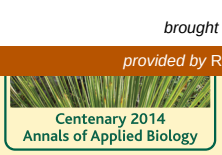

Annals of Applied Biology ISSN 0003-4746

CENTENARY EDITORIAL

The important legacy of the paper by Jones M.G.K. (1981) Host cell responses to endoparasitic nematode attack: structure and function of giant cells and syncytia. *Annals of Applied Biology*, 97, 353–372

I.G. Grove[1] & R.N. Perry[2]

1 Nematology/Agronomy Research, Crop and Environment Sciences, Harper Adams University, Newport, UK
2 Department of AgroEcology, Rothamsted Research, Harpenden, UK

Correspondence
I.G. Grove, Nematology/Agronomy Research, Crop and Environment Sciences, Harper Adams University, Newport, Shropshire TF10 8NB, UK. Email: igrove@harper-adams.ac.uk

doi:10.1111/aab.12103

All crop plants are parasitised by one or more species of plant-parasitic nematodes. The worldwide economic and social impacts are severe, especially in developing countries where loss of crops due to nematodes may be disastrous. In addition to the detrimental effects on the growth of the plants, causing stunting, early senescence and in severe cases total crop failure, the damage caused, especially to root crops, can render the produce unmarketable and eliminate income. Among the most economically important nematodes are those endoparasitic species that form complex feeding structures in the roots of their host plants; the most damaging are the root-knot (*Meloidogyne* spp.) and cyst (*Heterodera* and *Globodera* spp.) nematodes. With the worldwide spread of root-knot and cyst nematodes and the current estimates of loss of crop production from nematodes worldwide given as US$118 billion (Atkinson *et al.*, 2012), control of these injurious pests is imperative. In this context, the work of Jones (1981) helps us to understand how these obligate nematodes rely on the development of these specialised cells and how we can develop management or control strategies to reduce their impact on the world's food supply.

Although chemical control still remains an option (Haydock *et al.*, 2013), the decline in use or 'removal from use' of many nematicides because of adverse environmental impacts means that new strategies for nematode control and management are required. The need to find environmentally benign control or management options has provided the impetus for molecular studies of plant–nematode interactions, which will depend on a thorough understanding of the biology of the target species of nematode (Perry & Moens, 2011). In this context, perturbing the induction and maintenance of the feeding sites of endoparasitic nematodes, as is the method of the *Globodera* ssp. H1 resistance mechanism, may also provide other viable novel control strategies. Although root-knot and cyst nematodes have independently evolved the ability to make biotrophic feeding structures, the convergent evolution of the two groups has resulted in the same outcome: the nematodes become sessile within the roots and feed on self-induced nutrient sinks from the vascular system of the host plants. However, the method of achieving the end result and the feeding sites themselves show interesting differences.

Understanding the biology of the feeding sites underpins current research and the paper by Jones (1981) is a benchmark for detailing the structure and function of giant cells and syncytia. Although published over 30 years ago, this paper is still frequently cited and is directly relevant to research today. It summarises earlier work by Jones *et al.* on nematode-induced feeding sites (Jones & Northcote, 1972*a*,*b*; Jones & Payne, 1977, 1978) and extends the observations considerably to provide a seminal paper on the basic understanding of the feeding sites. The work focuses on the responses of susceptible hosts and also provides the essential comparative information for subsequent studies of the mechanisms of resistance that involve the inhibition of initiation, development or maintenance of feeding sites.

