Attachment of *Pasteuria penetrans* spores to the cuticles of root-knot nematodes

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SUMMARY

Several experiments were carried out to elucidate the nature of the process of attachment of *Pasteuria penetrans* spores to second-stage juveniles of *Meloidogyne javanica*. Sonication of spores for 5-30 mn disrupted the sporangial wall and significantly increased the rate at which spores attached to nematodes. Spores attached equally well to living and dead nematodes. Some spores attached to nematodes after either spores or nematodes were heated at 100° for 30 mn, but spores heated at 100° for 15, 30 or 60 mn attached in significantly fewer numbers than unheated spores. Spores also adhered to free living and parasitic second-stage juveniles but not to males and females, suggesting that recognition sites for spore adhesion were altered during moulting. The sites of spore adhesion were randomly distributed on the surface of the cuticle. Preliminary studies failed to implicate lectins in the adhesion process because a range of sugars, lectins and other compounds did not affect attachment. Sialic acid reduced the number of spores attached to nematodes in two experiments but other evidence suggested it was unlikely to be involved in the mechanism of attachment. Observation at high resolution under the TEM of sections cut through the spore on the surface of the cuticle showed that the glycocalyx broke down and the parasporal fibres from the lower half of the spore became inserted and entwined within the epicuticle.

RÉSUMÉ

La fixation des spores de Pasteuria penetrans *sur la cuticule du nématode des racines noueuses*

Plusieurs expériences ont été effectuées pour élucider le procédé de fixation des spores de *Pasteuria penetrans* sur les juvéniles de second stade de *Meloidogyne javanica*. Un traitement des spores aux ultrasons pendant 5 à 30 mn brise la paroi du sporange et augmente de façon significative le taux de fixation des spores sur les nématodes. Les spores se fixent sur les nématodes aussi bien vivants que morts. Certaines spores se fixent sur les nématodes après que celles-ci, ou les nématodes, aient été chauffés à 100° pendant 30 mn, mais les spores chauffées à 100° pendant 10, 30 ou 60 mn se fixent en moins grand nombre que les spores non chauffées. Les spores se fixent indifféremment sur les juvéniles de second stade, libres ou infestants, mais non sur les adultes mâles ou femelles, suggérant ainsi que l'identification des sites de fixation a été altérée durant la mue. Ces sites de fixation sont répartis au hasard à la surface de la cuticule. Des expériences préliminaires n'ont pas permis de démontrer le rôle des lectines dans la fixation des spores car l'action d'une série de sucres, de lectines et d'autres composés chimiques n'a pas affecté cette fixation. Dans deux expériences, l'acide sialique a réduit le nombre des spores fixées aux nématodes, mais d'autres observations laissent penser qu'il est peu probable que cet acide intervienne dans le mécanisme de la fixation. L'observation au TEM à haute résolution de coupes fines effectuées dans des spores en place à la surface de la cuticule a montré que le glycocalyx est brisé et que les fibres parasporales de la partie inférieure de la spore s'insèrent et s'entrelacent dans l'épicuticule.

Pasteuria penetrans Sayre & Starr, 1985, known previously as *Duboscqia penetrans* Thorne, 1940 and *Bacillus penetrans* (Thorne, 1940) Mankau, 1975, is an obligate endoparasite of nematodes with potential as a biological control agent against root-knot nematodes (Mankau, 1975; Sayre, 1980; Stirling, 1984; Brown, Kepner & Smart, 1985). Adhesion of spores to the cuticle plays a vital role in the activity of the pathogen when it is used as a biological nematicide (Stirling, 1984), but other than observations that spore attachment is host specific (Mankau & Prasad, 1977; Dutky & Sayre, 1978; Spaull, 1984; Sayre & Starr, 1985; Stirling, 1986), little is known about the process of attachment. In this paper we report on several factors that influence spore adhesion and report on observations of the nature of the attachment process.

Materials and methods

The nematode used was a single egg mass population of *Meloidogyne javanica* originally obtained from

Merbein, Victoria and maintained on tobacco *(Nicotiana tabacum)* or tomato *(Lycopersicon esculentum)*. Second-stage juveniles were obtained by dissecting egg masses from roots and placing them in shallow dishes of distilled water at 25°, or by placing roots containing egg masses in water and aerating them with an aquarium pump. Spores of *P. penetrans*, obtained originally from Dr. R. Mankau (University of California, Riverside) and increased on *M. javanica*, were obtained by squashing infected adult females into either distilled water or buffer. The spores were then counted in a haemocytometer.

