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Symposium paper [1]

STUDIES ON NEMATODE SENSORY PERCEPTION AS A BASIS FOR NOVEL CONTROL STRATEGIES

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Summary – The possibilities of perturbing nematode sensory perception as a basis for novel control strategies cannot be assessed effectively until more information is available on the chemosensory and secretory roles of the anterior sense organs of plant parasitic nematodes. This brief review presents some of the current research programmes at Rothamsted Experimental Station which investigate aspects of the role and functioning of the anterior sense organs, particularly the amphids.

Résumé – ***Études sur la perception sensorielle des nématodes en tant que base pour de nouvelles stratégies de contrôle*** – La possibilité de troubler la perception sensorielle des nématodes (envisagée comme base de nouvelles stratégies de contrôle) ne peut être établie avant que des informations ne soient disponibles concernant le rôle chémo-sensible et sécréteur des organes sensoriels de la partie antérieure des nématodes phytoparasites. La présente courte revue expose quelques-uns des programmes de recherche en cours à la Rothamsted Experimental Station, programmes visant à explorer différents aspects du rôle et du fonctionnement de ces organes sensoriels antérieures, et en particulier des amphides.

Key-words : nematode, amphid, sense organ, ultrastructure, electrophysiology, immunocytochemistry, secretion.

The life cycles of plant parasitic nematodes offer several target sites for possible control approaches but more information is required about the basic biology, physiology and biochemistry of these parasites before novel control strategies can be evaluated effectively. For example, sensory perception may be particularly susceptible to disruption during certain phases of the life cycle, such as host location, movement to the feeding site and mate finding behaviour, but little is known of the physiology of nematode sense cells. This brief review presents some of the approaches currently being explored at Rothamsted Experimental Station to investigate the role and functioning of the anterior sense organs, in particular the amphids, of plant parasitic nematodes.

The detailed ultrastructure of the sense organs of many nematodes has been comprehensively reviewed by McLaren (1976) and Wright (1980). The anterior sense organs, or sensilla, of nematodes consist of twelve labial sensilla, four cephalic sensilla and two amphids. It is generally accepted from ultrastructural evidence, that the amphids are the primary chemosensory organs. They are situated laterally on either side of the mouth, each opening to the exterior via a prominent pore, and they are the largest and most complex of the anterior sense organs. The amphidial cavity contains dendritic processes which are bathed in secretions, apparently produced by the amphidial sheath cell, which may be involved in functions other than chemoreception.

Chemosensory role

The orientation of plant parasitic nematodes to known stimuli is impaired by low concentrations of nematicides, although motility is not inhibited; the sensilla may be the primary sites of action of these nematicides. Following the description of the functional morphology of the anterior sensilla of *Pratylenchus* species (Trett & Perry, 1985 *a*), the effects of low concentrations of the carbamoyloxime, aldicarb, on adult female *P. penetrans* was examined (Trett & Perry, 1985 *b*). Treatment with 5 and 10 ppm aldicarb resulted in hypertrophy of the internal dendrite terminals within the amphidial sheath cell, reduction in the surface volume fractions of the dendritic processes and the appearance of large, electron-lucent granules in the amphidial sheath cell cytoplasm. The presence of the granules indicated that the sheath cell metabolism is affected by aldicarb but the adverse effect that these changes may have on sensory transduction is a matter of speculation.

Ultrastructural studies may help to elucidate the changing role of amphids during the nematode life cycle and the times when they are functional and thus can be targeted to disrupt sensory perception. For example, in some animal parasitic nematodes, such as *Syngamus tra-*

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chea (Jones, 1979) and *Necator americanus* (McLaren, 1974; McLaren *et al.*, 1974), the amphids become greatly altered at certain stages of the life cycle in order to fulfil a modified role; the sheath cell (referred to as the gland cell by the authors) becomes enlarged and changes in ultrastructure were observed, such as the formation of large quantities of endoplasmic reticulum and Golgi bodies, which are often associated with an increase in secretory activity. These changes occur as the nematode enters its primary host and moults to the adult parasitic stage and are thought to be associated with the onset of production of anticoagulants associated with the nematodes' blood feeding activities. Thus, in some nematodes, the amphids may become altered at a specific stage in the life cycle to serve a functional role other than chemoreception. In second stage juveniles (J2) of *Globodera rostochiensis* changes were observed in the structure of the amphids during the hatching process (Jones *et al.*, 1994). The absence of secretory material and the shrunken state of the sheath cell in unhatched nematodes indicated that the amphids may not be functional before hatching and, thus, have no role in the detection of the hatching stimuli. The change to a functional appearance is not associated specifically with exposure to the natural hatching stimulus, potato root diffusate, but is a more general characteristic of naturally hatched juveniles. The amphids may be used in the later stages of hatching; once the nematode water content has increased sufficiently for hatching to occur (Perry, 1987), the amphids may become prepared for a functional role of host location. The structure of the amphids of *G. rostochiensis* altered very little during subsequent phases of the life cycle (Jones *et al.*, 1994).

