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CHEMORECEPTION IN PLANT PARASITIC NEMATODES

Roland N. Perry
Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK; e-mail: Roland.Perry@bbsrc.ac.uk

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ABSTRACT

The role and functioning of the anterior chemosensory organs of plant parasitic nematodes is examined, with particular emphasis on the amphids. The morphology of the amphids is discussed primarily in the context of the changes in the ultrastructure associated with different life stages. The involvement of amphidial secretions in chemoreception and the behavioral and electrophysiological analyses of nematode responses to semiochemicals are discussed with special reference to research on sex pheromones. These research techniques, combined with the use of lectins and antibodies, provide information on nematode sensilla that may lead to novel control strategies for economically important plant parasitic nematodes based on perturbing nematode sensory perception to prevent host or mate location.

INTRODUCTION

Plant parasitic nematodes are typically cylindrical, approximately 0.3 mm to 10.0 mm in length and 20 to 60 mm in diameter. All species have stages in their life cycle in soil, but they are essentially aquatic organisms requiring for active existence at least a film of water on the surface of soil particles in air-filled pores. Thus, chemicals eliciting nematode responses in soil are likely to be water soluble. All plant parasitic nematodes have a flexible stylet or mouth-spear that can be inserted into plant cells for food uptake. Ectoparasitic nematodes frequently kill the cell on which they feed and then withdraw their stylet and move to another cell. Among endoparasitic nematodes are those species that induce the production of a feeding site that supports the development of the sedentary, saccate female. Nematodes are normally bisexual, but in some

species, where males are rare or unknown or nonfunctional, reproduction is parthenogenetic.

Plant parasitic nematodes cause > US$100 billion damage annually to crops worldwide (63). Several physical, chemical, and cultural methods are available for nematode control, usually in combination in an integrated management strategy. Those species with a restricted host range are effectively controlled by crop rotation, which remains the most widely practiced method of nematode control. However, rotation by itself may be uneconomically long for the control of species such as the potato cyst nematode, *Globodera rostochiensis*, which remain viable in the soil for many years. There is a great reliance on chemical control but many of the current nematicides are environmentally undesirable, and new, safer compounds are being developed by several companies. When available, resistant cultivars are widely used, but nematode populations able to overcome resistance are now more common. In the future, genetic engineering techniques to clone resistance genes from plants and transfer them to previously susceptible cultivars or species may provide commercially viable cultivars of major crops; more knowledge about the function of genes also may enable engineering of novel types of resistance (52).

However, there is an urgent need for additional control strategies. Although the life cycles of plant parasitic nematodes offer several putative targets for novel control approaches, the microscopic size of most species makes physiological and biochemical investigations difficult. Thus, there is a paucity of basic information necessary to evaluate the potential of novel control approaches. Chemoreception may be particularly sensitive to disruption during certain phases of the life cycle such as host location, movement to the feeding site, and mate finding (55). To achieve disruption, it may be possible to use naturally occurring compounds, such as nematode sex pheromones or host root diffusates, or their analogues. Unfortunately, although plant parasitic nematodes are thought to use chemoreceptors to locate their hosts and mates, little is known about the functional physiology of nematode sensilla (= sense organs).

This review focuses on functional aspects of the chemosensilla of plant parasitic nematodes, with occasional reference to information from research on free living and animal parasitic nematodes. Recent advances, using electrophysiological analyses of nematode responses to chemical stimuli, are emphasized. It is beyond the scope of this review to present details of the nervous system and neurotransmitters in nematodes, but the information available on plant parasitic nematodes is meager in comparison to the extensive and detailed research done on the free living nematode, *Caenorhabditis elegans* (73).

NEMATODE SENSILLA

Ideas on the function of nematode sensilla have been inferred largely from structural observations and comparisons with sense organs of known function in other animal groups, especially arthropods. As early as 1903, the anatomy of the sensilla of the animal parasitic nematode, *Ascaris lumbricoides,* was studied using light microscopy (28). The detailed ultrastructure of the sensilla of many nematodes has been comprehensively reviewed (14, 17, 74, 75). The main concentration of sensilla is at the anterior end of the nematode (Figure 1). These anterior sensilla are usually hexaradially arranged in subdorsal, subventral, and lateral positions; the six inner labial sensilla are closest to the mouth with the six outer labial sensilla just behind and four cephalic sensilla at the same level or just behind these; two lateral sensilla, the amphids, complete the complement of anterior sensory receptors (75).

