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Developmental expression of esophageal gland antigens and their detection in stylet secretions of *Meloidogyne incognita*

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Summary – Immunofluorescence microscopy with monoclonal antibodies (MAbs) was used to demonstrate developmentally-regulated synthesis of esophageal gland antigens in *Meloidogyne incognita* during parasitism of plants and the secretion of some of these antigens through the nematode's stylet. Several esophageal gland antigens were temporally expressed in the subventral and dorsal glands of preparasitic second-stage juveniles (J2), parasitic J2, and adult female life stages. Two MAbs that bound to the subventral glands of J2, and one MAb that bound to the dorsal and subventral glands of J2, also bound to stylet secretions produced by J2. Two MAbs that bound to the dorsal gland in adult females, and one MAb that bound to the subventral glands in adult females, bound to stylet secretions produced by adult females. These results demonstrate that secretions from the dorsal and subventral esophageal glands can be secreted through the stylet of *M. incognita,* and that their expression is developmentally regulated.

Résumé – ***Expression d'antigènes des glandes œsophagiennes durant le développement de* Meloidogyne incognita *et leur détection dans les sécrétions du stylet*** – La microscopie en immunofluorescence a été utilisée avec des anticorps monoclonaux pour montrer que la production d'antigènes dans les glandes œsophagiennes de *Meloidogyne incognita* est régulée par le développement durant la phase parasite et que quelques-uns de ces antigènes peuvent être excrétés au travers du stylet. Plusieurs antigènes des glandes œsophagiennes sont temporairement exprimés dans les glandes subventrales et dorsale des juvéniles préparasites de deuxième stade (J2), des juvéniles parasites (J2) et des femelles adultes. Deux anticorps monoclonaux reconnaissant les glandes subventrales de J2 et un anticoprs monoclonal reconnaissant la glande dorsale et les glandes subventrales de J2, reconnaissent également les sécrétions passant au travers du stylet des J2. Deux anticorps monoclonaux reconnaissant la glande dorsale des femelles adultes, et un anticorps monoclonal reconnaissant leurs glandes subventrales, reconnaissent aussi les sécrétions passant au travers du stylet des femelles adultes. Ces résultats montrent que les sécrétions des glandes dorsale et subventrales peuvent passer au travers du stylet de *M. incognita* et que leur expression est régulée par le développement.

Key-words : esophageal glands, monoclonal antibodies, plant-parasitic nematode, *Meloidogyne,* secretory proteins.

Secretory proteins synthesized in the esophageal glands and secreted through the stylet of plant parasitic nematodes have critical roles in plant-nematode interactions. Stylet secretions may function in penetration and migration of nematodes in plant tissue, modification and maintenance of plant cells as feeding sites, formation of feeding tubes, and digestion of host cell contents to facilitate nutrient acquisition by the nematode (Hussey, 1989 *a*; Hussey & Mims, 1991). In second-stage juveniles (J2) of *Meloidogyne* (Chitwood) species, a single dorsal gland cell overlaps the anterior end of the intestine and has a long cytoplasmic extension that extends anteriorly through the metacorpus to terminate in an ampulla that is connected via an elaborate valve to the esophageal lumen at the base of the stylet knobs. Two subventral gland cells also overlap the intestine but have short cytoplasmic extensions that terminate in ampullae. These ampullae are connected by valves to the esophageal lumen at the posterior end of the triradiate pump chamber in the metacorpus. The proximity of the dorsal gland valve to the stylet enables fluid secretions released from secretory granules of this gland to be secreted through the stylet (Hussey, 1989 *a;* Hussey *et al.,* 1990). In contrast, the location of the subventral gland valves at the base of the metacorpal pump chamber, and the rigid circular lumen of the esophagus anterior to the pump chamber, should both restrict anterior flow of subventral gland secretions during pumping of the metacorpus (Doncaster, 1971). The capacity for subventral gland products to be secreted through a nematode's stylet, and

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hence function in plant-nematode interactions, has not been observed or assayed directly (Hussey, 1989 *a;* Wyss *et al.,* 1992).