Preparation for microscopy

Nematodes with spores attached were photographed alive in buffer or distilled water under a coverslip sealed at its edges with nail varnish. The volume of water under the coverslip was enough to allow the nematodes to move. When this movement ceased, due to anoxia, the nematodes were photographed using an Olympus research microscope fitted with a differential interference contrast attachment for transmitted light. Ilford XPI 400 film, with high film speed and fine grain, was found to be the most suitable for this purpose.

Nematodes were prepared for transmission electron microscopy as follows : a clump of juveniles encumbered with *P. penetrans* spores were cooled to 5° and then fixed overnight at this temperature with 4 % paraformaldehyde buffered (0.1 M phosphate) to pH 7.3 and containing a drop of 0.01 % ruthenium red per ml of fixative. The clump of nematodes was then washed several times in 0.01 % aqueous ruthenium red before being fixed in buffered 1 % osmium tetroxide + ruthenium red (one drop of 0.01 % stain per ml of fixative) for 1 hour at 23°. The nematodes were then washed several times in dilute ruthenium red and stored overnight in this dilute aqueous solution. Prior to osmium tetroxide fixation some of the nematodes, in a tangled mass, were transferred to molten 1 % agar cooled to 60° in a Petri dish. When the agar had set these nematodes were cut out as a block and subsequently treated in this block for the remainder of the procedures. Other nematodes were treated individually. All specimens were dehydrated in an ascending ethanol series into absolute ethanol and then into a 1:1 mixture of ethanol : propylene oxide followed by pure propylene oxide. The material was then placed in a propylene oxide : Spurr's resin (1 : 1) mixture and left *in vacuo* at 23° for 1-2 days before being placed in pure resin in moulds and polymerized at 60° overnight. Thin sections were cut with glass knives using an LKB ultramicrotome and further stained in a filtered saturated aqueous solution of uranyl acetate for 20 mn at 23°, followed by lead citrate for 10 mn at the same temperature. Both of these solutions were filtered through 0.45 µm membrane filters before use. The sections were examined in a Philips EM 400 TEM at 80 kV.

Spores were prepared for scanning electron microscopy as follows : Spores were fixed in 4 % buffered paraformaldehyde, washed in buffer by centrifugation (1 700 *g* for 15 mn) three times. They were then post fixed in 1 % osmium tetroxide for 1 h at 20°, washed three times in distilled water by centrifugation and then left in freshly made, filtered saturated aqueous thiocarbohydrazide for 30 mn before being washed three times in distilled water by centrifugation. This process was then repeated. It utilizes the osmium-bridging properties of thiocarbohydrazide to bind additional osmium to tissue surfaces (Kelley, Dekker & Bluemink, 1973) and is used as an alternative to evaporative coating with heavy metals. Spores both treated and not treated by this technique were placed on a coverslip, attached to an aluminium stub, air dried, carbon and gold-palladium coated and examined in a Cambridge S250 Mk 3 scanning electron microscope (SEM) operating at 20 kV.

Effect of physical factors

During experiments in which spores were sonicated in an attempt to induce changes that might lead to germination, it was noticed that sonication increased the number of spores adhering to nematodes. To confirm this observation, aliquots of a suspension of *P. penetrans* spores were treated for either 0, 5 or 30 mn in a Biosonik IV (R) ultrasound generator (Bronwill Scientific, Rochester, New York) supplying 20 kHz modulated electrical output with a power setting of 100 W. Spores were observed for morphological changes following treatment and then freshly hatched second-stage juveniles of *M. javanica* were added and the suspensions agitated in an Ehrlenmeyer flask on an orbital shaker at 150 r.p.m. There were three replicates of each treatment and the spore concentration in each flask was 1.8×10^5 spores/ml. Samples were removed after 0.5, 1; 2, 4, 6 and 24 hours and the number of spores attached to each of a sample of ten nematodes was counted at a magnification of 400 ×.

To determine the effect of heat at 100° on the capacity of spores to attach to nematodes, 0.5 ml aliquots from a suspension containing 1.9×10^5 spores/ml were placed in thin-walled freeze-drying ampoules and four replicate samples heated in a boiling water bath for 0, 5, 10, 15, 30 or 60 mn. Spores from each ampoule and freshly hatched *M. javanica* juveniles were agitated on an orbital shaker at 150 r.p.m., the spore concentration in each flask being 0.95×10^4 spores/ml. After 19 hours, the number of spores attached to each of a sample of 20 nematodes was counted.