The amphids, situated on either side of the mouth, open to the exterior via a prominent pore. Although they are the largest and most complex of the anterior

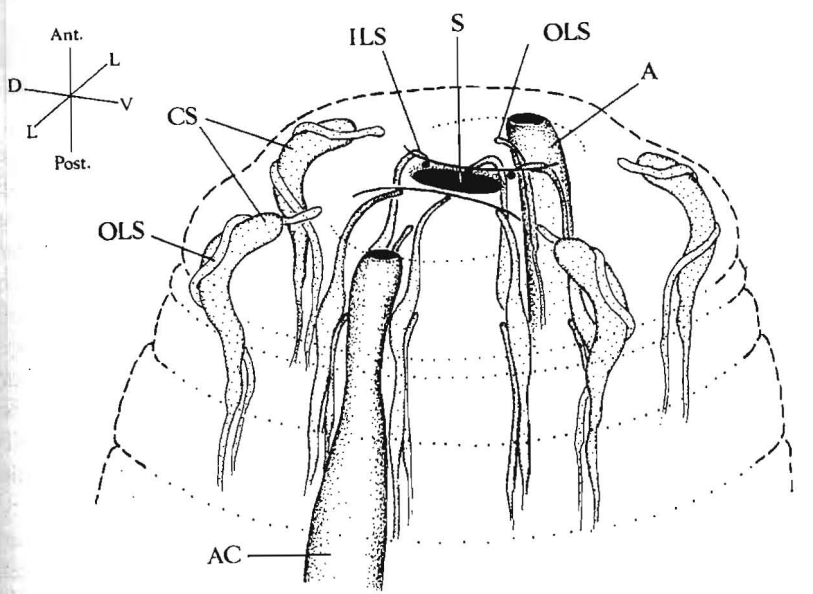

Figure 1 Diagrammatic reconstruction summarizing the form and arrangement of the anterior sensilla of adult *Pratylenchus penetrans*. *A*: amphid; *AC*: amphidial canal (= duct); *CS*: cephalic sensillum; *ILS*: inner labial sensillum; *OLS*: outer labial sensillum; *S*: stoma (= buccal cavity). From Trett & Perry (67).

sensilla, their structure is conserved in a wide range of plant parasitic nematodes including second-stage juveniles (J2) and adult males of the root-knot nematode, *Meloidogyne incognita* (10, 72), the soybean cyst nematode, *Heterodera glycines* (11, 24) and *G. rostochiensis* (39), and adults of *Pratylenchus* species, the root lesion nematodes (67). Each amphid comprises a glandular sheath cell, a supporting socket cell, and a number of dendritic processes that are bathed in secretions, apparently produced by the sheath cell. The number of dendritic processes differs between species although, in most, seven processes enter the amphid itself and between two and five processes originating from the same bundle of nerves pass through the amphid and form other structures at the anterior tip of the nematode.

Additional sensilla have been described associated with other parts of the nematode body. For example, ultrastructural studies on the pharyngeal nerve system of the virus vector nematode *Longidorus leptocephalus* (61) indicate the presence of proprioceptors (sensilla detecting pressure, position, or movement) at the base of the odontophore (posterior part of the mouth-spear), at the isthmus of the pharynx, and around the pharyngeal intestinal valve; chemoreceptors and/or mechanoreceptors are thought to be present in the anterior part of the pharynx and associated with the pharyngeal gland duct orifices. Of the caudal sensilla, the phasmids have received most attention. Phasmids are similar in general structure to the amphids, each consisting of an external pore, a cuticle lined duct, a socket cell, a sheath cell, and a dendritic receptor; it has been speculated that one of their functions may be as receptors for sex attractants (71). Baldwin (9) found two types of phasmids (termed A and B) in J2 of the beet cyst nematode, *H. schachtii*; in A, the larger type, the sheath cell was deeply invaginated to form a receptor cavity in which secretions accumulated, whereas in B no receptor cavity or secretions were present. The functional significance of these differences has not been investigated. Phasmids are taxonomically important, as the phylum Nematoda is divided into two major groups, the Adenophorea (or Aphasmidia) and the Secernentea (or Phasmidia), which contains most of the plant parasitic nematodes; the Adenophorea do not have phasmids. Information on the functional role of the phasmids may be derived, in the future, from comparative physiological and biochemical studies on representatives from both groups.