Incubation of second-stage juveniles of *Meloidogyne incognita* in resorcinol stimulates vigorous stylet thrusting and accumulation of stylet secretions at the lip region (McClure & von Mende, 1987). Associated with this secretory activity is forward movement and accumulation of secretory granules in the cytoplasmic extensions and ampullae of the subventral glands but not in the dorsal gland. Viable adult females of *Meloidogyne* species dissected from galled roots and incubated in an antibiotic-saline solution also produce viscous stylet secretions *in vitro* (Bird 1968), which can be separated by SDS-PAGE analysis into nine major protein bands (Veech *et al.,* 1987). Intrasplenic immunization of Balb/c mice with stylet secretions collected from adult females of *M. incognita* facilitated the isolation of two monoclonal antibodies (MAbs) that bound to secretory granules within the subventral glands (Davis *et al.,* 1992). Other MAb have been produced with specificity to secretory granules formed in the esophageal glands of preparasitic J2, gravid females, or both life stages of *Meloidogyne* species (Hussey, 1989 *b;* Davis *et al.,* 1992). One MAb that binds stylet secretions that accumulate on the lip region of *M. incognita* females incubated in perfusion chambers has been used to immunopurify a high molecular weight secretory glycoprotein from *M. incognita* (Hussey *et al.,* 1990).

The changes in esophageal gland morphology, morphology and movement of secretory granules, and secretory antigens during plant parasitism by *Meloidogyne* species (Bird, 1967; Bird, 1983; Hussey, 1989 *a, b;* Hussey & Mims, 1990; Davis *et al.,* 1992; Wyss *et al.,* 1992) indicate a changing role for the esophageal glands and their secretions throughout the nematode's life cycle. The binding of esophageal gland granule-specific MAbs to stylet secretions of preparasitic J2 and adult females of *M. incognita,* as first reported by Davis and Hussey (1992), and the developmental expression of these esophageal gland antigens in different parasitic stages of *M. incognita,* are reported here.

Materials and methods

Meloidogyne incognita was propagated on greenhouse-grown tomatoes (*Lycopersicon esculentum* Mill. cv. Rutgers). Eggs of *M. incognita* were collected from galled roots using 0.5 % NaOCl (Hussey & Barker, 1973) and either used as inoculum or placed on 25 μm-opening sieves to hatch J2. All gravid females of *M. incognita* used in this study were dissected from galled roots 30-35 days after inoculation with eggs. Collection of parasitic stages of *M. incognita* was based on the observations of post-infectional development by Triantaphyllou and Hirschmann (1960). Tomato roots infected with *M. incognita* were harvested at 6, 12, and 20 days after inoculation to provide sexually undifferentiated " early J2", sexually differentiated " late J2", and " early adult females " without eggs, respectively. Third and fourth stage juveniles were not observed since these parasitic stages do not feed. Early adult females were dissected from galled roots, while early and late J2 parasitic stages were recovered from infected tomato roots by a combination of root maceration and density flotation. Galled roots in water were chopped in a blender in three 30 s bursts at medium speed and the slurry was poured on a 250 μm-opening sieve nested over 75 μm-opening and 25 μm-opening sieves, respectively. Nematodes and some root debris were rinsed onto the 25 μm-opening sieve and collected in water. The nematode-root slurry was mixed 1:1 with 75 ml of 70 % sucrose solution in a 250-ml glass centrifuge bottle, overlaid with 50 ml of water, and centrifuged at 1 000 *g* for 5 min. Nematodes were recovered from the upper two-thirds of the suspension, then concentrated and rinsed with water on a 25 μm-opening sieve. Early J2, late J2, and early adult female parasitic stages were fixed for immunofluorescence in 2 % paraformaldehyde in phosphate-buffered saline (PBS; 137 mM NaCl, 1.4 mM KH_2PO_4, 2.6 mM KCl, and 8.1 mM Na_2HPO_4), pH 7.4, at 4 °C for 4 days. Fixed parasitic stages of *M. incognita* were rinsed and stored in M 9 buffer (22 mM KH_2PO_4, 42.3 mM Na_2HPO_4, 85.6 mM NaCl, and 1 mM $MgSO_4$) at – 20 °C until used in immunofluorescence assays.

