled water for 3 d and stimulated to hatch in potato root diffusate 1:3 in distilled water. Freshly hatched juveniles were collected daily and processed for microtomy by rapid freezing of a block of J2 in liquid nitrogen. The nematode block was sectioned using a cryostat and sections 7-8 µm thick were collected on poly-prep slides (Sigma), air dried and fixed in cold acetone. The post-fixed cryostat sections were soaked in 0.2% Triton X-100 in PBS for 30 min., and then incubated with goat serum diluted 1:50 in PBS for 20 min. The slides were then incubated with cell line supernatant (MAbs), overnight at room temperature. The sections were rinsed 3 times with PBS and incubated for 45 min. at room temperature with goat anti-mouse FITC Conjugate (Sigma) diluted 1:50 in PBS. After rinsing, the slides were mounted with an anti-quenching agent, Citifluor (Agar Scientific). Visualisation of bound antibodies was achieved using a microscope with an epifluorescent attachment (Olympus BH-2) fitted with a 455nm excitation filter and a 460nm secondary filter. Micrographs were obtained using an Olympus OM4 camera with Ilford XP2 400 film.
(results are shown in tables and histograms).
ELISA - Indirect ELISA was performed using the procedure described by Robinson *et al.*, 1993.

RESULTS

In the preliminary screening of 132 hybridomas raised against J2 of PCN *G. rostochiensis* and *G. pallida*, some hybridomas had shown positive reaction with more than one structure in the nematode body (Table 1). The indirect immunofluorescence test was performed more than once for the majority of hybridomas tested.

TABLE 1. Preliminary screening of 132 hybridomas by indirect immunofluorescence on cryostat sections of second stage juveniles (J2) of *G. rostochiensis* and *G. pallida*

Nematode structure	Number of hybridomas
amphids	05
anterior sense organs	01
stylet	03
stylet protractor muscle	03
stylet knob	06
procorpus membrane	04
dorsal gland amullae	07
dorsal gland extension	05
oesophageal lumen	02
metacorpus membrane	03
metacorpus pump chamber	02
subventral gland ampullae	01
nerve ring	03
dorsal gland	02
subventral glands	01
intestine canal	08
intestine granules	18
reproductive system	02
excretory system	04
nervous system	03
somatic musculature	19
cuticle	04
other structures	14
negatives	12

Thus, a variety of hybridomas binding to different nematode structures was obtained following standard immunizations and, from this panel of antibodies, six cell lines were repeatedly cloned and sub-cultured in order to obtain cell lines with a defined specificity. Indirect immunofluorescence was used to detect the range of nematode structures to which the MAbs bound (Table 2).

TABLE 2. Indirect immunofluorescence reaction of monoclonal antibodies (MAbs) raised against antigens from second-stage juveniles (J2) of *G. rostochiensis* and *G. pallida*.

Antibody	Nematode structure	Specificity of antibody
MAb GR-j5	Anterior part of the nematode head	Microvillar nerve process, dendritic and nerve processes.
	Median region of the nematode body	Nerve cord which contains neuronal process and associated neuronal cell bodies. Longitudinal nerve cords: nerve processes and cell bodies.
MAb GR-j6	Anterior part of the nematode body	Pharyngeal region, possibly related to the dorsal oesophageal gland extension and ampullae.
MAb GR-j9	Anterior part of the nematode body	Pharyngeal region, possibly related to the dorsal oesophageal gland extension or to the oesophageal lumen.
MAb GR-j7	Anterior end of nematode head	Possibly related to the style protractor muscles or to the nerve processes of the dorsal oesophageal gland ampullae.
MAb GR-j8	Cuticle	Annulations and lateral line.
MAb GR-j12	Intestine	Granules (in the gut wall).