Of all the nematode sensilla, the amphids are considered to be the primary chemosensilla; much of the subsequent discussion of chemoreception centers on the amphid and examines its function as a secretory organ and its role in sensory perception.

INVOLVEMENT OF AMPHIDIAL SECRETIONS IN CHEMORECEPTION

Molecules of chemical substances initially come into contact with the amphidial secretions filling the amphidial duct. Thus, information on the specific nature of these secretions may enhance understanding of nematode sensory perception. Evidence that the secretions contain protein derived from the observation that the protein-specific dye, Coomassie Brilliant Blue R-250, bound the amphidial secretions of a number of nematodes including *M. incognita* J2 (8, 56). Several studies used lectins (carbohydrate-binding proteins or glycoproteins) to demonstrate the presence of carbohydrate residues in amphidial secretions of *Meloidogyne* species (15, 48), in *Globodera* species (27), and in *H. schachtii* (7, 8). The alterations in binding patterns observed after digestion with certain proteases suggested that at least some of these residues were constituents of glycoproteins (1, 26). Components of amphidial secretions are thought to include *N*-acetylgalactosamine and fucose in *M. incognita* J2 (48, 64) and mannose or glucose, *N*-acetylglucosamine, and galactose and/or *N*-acetylgalactosamine in males of *H. schachtii* (4).

Zuckermann (77) suggested that nematode host-finding behavior could be modified either by blocking chemotactic signals emanating from host roots or by blocking the chemoreceptors. Certain lectins inhibited the attraction of *C. elegans* to its food source, indicating that the lectins bind to membrane receptors of dendritic nerve extensions. However, sex pheromone reception by males of *H. schachtii* could not be inhibited by lectins (5), perhaps because the lectins did not penetrate the amphidial secretions. At present, information from these and other lectin studies is conflicting and inconclusive (78). Treating males of *H. schachtii* with the sulfhydryl reagent, mersalyl acid, inhibited their ability to move to a pheromone-emitting source; including dithiothreitol (a sulfhydryl group protectant) in the treatment blocked the effect, indicating that sulfhydryl groups may be involved in pheromone reception (2). The penetration of sensilla secretions by putative blocking agents needs to be investigated in more detail using various species of plant parasitic nematodes on a comparative basis. This is especially important as there are indications of fundamental differences in the composition of sensilla secretions between species of nematodes (3).

Other research techniques have also demonstrated major differences in amphidial secretions. Indirect immunofluorescence studies using a rabbit polyclonal antiserum were used to localize the presence of a glycoprotein in the region of the amphids of *M. incognita* J2 (65). 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*. This indicates a more specialized function for this protein in

Meloidogyne. Biochemical studies on homogenates of *M. incognita* showed that the antigen was a 32-kDa glycoprotein (termed gp32), and the immunoreactivity (determined using immunoelectron microscopy) was associated with the secretions filling the amphidial duct 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 duct (65). 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 nonfunctional. In addition, electron microscopy indicated a difference in the morphology of amphidial secretions in the J2 and the adult female (66). Differences in the composition of amphidial secretions between J2 and females of *M. incognita* have also been demonstrated by Davis et al (18), who used a monoclonal antibody that reacted with the amphids of adult females but not with the amphids of J2. Thus, there are indications that at separate stages of the life cycle the amphids of some plant parasitic nematodes may have a different function or a different combination of functions or may be redundant.

Incubation of infective *M. javanica* J2 in the polyclonal antiserum for gp32 significantly retarded orientation of the nematode to host roots (66). Future work on gp32 needs to center 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*. From the work so far, it appears that this glycoprotein is involved directly or indirectly in the primary transduction of chemical stimuli.