Immunofluorescence of parasitic nematodes

Indirect immunofluorescence assays of nematode specimens were performed similar to methods described previously (Atkinson *et al.,* 1988; Davis *et al.* 1992). Anterior portions that contained the esophageal glands were dissected from thawed fixed parasitic stages of *M. incognita* and incubated at 37 °C in proteinase K (Sigma Chemical Co.) at 2 mg enzyme/ml buffer (100 mM Tris-HCl, pH 7.4, 1 mM $CaCl_2$, and 0.1 % Triton X-100). Anterior sections of early and late J2 and of early adult females were incubated in proteinase K for 20 min and 45 min, respectively. Nematode sections, rendered permeable by proteinase K treatment, were microcentrifuged at 3000 *g,* the supernatant fluid was removed, and the pellet was frozen on dry ice for 20 min. Thawed nematode sections were suspended in methanol on dry ice for 30 s, pelleted, resuspended in acetone on dry ice for 1.5 min, pelleted, and the acetone was removed. A stream of air was used to quickly bring the pellet near dryness and the nematode sections were suspended in PBS that contained 10 % goat serum to block nonspecific antibody-binding sites, 0.02 % sodium azide, and 1 mM phenylmethylsulfonyl fluoride. Blocked sections of the different parasitic stages were stored at 4 °C for at least 3 days but no longer than 1 month prior to use in immunofluorescence assays.

Table 1. *Binding of monoclonal antibodies [1] to parasitic stages and stylet secretions of* Meloidogyne incognita.

Monoclonal Antibody	Preparasitic[2] J2	Early J2	Late J2	Early Adult female	Gravid female	Stylet[3] secretions
$3F_4$	subventral glands[4], esophageal lumen	subventral glands, esophageal lumen	subventral glands, esophageal lumen	subventral glands, esophageal lumen	subventral glands, esophageal lumen	J2, Female
$6D_4$	dorsal gland, subventral gland	dorsal gland, subventral gland	dorsal gland, ND	dorsal gland, ND	dorsal gland, ND	J2, Female
$3F_{11}$	subventral glands	ND	ND	ND	ND	J2
$4B_6$	ND	ND	ND	dorsal gland	dorsal gland	ND
$12H_7$	hypodermal chords	hypodermal chords	hypodermal chords	dorsal gland	dorsal gland	Female
$7A_9$	muscle	muscle	subventral glands	subventral glands	subventral glands	ND

(1) Monoclonal antibody binding was confirmed by immunofluorescence microscopy using fluorescein isothiocyanate (FITC) conjugated anti-mousse IgG and IgM as second antibody.
(2) Preparasitic second-stage juveniles (J2) and mature females were tested previously (Davis *et al.,* 1992; Hussey, 1989). Early J2 = sexually undifferentiated J2 removed from tomato roots 6 days after inoculation; Late J2 = sexually differentiated J2 removed from tomato roots 12 days after inoculation; Early female = adult female without eggs removed from tomato roots 20 days after inoculation.
(3) Binding of monoclonal antibodies to stylet secretions isolated *in vitro* from preparasitic J2 and females of *M. incognita.*
(4) Antibody binding localized to dorsal or subventral esophageal glands, other structure, or not detected (ND) in *M. incognita.*

The monoclonal antibodies used in these assays were generated previously (Hussey, 1989 *b;* Davis *et al.,* 1992). The binding specificity of these MAbs to secretory granules in the esophageal glands of *M. incognita* is listed in Table 1. Testing of individual MAbs was conducted by adding 50 µl of MAb hybridoma supernatant to 10-20 nematode sections placed in a 500 µl microcentrifuge tube. Each tube was brought to a final volume of 350 µl per tube with primary antibody diluent (PBS plus 1.0 % bovine serum albumin, 0.05 % Tween 20, and 0.02 % sodium azide), and the mixture was agitated overnight at room temperature. Primary antibody-treated nematode sections were pelleted at 3000 *g,* rinsed three times with PBS containing 0.5 % Triton X-100 (PBST), and resuspended in fluorescein isothiocyanate (FITC) conjugates of goat anti-mouse IgG and goat antimouse IgM (Sigma Chemical Co.) combined and each diluted 1 : 500 in FITC diluent (10 mM Tris-HCl, pH 7.2, 149 mM NaCl, 3.0 % BSA, 0.2 % Triton X-100, and 0.02 % sodium azide). Specimens were agitated in FITC second antibody for 3 h in the dark at room temperature, pelleted at 3000 *g,* rinsed three times in PBST, and resuspended in water. Early and late J2 specimens were pelleted and transferred in 10-µl drops of water to individual wells on multitest slides (ICN-Flow) that were previously coated with 2 µl of 1.0 % poly-L-lysine. Sections of antibody-treated J2 were dried on slides, covered with a 2.5-µl drop of antiquenching agent (0.02 mg/ml of phenylenediamine in 0.5 M carbonate buffer, pH 8.6, mixed 1:1 with nonfluorescent glycerol), and a coverslip applied. Fine forceps were used to transfer the antibody-treated anterior sections of early adult females under a stereomicroscope to 2.5-µl drops of antiquenching agent in wells of uncoated multitest slides, and the slides were covered with a coverslip. Specimens were viewed with the 40 × objective of an Olympus fluorescence microscope. Control treatments to determine nonspecific antibody binding to nematode sections included no primary MAb or preimmune mouse serum.