Electron microscopical observations have provided information about the structure and development of the amphids and other sense organs (see review by Wright, 1991) but their function can only be inferred. A link between structure and function is often difficult to make and may depend on specific circumstances as, for exampe, the occurrence of behavioural mutants of *Caenorhabditis elegans* with structurally altered amphids (Lewis & Hodgkin, 1975). Recent laser microbeam studies involving ablation of individual amphidial neurons of *C. elegans* have demonstrated the chemosensory role of the amphids; it was shown that the accessory cilia were responsible for sensing certain volatile compounds (Bargmann *et al.*, 1990). Direct electrophysiological recordings from nematode sense organs will allow detailed analysis of responses to semiochemicals in an approach similar to that used to record from insect gustatory receptors (Wadhams *et al.*, 1982). Using these techniques, the first electrophysiological recordings of responses from an intact nematode were made (Jones *et al.*, 1991). The small size of adult male *G. rostochiensis* made direct recordings from individual sense organs impossible and intracellular recordings similar to those made by Davis and Stretton (1989 *a, b*) on *Ascaris* were also not possible. Extracellular recordings of electrical activity inside the body of male *G. rostochiensis* were obtained and changes in electrical activity were recorded in response to stimulants such as the female sex pheromone from adult female *G. rostochiensis* (Jones *et al.*, 1991). As well as making possible the detailed analysis of responses, the electrophysiological technique could be used as an efficient bioassay system to determine the active fractions of compounds such as sex pheromones, thus obviating the use of frequently unreliable agar plate behavioural assays. Direct recordings from individual sense organs are also feasible. The first recordings from a nematode sense organ were obtained directly from the cephalic papillae of the animal parasitic nematode *Syngamus trachea* and changes in spike activity were monitored in response to blood when it was introduced into the liquid surrounding the nematode (Jones *et al.*, 1991).

Studies on arthropods should provide useful models for work on nematodes. Insect and nematode sense organs share many structural similarities and are also thought to have similar roles. Like amphids, the uniporous chemosensilla of insects have an opening in the cuticle for chemical communication with the external environment, sheath cells are present and the dendritic processes are bathed in secretions. The amphidial secretions of nematodes may serve to maintain electrical continuity between the bases and tips of the dendritic processes (Trett & Perry, 1985 *a*); this is thought to be important in the generation of receptor currents in some insect sensilla (Zacharuk, 1980). Vogt *et al.* (1990) considered that two main types of protein exist in the secretions of insect sense organs : olfactory binding proteins (OBP) and odorant degrading enzymes (ODE). Odorant molecules bind to the OBP and are transported to the site of the receptor molecule on the dendritic process. Once released from the receptor molecule, the odorant molecule is broken down by the ODE to prevent repeated stimulation of the dendritic process. DNA probes complementary to two conserved regions of the DNA coding for insect OBP were synthesised and used as primers for PCR using nematode DNA. Analysis of the genomes of various nematodes, including *Meloidogyne incognita, Globodera pallida* and *Heterodera glycines,* indicated that they contained genes similar to those coding for olfactory binding proteins in insects (Jones *et al.*, 1992). Sequence data following cloning will confirm whether the fragments amplified from nematodes code for OBP. Thus, the secretions of nematode and insect sense organs may contain similar molecules and have a similar role. However, as well as involvement with sensory perception, the secretions may have additional roles several of which are discussed by Wright (1983) and Bird and Bird (1991).