The amphidial secretions of nematodes may serve to maintain electrical continuity between the bases and tips of the dendritic processes (67); this is thought to be important in the generation of receptor currents in some insect sensilla (76). Vogt et al (70) 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 synthesized and used as primers for PCR using nematode DNA. Analysis of the genomes of various nematodes, including *M. incognita*, *G. pallida*, and *H. glycines*, indicated that they contained genes similar to those coding for olfactory binding proteins in insects (40); sequence data following cloning are required to confirm whether the fragments amplified from nematodes code for OBP. Although this work indicates that the secretions of nematode and insect sense organs may contain similar molecules and have a similar role, more work is needed to resolve the apparent paradox between the presence of proteins binding olfactory molecules and the need for signal molecules to move through the aqueous environment inhabited by plant parasitic nematodes. In the amphidial ducts, signals may reach the receptors exclusively by diffusion (3), but recent laser microbeam studies involving ablation of the amphidial neurons of *C. elegans* have demonstrated that the accessory cilia were responsible for sensing certain volatile compounds (12). Sex pheromones of species of *Heterodera* and *Globodera* include volatile as well as nonvolatile fractions (31).

Direct biochemical analyses of amphidial secretions have been done on the animal parasitic nematode, *Syngamus trachea* (58). The amphidial gland secretes two major proteins of 36.0 and 41.5 kDa; antibodies to these secretions may be used to determine how conserved the proteins are by screening other nematode species. Given the large volume of secretions that were produced by amphids following chemical induction (56), direct biochemical analyses may also be possible on species of plant parasitic nematodes.

CHANGES IN THE ULTRASTRUCTURE OF THE AMPHIDS

As well as involvement with sensory perception, amphidial secretions may have additional roles (14, 74). For example, in some animal parasitic nematodes, such as *S. trachea* (37) and *Necator americanus* (49, 50), the amphids become greatly altered at certain stages of the life cycle: The sheath cell becomes enlarged and changes in ultrastructure have been 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 molts 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 function other than chemoreception.

Information is needed on the possible changes in the structure and function of amphids of plant parasitic nematodes at different stages of their life cycles, especially as research with antibodies, discussed in the previous section, has demonstrated differences in composition of amphidial secretions between active and sessile stages. Ultrastructural studies may help to elucidate the changing function of amphids during the nematode life cycle and the times when they are functional and thus can be targeted to disrupt sensory perception. In *G. rostochiensis* J2 changes were observed in the structure of the amphids during the hatching process (39). The absence of secretions 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 present in potato root diffusates (PRD). The change to a functional appearance is not associated specifically with exposure to PRD 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 (53), 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 (39).

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. Treatment of adult female *P. penetrans* with 5 and 10 ppm of the nematicide aldicarb resulted in hypertrophy of the internal dendrite terminals within the amphidial sheath cell and the appearance of large, electron-lucent granules in the amphidial sheath cell cytoplasm (68). 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.

Electron microscopical observations have provided information about the structure, development, and modification of the amphids and other sensilla, but their function can only be inferred. A link between structure and function is often difficult to make and may depend on specific conditions such as, for example, the occurrence of behavioral mutants of *C. elegans* with structurally altered amphids (44). 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 (12).

BEHAVIORAL ANALYSES OF NEMATODE RESPONSES

Chemicals that mediate inter- and intraspecific interactions between organisms are termed semiochemicals. Numerous bioassays have been designed to measure the responses of nematodes to semiochemicals (see 21–23, 33 for reviews). No single technique is superior; each has advantages and disadvantages. In the soil, nematodes move through a three-dimensional matrix and respond to gradients of a variety of stimuli. Ideally, test arenas should allow the nematode to move as naturally as possible, and some authors have attempted to use this approach. In the first demonstration of sex attractants in nematodes, Greet (30) used tubes of agar with males and females of the free living nematode *Panagrolaimus rigidus* separated from each other by a cellophane barrier and found that both sexes accumulated at the barrier. In order to assess movement more efficiently in a layer of soil, Luc et al (47) labeled *Hemicycliophora paradoxa* with radioactive phosphorus and then traced the nematodes by measuring the

variations in radioactivity in the soil at different places and times. Using this technique, they confirmed earlier observations (46) that host root diffusates were attractants and stimulated nematode activity. Since these pioneering studies, a considerable amount of information has accumulated on the behavioral responses of various species of plant parasitic nematodes to sex attractants and to roots. Factors other than those being tested may affect the distribution of nematodes in these and other three-dimensional systems, and the most frequently used behavioral bioassays are based on radial two-dimensional attraction gradients established in thin layers of agar. The nematode responses to the stimuli (usually evaluated on a time basis) can be seen as tracks in the agar, and contact prints can be made to record the tracks for subsequent analysis. Detailed analyses of movement patterns, together with the rate of movement away from or toward the test compound, can be obtained. Important differences in orientation behavior can be defined as taxis (directed movement towards the stimulus source) or kinesis (change of rate of movement in relation to stimulus intensity), for example.