Immunofluorescence and ultrastructure of stylet secretions

Stylet secretions were produced and collected *in vitro* from the lip regions of adult females of *M. incognita* as reported previously (Davis *et al.,* 1992; Veech *et al.,* 1987). Viable adult females were dissected from galled tomato roots and incubated for 3 days at room temperature in 0.9 % NaCl containing 1.5 mg/ml gentamycin sulfate and 0.05 mg/ml nystatin to produce stylet secretions. Stylet secretions from preparasitic J2 of *M. incognita* were produced *in vitro* as described by McClure and von Mende (1987). A 24-h cohort of freshly-hatched J2 was incubated at room temperature in 0.4 % resorcinol containing 0.001 % Coomassie Brilliant Blue G (Sigma Chemical Co.) for 4 h to produce (blue) stylet secretions. A fine glass needle held by a micromanipulator was used under a stereomicroscope to collect the viscous stylet secretions that accumulated on the lip region of both stages. Several stylet secretions collected from either adult females or J2 were immediately deposited within a designated area on the surface of a (12 000-14 000 MWCO) dialysis membrane and used for immunofluorescence assays. Stylet secretions adhered to the dialysis membrane, and each membrane was carefully placed in two changes of PBS for rinsing. The membrane carrying the secretions was then placed in a mi-

crocentrifuge tube that contained hybridoma fluid of a MAb diluted 1:10 with PBS and incubated overnight at room temperature. The membrane carrying secretions was then rinsed in two changes of PBS and incubated in the FITC anti-mouse IgG/IgM second antibody described above (minus BSA and Triton X-100) for 3 h in the dark. The membrane was rinsed in two changes of PBS and then water, placed on a glass slide with secretions facing up, and a coverslip applied. The secretions were viewed at 40 × with a fluorescence microscope. Control treatments were the same as described above for the nematode specimens.

Some adult females that had accumulated stylet secretions were fixed in 3.0 % glutaraldehyde in 0.05 M phosphate buffer, *p*H 7.2, in preparation for transmission electron microscopy. After 4 h in fixative at room temperature, females were punctured with a fine needle to reduce shrinkage during processing, and embedded in 2.0 % Noble agar. Agar blocks containing females with stylet secretions were excised and transferred to vials containing phosphate buffer. Specimens were processed further for transmission electron microscopy as described previously (Hussey & Mims, 1990).

Results

Immunofluorescence observation of MAb binding to esophageal gland granule antigens differed among the MAb and parasitic stages of *M. incognita* (Table 1). MAb $3F_4$ bound to secretory granules within the subventral esophageal glands and to the esophageal lumen of all parasitic stages of *M. incognita* observed. Binding of MAb $6D_4$ to dorsal esophageal gland granules was observed in all stages examined, but binding of $6D_4$ to subventral gland granules was observed only in preparasitic and early J2 stages. MAb $3H_{11}$ bound to subventral gland granules of preparasitic J2, but not to secretory granules in any parasitic stage of *M. incognita* examined. Binding of MAb $4B_6$ was limited to dorsal gland granules of early and gravid adult females. MAb $12H_7$ bound to the lateral hypodermal chords of all juvenile stages, but bound exclusively to dorsal gland granules of early and gravid adult females. Weak binding of MAb $7A_9$ to somatic muscles was observed in preparasitic and early J2, but this antibody bound only to subventral gland granules in late J2 and further parasitic stages.

