Characterization of *Meloidogyne javanica* surface coat with antibodies and their effect on nematode behaviour

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SUMMARY

The surface coat of the 2nd-stage juveniles (J2) of plant-parasitic nematodes is considered to be involved in interactions with microorganisms in the soil and rhizosphere, as well as with the host plant. Characterization of surface antigens might be important in the development of new nematode control strategies. In this study, polyclonal and monoclonal antibodies raised against *Meloidogyne javanica*, *M. incognita* and other plant-parasitic nematodes were tested for their binding to the surface coat and secreted–excreted products of *M. javanica*. Some of the monoclonal and polyclonal antibodies raised against *M. incognita* showed cross-reactivity with the surface coat of *M. javanica*. Western blot analysis of *M. javanica* surface coat extracts revealed labelling of several polypeptides with a 48 kDa main band for the polyclonal antibody IACR-PC Mi 373, and a 55 kDa main band for PC Mj E2. Further characterization of the antigens recognized by the polyclonal antibody PC Mj E2, *in planta*, showed that they were present in the parasitic stages J2 and J3 and that the surface coat was shed during root penetration. The hypodermis of the infective juveniles was labelled by PC Mj E2 and the monoclonal antibody IACR-Misec 3F.4, suggesting that these surface antigens are produced in the hypodermis. Nematode behaviour was affected by all the antibodies that bound to the surface coat of the pre-parasitic J2, and we demonstrated that the movement pattern of the *M. javanica* J2 was affected by these antibodies. Continuous binding of the antibodies to the *M. javanica* surface inhibited the infection of *Arabidopsis thaliana* roots on agar plates.

Key words: *Arabidopsis thaliana*, cuticle, *Meloidogyne incognita*, plant-parasitic nematodes, secreted–excreted products, surface antigens.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are among the most damaging agricultural pests; they attack nearly all crops. These sedentary plant-parasitic nematodes manipulate with host gene expression in order to establish their permanent feeding site (Bird, 1996). In many species of nematodes the external cuticular layer, the epicuticle, is covered with a fuzzy material termed the 'surface coat' (SC), that is composed mainly of proteins, carbohydrates and lipids (Spiegel & McClure, 1995). The nematode surface coat, being the outermost layer of the cuticle, is considered one of the major targets for developing new control strategies against plant-parasitic nematodes. Therefore, characterization of surface antigens and their roles in different life-stages may be an important step towards the development of such alternative controls. Nematode secretions and surface coat antigens are likely to be the first signals perceived by the plant and they probably have important roles in the host–parasite relationship (Jones & Robertson, 1997). The nematode surface is probably also involved in protection of the parasite from plant defence responses (Robertson *et al*. 2000).

Meloidogyne spp. surface coats have been partially characterized by various methods (Lin & McClure, 1996; Spiegel *et al*. 1995, 1997) including several based on monoclonal and polyclonal antibodies (Curtis, 1996; Gravato-Nobre & Evans, 1998; Lopez de Mendoza, Curtis & Gowen, 1999). The role of the SC in the interactions between root-knot nematodes and microorganisms has been intensively investigated e.g. with the obligate bacterial parasite *Pasteuria penetrans* (Davies & Danks, 1993; Spiegel, Mor & Sharon, 1996) and with nematophagous fungi (Dijksterhuis *et al*. 1994).

One of the most interesting features of the nematode SC is its dynamic nature; there is a continuous turnover that involves shedding and replacing of the surface antigens (Blaxter & Robertson, 1998). In plant-parasitic nematodes this was demonstrated for the pre-parasitic juveniles of *Meloidogyne* spp. (Spiegel *et al*. 1997; Lin & McClure, 1996). However, the information about the fate and role of surface antigens in the parasitic stages within the roots is very limited (Gravato Nobre & Evans, 1998). *Arabidopsis thaliana* roots infected with *M. incognita* were immunolabelled,

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and shedding of surface antigens was observed during the migration of the juveniles within the roots (Gravato-Nobre *et al*. 1999). Root sections were also immunolabelled with a polyclonal antibody at the interface formed by the cells adjacent to the nematode (Gravato-Nobre *et al*. 1995). A monoclonal antibody reactive with the surface of *M. incognita* was immunolocalized *in planta* around the plasma membrane of root cells in the vicinity of giant cells of *A. thaliana* (Curtis, 1996). Molecules secreted or shed by the nematodes might actively participate in the infection by binding to receptors on the plant cells to trigger signal transduction cascades leading to the formation of the feeding sites. They may also help with the initial penetration, migration of the juvenile in the plant tissues and digestion of the host cell contents (Jones & Robertson, 1997). As yet, little is known about these molecular events.