Space constraints make it impossible to detail all the research on behavioral analysis of plant parasitic nematodes, but the two aspects already mentioned, the responses to sex pheromones and the responses to components of root activity, are important because of their relevance to possible novel control strategies based on the disruption of nematode sensoryperception.

Response to Sex Pheromones

The term pheromone refers to a chemical released by an organism that causes physiological or behavioral responses in another organism, usually of the same species. Although the original definition implied species specificity (42), interspecific communication between closely related species has also been classed as a pheromone response (33), and the more flexible definition may be appropriate to research on the sex pheromones of plant parasitic nematodes. Research on nematode sex pheromones has been extensively reviewed (16, 29, 32, 34), and most studies have been on the animal parasitic nematode, *Nippostrongylus brasiliensis*, and species of *Heterodera* and *Globodera*. Of ten species in *Heterodera* and *Globodera*, most females attracted more than one species of male, and most males responded to more than one species of female; probably at least six different male attractants exist in these genera (29).

Little is known about the chemical nature of the sex pheromones of plant parasitic nematodes. Early research indicated that they are polar, probably organic materials with several active components and are probably nearly neutral or amphoteric and physically stable. A substance with sex pheromone activity from females of *H. glycines* was isolated and identified as vanillic acid (35, 36), and the analog, syringic acid, has been used in preliminary field trials to disrupt

mating of this species. Vanillic acid did not attract males of *H. schachtii* (6) or *G. rostochiensis* (RN Perry & J Beane, unpublished information).

Response to Roots

The term allelochemic describes a chemical substance that causes a physiological or behavioral response between members of different species; thus, chemicals in plant root diffusates that elicit nematode responses are allelochemics. Studies on the ability of plant parasitic nematodes to locate host roots have used a variety of experimental conditions, and it is difficult to compare results from different behavioral bioassays. It is generally accepted that host roots are attractive to plant parasitic nematodes, but some authors have reported repulsion or a total absence of any effect (57). Although, in general, the attraction is nonspecific, there are indications that the attractiveness of a host to the pest species is correlated with its efficiency as a host (43, 69). Other factors, such as age of the root or presence of microorganisms, also condition the root's attractiveness; for example, attraction is lost when the root's growth is stopped or limited (45), but the reason for this is unknown. Bird (13) was the first to suggest that nematodes orientate along a potential gradient created by a lower redox potential at the root's surface; subsequent studies have demonstrated that nematode movement can be orientated by redox potential or an electric field created by the roots (62). The relative importance of chemical and electrical attractants has not been assessed.

Research on the chemical analysis of PRD has been motivated by the dependence of *G. rostochiensis* and *G. pallida* on PRD for hatching (54). Identification of specific hatching factors (54) still remains a possible precursor to novel control strategies for these nematodes and for *H. glycines*. Although the initiation of the hatching sequence may not involve chemoreception (39), PRD also stimulates movement of hatched J2 and may aid in host location, so chemical analysis of diffusates may have broader implications.

ELECTROPHYSIOLOGICAL ANALYSES OF NEMATODE RESPONSES

There are limitations and disadvantages to agar plate behavioral bioassays. The recent use of electophysiological techniques to analyze the response of intact nematodes to chemical stimulation is examined in detail here because it will support and considerably extend knowledge of nematode sensory perception. Direct electrophysiological recordings allow detailed analysis of responses to semiochemicals. The first electrophysiological recordings of responses from an intact nematode were made by Jones et al (38); 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 sex pheromone from adult female *G. rostochiensis*.

The small size of plant parasitic nematodes will make intracellular recordings similar to those obtained by Davis & Stretton (19, 20) from *Ascaris* and direct recordings from individual sense organs extremely difficult. However, the use of larger nematodes, such as *S. trachea*, enables direct extracellular recordings from individual sense organs. *Syngamus trachea* is a cosmopolitan parasite feeding on blood in the trachea of some species of birds belonging to the orders Galliformes and Passeriformes. The first recordings from a nematode sense organ were obtained directly from the cephalic papillae of this nematode, and changes in spike activity were monitored in response to blood when it was introduced into the liquid surrounding the nematode (38). This also demonstrated the chemosensory function of the cephalic papillae.