Using electron microscopy, Jones described the early stages of giant cell development in *Meloidogyne* after the nematode becomes sessile in the root. He demonstrated that the giant cells expand by repeated mitosis without cytokinesis and that the earlier reports of cell wall breakdown and coalescence of adjoining cells were inaccurate. Several large multinucleate giant cells result,

and cells surrounding them also enlarge resulting in a gall or root-knot. By contrast, a cyst nematode selects a cell, becomes sessile, and a multinucleate feeding site, termed a syncytium, is formed by cell wall breakdown resulting in the gradual incorporation of hundreds of adjacent cells as the intervening cell walls disintegrate. The detailed examination of the feeding sites of root-knot and cyst nematodes, and also sites induced by other endoparasitic species, such as the false root-knot nematodes (*Nacobbus* spp.), the reniform nematode (*Rotylenchulus reniformis*) and the citrus nematode (*Tylenchulus semipenetrans*), enabled Jones to present comparative information of the cytoplasmic changes, wall ingrowths and plasmodesmatal frequency. However, the paper also highlighted that knowledge on the cystoid nematode *Meloidodera* was severely lacking and only one paper related to cell response for that nematode could be cited.

Another aspect of the paper that deserves to be emphasised is that the feeding sites induced by various species of nematodes may be similar to, or part of, normal plant development. Jones details the cell modifications caused by root-knot and cyst nematodes and other endoparasitic nematodes and convincingly demonstrates that the feeding sites are derived from cell modifications similar to those found at various stages of plant growth. For example, nematode-induced feeding sites with cell wall ingrowths had been called 'transfer cells'; such transfer cells are a normal part of plant development, with transfer cells frequently modified from undifferentiated plant cells to facilitate short-distance nutrient transport. Thus, from this and other evidence, Jones concluded that endoparasitic nematodes directly influence normal cell function and suggested that the substances injected by the nematode into the plant cells were responsible for initiating cell modifications that are part of normal plant development; this leads to development of extensive feeding sites or nutrient sinks. It is now clear that the formation of feeding sites is the result of changes in gene expression and resultant reprogramming of undifferentiated plant cells.

Substantial discussion within the paper also highlighted the importance of the nematode–phytohormone interactions and it is now clear that there is an evident role for phytohormones in facilitating changing gene expression profiles (Goverse & Bird, 2011) and any changes to hormone levels or balance could affect this. Ithal *et al.* (2007) not only acknowledge the role of auxins and ethylene in feeding cell development but also suggest roles for jasmonic and gibberellic acids. Consequently, beyond the feeding cell itself, any researcher investigating plant growth effects of nematode invasion must acknowledge their potential to alter growth patterns and physiological responses. Similarly, when studying plant hormones within plants, it would be pertinent to ensure that any growing media is either sterilised or checked for the presence of nematodes.

The importance of nematode-derived secretions, indicated by Jones and earlier by Bird (1968), formed the basis for justifying research into the secretions from the dorsal and subventral pharyngeal glands. We now know that in both root-knot and cyst nematodes, secretions of proteins and other components, principally from the dorsal pharyngeal gland, play a central role in the induction and maintenance of the feeding sites (Sobczak & Golinowski, 2011; Jones & Goto, 2011). The genes whose products enable the nematode to invade host plants and set up feeding sites have been called 'parasitism genes' or the 'parasitome' (Gao *et al.*, 2003) but are now called effectors. They offer attractive targets via RNA interference (RNAi) to disrupt the nematode life cycle. Identifying such parasitism genes is essential to understand the molecular basis of nematode parasitism of plants. Hussey *et al.* (2011) cloned parasitism genes by directly microaspirating the cytoplasm from the pharyngeal gland cells of different parasitic stages of cyst or root-knot nematodes to provide mRNA to create a gland-cell-specific cDNA library and confirmed gland-specific expression by *in situ* hybridisation.

Much research has been done on the dorsal pharyngeal glands but the subventral glands play a major role in parasitism, including during migration of endoparasitic nematodes through the root. Investigation of secretions from the subventral glands has revealed cell-wall-degrading enzymes in both root-knot and cyst nematodes, which are secreted through the stylet to facilitate migration by weakening or breaking down cell walls. Among the enzymes identified in both nematode groups are cellulases and pectate lyases, and in root-knot nematodes xylanase and polygalacturonase (Davis *et al.*, 2000; Gheysen & Jones, 2006). Interestingly, these enzymes had been reported previously only from plants and pathogenic bacteria and fungi and it is probable that they were acquired by horizontal gene transfer from bacteria to plant-parasitic nematodes (Jones *et al.*, 2005).