To determine whether *P. penetrans* spores attached to all stages of *M. javanica*, free living and parasitic second-stage juveniles, males and females were added to

0.1 M phosphate buffer containing 3×10^5 spores/ml. Sedentary stages of the nematode were obtained by dissecting them from roots and the phosphate buffer was used to prevent those stages from bursting. The females used included immature adults dissected from roots soon after the fourth moult. After bubbling air through the suspension for 20 hours, nematodes were examined for adhering spores. A further experiment was carried out to compare the attachment of spores to living nematodes with attachment to nematodes killed either in water for 5 mn at 60°, by immersion in 100 µg/ml fenamiphos or by contact with 1, 3 dichloropropene in a closed chamber. The nematodes were added to four replicate suspensions containing 2.9×10^4 spores/ml and the number of spores attached to a sample of 20 nematodes was counted after agitation for 24 hours on an orbital shaker at 150 r.p.m.

Effect of chemicals

To test the effect of various sugars, lectins and other compounds on spore attachment, several experiments were carried out using spores sonicated for 5 mn as described above. The spore concentration differed in each experiment, ranging from 0.6×10^6-1.7×10^6 spores/ml and treatments were replicated three or four times.

Experiments 1 and 2

Replicate 50 µl samples in water of sonicated spores, freshly hatched second-stage juveniles of *M. javanica* and the test compound prepared at three times the desired concentration were mixed together in 0.35 ml wells of a micro-titration plate. After incubation for 30 mn, the number of spores attached to a sample of 10-20 nematodes was counted. Spores incubated in water served as controls.

Experiments 3 A, 3 B and 3 C

In experiment 3 A the above procedure was used to examine the effect of five preparations of N-acetyl neuraminic acid (sialic acid) and N-acetyl galactosamine (Sigma Chemical Company, St-Louis, Mo, USA). Since 50 mM sialic acid caused inactivity and then death of nematodes in this experiment, the experiment was repeated (experiment 3 B) but the three sialic acid preparations, spores and nematodes were prepared in phosphate buffer (pH = 7.0) and the acidity of the sialic acid was neutralized with NaOH. This procedure also was used in experiment 3 C, except that nematodes were killed by heating at 70° for 5 mn and spores were brought into contact with the dead nematodes by agitating the plates on an orbital shaker at 100 r.p.m. Spores incubated in water (experiment 3 A) or phosphate buffer (experiments 3 B and 3 C) served as controls.

Experiment 4

Sonicated spores were incubated in 50 mM sialic acid for 20 hours and then the sialic acid concentration was reduced to 5 mM by adding second-stage juveniles of *M. javanica* in water. After 30 mn, the number of spores attached to samples of 20 nematodes was counted. The untreated control consisted of spores incubated in water.

Experiment 5

This experiment aimed to determine whether the capacity of spores to attach to nematodes was affected by treatment with glutaraldehyde. Sonicated spores incubated in 1 % glutaraldehyde for 15 mn were pelleted by centrifugation at 10 000 *g* for 15 mn and the glutaraldehyde removed by twice decanting the supernatant, re-suspending the spores in water and re-centrifuging. The concentration of spores in the final suspension, and in a suspension of spores in water which had been subjected to the same centrifugation procedure, was determined with a haemocytometer and the concentration of each adjusted to 0.5×10^6 spores/ml. Three replicate 0.5 ml samples of either glutaraldehyde-treated or untreated spores were added to 3.5 ml wells of a tissue culture plate containing second-stage juveniles of *M. javanica* in 2 ml water, and the number of spores attached to samples of 10 nematodes was counted after 30 mn.

Experiment 6

Limulin III (Sigma Chemical Company, St-Louis, Mo, USA) and second-stage juveniles of *M. javanica* were incubated for 90 mn in 50 mM HEPES buffer. Sonicated spores were added and the number of spores attached to a sample of ten nematodes was counted after 6 hours. During the incubation phase, the concentration of Limulin III was 45 µg/ml but this was reduced to 35 µg/ml after spores were added. Nematodes incubated in 50 mM HEPES buffer without Limulin III served as controls.

Results

Effect of physical factors

Sonication caused the disruption of the sporangial wall of *P. penetrans* spores (Fig. 1); only 12 % of unsonicated spores lacked sporangial walls but this percentage increased to 50 and 96 % respectively after 5 and 30 mn sonication. Sonicated spores attached readily to nematodes and within a few hours the number of spores attached was so great that they could not be counted accurately. The number of unsonicated spores attached had not reached this level after 24 hours (Fig. 2). Regressions of *log (x + 1)* number of spores per nematode against time were significant for all three treatments. The slopes of the regression lines for 5 and