In the present study, several polyclonal and monoclonal antibodies raised against *M. incognita* or *M. javanica*, were used to characterize the binding to the surface coat antigens and to secreted–excreted (SE) products of *M. javanica* various life-stages *in vitro* and *in planta*. The effect of the antibody binding to the surface of infective juveniles was assayed by tracing the movement patterns of the juveniles on agar plates and by assessing their ability to infect *A. thaliana* roots.

MATERIALS AND METHODS

Nematodes

Meloidogyne javanica egg masses were collected from infected roots of glasshouse-grown tomatoes with 1% hypochlorite (NaOCl) solution and juveniles (J2) were hatched from the eggs in water. Tomato roots were grown monoxenically and were infected with *M. javanica* as described by Huettel (1990). These infected roots were used for cryosections and to obtain J2 hatched in sterile conditions for the bioassay experiments.

Antibodies

The monoclonal antibodies (MAbs) IACR-Misec 8D.3, IACR-Misec 1C.3, IACR-Misec 8E.2, IACR-Misec 9F.5, IACR-Misec 7E.1 and IACR-Misec 3F.4 were previously raised to *M. incognita* J2 SE products (Lopez de Mendoza *et al*. 1999). The polyclonal antibodies (PAbs) were raised in rabbits to several plant-parasitic nematodes: IACR-PC 373 (live pre-parasitic J2 and SC extract of *M. incognita*), IACR-PC 353 (live pre-parasitic J2 of *Heterodera avenae*), IACR-PC 320 (*Globodera rostochiensis* purified protein), IACR-PC 266 and IACR-PC 267 (homogenates of J2 of *G. rostochiensis* and *G. pallida*, respectively) and IACR-PC 389 (secretions from *M. incognita*). The PAb Mj PC K1 was previously raised in mouse against *M. javanica* SC extract (Spiegel *et al*. 1996) and the PAb Mj PC E2 was raised in rabbit against *M. javanica* live pre-parasitic J2.

Immunofluorescence of live pre-parasitic J2

Live pre-parasitic J2 were incubated for 20 min in 1:50 diluted goat serum and then treated for 90 min with the primary MAbs or PAbs. The MAbs were diluted 10-fold in phosphate buffered saline (PBS) (0·15 M, pH 7·2), the PAbs Mi PC 373 and Mj PC K1 were diluted 1:500 and the Mj PC E2 was used at 1:5000 dilution. The nematodes were washed 3 times with PBS and were then incubated with the FITC-conjugated secondary antibodies: goat anti-mouse polyvalent immunoglobulin (Sigma) diluted 1:50 (for the MAbs), goat anti-rabbit IgG (Sigma) diluted 1:50 (for the PAbs raised in rabbit) and goat anti-mouse IgG (Sigma) diluted 1:50 (for the PAb raised in mouse). Negative controls were treated with the appropriate non-immune serum for the PAbs and with Dulbecco's Modified Eagle's medium containing 20% fetal calf serum (20D medium) for the MAbs. Controls with the secondary antiserum alone were also tested. To stimulate production of SE products by the pre-parasitic J2, live nematodes were treated for 90 min with 0·1 mg/ml of the neurotransmitter 5-methoxy-*N*,*N*-dimethyl tryptamine (DMT) (Sigma) prior to incubation with the antibodies.

Immunofluorescence of cryosections

Cryosections (30 μm) of *M. javanica* pre-parasitic J2, and of infected and non-infected tomato roots were prepared and processed as described by Curtis (1996). The monoclonal antibody Misec 3F.4 and two PAbs (Mi PC 373 and Mj PC E2) which had been shown to be reactive to the surface of live pre-parasitic J2 of *M. javanica*, were used to probe the cryosections of the pre-parasitic J2. Sections of infected tomato roots containing the parasitic stages J2 and J3 were taken 3 and 6 days after infection and labelled with Mj PC E2 and with a pool of the 3 MAbs (Misec 3F.4, Misec 7E.1 and Misec 9F.5). The immunolabelling procedure was as described above for the live J2. The sections were then mounted with Citifluor (Agar Scientific) and examined under an epifluorescence microscope. Negative controls of non-immune serum or 20D medium and secondary conjugates were also examined.

Nematode extract

The method used was based on SDS extraction of somatic antigens, as described by Maizels *et al*. (1991). A pellet of 0·2 ml of *M. javanica* J2 was mixed with 120 μl of PBS and 80 μl of 5-fold-concentrated SDS–PAGE sample buffer (Bollag, Rozycki & Edelstein, 1996) containing 2-mercap-

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toethanol, and boiled for 10 min. The tubes were centrifuged for 10 min at 10000 ***g*** and the supernatant fraction was collected and stored at −20 °C until needed.