The most recent work on sensory responses of intact nematodes uses a modified electrophysiological assay, enabling more effective movement restriction and more detailed analysis of the extracellular recordings (59, 60). The research, outlined below, derives from past and current collaborative projects between IACR-Rothamsted and the University of Wales, Aberystwyth. The test nematode is placed in a plastic well containing the appropriate physiological buffer on an inverted microscope and is immobilized using a suction pipette, drawn out approximately to the diameter of the nematode, connected to a vacuum pump. The posterior part of the nematode's body is sucked inside the pipette to reduce movement, while the anterior end remains outside. The indifferent electrode is placed in the buffer solution close to the cephalic region of the nematode and, when plant parasitic or other microscopic nematodes are being used, the recording electrode, mounted on a micromanipulator, is inserted into the test nematode as close as possible to the cephalic region. It is difficult to ensure that the same cells are pierced every time, but, for each nematode species, individuals of the same age, sex, and size are used and the electrode is always inserted in the same site. With larger nematodes, such as *S. trachea*, the recording electrode can be inserted into an individual sensillum.

Each test solution is pipetted into the well where the nematode is immobilized. The test solution can be removed by perfusing the well with the appropriate buffer solution and then restarting the recording in the presence of buffer only. Cellular activity in the form of action potentials was recorded as a marked change, or spike, of electrical potential with time. These electrical signals were amplified, displayed on an oscilloscope, and stored on a digital audio tape recorder for subsequent analysis.

Extracellular recordings were obtained of the intra- and interspecific responses of *G. rostochiensis* and *G. pallida* males to sex pheromones from virgin

females (60). Electrical activity was recorded from individuals before, during, and after stimulation at 20°C, and differences in mean spike activity (from at least ten individuals) were taken to be significant when $P < 0.05$. There was a significant increase in spike frequency from males of *G. pallida* in response to sex pheromones from females of *G. pallida* and *G. rostochiensis*. The mean number of spikes/s before and after stimulation was 94 ± 2.2 and 480 ± 11.9, respectively, when stimulated with *G. pallida* sex pheromone and 80 ± 3.4 and 196 ± 7.9 spikes/s, respectively, when stimulated with *G. rostochiensis* sex pheromone.

The spike frequency produced by *G. rostochiensis* males increased significantly after the application of their homospecific pheromone. A typical recording (Figure 2) shows that when a male *G. rostochiensis* was stimulated with sex pheromone from female *G. rostochiensis* 30 s into the recording (arrow *A*), the frequency of the spikes produced by the cells of the cephalic region increased markedly from 80 spikes/s before stimulation to 249 spikes/s during exposure to sex pheromone; replacing the pheromone with buffer solution (arrow *B*, Figure 2) caused the spike frequency to reduce to 92 spikes/s. The mean values for spike activity before and during stimulation (86 ± 3.0 and 289 ± 12.5 spikes/s, respectively) were significantly different. This contrasted with the response of *G. rostochiensis* males to sex pheromone from female *G. pallida* where the mean values before and after stimulation (93 ± 3.2 and 103 ± 4.7 spikes/s, respectively) were not significantly different.

Thus, only *G. rostochiensis* males exhibit specific mate recognition; as *G. rostochiensis* is nearly sympatric worldwide with *G. pallida*, it is surprising that this is a facet of the reproductive strategy of one species and not the other. These results also demonstrate that sex pheromones of *G. rostochiensis* females can elicit both inter- and intraspecific responses, which is contrary to the strict definition of pheromones mentioned above. Further work examining responses to dilutions of the sex pheromones is necessary, and, for this type of study, the electrophysiological system has an advantage over agar plate behavioral bioassays. In agar plate assays, the exact concentrations of a test compound along a gradient are unknown, whereas in the electrophysiology system the concentration of a chemical to which the nematode is exposed in the plastic well can be determined. As many of the species of nematodes used so far will continue to respond to stimuli for up to 40 min, sequential exposure to stimuli is possible and is useful for evaluating the comparative responses to different fractions of a compound. Current work at Rothamsted is examining the relative response of males to fractions of the sex pheromone, separated using reverse phase high performance liquid chromatography.

