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Forum article

HOST PLANT INFLUENCES ON THE HATCHING OF CYST NEMATODES

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Summary – The hatching pattern of selected cyst nematodes is compared with especial emphasis on species having several generations during a single host growing season. The changing hatching pattern of cysts produced at different stages of plant growth is an important attribute in relation to future research on the influence of the host plant, and particularly the syncytium, on the biology of the developing nematodes.

Résumé – ***Influences de la plante-hôte sur l'éclosion des nématodes à kyste*** – Une comparaison est faite concernant le schéma d'éclosion entre certains nématodes à kyste et plus particulièrement entre espèces présentant plusieurs générations pendant une seule période de croissance de l'hôte. Les modifications du schéma d'éclosion des kystes lors des différents stades de la croissance de la plante est un point important pour de futures recherches concernant l'influence de la plante-hôte – et plus particulièrement des syncitiums nutritionels – sur la biologie des nématodes en cours de développement.

Key-words : cyst nematodes, dormancy, hatching, host plant, nematodes, survival, syncytium.

Co-evolution of cyst nematodes with their hosts has resulted in a sophisticated interrelationship which enhances the chances of nematode invasion, multiplication and survival. The nematode life cycle is frequently synchronised with that of the host to optimise the chances of successful invasion. The dependence of species like *Globodera rostochiensis* on host root diffusates to stimulate hatch is well documented and the sequence of events in the hatching process has been the subject of extensive research (Perry, 1987, 1989). Potato root diffusate has a bimodal effect on *G. rostochiensis* : it causes a Ca^{2+} – mediated change in the permeability of the lipid layer of the eggshell (Perry, 1986) and it directly affects the second stage juveniles (J2) by stimulating movement (Weischer, 1959; Clarke & Hennessy, 1984) and the dorsal oesophageal glands (Atkinson *et al.*, 1987; Perry *et al.*, 1989).

It is also evident that the activity of the hatching factors in root diffusates is not constant throughout the life of some host plants but declines as the plant ages. This is apparent from the results of *in vitro* hatching tests with *Heterodera goettingiana,* for example, where substantial hatch occurred in diffusates from 4- and 6-week-old pea plants but diffusates from 2- or 10-week-old plants elicited hatches of less than 10 % (Perry *et al.*, 1980). Clearly, this aspect, plus the fact that a percentage of cyst contents are refractory to hatch stimulation initially, ensures that there is a " carry over " population after host senescence or crop harvest.

The cyst is central for the survival of species of cyst nematodes; even J2 of species such as *Heterodera sacchari, H. oryzae* and *Heterodera oryzicola,* endemic in areas where drying conditions are present, have no intrinsic ability to survive desiccation once hatched and, like temperate species, are dependent for survival on the protection afforded by the eggshell and cyst (Ibrahim & Perry, 1992). Thus, modifications to enhance survival and persistence have centred on the hatching response.

In this short article, the hatching pattern of selected cyst nematodes will be contrasted to illustrate the range of hatching strategies which ensures infection and development and also enables the populations to survive the intercrop period. It is not intended to be an exhaustive review of the literature but aims to focus on species of cyst nematodes in which the hatching pattern of juveniles from cysts produced at different stages of host plant growth varies, even when exposed to root diffusates with optimum hatching activity. Consideration of this aspect of the complex host-parasite relationship may indicate ways of investigating the host effects, presumably via the feeding female, on J2 hatching.

Cyst nematode hatching strategies

The contrasting hatching behaviour of cyst nematodes may be related to different strategies for survival (Perry, 1989). Cysts of *G. rostochiensis* and *G. pallida* are resistant to environmental extremes and unhatched J2

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can survive for 20 or more years in the absence of a host crop. Hatched J2 are susceptible to environmental extremes and their infective life is only 6-11 days under optimal conditions for motility (Robinson *et al.,* 1987). Hatching is dependent on host root diffusates, ensuring synchrony between emergence of infective J2 and the presence of roots at the initial stages of host growth (Perry, 1987). In contrast, J2 of the beet cyst nematode, *H. schachtii,* hatch well in water without the need for stimulation by host root diffusates; under non-host crops this results in population decline rates of about 50-60 % per year (Cooke, 1987). In a careful analysis of the hatching of *H. schachtii,* Zheng and Ferris (1991) identified different types of dormancy in encysted eggs. Some J2 hatch very readily and infect any host plants present, in others hatching is delayed which permits infection of any surviving plants; the remaining J2 do not hatch readily, thus increasing their chances of survival in the absence of a host. However, the primary attribute of *H. schachtii* ensuring persistence must be that, compared to *Globodera* species, *H. schachtii* has a wide host range, encompassing 218 hosts within 95 genera from 23 families (Steele, 1965), and populations can be supported on weeds and other alternative hosts between crops.