For the cyst nematodes, *Heterodera* and *Globodera* spp., it was also speculated that the material that formed the feeding plug where the stylet pierces the cell wall appeared to originate from the amphids of the nematodes. Jones *et al.* (1994), working with *Globodera rostochiensis*, found no evidence to support this but Maule & Curtis (2011) report that for *Heterodera glycines* the amphids are still postulated as the source for this plug, although noting that even now it is an area still under debate.

Jones (1981) speculated on the mechanism whereby feeding sites provide continuing sinks for water and nutrient provision for the developing nematodes. There have been detailed studies on the chronological

changes in syncytia induced by the cyst nematode *Heterodera schachtii* in *Arabidopsis*, including plasmodesmata appearance (Hofmann *et al.*, 2010) and symplasmic phloem-unloading (Hofmann *et al.*, 2007), but there is a paucity of information on other nematode–host associations. Although it is evident that short-distance and long-distance transport of water and nutrients into the giant cells and syncytium occurs, Grundler & Hoffmann (2011) point out that it is still unclear what compels the plant continually to supply the feeding sites with large amounts of nutrients and how the switch from apoplastic to symplastic solute supply is regulated.

An understanding of the intimate relationship between the nematode, its modified feeding cell and the plant is also of great importance when investigating and quantifying the effects of the nematode on the plant. The majority of the nematodes cited are suggested to draw their nutrients directly from vascular tissues, whereas the nurse cells of *T. semipenetrans* appeared to draw nutrients from the symplastic cortical cells. The work by Kallel *et al.* (2005) now supports this cortical cell role and these authors have reported the presence of tubular structures forming a trophic network extending beyond cells adjacent to the nematode that ensure the exchange of nutritive materials. Consequently, nematodes that utilise giant cells or syncytia surrounding the vascular system, such as *Globodera* and M*eloidogyne* spp., will be taking sucrose directly into their feeding cells and so will have a direct draw on carbohydrate that is intended for root growth and development, thus greatly decreasing crop yields, disrupting normal root growth and restricting plant water and nutrient uptake; all effects reported by De Ruijter & Haverkort (1999) and Melakeberhan *et al.* (1987) and others. With *T. semipenetrans*, it could be more complicated as by utilising starch outside the endodermis, and possibly the endodermal cells, it is not tapping into the direct delivery of sucrose from leaf to root growing points, so could be seen as having less direct effect on growth and development. However, there may be feedback from the plant, responding to reduced starch in the cortex and/or endodermis by directing sucrose to replace the starch losses. This feeding cell variation of *T. semipenetrans* could therefore be a reason for the slow decline of the citrus host even under high nematode pressure. Also, as starch in the roots is a source of energy for regrowth (for instance after winter in non-annual species), *Tylenchulus* may have an effect on the speed of regrowth.

Jones also draws attention to the lack of work connected with endodermis damage by the nematodes and how this may lead to a predisposition to attack by other plant pathogens. The recent work of Bhattarai *et al.* (2010) with *Globodera pallida* and Walker (1997) with *Meloidogyne* spp. demonstrates how this is indeed the case when *Rhizoctonia solani* infection is increased in the presence of these nematodes. Several reports show that *Meloidogyne* spp. increase the prevalence of *Verticillium* and *Fusarium* disease, while Jonathan *et al.* (1996) demonstrate interactions between *Meloidogyne incognita* and *Phytophthora*. Jones also refers to the metabolite leakage accompanying the root damage. If this leakage has similar effects to, or alters, root exudates, it could be influential in complex disease development as affected by changes to the rhizosphere flora mediated by changes to the nutritional quantity and quality of the exudates (Riedel, 1988).