Adult females of *M. incognita* incubated in antibiotic saline solution thrusted their stylets five to ten times per minute and accumulated viscous stylet secretions at their lip region within 3 days (Fig. 1 A). The stylet tip barely extended past the oral aperture during thrusts and appeared to deposit secretory material into the center of the accumulating secretions. Little movement of the metacorpus, except for infrequent twitching of the metacorpal muscles, was observed during the secretory activity. The fine structure of stylet secretions from females consisted of a network of intertwining electron-dense strands that usually surrounded a granular central core which was adjacent to the lip region (Fig. 1 B). Stylet secretions from mature adult females placed on dialysis membranes for immunofluorescence assays (Fig. 1 C) labeled with several MAbs that bound to secretory granules within the esophageal glands of *M. incognita* females (Table 1). A MAb, $6D_4$, that bound to dorsal gland granules labeled only the interior of stylet secretions accumulated by adult females (Fig. 1 D). A second MAb, $12H_7$, that labeled dorsal gland granules of females only bound to the central core of stylet secretions that came in contact with the stylet tip. Another MAb, $3F_4$, that bound to subventral gland granules (Fig. 1 E) labeled entire stylet secretions of *M. incognita* females (Fig. 1 F). Two MAbs that bound to secretory granules in the esophageal glands of *M. incognita* females, $4B_6$ and $7A_9$, did not bind to stylet secretions.

Freshly-hatched J2 of *M. incognita* incubated in resorcinol exhibited more frequent stylet thrusting than adult females and accumulated large amounts of Coomassie Blue-stained stylet secretions within 2-4 h (Fig. 2 A). Within minutes after the J2 were placed in resorcinol, secretory granules rapidly moved anteriorly in the subventral gland cells to pack and distend the cytoplasmic extensions and ampullae (Fig. 2 A). Secretory granule movement or accumulation in the ampulla of the dorsal gland cell were not observed. Pumping of the metacorpus did not occur during the secretory activity, but the pump chamber appeared slightly open and secretory granules within the subventral gland ampullae moved with each stylet thrust. Nematodes in resorcinol became inactive within 4-6 h when stylet secretions were collected for immunofluorescence assays. One MAb, $6D_4$, that bound to the dorsal and subventral gland granules of J2 and two MAbs, $3H_{11}$ and $3F_4$, that bound only to subventral gland granules of J2, labeled J2 stylet secretions (Table 1) in a general pattern (Fig. 2 B).

Discussion

The specificities of MAbs used in this study indicate clearly that secretions synthesized in both the dorsal and subventral esophageal glands can be secreted through the stylet of *M. incognita*. Previously, labeling of stylet secretions produced by adult female *M. incognita* with a MAb specific for dorsal gland granules (Hussey *et al.*, 1990) and video-enhanced observations of nematode secretory activity *in vivo* (Wyss & Zunke, 1986) provided evidence that dorsal gland secretions can be secreted through stylets of plant-parasitic nematodes. In the present study, we used MAbs to establish conclusively that secretions from subventral esophageal glands also can be secreted through a nematode's stylet. Specifically, MAbs that only bound secretory granules in the subventral glands of *M. incognita* J2 and adult females also labeled stylet secretions produced by these life stages.