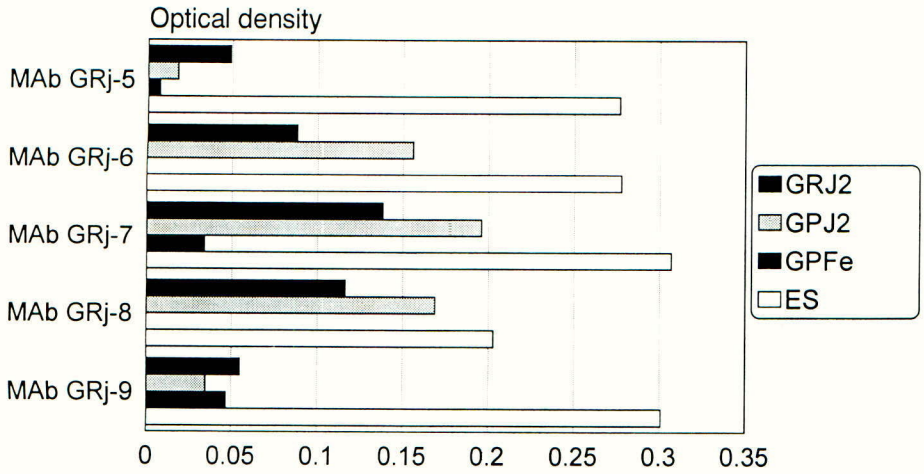
The MAbs which reacted with the nematode nervous system were tested for reactivity with several neurotransmitters. Mabs GRj-10 and 11 reacted with adrenaline and MAb GRj-11 had an additional reactivity with GABA (gamma-amino butyric acid) and a weak reaction with acetylCoA, noradrenaline and octopamine (Table 3).

TABLE 3. Binding of MAbs to neurotransmitters in indirect ELISA (optical densities less than 3X negative control value are recorded as negative).

MAbs	GABA	5-HT	acetylCoA	Adrenaline	Noradren	Octopamine
MAb GR-j5	neg.	neg.	neg.	neg.	neg.	neg.
MAb GR-j10	neg.	neg.	neg.	0.058	neg.	neg.
MAb GR-j11	0.067	neg.	0.032	0.089	0.037	0.030

MAbs reacted with homogenates prepared from cysts containing juveniles of *G. rostochiensis* and *G. pallida*, adult females of *G. pallida* and excreted/secreted antigens produced *in vitro* by juveniles of both species (Fig. 1). Negative controls consisted of PBS pH 7.2 and 5-Methoxy DMT 0.2 mg/ml. MAbs GRj 5,6,7 showed most reactivity with the antigens tested, and none of the MAbs tested reacted well with *G. pallida* adult females.

Fig.1 Binding of MAbs in indirect ELISA to PCN antigens prepared from cysts (containing juveniles) of *G. rostochiensis* and *G. pallida* (GRJ2 and GPJ2 respectively), adult females of *G. pallida* (GPFe) and excreted/secreted antigens collected from juveniles of both species (ES).



DISCUSSION

Nematode sections were screened by immunofluorescence with 132 Mabs. Mabs were found that, in preliminary tests, appeared to react with nematode structures including the nervous system, oesophageal glands, oesophageal lumen, cuticle/epicuticle, stylet protractor muscles, granules in the gut. The reactivity of five selected Mabs with PCN antigens was tested by ELISA and binding was obtained with homogenates prepared from *G. rostochiensis* and *G. pallida* J2 and excreted/secreted antigens. However, the reactivity of these antibodies with a homogenate prepared from *G. pallida* gravid white females was low.

Three Mabs reacted with the nematode nervous system, showing immunofluorescence staining of longitudinal nerves and cell bodies. They also seem to react with neural processes present in the nematode dorsal oesophageal gland ampullae and amphids. The reactivity of these MAbs with several neurotransmitters was tested in ELISA and most binding was obtained with GABA (MAb GRj-11) and adrenaline (MAbs GRj-5 and 11). The cell lines producing antibodies of interest were subcloned and stored in liquid nitrogen. Further work will include characterisation of the antigens

recognised by the selected MAbs by Western blotting, isoelectric focusing, immunofluorescence using nematodes 'pieces' and immunogold labelling at the EM level. Attempts will be made to devise a system to assess antibody efficacy in interfering with the nematode life-cycle *in vitro*. The eventual aims of this programme of work are to understand more about the interactions between PCN and their potato hosts and to obtain resistance to PCN by expressing antibodies that interfere with nematode development in transgenic plants.

ACKNOWLEDGEMENTS

IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. Bruno Ramos is in receipt of a research studentship funded by the Higher Education Funding Council for England. Financial help from Harper Adams University Sector College and MAFF is gratefully acknowledged.

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