Thus, many questions remain to be answered in order to fully understand the sophisticated host–parasite interaction of sedentary endoparasitic nematodes. Jones (1981) in his seminal paper has provided the basis for such research and with the increasing number of plant-parasitic nematode genomes and the importance of comparative genomics more information will be available, not just for root-knot and cyst nematodes but also for other endoparasitic nematodes, such as *Nacobbus* spp., *R. reniformis* and *T. semipenetrans*, whose feeding sites also Jones had studied in detail.

References

Atkinson H.J., Lilley C.J., Urwin P.E. (2012) Strategies for transgenic nematode control in developed and developing world crops. *Current Opinion in Biotechnology*, **23**, 251–256.

Bhattarai S., Haydock P.P.J., Back M.A., Hare M.C., Lankford W.T. (2010) Interactions between field populations of the potato cyst nematode *Globodera pallida* and *Rhizoctonia solani* diseases of potatoes under controlled environment and glasshouse conditions. *Nematology*, **12**, 783–790.

Bird A.F. (1968) Changes associated with parasitism in nematodes IV. Cytochemical studies on the ampulla and dorsal esophageal gland of *Meloidogyne javanica* and on exudations from the buccal stylet. *Journal of Parasitology*, **54**, 897–890.

Davis E.L., Hussey R.S., Baum T.J., Bakker J., Schots A. (2000) Nematode parasitism genes. *Annual Review of Phytopathology*, **38**, 365–396.

De Ruijter F.J., Haverkort A.J. (1999) Effects of potato cyst nematodes (*Globodera pallida*) and soil pH on root growth, nutrient uptake and crop growth of potato. *European Journal of Plant Pathology*, **105**, 61–76.

Gao B., Allen R., Maier T., Davis E.L., Baum T.J., Hussey R.S. (2003) The parasitome of the phytonematode *Heterodera glycines*. *Molecular Plant–Microbe Interactions*, **16**, 720–726.

Gheysen G., Jones J.T. (2006) Molecular aspects of plant–nematode interactions. In *Plant Nematology*, pp. 234–254. Eds R.N. Perry and M. Moens. Wallingford, UK: CABI.

Goverse A., Bird D. (2011) The role of plant hormones in nematode feeding cell formation. In *Genomics and Molecular Genetics of Plant–Nematode Interactions*, pp. 325–327.

Eds J. Jones, G. Gheysen and C. Fenoll. Dordrecht, the Netherlands: Springer.

Grundler F.M.W., Hoffmann J. (2011) Water and nutrient transport in nematode feeding sites. In *Genomics and Molecular Genetics of Plant–Nematode Interactions*, pp. 423–439. Eds J. Jones, G. Gheysen and C. Fenoll. Dordrecht, the Netherlands: Springer.

Haydock P.P.J., Woods S.R., Grove I.G., Hare M.C. (2013) Chemical control of nematodes. In *Plant Nematology*. 2nd edn, pp. 459–477. Eds R.N. Perry and M. Moens. Wallingford, UK: CABI.

Hofmann J., Wieczorek K., Blochl A., Grundler F.M.W. (2007) Sucrose supply to nematode-induced syncytia depends on the apoplasmic and symplasmic pathways. *Journal of Experimental Botany*, **58**, 1591–1601.

Hofmann J., Ashrey A.R.N., Anwar S., Erban A., Kopka J., Grundler F.M.W. (2010) Metabolic profiling reveals local and systemic responses of host plants to nematode parasitism. *Plant Journal*, **62**, 1058–1071.

Hussey R.S., Huang G., Allen R. (2011) Microaspiration of esophageal gland cells and cDNA library construction for identifying parasitism genes of plant-parasitic nematodes. *Methods in Molecular Biology*, **712**, 89–107.

Ithal N., Recknor J., Nettleton D., Maier T., Baum T.J., Mitchum N.G. (2007) Developmental transcript profiling of cyst nematode feeding cells in soybean roots. *Molecular Plant–Microbe Interactions*, **20**, 510–525.