Comparison between *G. rostochiensis* and *H. schachtii* illustrates the two extremes of host influence on hatching response and usually relates to the situation of a single generation during the host growing season. However, the 8-month duration of the sugar-beet crop enables *H. schachtii* to complete two generations per year in Western Europe and five in California (Williams, 1978). Other species of cyst nematodes, especially those from the tropics, complete several generations during the host growing season and demonstrate different strategies for survival. Some tropical species are similar to *G. rostochiensis* and *G. pallida* in their hatching response. For example, J2 of *H. oryzicola* are always dependent on host root diffusate for substantial hatch, irrespective of generation, and can remain dormant until the subsequent growing season (Ibrahim *et al.,* 1993). In contrast, research on *H. cajani, H. sacchari* and *H. sorghi* has demonstrated a change in dependence of successive generations on root diffusates during a crop growing season (Gaur *et al.,* 1992, 1995; Ibrahim *et al.,* 1993). This is an important facet of the host-parasite interaction that has not been studied extensively. It will be examined in detail in the next section as it could be used as a basis for examining changes in the syncytia of different age plants which may relate indirectly to changes in juvenile biochemistry and physiology.

Hatch of cyst nematodes with several generations per year

Heterodera cajani, found on plant species of the families Leguminosae and Pedaliaceae in India, has a short life cycle of 17-22 days at 29 °C and completes several generations in a single crop season. Many eggs are deposited into the large eggsac which is sometimes almost double the size of the cyst. An analysis of the hatching from cysts and eggsacs of six successive generations of *H. cajani* produced on cowpea during a single growing season in glasshouse pot cultures demonstrated that the majority of eggs in eggsacs hatched within 7 days in distilled water, soil leachate or host root diffusates irrespective of generation (Gaur *et al.,* 1992). The eggs in eggsacs hatched more readily than those in cysts and probably provided J2 for rapid further invasion and multiplication; this is also the situation with *H. oryzae* (Merny, 1966), *H. glycines* (Ishibashi *et al.,* 1973), *H. carotae* (Greco, 1981) and *H. cruciferae* (Koshy & Evans, 1986). It is unclear whether the more rapid hatch from eggsacs is due to differences in maturation of J2 in cysts and eggsacs or to differences in the physiology of the J2 from the two sources or is merely a reflection of the extra time needed after hatch from encysted eggs for the J2 to escape from the cyst.

In contrast to the hatching of eggs from eggsacs, the hatching response of J2 in cysts of *H. cajani* varied according to the age of the host plant on which they were produced. Hatch from cysts was similar in all solutions over the first four generations but in the fifth and sixth generations, produced on senescing plants, 18-22 % of the eggs in the cysts required host root diffusate to stimulate hatch; in the final generation, the encysted J2 contained more lipid reserves than those in the eggsac (Gaur *et al.,* 1992). The advantage of using *H. cajani* as an experimental model is that the generation time is short and cysts from sucessive generations can be separated. Although it is not possible to separate generations quite so readily with other species, the change in hatching response can be assessed by selecting new cysts from host plants of different age. Using this approach, a change in the hatching pattern was found with *H. sacchari.* With the onset of plant senescence, females of this species developed into cysts which contained approximately 20 % more eggs which were refractory to hatching stimuli and an additional 10-15 % which depended on host root diffusate for hatch stimulation, compared to cysts produced on younger plants (Ibrahim *et al.,* 1993); this 30-35 % of viable, dormant J2 provides a " carry-over " between crops.

Dormancy has been separated into quiescence and diapause : quiescence is an arrest in development induced in response to unfavourable conditions and development is resumed soon after the return of favourable conditions whilst diapause is a condition in which development has been arrested and cannot be resumed until specific requirements have been recognised, even if favourable conditions return (Evans & Perry, 1976). The J2 of *H. sacchari* and *H. cajani* which hatch immediately on stimulation with host root diffusate are quiescent and those J2 of *H. sacchari* which are refractory to

host diffusates, even under favourable conditions, are likely to be in a state of diapause. There are at least three kinds of eggs in cysts of *H. sorghi* : ones that hatch freely in soil leachates, those that require stimulation from host root diffusates to hatch and a large percentage which do not hatch immediately; the proportions of these three types of eggs changed with successive generations (Gaur *et al.,* 1995). The change in hatching behaviour of *H. cajani, H. sacchari* and *H. sorghi* ensures that a large proportion of J2 from the later generations do not hatch and are protected by the egg and cyst during the intercrop period. The increased lipid reserves found in encysted J2 of *H. cajani* in the final generation, combined with the lowered metabolism associated with quiescence (Evans & Perry, 1976), are likely to enhance considerably the period that encysted J2 can remain viable.