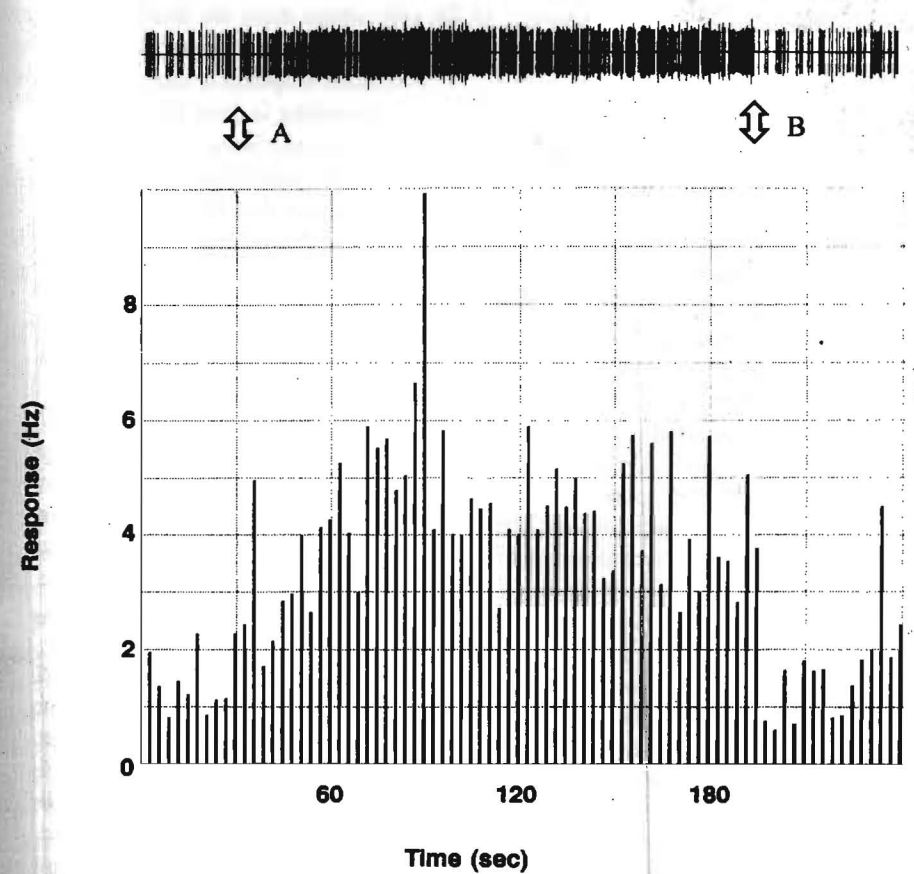

Figure 2 Extracellular recordings from the cephalic region of an adult male *Globodera rostochiensis* in response to sex pheromones from virgin females of the same species. Arrow *A* indicates when the stimulus was introduced and arrow *B* indicates when the stimulus was removed. The top of the figure shows actual spike events, while the histogram gives the accurate total spike frequency. From Riga et al (60).

Although there was a significant response by males of *G. pallida* to the sex pheromones from both *G. pallida* and *G. rostochiensis* females, the homospecific response, 480 ± 11.9 spikes/s, was greater than the heterospecific response, 196 ± 7.9 spikes/s. In the soil, where there are likely to be many semiochemicals eliciting responses and the weaker heterospecific recognition may be swamped, the very strong homospecific response is likely to be dominant. Thus, out-breeding is possible but in-breeding is more likely.

In contrast to the response to sex pheromones, exposure of males of both *G. rostochiensis* and *G. pallida* to PRD elicited no significant response (E Riga & RN Perry, unpublished information). It appears that PRD plays no part in orientating males. Males exit from the roots into the soil but probably remain in close proximity to the roots, apparently needing only sex pheromones to attract them to the sessile females protruding out of the roots.

Responses of males of *G. rostochiensis* and *G. pallida* to various chemicals have been tested and some interesting contrasts have been identified (E Riga & RN Perry, unpublished information). For example, when a male of *G. pallida* was exposed to L-glutamic acid the frequency of spikes increased from 17 spikes/s before stimulation to 266 spikes/s during stimulation; the frequency decreased to 12 spikes/s when the stimulus was removed. In contrast, exposure to D-glutamic acid did not elicit a similar response, with values for spikes/s before, during, and after stimulation of 30, 36, and 30, respectively. In insects, the D-isomer of many amino acids usually elicits a phagostimulatory response whereas many L-amino acids are feeding deterrents (51). Adult males of *G. rostochiensis* exit from the root and remain active for about 9 to 10 days during which time they mate with the females (25); the males probably have no need for food, but the stylet and pharyngeal glands appear functional, and it is possible that alternative food sources, such as fungi, are used. Chemoreception is essential for suitable food selection and the electrophysiological analysis of responses of various species of nematodes to specific plant compounds will provide information about feeding deterrents and stimulants that may relate to plant host suitability and the biochemical nature of the resistant response.