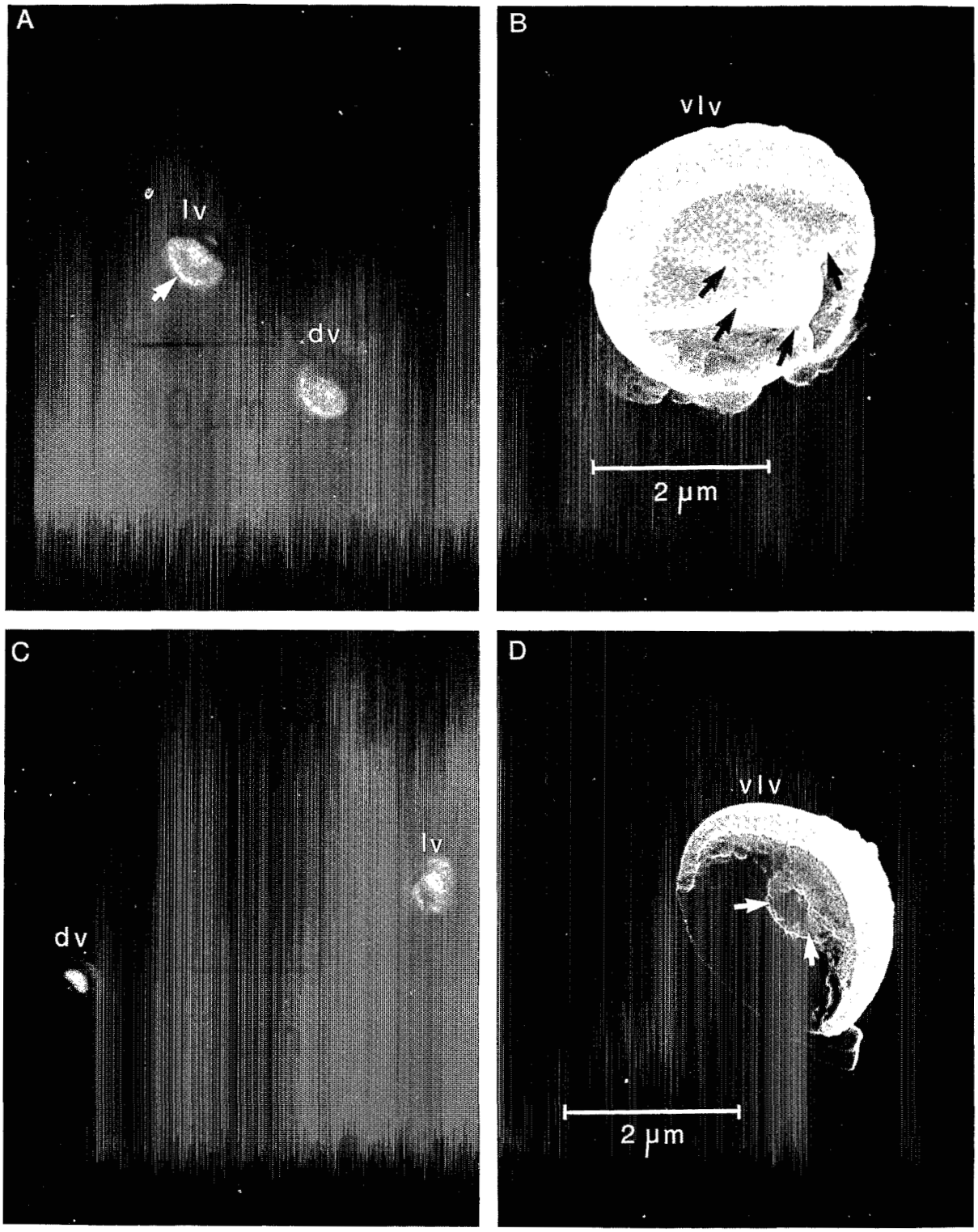

Fig. 1. Comparison of unsonicated and sonicated spores of *Pasteuria penetrans*. A & B - Unsonicated spores. A : fixed in 4 % paraformaldehyde and viewed under differential interference contrast optics showing dorsal (dv) and lateral (lv) views. The intact sporangial wall is clearly visible in lateral view (arrow); B : treated with the osmium-bridging technique and examined under the SEM, showing a ventro-lateral view (vLv) of the spore with its collapsed sporangial wall (arrows). C & D - Sonicated spores. C : treated as for A, the absence of the sporangial wall is shown in the lateral view of the spore (lv); D : treated as for B, the sporangial wall has been removed and the basal ring (arrows) is exposed on the ventral surface.