In these experiments, females produced on the plant at various intervals experience host tissue which changes physiologically and biochemically, not only from the effects of senescence but also from the adverse effects of invading and feeding nematodes (Wallace, 1987). In an alternative experimental approach, Singh and Sharma (unpubl.) inoculated pigeon pea plants of different ages with *H. cajani.* Emergence of J2 from first generation cysts produced on 30-day-old plants was 15-25 % greater than hatched from equivalent cysts produced on 60-, 90-, 120-, and 150-day-old plants. This supports the view that changes related to the host affect developing nematodes and their subsequent hatch.

Possible factors involved in hatch variations

Changing conditions during the host growing season clearly influence hatch, and variations in the pattern of hatching reflect the requirements of the nematode to multiply during host growth and survive after host senescence. Age of the host plant is not the only factor affecting hatch and inducing dormancy. For example, Hill and Schmitt (1989) found that eggs in cysts of *H. glycines* produced on senescing soybeans hatched more freely than those produced on vegetative plants. The tenor of the present review indicates that this is the converse of what might be expected but Hill and Schmitt (1989) suggested that decreasing temperatures may be more important than soybean phenology in inducing dormancy. However, it is useful to focus on the host influence on hatching because it points to some interesting questions.

The factors directly causing the change in hatching response, even under optimal conditions, are unknown. Compared to eggs which hatch in water, the eggs of *H. cajani, H. sacchari* and *H. sorghi* which are dependent on root diffusates for hatch either have a different structure or contain unhatched J2 which are in a modified physiological state, perhaps involving the induction of obligate quiescence (Evans & Perry, 1976); both possibilities warrant further investigation. There is relatively little variation in the average size of eggs throughout the Nematoda and egg size appears not to be related to the size of the adult. However, there is considerable variation in thickness and complexity of nematode eggshells and the most complex eggshells are found in species where eggs need to survive adverse environmental conditions (Wharton, 1980). Nematodes may increase the chance of survival by providing a resistant eggshell rather than by increasing the size of the embryo. Quantitative information on the partitioning of resources into eggshell and egg content are required. If the change in hatching response was a consequence of change in eggshell structure, then this would be an intraspecific variation caused by biotic factors influencing the egg-laying female. Such a change in eggshell structure would presumably involve the presence of lipoprotein membranes whose permeability has to be altered by diffusates before hatching can commence; an increased trehalose content of the perivitelline fluid would also be involved.

The possible changes in juvenile physiology and eggshell structure are clearly worth investigating but, perhaps more importantly, the possible changes in the syncytium with age of host plant may be crucial to the feeding female and the development of the dependent juveniles. The presence of compounds and/or the absence of key nutritional elements in the syncytium associated with plant senescence, may be the trigger for induction, in the J2, of diapause and concomitant preparation for a period of survival in the absence of a suitable host. Diapause is not an intrinsic property of the eggs of *G. rostochiensis* and Hominick (1986) considered that photoperiod, acting on the potato plant, affected the developing females of *G. rostochiensis* and influenced the hatching mechanism of the developing juveniles.

The effect of food availability on nematode development

The physiological state of the host plant is known to influence egg deposition and hatching. Retardation of plant growth by no-nutrient or short photoperiod treatments resulted in fewer eggs being deposited into smaller eggsacs on cysts of *H. glycines* compared with control plants receiving full nutrients under long-day treatment (Ishibashi *et al.,* 1973). The number of J2 per cyst changes during the host growing period. This has been noted with *H. cajani* (Gaur *et al.,* 1992), *H. sacchari* and *H. oryzicola* (Ibrahim *et al.,* 1993). Although the mean number of eggs per cyst varies between species, with some species having consistently fewer eggs per cyst than others, the females of *H. cajani,* for example, do not continue feeding until they reach a certain size or until they contain a set number of eggs. In general, under constant environmental conditions for plant growth, there is a set developmental time from initiation of feeding to the end of egg deposition. Thus, differences in the