Western blots

SDS–PAGE gradient gels 4–15 % (Bio-Rad) were loaded with 15 μl of the nematode extract. After the gels had been run on a Mini-Protean II apparatus (Bio-Rad), they were electro-blotted onto a 0·2 μm nitrocellulose (NC) membrane (Schleicher & Schuell) in a semi-dry apparatus (Gelman Sciences). Pre-stained molecular weight standards were used (Sigma). Immunolabelling was performed after blocking the NC membrane with 5 % powdered milk (Bio-Rad) in PBS containing 0·05 % Tween 20 (PBST) for 1 h at room temperature (RT). The NC membranes were incubated with the primary PAbs (Mi PC 373 1 : 2000 and Mj PC E2 1 : 10000) for 1 h at RT under agitation. The membranes were then washed twice with PBST and once with TBS (50 mM Tris–HCl and 150 mM NaCl) and incubated for 90 min at RT with alkaline phosphatase-conjugated goat anti-rabbit IgG 1 : 10000 (Sigma). The membrane was washed twice with TBS and once with distilled water. The immunolabelling was visualized with tablets of the substrate BCIP/NBT (Sigma). Negative controls of the non-immune serum and secondary antibody alone were also tested.

Effect of antibodies on nematode movement

The assays that were developed to assess nematode movement following antibody binding to their surface were performed in Petri dishes (55 mm) containing 4 ml of autoclaved agar substitute medium that contained 0·75 % Phytagel (Sigma) and 0·1 % $MgSO_4.7H_2O$. *M. javanica* J2 were incubated for 20 min at RT with the PAbs (Mj PC K1 1 : 500; Mj PC E2 1 : 10000; Mi PC 373 1 : 500) and the MAbs (Misec 9F.5, Misec 7E.1 and Misec 3F.4, all diluted 1 : 10). Controls consisted of non-immune sera from mouse and rabbit and 20D medium. The nematodes were washed with PBS and 10 μl aliquots containing about 100 J2 were placed in the centre of the agar plates to monitor nematode movement for 24 h. Continuous presence of the antibodies in the plates was achieved by placing 100 μl of each PAb solution in PBS on the centre of the Petri dish and then spreading it evenly over the plate with a sterilized glass rod. The antibody solution was then allowed to penetrate the agar medium for about 1 h before the antibody-treated or non-treated pre-parasitic J2 were introduced. The final dilution of the antibody incorporated in the agar medium was calculated to be equal to the dilution used for the antibody binding to live J2. Fifty microlitres of each MAb were spread on the agar medium in 22-mm wells (Corning), and 5 μl aliquots containing about 50 treated and untreated pre-parasitic J2 were added to the wells. At least 4 replicates were used for each treatment. The movement of antibody-treated nematodes was compared with that of untreated nematodes incubated with non-immune serum, 20D medium or PBS, which were used as controls.

Effect of antibodies on nematode penetration of Arabidopsis thaliana

Arabidopsis thaliana seeds were surface sterilized for 2 min with 70 % ethanol and then treated for 4 min with 5 % hypochlorite (NaOCl). They were then washed with sterilized water and germinated on Phytagel-water medium. After germination the seeds were transferred to Gamborg's B-5 basal medium with minimal organics (Sigma) containing 1·5 % sucrose and 0·75 % Phytagel, in 55-mm plates (for the assay with the PAbs) or in 22-mm wells (for the assay with the MAbs). Seedlings were grown at 24 ± 2 °C under a 16 h light/8 h dark lighting regime for 2–3 weeks and then inoculated with approximately 60 J2 per 55-mm plate and 40 J2 per 22-mm well, by adding 5–10 μl aliquots containing the nematodes, as far as possible away from the roots. The medium and/or the J2 were pre-treated with the various antibodies as described above, or with the controls. Each treatment had 6 replicates. Nematode behaviour was monitored and nematode infection was demonstrated by staining the *Arabidopsis* roots 4 days after infection. The leaves were removed and the agar was covered with acid fuchsin solution (containing 0·01 % acid fuchsin, 25 % lactic acid and 25 % glycerol in distilled water) and incubated at 60 °C for 1–2 days until the J2 within the roots were stained. Nematode development within the roots was observed for a longer period (10 days) when only the J2 were pre-treated with the antibodies.

Statistical analysis

Experiments were conducted at least twice. Analysis of variance was performed according to Tukey's test ($P = 0{\cdot}05$).