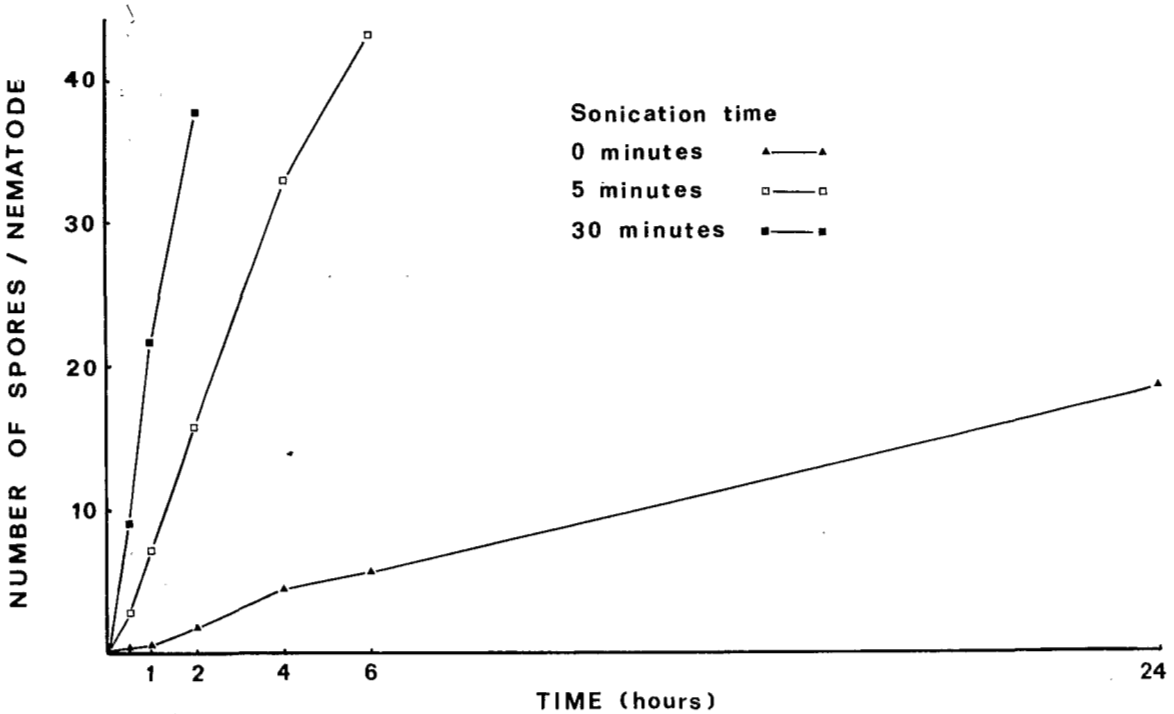

Fig. 2. Rate of attachment of *Pasteuria penetrans* spores to *Meloidogyne javanica* juveniles after spores were sonicated for 0, 5 or 30 mn.

30 mn sonication were not significantly different, but both these slopes differed significantly from that of the unsonicated treatment.

The sporangial wall of unsonicated spores was seen best when the spore was viewed laterally (Fig. 1 A, 1 B). This structure is shown under oil immersion using differential interference contrast optics (Fig. 1 A) and under the SEM (Fig. 1 B) where it has collapsed inwards. The absence of the sporangial wall is shown clearly in lateral views of the spore under differential interference contrast optics (Fig. 1 C) and is most obvious when viewed under the SEM (Fig. 1 D) where its absence exposes the basal ring of wall materials (arrows) that rests on the surface of the nematode cuticle during attachment.

Fewer spores adhered to nematodes as heating time at 100° increased, the number of spores attached per

Table 1

Attachment of *Pasteuria penetrans* spores to second-stage juveniles of *Meloidogyne javanica* after spores were heated at 100° for various times.

Heating time (mn)	*Spores attached/nematode**
0	18.2 (3.0)
5	15.3 (2.8)
10	14.3 (2.7)
15	10.3 (2.4)
30	5.5 (1.9)
60	0.1 (0.1)
LSD (P = 0.05)	(0.4)

* Equivalent means with transformed means *(log, x + 1)* in parentheses.

Table 2

Attachment of *Pasteuria penetrans* spores to second-stage juveniles and adults of *Meloidogyne javanica.*

	Stage in life cycle of the nematode			
	Second-stage juveniles		*males*	*females*
	Free living	*parasitic*		
Numbers of nematodes	40	10	22	21
Percentage of nematodes with spores adhering	78	90	0	0

nematode being significantly reduced after heating for 15, 30 and 60 mn (Tab. 1).

The response of various stages of *M. javanica* to *P. penetrans* spores showed that spores adhered readily to both free living and parasitic second-stage juveniles. Spores did not adhere to males or females (Tab. 2). Comparisons of attachment to living and dead nematodes showed that 29.2, 19.5, 19.1 and 17.1 spores attached per nematode to healthy, heat killed, 1, 3 dichloropropene killed and fenamiphos killed nematodes respectively. These numbers did not differ significantly between treatments (P = 0.05).