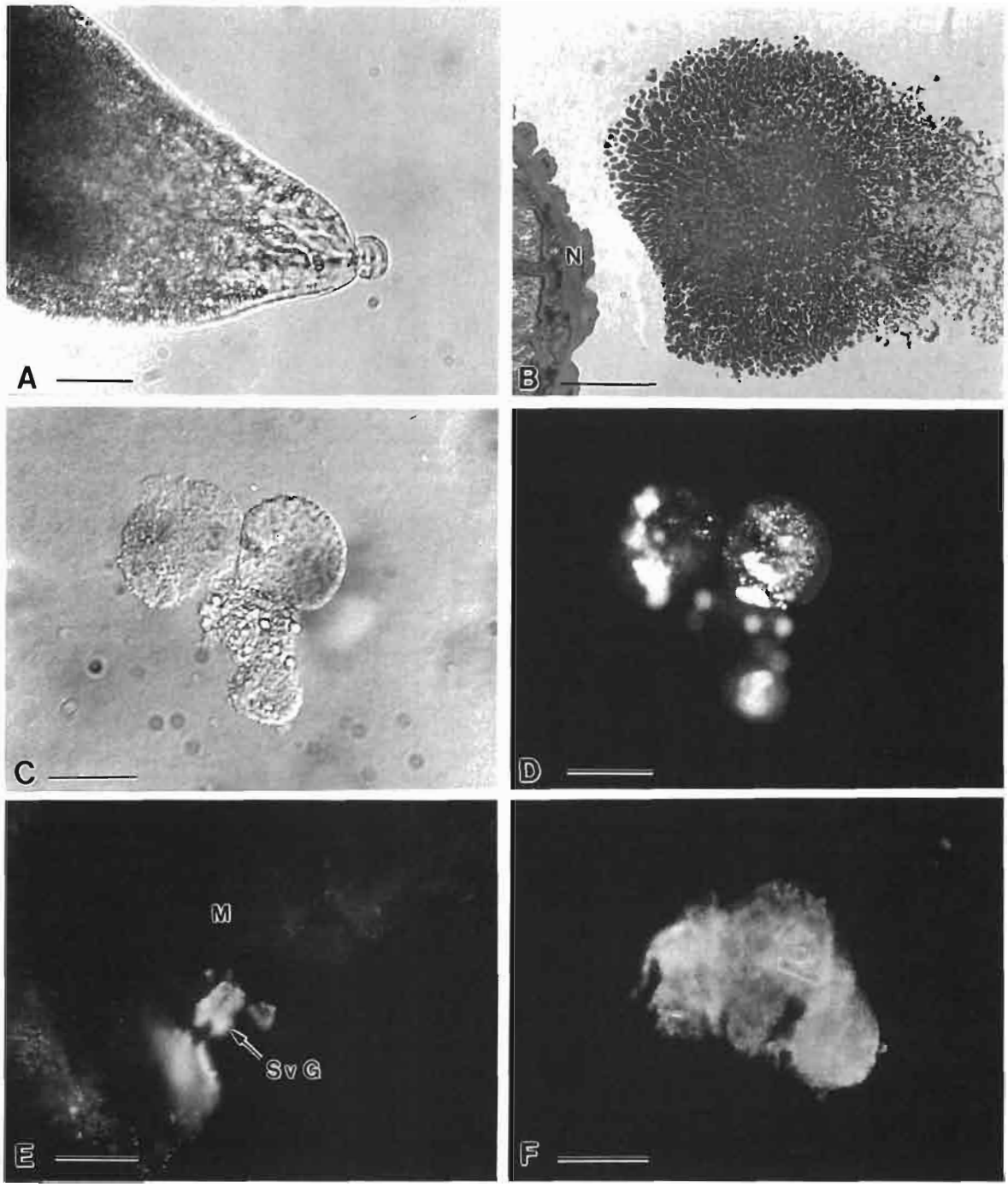

Fig. 1. *Light, immunofluorescence, and electron micrographs of adult females of* Meloidogyne incognita *and their stylet secretions. A : Light micrograph of stylet secretion attached to the lip region; B : Transmission electron micrograph of a longitudinal section of a stylet secretion and nematode (N) lip region; C : Nomarski micrograph of stylet secretions on a dialysis membrane; D : Immunofluorescence (FITC) labeling of the stylet secretions in C with a monoclonal antibody ($6D_4$) that bound to secretory granules in the dorsal esophageal gland; E : FITC labeling of a monoclonal antibody bound to secretory granules within the subventral esophageal gland (SvG) extensions and ampullae in the metacorpus (M); F : Immunofluorescence labeling of stylet secretions on a dialysis membrane with a monoclonal antibody ($3F_4$) that bound secretory granules in the subventral glands.* (Bar equivalent : A = 50 µm; B = 3 µm; C-F = 20 µm.)