RESULTS

Immunofluorescence of live pre-parasitic J2, eggs and pre-parasitic J2 cryosections

Only 3 MAbs (Misec 9F.5, Misec 7E.1 and Misec 3F.4), out of 6 tested, labelled live J2 of *M. javanica*, and the labelling appeared on the annulations and lateral fields of the cuticle surface. Differences were observed among the labelling patterns of the head region, the head cap and the amphids (Table 1). None of the tested MAbs labelled live eggs. Only 1 (Mi PC 373) out of 6 PAbs labelled the *M. javanica* J2 cuticle surface, with a weak labelling of the amphids. This PAb also labelled the *M. javanica* live

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Table 1. Immunofluorescence of *Meloidogyne javanica* juveniles (J2) and eggs treated with monoclonal and polyclonal antibodies

((+) Positive reaction; (−) negative reaction; (±) weak reaction. N.T., Not tested. SC, Surface coat along the J2 body, annulations and lateral cords; H, head region; HC, head cap; Am, amphids; SE, secreted–excreted products after stimulation with the neurotransmitter 5-methoxy-*N*,*N*-dimethyl tryptamine (DMT); E, eggs were extracted with hypochlorite; Hy, 'spots' on the hypodermis.)

	Live J2					Eggs	J2 Cryosections				
Antibody	SC	H	HC	Am	SE	E	SC	H	HC	Am	Hy
IACR-Misec 9F.5	+	−	−	−	−	−	N.T.	N.T.	N.T.	N.T.	N.T.
IACR-Misec 7E.1	+	−	−	−	−	−	N.T.	N.T.	N.T.	N.T.	N.T.
IACR-Misec 3F.4	+	+	−	−	−	−	+	+	−	−	+
IACR-Mi PC 373	+	+	−	±	−	+	+	+	−	+	−
Mj PC E2	+	+	+	−	−	−	+	+	−	−	+
Mj PC K1	+	−	−	+	N.T.	−	N.T.	N.T.	N.T.	N.T.	N.T.

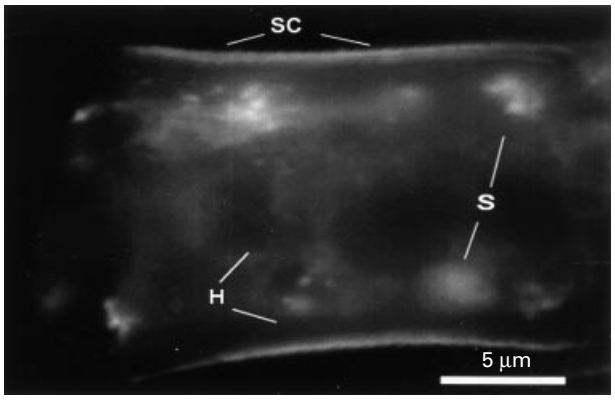


Fig. 1. Immunofluorescent labelling of a longitudinal cryosection of *Meloidogyne javanica* infective 2nd-stage juvenile showing reactivity of the MAb Misec 3F.4 with the surface coat and with regions on the hypodermis ('spots'). H, hypodermis; S, 'spots'; SC, surface coat.

eggs (Table 1). The PAbs Mj PC K1 and Mj PC E2 raised against *M. javanica* J2 in mouse and in rabbit, respectively, labelled live J2 differently (Table 1). The negative control treatments presented no fluorescence. Antibodies that revealed positive labelling on live pre-parasitic J2 were further tested on pre-parasitic J2 treated with DMT but none of the antibodies tested reacted with the SE products of *M. javanica* J2 (Table 1).

The immunolabelling of cryosections of *M. javanica* pre-parasitic J2 was similar to the labelling of live J2 except for the labelling of the amphidial pore with Mi PC 373, which was shown to be more intense on the cryosections (Table 1). MAb Misec 3F.4 and PAb Mj PC E2 labelled 'spots' at regular intervals along the J2 hypodermis (Fig. 1). All the control treatments were negative.

Immunolabelling of Western blots

Western blots of *M. javanica* extracts on SDS–PAGE were labelled with 2 PAbs, one of which was

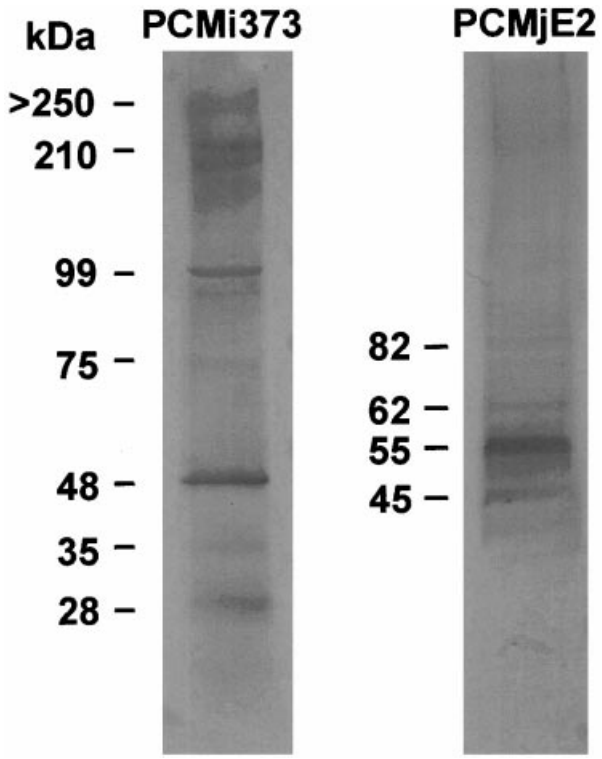


Fig. 2. Western blot of extracts of *Meloidogyne javanica* 2nd-stage juveniles labelled with PAb Mi PC 373 and PAb Mj PC E2.