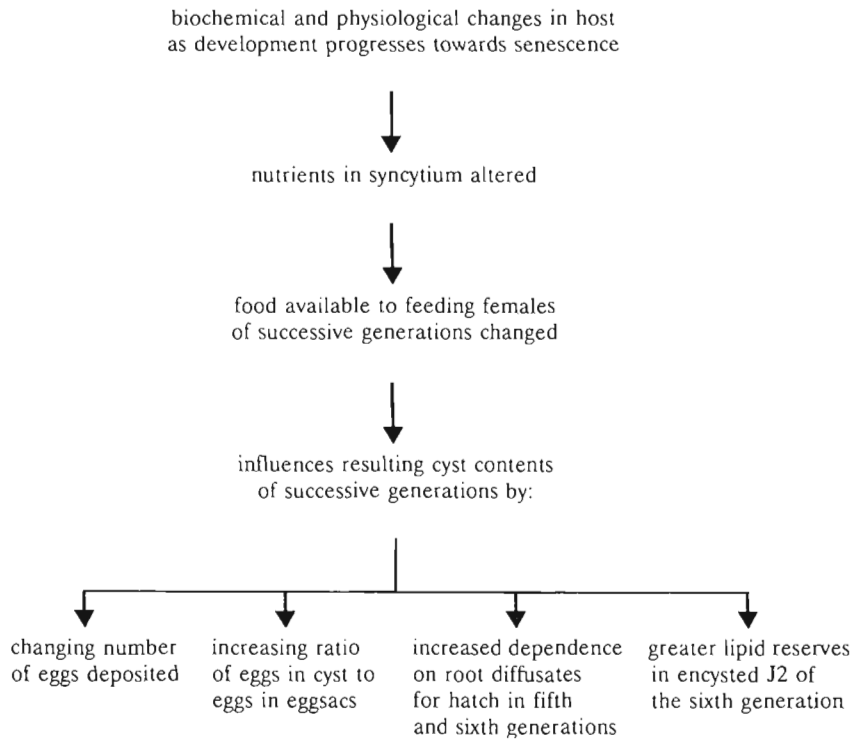

Fig. 1. *Known changes in successive generations of* Heterodera cajani *with possible preceding causes. It may be possible to elucidate nutrient changes by comparing syncytia induced by successive generations.*

number of J2 are more likely to relate to the food intake and lower numbers in the earlier generations may relate to food limitation. In the experiments by Gaur *et al.* (1992) with *H. cajani,* the mean number of eggs produced per female progressively increased from 125 in the first generation to a maximum of 477 in the fifth generation; this declines to 343 in the sixth generation produced on senescing plants. Although the plant energy requirements will be considerable, especially during the initial weeks of growth, it is difficult to envisage that this will effectively limit the food available from the syncytium. However, this appears the most likely explanation : the availability of nutrients, or the balance of nutrients, that the feeding female is able to extract from the syncytium at the early stages of plant growth is less than ideal for the maximum number of eggs per female.

Nematode development is clearly dependent on plant metabolism and Van Haren *et al.* (1994) demonstrated that the energy available to feeding *H. schachtii* from its syncytium determines its growth and fecundity. A large feeding site will have a large area of contact with tissues of the host and conduct more nutrients. Essentially, as Bird (1972) demonstrated with *Meloidogyne,* large feeding sites result in large nematodes and small sites in small nematodes; egg size is unaffected. Future studies on cyst nematodes with multiple generations during a host growing season could examine this basic premise further by determining changes in the syncytium of different nematode generations or at different stages of plant growth and relating this to J2 biomass produced by the feeding females. The apoplastic fluorescent tracer, fluorescein, has been used to quantify ingestion rates of *G. pallida* (van Haren, 1995) and this approach may be useful in a comparative study of ingestion rates of females of different generations of species such as *H. cajani.*

Although quantity of food may relate to the number of eggs per cyst, the quality of food is important and is likely to influence directly nematode biochemistry and physiology. Elements present in syncytia have been investigated by scanning electron microscopy and energy dispersive X-ray microanalysis and changes were found in the elemental composition of syncytia compared to unmodified tissue (Cook *et al.*, 1992). For example, syncytia induced by *H. trifolii* in white and red clover had more Mg and P than unmodified tissue; syncytia induced in wheat by *H. avenae* had greater P and S

concentrations but Mg was unchanged (Cook *et al.*, 1992). There were variations in elemental composition with age of syncytia induced by *H. trifolii* and the greatest changes were associated with maturing nematodes during egg production. It may be feasible to use this technique to compare the elemental composition of syncytia at a comparable developmental phase but from host plants of different ages. The composition of nutrients available to the feeding nematode is unlikely to remain constant but may vary during senescence and under the influence of other established nematodes. Amino acids have been known for some time to influence the development of nematodes and Betka *et al.* (1991) demonstrated that glutamine enhanced development of *H. schachtii* whereas methionine, phenylalanine, lysine and tryptophan inhibited development. Techniques such as the microinjection method (Böckenhoff & Grundler, 1994), have great potential for examining aspects of nutrient uptake and it may be possible to use this method to examine changes in the syncytium. For modelling population dynamics of cyst nematodes with annual multiple generations, it should be possible to adapt the dynamic energy budgets model used by van Haren (1995) for *G. pallida.*

Conclusion

The study of host-cyst nematode interactions and the influence of the host plant on the biology of the developing nematodes can be considerably advanced if research focuses on the syncytium (Fig. 1). This article illustrates possible productive avenues for future work with cyst nematodes and demonstrates the advantages of using as the experimental model cyst nematodes which have several generations during a host growing period.

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