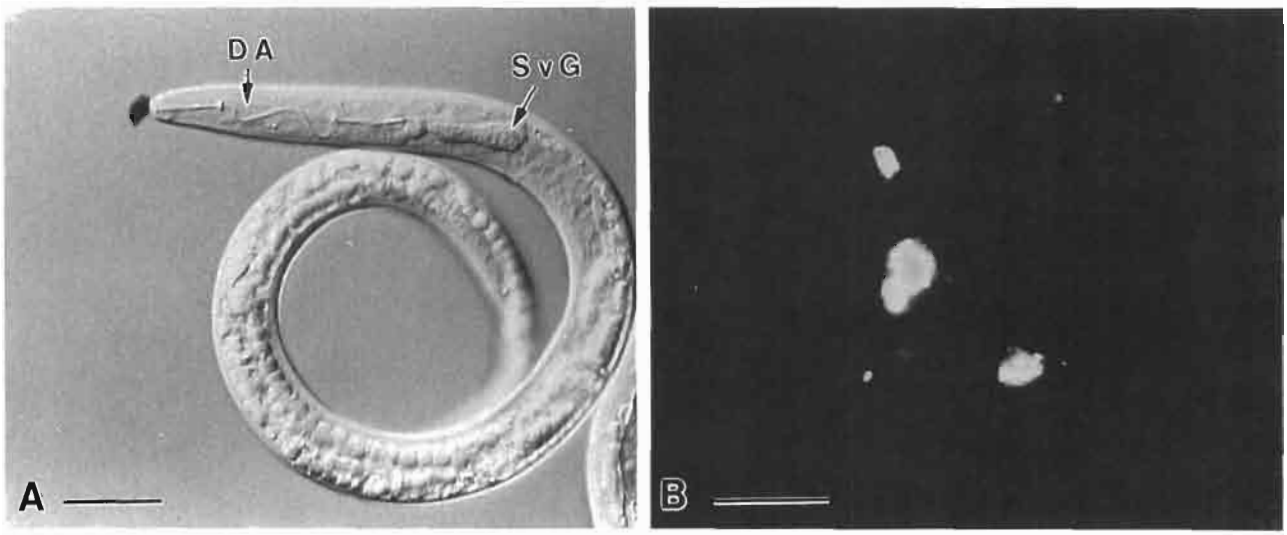

Fig. 2. *Micrographs of stylet secretions from preparasitic second-stage juveniles of* Meloidogyne incognita. *A : Nomarski micrograph showing stylet secretion attached to the lip region following incubation in 0.4 % resorcinol containing Coomassie Brilliant Blue G. Secretory granules accumulated in and distended the subventral esophageal gland (SvG) extensions and ampullae but not the dorsal esophageal gland ampulla (DA); B : Immunofluorescence labeling of second-stage juvenile stylet secretions on a dialysis membrane with a monoclonal antibody ($3F_4$) that bound to secretory granules within the subventral glands in second-stage juveniles.* (Bar = 20 µm.)

Additionally, MAbs with specificity to secretory granules in the subventral glands have been generated by immunizing mice with stylet secretions collected from *M. incognita* adult females (Davis *et al.*, 1992) and preparasitic J2 (Davis & Hussey, unpubl.). Also, as described by McClure and von Mende (1987), we observed here, movement and accumulation of secretory granules in the ampullae of the subventral glands but not within the dorsal gland, and subsequent production of stylet secretions in resorcinol-treated J2 of *M. incognita,* further support the notion that stylet secretions can originate from subventral glands.

We also suggest that the predicted morphological resistance to anterior flow of subventral gland secretions in the esophageal lumen that occurs during pumping of the metacorpal pump chamber (Doncaster, 1971) may be minimal during the secretion phase of a feeding cycle. Production of stylet secretions *in vitro* by preparasitic J2 and adult females involves very little movement and only a slight opening of the metacorpal pump chamber, although this was not always clear. However, even when the pump chamber is closed there is a slight gap between the sclerotized walls of the triradiate pump chamber that might allow for anterior flow of subventral gland secretions during the secretion phase (Endo & Wergin, 1988). Wyss *et al.* (1992) observed the release of subventral gland granule contents just prior to and during intermittent pumping of the metacorpus by *M. incognita* J2 infecting *Arabidopsis thaliana* and suggested that secretions from these glands may assist in root invasion and migration by the J2. During the secretion phase of the feeding cycle of *Criconemella xenoplax,* intermittent twitching of muscles in the posterior part of the metacorpus is accompanied by a slight opening of the pump chamber which could accommodate anterior flow of subventral gland secretions in the esophageal lumen for injection into host root cells (Westcott & Hussey, 1992).