raised against *M. incognita* and the other against *M. javanica*. The 2 labelling patterns differed, with main bands of 55 kDa and 48 kDa for Mj PC E2 and Mi PC 373, respectively (Fig. 2). Mi PC 373 labelled a broader range of proteins of the *M. javanica* J2 extract, although this antibody was originally raised against *M. incognita* J2 and SC extract.

Immunofluorescence of infected root cryosections

The PAb Mj PC E2 labelled the cuticle surface of parasitic J2 and J3 in cryosections of *M. javanica*-

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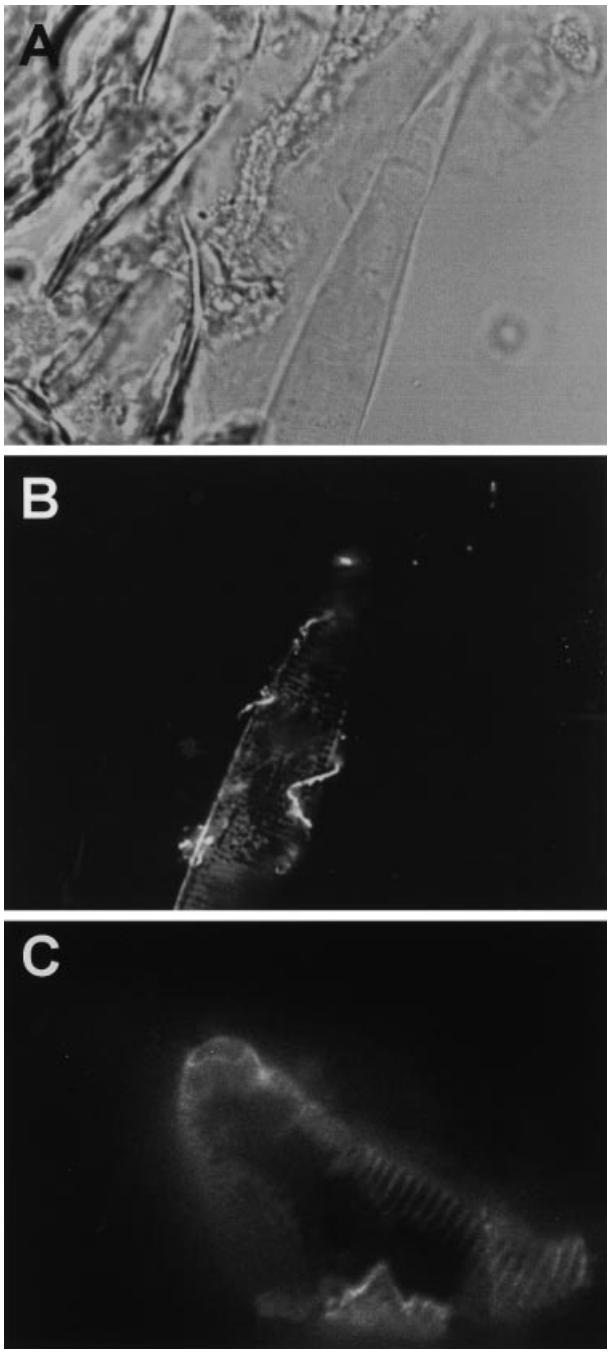


Fig. 3. Immunofluorescent labelling of cryosections of *Meloidogyne javanica*-infected tomato roots. (A) Bright-field image of an infective juvenile at root penetration; (B) immunofluorescence of infective juvenile labelled with a pool of MAbs (Misec 9F.5, 7E.1 and 3F.4) showing abundant shedding of surface coat during penetration of the root; (C) PAb Mj PC E2 labelling the anterior part of a 3rd-stage juvenile.

infected tomato roots (Fig. 3). Other developmental stages were not tested. The 'spots' on the hypodermis were also detected in the parasitic J2, with the same pattern as that observed on cryosections of pre-parasitic J2 (data not shown). Abundant shedding of surface antigens was observed when a pool of 3 MAbs was used to probe a section of infected