EFFECT OF CHEMICALS

During experiments on the effect of various compounds on spore attachment, sialic acid was the only compound tested which inhibited attachment. In experiments 3 A and 3 B, 50 mM sialic acid generally reduced the number of spores attached to nematodes (Tab. 3), but also affected nematode behaviour. In experiment 3 A, some nematodes were inactive and the rest moved sluggishly after 30 mn and all were dead 1 1/2 hours later. Sialic acid neutralized with NaOH (experiment 3 B) was less toxic; nematodes moved erratically and appeared over-active after 30 mn and

Table 3

Attachment of *Pasteuria penetrans* spores to second-stage juveniles of *Meloidogyne javanica* in the presence of various sugars, lectins and other compounds.

	Compound		*Spores attached per nematode***		
Experiment 1	Fucose	50 mM	5.8		
	Glucose	50 mM	5.6		
	Mannose	50 mM	7.1		
	N-acetyl glucosamine	50 mM	7.6		
	Galactosamine	50 mM	6.5		
	Galactose	50 mM	5.3		
	Water		7.9		
Experiment 2	Concanavalin A	0.4 mg/ml	7.0		
	Bovine serum albumin	0.4 mg/ml	5.8		
	Sodium metaperiodate	0.5 M	6.0		
	Water		6.9		
Experiments 3 A, B and C			(3 A)	(3 B)	(3 C)
	Sialic acid Type II	50 mM	1.5	—	—
	Sialic acid Type IV	50 mM	0.9	2.1	1.6
	Sialic acid Type VI	50 mM	2.0	4.4	1.5
	Sialic acid Type VII	50 mM	1.6	—	—
	Sialic acid Type VIII	50 mM	1.6	1.8	2.0
	N-acetyl galactosamine	50 mM	5.0	—	—
	Water/Phosphate buffer		5.7	5.5	1.9
		LSD (P = 0.05)	1.3	2.2	
Experiment 4	Sialic acid Type IV	5-50 mM*	14.8		
	Sialic acid Type VI	5-50 mM*	12.7		
	Sialic acid Type VII	5-50 mM*	12.4		
	Sialic acid Type VIII	5-50 mM*	15.7		
	Water		16.8		
Experiment 5	Glutaraldehyde		9.6		
	Water		8.4		
Experiment 6	Limulin III in 50 mM HEPES buffer	35-45 µg/ml*	6.6		
	50 mM HEPES buffer		5.4		

* Details in text.

** Differences between treatments were not significant except in experiments 3 A and 3 B.

were still alive after 2 hours. When heat-killed nematodes were agitated with spores in experiment 3 C, sialic acid did not reduce the number of spores attached (Tab. 3).

THE NATURE OF ADHESION

When exposed to spores of *P. penetrans* in a non-agitated situation, larvae of *M. javanica* stuck together in clumps (Fig. 3 A) because spores adhering to cuticles also adhered readily to each other. Spore adhesion appeared to be quite random (Fig. 3 B). Although spores attached to sites such as lateral alae and to areas such as amphids (Fig. 3 C), where chemicals may be exuded, such sites did not appear to be favoured. At the sites of adhesion between the spore and the surface of the nematode the cuticle was slightly indented (Fig. 3 D). Where this close contact had not been made the glycocalyx remained intact and clearly visible (Fig. 3 E) but at the site of contact the parasporal fibres from the lower half of the spores become inserted and entwined with the epicuticle (Fig. 3 F) and the continuity of the glycocalyx was broken. The " hair-like " or " microfibrillar " external coat of the sporangium (Fig. 3 D, F), whose carbohydrate content had been stained with ruthenium red, did not appear to be involved in this adhesion.

Discussion

The adhesion of *P. penetrans* spores to second-stage juveniles of *M. javanica* and corynebacteria to infective stages of *Anguina agrostis* are the only examples where bacterial adhesion to plant-parasitic nematodes has been studied in detail. In both cases, the capability of the bacteria to adhere to their hosts varied with different nematode populations (Bird & Riddle, 1984; Bird, 1985; Stirling, 1986). Also, there were differences in attachment for corynebacteria to infective second-stage dauer larvae of *A. agrostis* and to their adults (Bird & Riddle, 1984; Bird, 1985) and our results indicated similar changes in the capacity of *P. penetrans* spores to attach to root-knot nematodes as they developed. Since *P. penetrans* spores attached to free living and parasitic second-stage juveniles but not to adults, chemical changes in the epicuticle involving recognition sites for spore adhesion must have taken place at moulting. Changes in the chemistry of the cuticle probably occur at this time because morphological changes have been observed (Bird & Rogers, 1965) and changes in cuticle morphology were associated with changes in its chemical composition in nematodes such as *Caenorhabditis elegans* (Cox, Staprans & Edgar, 1981; Cox & Hirsh, 1985).