Secretory role

Understanding the specific nature of the amphidial secretions, including the characterisation of the amphid

specific proteins, is essential to define possible novel control strategies based on perturbing nematode sensory perception. Indirect immunofluorescence studies using a rabbit polyclonal antiserum have been used to localise the presence of a glycoprotein in the region of the amphids of J 2 of the rootknot nematode, *M. incognita* (Stewart *et al.,* 1993 *a*). Similar immunoreactivity was found in five other species of *Meloidogyne,* but appears to be genus-specific as it was not found in representatives from eight other genera, including *Globodera* and *Heterodera,* indicating a more specialized function for this protein in *Meloidogyne.* SDS-PAGE, Western-blotting and lectin-binding studies on homogenates of *M. incognita* showed that the antigen was a 32kDa glycoprotein (termed gp32). Immunoelectron microscopical studies demonstrated that the immunoreactivity in *M. incognita* was associated with the secretory material filling the amphidial channel and with the sheath cell, indicating that gp32 is produced by the sheath cell and secreted into the receptor cavity from where it passes up the amphidial canal (Stewart *et al.,* 1993 *a*). Gp32 is expressed in all stages of the *Meloidogyne* life cycle, including males of *M. javanica,* but not in the sedentary adult female where the amphids appear to be non-functional. Incubation of infective J2 in the polyclonal antisera for gp32 significantly retarded orientation of the nematode to host roots (Stewart *et al.,* 1993 *b*). This glycoprotein is very important as it appears to be involved directly or indirectly in the primary transduction of chemical stimuli. Differences in the composition of amphidial secretions between J2 and females of *M. incognita* have also been demonstrated by Davis *et al.,* (1992), who used a monoclonal antibody which reacted with the amphids of adult females but not with the amphids of J2.

Future work on gp32 will centre on the elucidation of its function using molecular biological techniques to isolate and sequence the " gp32 gene " and to determine the time course of expression over the life cycle of *Meloidogyne.* It is important to localise and characterise additional amphidial molecules from a variety of nematodes, perhaps using induced amphidial secretions (Premachandran *et al.,* 1988). An alternative approach is to use a nematode where the amphids can be dissected out. The avian parasitic nematode, *S. trachea,* is a useful model nematode with large sense organs which are easy to dissect. As part of a project to compare secretions from the amphidial gland, the secretory-excretory gland and the arcade cells, the amphidial glands were dissected from *S. trachea* at known postinfection times and analysed by electrophoresis on mini gels followed by differential staining for proteins and specific enzyme activity (Riga *et al.,* 1993). Figure 1 is an example of the protein profiles of the amphidial gland and the secretory-excretory gland of *S. trachea* obtained from avian hosts 15 day post infection. Distinctive protein profiles were consistently obtained from both glands and their secretions at known post infection ages. There are many proteins present in the amphidial glands but there is no dominant protein. The use of monoclonal antibodies raised against amphidial proteins will facilitate investigations of the role of the amphidial secretions, the changes associated with specific life cycle events and the degree of conservation of amphidial proteins in free living and animal and plant parasitic nematodes.

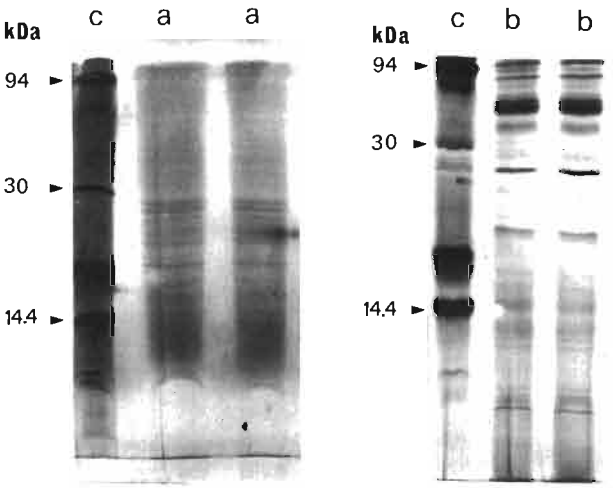

Fig. 1. *SDS-PAGE protein profiles of an amphidial gland (a) and an excretory-secretory gland (b) dissected from adult* Syngamus trachea, *15 day post infection. Proteins were silver stained; track c are markers.*

Conclusion

Plant nematologists are far behind entomologists and *C. elegans* research groups in studies on sensory physiology and biochemistry. As with many other areas of plant nematology research, progress will be enhanced by an awareness of literature from these groups and from animal nematologists. Clearly, the research programmes outlined above represent only a few of the many approaches which will be needed to examine the role and functioning of the anterior sense organs and related aspects, such as the presence and distribution of various neurotransmitters. Only when information is available in these areas will it be possible to evaluate novel control strategies aimed at perturbing sensory perception and/or neurotransmission.

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