Jonathan E.I., Sivakumar M., Padmanabhan D. (1996) Interaction of *Meloidogyne incognita* and *Phytophthora palmivora* on betelvive. *Nematologia Mediterranea*, **24**, 341–343.

Jones M.G.K. (1981) Host cell responses to endoparasitic nematode attack: structure and function of giant cells and syncytia. *Annals of Applied Biology*, **97**, 353–372.

Jones M.G.K., Goto D.B. (2011) Root knot nematodes and giant cells. In *Genomics and Molecular Genetics of Plant–Nematode Interactions*, pp. 83–100. Eds J. Jones, G. Gheysen and C. Fenoll. Dordrecht, the Netherlands: Springer.

Jones M.G.K., Northcote D.H. (1972*a*) Multinucleate transfer cells induced in coleus roots by the root-knot nematode, *Meloidogyne arenaria*. *Protoplasma*, **75**, 381–395.

Jones M.G.K., Northcote D.H. (1972*b*) Nematode-induced syncytium – a multinucleate transfer cell. *Journal of Cell Science*, **10**, 789–809.

Jones M.G.K., Payne H.L. (1977) The structure of syncytia induced by the phytoparasitic nematode *Nacobbus aberrans* in tomato roots, and the possible role of plasmodesmata in their nutrition. *Journal of Cell Science*, **23**, 299–313.

Jones M.G.K., Payne H.L. (1978) Early stages of nematode-induced giant cell formation in roots of *Impatiens balsamina*. *Journal of Nematology*, **10**, 70–84.

Jones J.T., Perry R.N., Johnston M.R.L. (1994) Changes in the ultrastructure of the amphids of the potato cyst nematode, *Globodera rostochiensis*, during development and infection. *Fundamental and Applied Nematology*, **17**, 369–382.

Jones J.T., Furlanetto C., Kikuchi T. (2005) Horizontal gene transfer from bacteria and fungi as a driving force in the evolution of plant parasitism in nematodes. *Nematology*, **7**, 641–646.

Kallel S., Louhichi A., B'Chir M.M., Van Oostveldt P. (2005) Structure du site trophique induit par *Tylenchulus semipenetrans* Sur bigaradier observé en microscopie photonique, Confocale et électronique à transmission. *Nematologia Mediterranea*, **33**, 171–178.

Maule A.G., Curtis R. (2011) Parallels between plant and animal parasitic nematodes. In *Genomics and Molecular Genetics of Plant–Nematode Interactions*, pp. 221–253. Eds J. Jones, G. Gheysen and C. Fenoll. Dordrecht, the Netherlands: Springer.

Melakeberhan H., Brooke R.C., Webster J.M., D'Auria J.M., Cackette M. (1987) Effect of *Meloidogyne incognita* on plant nutrient concentration and its influence on the physiology of Beans. *Journal of Nematology*, **19**, 324–330.

Perry R.N., Moens M. (2011) Introduction to plant-parasitic nematodes; modes of parasitism. In *Genomics and Molecular Genetics of Plant–Nematode Interactions*, pp. 3–20. Eds J. Jones, G. Gheysen and C. Fenoll. Dordrecht, the Netherlands: Springer.

Riedel R.M. (1988) Interactions of plant-parasitic nematodes with soil-borne plant pathogens. *Agriculture, Ecosystems and Environment*, **24**, 281–292.

Sobczak M., Golinowski W. (2011) Cyst nematodes and syncytia. In *Genomics and Molecular Genetics of Plant–Nematode Interactions*, pp. 61–82. Eds J. Jones, G. Gheysen and C. Fenoll. Dordrecht, the Netherlands: Springer.

Walker G.E. (1997) Effects of *Meloidogyne* spp. and *Rhizoctonia solani* on the growth of grapevine rootings. *Journal of Nematology*, **2**, 190–198.