The amphids of *S. trachea* respond to serum collected from bird blood; these findings are consistent with a chemosensory role for the amphids (59). After the introduction of the stimulus, it took approximately 5–10 s before a neuronal response was visible when recording from the amphids. Two different spike types were then evident and could be distinguished easily by their amplitude, each representing the activity of an individual neuron: one had a small amplitude and started to respond 5 s after application of the serum, while the other had a large amplitude and responded approximately 12 s after stimulation. Thus, at least two amphid neurons respond to the presence of bird serum. The same pattern

of response from amphids of *S. trachea* was found when they were exposed to 100 mM D-tryptophan, but the responding cells started to show adaptation approximately 17 s after application of the solution (59). Chemosensory neurons of *C. elegans* have been reported to be multifunctional, with each neuron bearing more than one type of receptor (41).

The electrophysiological assays have the advantage of demonstrating that the difference in recognition of a stimulus is due to direct differences in sensory responses rather than to other factors, such as differences in mobility or activity of the nematodes, that can complicate interpretation of agar plate bioassays. Differences in sensory response can be quantified, spike activity before, during, and after stimulation can be compared, and the occurrence of sensory adaptation can be demonstrated. Control tests confirmed that the responses were to the stimulus and not to slight variations in temperature, osmotic pressure, or oxygen content, for example, associated with exposure to a fresh solution.

The electrophysiological technique offers exciting potential to analyze fractions of nematode excretory-secretory products, and subsequently it may be possible to purify sex pheromones and evaluate inter- and intraspecific responses as a basis for modifying, if necessary, the classification of chemicals that attract nematodes. Detailed evaluation of compounds responsible for attracting nematodes to plant roots will be possible, and the technique will also enhance understanding of the mode of action of control agents.

FUTURE RESEARCH

This article summarizes several current research approaches and indicates some potentially productive avenues for future work on sensory physiology and biochemistry. It is particularly important that research on sensory perception in plant parasitic nematodes is enhanced by an awareness of the literature from entomology, animal nematology, and *C. elegans* research groups.

Much of the information on the sensilla of plant parasitic nematodes is confined to the anterior sensilla, particularly the amphids. Research should expand to include detailed examination of other sensilla such as the phasmids that are present in some nematodes, including most of the plant parasitic forms, but not in others. Little is known of the likely specialized function of the phasmids. The secretory roles of the phasmids and amphids need to be defined in the context of possible functional changes at different stages of the life cycle. Although the main functions of the secretions are likely to be associated with the initial sensory events, it is especially important to determine if the amphidial secretions are involved in host-parasite interactions, particularly in the initiation and maintenance of the feeding site in sendentary endoparasitic nematodes. Immunology

and molecular biology provide the potential for identifying components of the secretions and cloning the encoding genes. However, it is important to recognize that pronounced secretory activity may not be a function of the sheath cell in the majority of nematodes (17) so comparative studies, using several species of nematodes from different genera, will be necessary to identify secretory components and to determine their degree of conservation.

Many research approaches will be required to examine the role and functioning of the sensilla and related aspects such as the presence and distribution of various neurotransmitters. The use of electrophysiological techniques will expand knowledge of sensory responses of plant parasitic nematodes. Several applications for this approach have been indicated in this review, and an additional one would involve laser microsurgery of individual sensilla and subsequent electrophysiological analyses of responses to known chemical stimulants. Laser microsurgery has been used successfully with *C. elegans* to ablate individual neurons and then to determine the effects on behavior.

Information on the role and functioning of the chemoreceptors of plant parasitic nematodes should be allied to research on the fractionation of nematode attractants, such as root diffusates and sex pheromones, to determine which fractions are active. Only when there is a comprehensive understanding of nematode chemoreception and of semiochemicals involved in the life cycles of plant parasitic nematodes, will it be possible to evaluate novel control strategies aimed at perturbing sensory perception and/or neurotransmission.

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