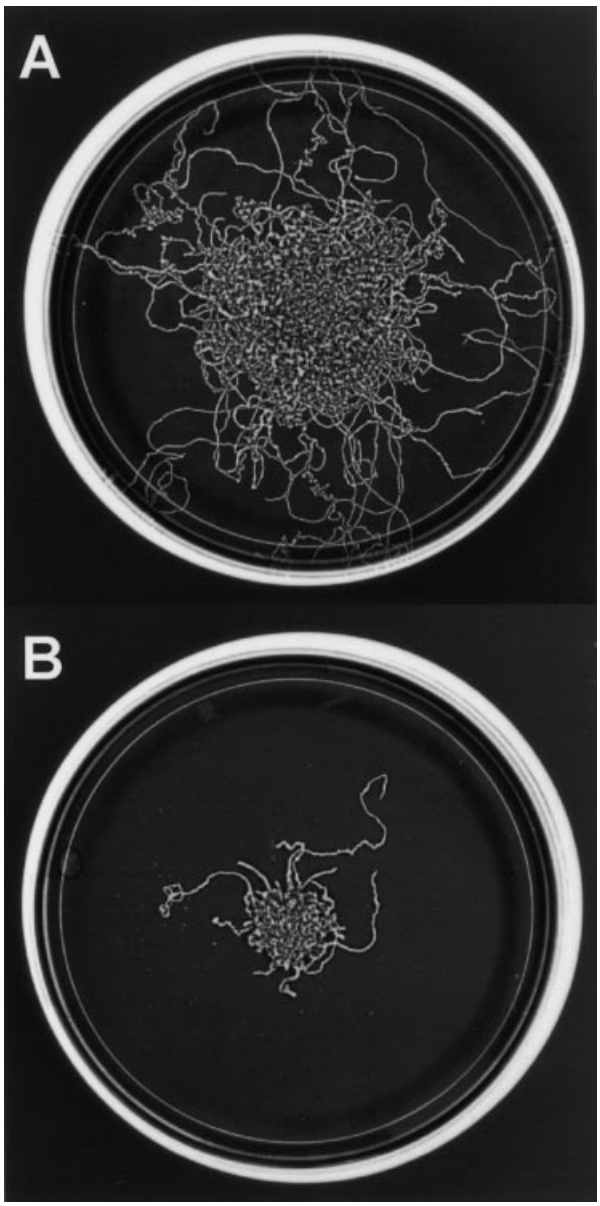


Fig. 4. *Meloidogyne javanica* juvenile tracks on agar plates 1 h after nematode application. (A) Controls of untreated or non-immune serum-treated juveniles; (B) antibody-treated juveniles (Mj PC E2).

tomato roots that contained a J2 during the penetration stage inside the root tissue (Fig. 3).

Effect of antibodies on nematode movement and host penetration

The MAbs, Misec 9F.5, Misec 7E.1 and Misec 3F.4, and the PAbs, Mi PC 373, Mj PC E2 and Mj PC K1, all of which labelled the cuticle surface of live pre-parasitic J2 of *M. javanica* (Table 1), were further tested for their effects on nematode behaviour. All of them affected the movement pattern of the pre-parasitic J2. Monitoring of nematode tracks showed that, while J2 in the control treatments moved normally in a wave pattern and spread quickly all over the plates (Fig. 4A), the antibody-treated J2 remained in the same area for several hours (Fig.

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Fig. 5. *Meloidogyne javanica* juvenile tracks on agar plates containing *Arabidopsis thaliana* seedlings, 1 h after nematode introduction onto the plate. (A) Control with untreated juveniles; (B) juveniles treated with antibody Mj PC E2.

4B). About 3–6 h later, the J2 started to recover from this effect and after 24 h they moved normally. In the treatments where antibodies were incorporated in the medium, the J2 remained stationary and completely stopped moving after 2–3 days, whereas in the control treatments they were still mobile after 3 days. Observations showed that nematode mobility was most affected by the MAb Misec 3F.4. The inhibitory effect of the antibodies on nematode movement was not affected by the presence of *Arabidopsis* seedlings in the plates (Fig. 5). When the antibody-treated J2 recovered their movement they were able to infect the roots with a delay of several hours. No significant difference in nematode penetration was recorded after 4 days with antibody-treated J2 (Tables 2 and 3). Nematode development