We acknowledge that *in vitro* production of stylet secretions, especially when stimulated by resorcinol, may not represent actual secretory activity or secretions produced *in planta.* The detrimental effects of resorcinol appear to be minimal, however, since J2 treated with resorcinol for 4 h and allowed to recover in water successfully infected tomato roots (Davis & Hussey, unpubl.). Stylet secretions produced by adult females of *M. incognita* should be more natural since these nematodes were not exposed to resorcinol. Secretions that accumulated on the nematode lip region also could have originated from other orifices such as the amphids, but the specificity of MAb binding indicated that at least some, if not all, of the secretions originated in the esophageal glands.

The immunofluorescence assay with isolated stylet secretions on dialysis membranes was more informative about antibody binding patterns and more efficient than previous methods that utilized perfusion chambers (Hussey *et al.*, 1990). The reason for the inability of several secretory granule-specific MAbs to bind to stylet secretions is unknown. These secretory granule antigens may be transport proteins important in the secretory process and not the passenger proteins which are secreted from the esophageal glands (Hussey, 1989 *a*). How-

ever, this may not account for the lack of stylet secretion binding by MAb $7A_9$, which binds specifically to subventral gland granules of *M. incognita* adult females and was derived from immunizations with stylet secretions from females. Perhaps epitopes available within secretory granules in the esophageal glands, or epitopes made available when immunogens are introduced into mice, are not accessible for binding by some MAbs to stylet secretions *in vitro*. Epitope accessibility may be further complicated when nematode secretions are injected into the cytoplasm of plant cells (Davis & Hussey, 1992).

Parasitic stages of *M. incognita* removed from tomato roots demonstrated temporal and spatial expression of several esophageal gland antigens. Epitope accessibility and MAb binding specificity were confirmed by the ability of two MAbs to bind to esophageal glands of all parasitic stages examined and the correlation of stage-specific binding of MAbs to stylet secretions. Monoclonal antibodies also have been used to show changes in dorsal and subventral gland antigens in *Heterodera glycines* during infection of soybean (Atkinson & Harris, 1989). In *M. incognita,* MAb $3H_{11}$ bound to the subventral glands and stylet secretions of preparasitic J2 but did not bind to the esophageal glands of early parasitic J2 or any later parasitic stage. The expression of this antigen is similar to the reduction in subventral gland contents observed after penetration and migration of J2 of *M. incognita* in *Arabidopsis thaliana* (Wyss *et al.,* 1992). The secretory antigen recognized by MAb $6D_4$ was present in both the dorsal and subventral glands in preparasitic and early parasitic J2 but was absent in the subventral glands of late parasitic J2 and increased in the dorsal gland in later stages of the nematode's life cycle. This antigen may function in J2 migration and subsequently in food utilization or possibly have a role in the initiation and maintenance of giant-cells. The antigens bound by MAbs $12H_7$ and $4B_6$were present in secretory granules in the dorsal gland in only adult females of *M. incognita* and therefore, could be involved directly in food utilization or formation of a feeding tube (Hussey & Mims, 1991). The expression of the antigen recognized by $7A_9$ in the subventral glands of only later parasitic stages of *M. incognita* is intriguing. This esophageal antigen may be important in the secretory process or internal digestion of food since no binding of $7A_9$ to stylet secretions was observed. The binding of MAb $7A_9$ and $12H_7$ to muscle and lateral hypodermal chords, respectively, of early life stages, but only to the subventral and dorsal glands, respectively, in later life stages is puzzling. Genes encoding these esophageal gland antigens may be pleiotropic. Still, the function of any of the antigens mentioned above has not been identified and other potential roles of these antigens must be considered in future investigations (Hussey, 1989 *a*).

Monoclonal antibodies have proven to be valuable tools for demonstrating the presence of nematode esophageal gland antigens in stylet secretions. Monitoring of esophageal gland antigens with MAbs in several developmental stages of the nematode life cycle has demonstrated the differential expression of these secretions and provided insights into the functioning of the esophageal glands during parasitism. The localization of these nematode secretions *in planta,* and the identity of the secretory molecules and their corresponding genes, is currently under investigation.

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