TEM photographs of sections through adhering cells showed that both *P. penetrans* (Sayre & Wergin, 1977) and *Corynebacterium rathayi* (Bird, 1985) intruded into the cuticle of nematodes to which they were attached. Capsular material of corynebacteria intruded markedly into the cuticle but *P. penetrans* is less destructive and the continuity of the glycocalyx is barely disrupted as the parasporal fibres become inserted and entwined with the epicuticle (Fig. 3 F) and anchor the spore to the surface of the nematode.

Behavioural responses of nematodes to adhering parasites is similar in a number of instances where this has been studied. Thus clumping of nematodes occurred when corynebacteria adhered to *A. agrostis* (Bird & Riddle, 1984) or when conidia of *Dilophospora alopecuri* attached themselves to *Anguina tritici* (Atanasoff, 1925). Clumping of *M. javanica* juveniles encumbered with *P. penetrans* spores probably is caused by spores sticking to each other by the interlocking of their hair-like external coats. Such clumping may not occur in nature where spore-encumbered nematodes are unlikely to be in such close contact with each other.

The marked increase in the number of spores attaching to second-stage juveniles of *M. javanica* following sonication was apparently due to its disruptive effect on the sporangial wall. Sonication removed the sporangial wall and perhaps the exosporium, exposing the parasporal fibres and allowing them to make contact with the nematode cuticle. Under natural conditions in soil, this disruption probably occurs through microbial degradation.

Specific recognition between *P. penetrans* and *M. javanica* is still not understood. The ready adhesion of spores to dead nematodes suggested that the adhesion process was not dependent on a living system in which the cuticle was selectively permeable to chemicals. Also, the adhesion process was not markedly affected by heat because spore attachment still occurred after either spores or nematodes were heated at 100° for 30 mn. Many host-specific interactions between micro-organisms and their hosts are mediated by lectins, *coniospora* to nematodes (Zuckerman & Jansson, 1984; Jansson, Jeyaprakash & Zuckerman, 1985); Zuckerman, 1985; Zuckerman & Jansson, 1984). However, preliminary studies have failed to implicate lectins in the adhesion of *P. penetrans* spores to *M. javanica* juveniles. O'Brien (1980 and pers. comm.) tested twenty-one sugars and three lectins at concentrations of 100-150 µM and found that none of them inhibited attachment. Our tests with six sugars at higher concentrations gave similar results. Spores treated with glutaraldehyde, which cross-links proteins, were able to attach to nematodes, suggesting that proteins on the surface of *P. penetrans* spores were not involved in the attachment process. Also, 0.05 M sodium metaperiodate, which is capable of oxidizing terminal and internal monosaccharide residues other than those in non-terminal positions joined in 1-3 glycosidic