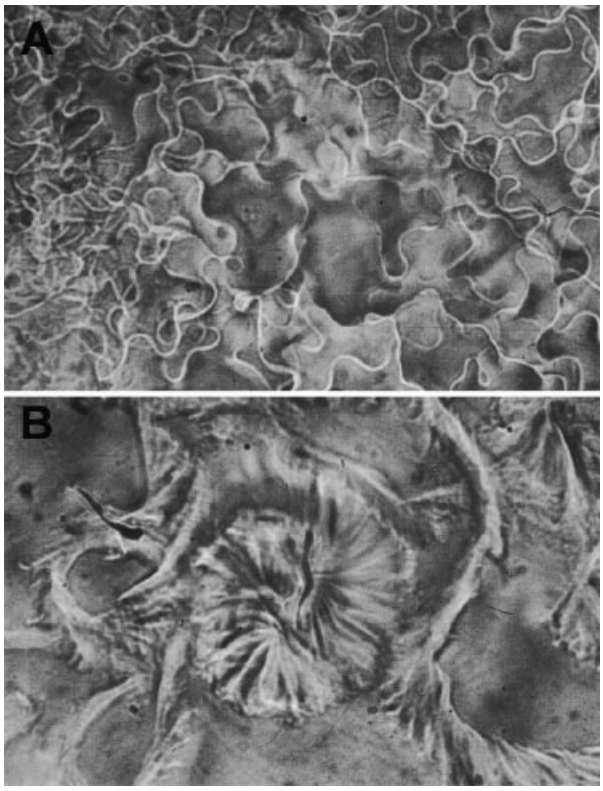


Fig. 6. Tracks of *Meloidogyne javanica* juveniles on agar plates. (A) Typical wave pattern movement of untreated or non-immune serum-treated controls; (B) movement pattern of juveniles treated with antibody Mj PC E2, showing rounded and forward movement.

within the roots was monitored for 10 days and it too was not affected by pre-treatment of J2 with the antibodies (data not shown). However, when the antibodies were incorporated into the agar plates the movement inhibitory effect lasted for several days and it significantly decreased the penetration of the antibody-treated J2 into the roots (Tables 2 and 3). Similar effects were obtained when untreated nematodes were placed in agar plates in which antibodies were incorporated (data not shown). Observations under a stereomicroscope showed that the antibody-treated J2 moved very slowly (Fig. 6B) with a different wave pattern of movement from that shown by the untreated J2 or by J2 treated with non-immune serum or 20D medium (Fig. 6A).

DISCUSSION

In this study we showed that monoclonal and polyclonal antibodies that are reactive with the surface coat of *M. javanica* can be used as a tool to alter nematode behaviour. Several antibodies which had been raised against *M. incognita* presented antigenic cross-reactivity with *M. javanica*. However, none of the antibodies which had been raised against other plant-parasitic nematodes showed such cross-reactivity. The MAbs, which reacted with the *M. javanica* SC, had recognized carbohydrate epi-

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Table 2. Effects of polyclonal antibodies on infection of *Arabidopsis thaliana* roots by *Meloidogyne javanica* 2nd-stage juveniles (J2)

(Antibody or control solutions were spread on the plates and/or J2 were treated prior to the inoculation of *A. thaliana* seedlings in Petri dishes. Values in columns followed by different letters are significantly different at $P = 0{\cdot}05$ according to Tukey's test.)

	No. J2/root after 4 days	
Treatment	Plate and J2 treatment	J2 treatment
Mj PC K1 (mouse) 1:500	—	19·0[ab]
Non-immune serum (mouse) 1:500	20·5[a]	21·3[a]
Mj PC E2 (rabbit) 1:10000	3·2[b]	18·5[ab]
Non-immune serum (rabbit) 1:10000	19·8[a]	22·7[a]
IACR-Mi PC 373 1:500	4·5[b]	17·5[ab]
Non-immune serum (rabbit) 1:500	20·0[a]	22·3[a]
Control (PBS)	21·5[a]	23·0[a]

Table 3. Effect of monoclonal antibody treatments on infection of *Arabidopsis thaliana* roots by *Meloidogyne javanica* juveniles (J2)

(Antibody or control solutions were spread on the plates (final dilution 1:10) and/or J2 were treated with antibodies (1:10) prior to the inoculation of *A. thaliana* seedlings in Petri dishes. Values in columns followed by different letters are significantly different at $P = 0{\cdot}05$ according to Tukey's test.)

	No. J2/root after 4 days	
Treatment	Plates and J2 treatment	J2 treatment
IACR-Misec 9F.5	2·5[b]	12·5[ab]
IACR-Misec 7E.1	3·0[b]	13·0[ab]
IACR-Misec 3F.4	2·3[b]	12·5[ab]
Control medium 20D	15·8[a]	15·5[a]
Control PBS	17·0[a]	16·0[a]

topes of *M. incognita* (Lopez de Mendoza *et al.* 1999), this is probably also the case for *M. javanica* J2. Interestingly, the antibodies tested did not react with SE products of *M. javanica* obtained after stimulation with the neurotransmitter DMT, although all 3 MAbs raised against *M. incognita* SE products had shown a positive reaction with SE products of *M. incognita* and of animal parasitic nematodes (Lopez de Mendoza *et al.* 1999).

The antibodies Misec 3F.4 and Mj PC E2, reactive with the SC, also showed reactivity with the hypodermis, which perhaps indicated the origin of these surface antigens. Antigens present in the hypodermis of *M. incognita* race 2 females were also labelled with a monoclonal antibody that labelled surface-associated antigens (Lopez de Mendoza, 1999). Nevertheless, the origins of surface-associated antigens on nematodes may differ for various antigens and is still not clear in most cases (Blaxter & Robertson, 1998). These non-structural proteins could originate from gland cells such as excretory cells, pharyngeal glands, amphids and phasmids as well as from the hypodermis (Blaxter & Robertson, 1998). The rectal glands have also been suggested as the origin of a surface antigen of *M. incognita* race 3 (Hu, McLure & Schmitt, 2000).

PAbs Mi PC 373 and Mj PC E2, originally raised against different *Meloidogyne* species, labelled the body surfaces of live *M. javanica* J2 equally, but presented very different labelling patterns on the J2 head region, eggs and hypodermis. Probing of Western blots of *M. javanica* J2 extract by these antibodies also revealed very different labelling patterns, with no bands in common. Interestingly, the antibody raised against *M. incognita* reacted with more bands than the one raised against *M. javanica*, indicating a high level of cross-reactivity between the two species, which share many epitopes. The PAb Mj PC K1 used in this study had labelled a 250 kDa antigen from *M. javanica* SC extracted with the detergent CTAB (cetyltrimethyl ammonium bromide). This antibody interfered with the attachment process of the bacterial spores of *Pasteuria penetrans* to *M. javanica* J2 (Spiegel *et al.* 1996).

Immunolabelling of early parasitic life-stages (parasitic J2 and J3) *in planta* demonstrated that

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surface antigens detected on the pre-parasitic J2 were still present in these stages, which showed similar labelling patterns. However, abundant shedding of surface antigens was observed during penetration of the root by the J2, and the same phenomenon has been observed for *M. incognita* (Lopez de Mendoza, 1999). Although the biological role of the surface coat in plant-parasitic nematodes is still not fully understood, its dynamic properties are probably very important at all life-stages, and especially so at the critical stage of penetration of the host plant.

Regardless of the binding pattern, antibodies that were able to bind to the nematode surface all had inhibitory effects on juvenile movement, as demonstrated by monitoring J2 tracks on agar plates. Similar effects on J2 movement were obtained, using this agar-tracks method, when pre-parasitic juveniles of *Globodera pallida* (potato cyst nematodes) were pre-incubated with antibodies reactive with their surface cuticle, indicating that antibodies can affect nematode movement also in other plant-parasitic nematodes (Fioretti, 2000). When continuous antibody binding was facilitated by incorporating the antibodies into the agar, infection of the host by J2 was decreased. For the bioassays, nematodes treated with MAbs were placed nearer to the roots than those treated with PAbs, but these antibody treatments inhibited nematode infection to similar extents, regardless of the distance that the nematodes were placed from the roots. Although there was weak binding of some of the antibodies to the nematode amphidial pores, the effects of antibodies on nematode behaviour were probably not due to blocking of chemoreception binding sites, since the same effect on nematode movement was observed with antibodies that bound exclusively to the nematode cuticle surface. Nowell, di Pomerai & Pritchard (1999) showed that antibody binding to the surface coat of *Caenorhabditis elegans* caused immunological stress responses in the nematode, which were monitored by increase of β-galactosidase activity and by heat shock protein hsp-16 expression; binding of cationized ferritin also resulted in increased induction of β-galactosidase activity. The presence of the antibodies also reduced worm growth and fecundity (Nowell *et al*. 1999). The concept of immunological stress may account for the effects of the surface reactive antibodies on *M. javanica* J2 behaviour. Moreover, the fact that immunological stress can affect nematode development suggests that, in accordance with the plantibody approach (Stiekema *et al*. 1997) transgenic plants expressing surface reactive antigens might be able to suppress the parasitism of plant-parasitic nematodes.

Examination of nematode tracks is a useful tool for monitoring the effect of antibodies on nematode movement patterns. The bioassay experiments allowed us to demonstrate that there was active turnover of the surface layer that involves shedding and renewal of the surface antigens. Antibody-treated nematodes recovered after several hours. However, when antibodies were allowed continuously to bind to the nematode cuticle surface, the inhibitory effect lasted for days. Although antibody-mediated shedding has been reported previously, for other nematode-antibody interactions (Page *et al*. 1992), it seems that the antibodies in our assay did not enhance the shedding process. MAb Misec 8D.3 has been shown to bind to surface antigens of *M. incognita*, which were shed after a brief incubation of the J2 with this antibody (Lopez de Mendoza *et al*. 1999); no reactivity of this antibody was detected with the surface of live pre-parasitic J2 of *M. javanica*.

The present study showed that the specific binding of antibodies to the nematode cuticle surface can affect nematode movement, and so lead to an inhibitory effect on penetration of *A. thaliana* roots by nematodes.

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