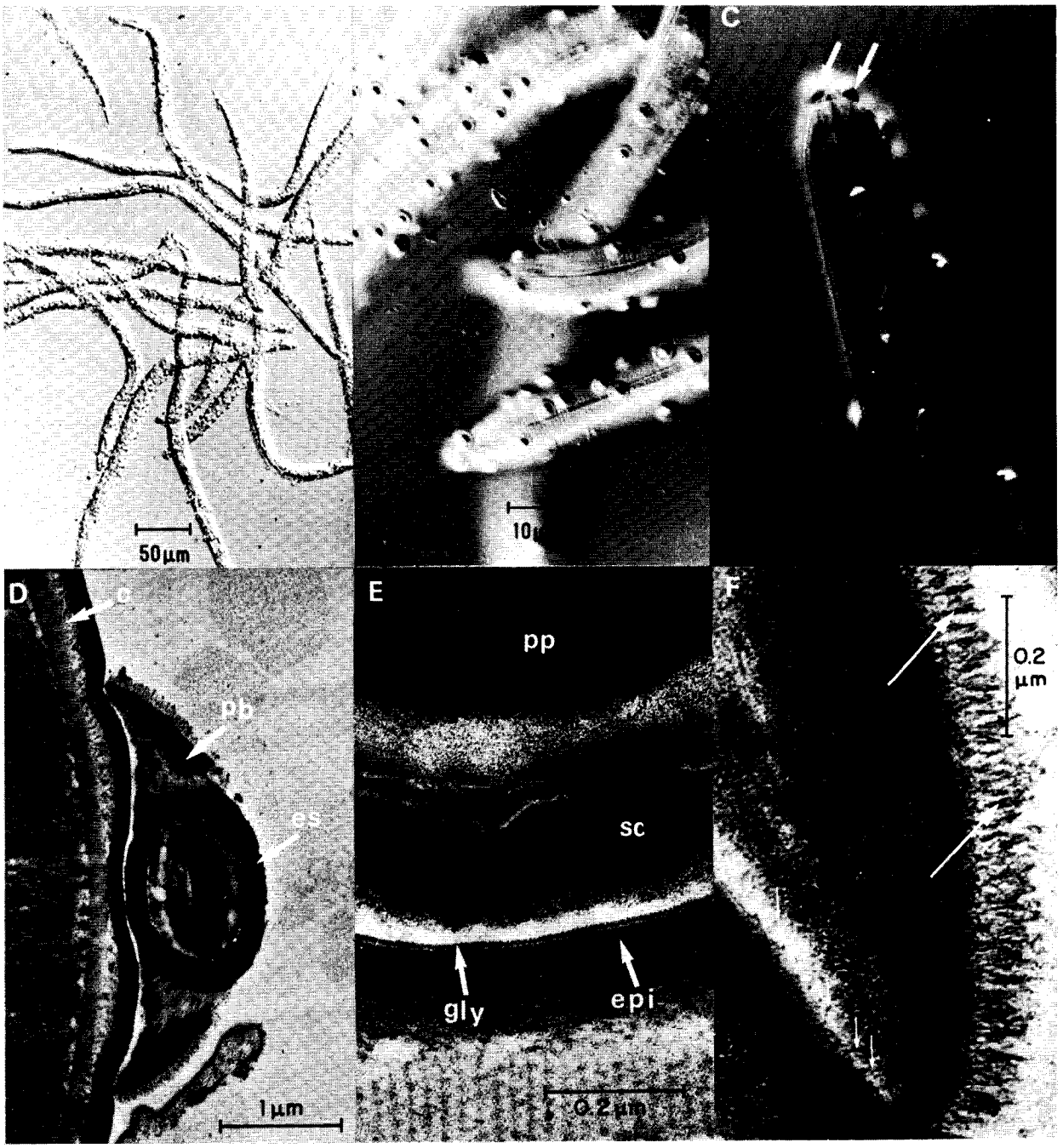

Fig. 3. Adhesion of spores of *Pasteuria penetrans* to the cuticles of infective juveniles of *Meloidogyne javanica*. A, B & C - Living specimens viewed under differential interference contrast optics showing A : clumping of heavily infested juveniles; B : random distribution of spores on surface of cuticle; C : spores covering amphidial pores (arrows). D, E & F - Electron micrographs of sections cut through an attached spore. D : nematode cuticle (c), endospore (es), and parasporal body (pb); E : nematode epicuticle (cpi), glycocalyx (gly), protoplast (pp), and spore coat (sc); F : microfibrillar external coat with hair-like fibres (large arrows), showing parasporal fibres from the lower half of the spore entwined with the epicuticle (small arrows).

linkages (Hinch & Clarke, 1980), the lectin concanavalin A which binds α-mannosyl and α-glucosyl residues and bovine serum albumin, which binds specifically to cell surfaces did not inhibit adhesion.

Studies with sugar residues known to occur on the surface of nematodes failed to implicate them in the mechanism of attachment. N-acetyl galactosamine, which has been found on the cuticles of some nematodes (Spiegel, Cohn & Spiegel, 1982) and plays a role in their recognition and capture by the nematode-trapping fungus *Arthrobotrys oligospora* (Premachandram & Pramer, 1984), did not inhibit attachment. The results of experiments with sialic acid, which is found all over the body surface of *M. javanica* (Spiegel, Cohn & Spiegel, 1982) were equivocal because sialic acid reduced the number of spores attached in experiments 3 A and 3 B but also affected nematode behaviour. However, spore adhesion was not inhibited when spores were agitated with heat-killed nematodes, suggesting that the former result was due to an effect on nematode mobility rather than on the attachment process. Also, spore adhesion was never completely inhibited by sialic acid, adhesion of sialic acid-treated spores was comparable with that of untreated spores while limulin, which binds specifically to sialic acid, did not affect attachment. Since *P. penetrans* spores attach to second-stage juveniles but not to males of *M. javanica* and sialic acid is found in both males and juveniles (Spiegel, Cohn & Spiegel, 1982), it is unlikely that sialic acid is involved in the attachment process.

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