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# Host plant effects on hatching of root-knot nematodes

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**Summary.** Dormancy and the hatching response enable hatching of many plant-parasitic nematodes to be synchronised with the presence of host plants. There is evidence that the host plant, especially during the onset of senescence, influences the physiology of the developing, unhatched juveniles. Hatching of juveniles of *Meloidogyne* is primarily temperature driven and usually occurs without requiring stimulus from host root diffusates. However, root diffusates sometimes stimulate hatching and research has demonstrated that hatching of some species of *Meloidogyne* is influenced by the host plant. Thus, there are parallels between some root-knot and cyst nematodes in aspects of their hatching response in relation to survival, and understanding these characteristics is an important basis for effective management strategies.

**Key words:** diapause, dormancy, hatch, host-parasite interaction, *Meloidogyne*, quiescence.

## INTRODUCTION

The hatching behaviour of many species of parasitic nematodes is an essential component of the life cycle for optimising the chances of successful infection by synchronization with host availability (Perry & Clarke, 1982; Perry, 2002). In addition, the ability to remain unhatched and protected within the egg when hosts are not available is an integral part of the survival strategies of nematodes. Investigating the hatching response in relation to the survival attributes of economically important plant-parasitic nematodes is important for effective management strategies. Although there is considerable information about the hatching responses and the cascade of events after hatch stimulation of cyst nematodes, especially *Globodera* spp. (reviewed by Perry, 2002), there is less information about the hatching biology of species of *Meloidogyne*.

This brief article, based on an invited plenary paper given by the first author at the Meeting of the Russian Society of Nematologists (Petrozavodsk, July 2007), describes the hatching biology of some species of root-knot nematodes to illustrate important parallels between the root-knot and cyst nematodes in their hatching responses in the context of the host influence on their survival attributes.

## HATCHING RESPONSE OF *MELOIDOGYNE* SPP.

**General.** The simplistic description of the hatching of *Meloidogyne* is that hatch of the second-stage juveniles (J2) occurs if environmental conditions, such as appropriate temperature, oxygen availability and soil moisture levels, are suitable and there is an absence of physiological barriers, such as diapause. In the context of hatching in relation to survival, diapause is an important component. Diapause is defined as a state of arrested development where development does not continue until specific requirements have been satisfied, even if favourable conditions return; it is contrasted with quiescence, which is a spontaneous reversible response to unfavourable environmental conditions and is readily reversible when favourable conditions return (Evans & Perry, 1976). Both diapause and quiescence are forms of dormancy. The existence of diapause in some species of *Meloidogyne* has been demonstrated and this area of research has been summarised by Jones *et al.* (1998) and Wright & Perry (2006). However, in this review we shall focus on the effects of host root diffusates and show that the physiological barriers to hatching of *Meloidogyne* include factors other than diapause.

**Hatching mechanism.** Females of root-knot nematodes lay eggs into a gelatinous matrix that shrinks and hardens when dried (Bird & Soeffky, 1972), thus exerting mechanical pressure on the eggs to inhibit hatching of J2 during drought conditions. Hatched J2 are vulnerable to environmental stresses and they are viable in the soil for periods much shorter than if they had remained unhatched. In addition to the gelatinous matrix, the eggshell affords protection to the enclosed J2. Species of *Meloidogyne* have been separated into two survival groups, thermophils and cryophils, based on their ability to survive lipid-phase transitions that occur at 10°C (Van Gundy, 1985). This grouping also relates to hatching; for example, *M. chitwoodi and M. hapla* are cryophils and can hatch at temperatures below 10°C, whereas *M. incognita and M. javanica* are thermophils and do not hatch at temperatures below 15°C.

The hatching process of cyst nematodes has been divided into three phases: changes in the eggshell, activation of the J2, and eclosion (Perry, 2002). In *Meloidogyne*, activation of the J2 precedes, and probably causes, changes in eggshell structure, in contrast to cyst nematodes, such as *Globodera rostochiensis*, where changes in the eggshell initiate the hatching sequence (Perry, 2002). Bird (1968) suggested that in *M. javanica* enzymes from the pharyngeal glands of the J2 caused hydrolysis and flexibility of the eggshell. Subsequently, Perry *et al.* (1992) demonstrated that the hatch of *M. incognita* was positively correlated with lipase activity in the hatching fluid (Fig. 1) and proteinase, including collagenase, and chitinase activity also were detected; these enzymes are likely to erode layers of the eggshell resulting in increased flexibility of the eggshell prior to eclosion. The flexible egg of *M. incognita* has about 30% internal free space, which allows the J2 to be fully hydrated and active by the time of eclosion (Ellenby, 1974). In *M. javanica*, the anterior end of the J2 projects into the flexible eggshell and stylet thrusts cause a tear through which the J2 escapes. In this scenario, hatching of J2 of *Meloidogyne* occurs once the J2 has fully developed. In contrast to many cyst nematodes, where root diffusates are required to change the eggshell permeability characteristics and initiate hatching, it is the *Meloidogyne* J2 itself that changes the eggshell structure prior to hatching.

Thus, in general, hatch of *Meloidogyne* occurs without requiring specific cues from host roots (Perry, 1987). As with all generalisations, there are exceptions and responses to root diffusates may be more important than previously realised. Root diffusates can affect the rate of hatch (i.e., the number of J2 that hatch per unit time), and some J2 depend on root diffusate to initiate the hatching process.

**Dependence of some J2 of *Meloidogyne* spp. on root diffusate.** There is evidence that root diffusates can enhance the rate of hatch of some species of *Meloidogyne*. Brzeski & Hendricks (1971) were among the first to note this when they reported that hatch of *M. hapla* was enhanced when stimulated by host root diffusates. However, enhancement of the rate of hatch is less well documented than the changes in hatch in relation to host age. The sophisticated host-parasite interaction of *Meloidogyne* extends to modifications in hatching response as a reflection of changes in cues from host root diffusates.

For cyst nematodes with multiple generations during a crop growing season, it is well established that a large proportion of J2 from later generations do not hatch but remain protected by the egg and cyst during the period between crops (Perry & Gaur, 1996; Perry, 2002). An increasing body of evidence demonstrates that a similar variation in hatching between generations occurs with some species of *Meloidogyne*. As long ago as 1969, Ishibashi considered that old or poorly nourished females of *M. incognita* produce brown egg masses containing eggs with dormant J2, which are resistant to environment stresses and nematicides and which depend on hatch stimulation by host root diffusates. By contrast, young and well nourished females produce white egg masses susceptible to environmental stresses but from which J2 hatch readily in water without needing diffusate stimulation. Immediate hatching of J2 from white egg masses ensures more than one generation per host growing season, whilst the dormant J2 in brown egg masses ensures carry-over of infective J2 from one season to the next. Gaur *et al.* (2000) showed that females of *M. triticoryzae* from India produced three types of eggs: those that hatch in water, those that hatch in host root diffusates and those that do not hatch even in the presence of diffusate. The proportion of these three types varies with generation, with the final generation produced on senescing plants having a large proportion of unhatched juveniles of the third type, which is likely to equate with diapause. The difference between generations in hatching of J2 from egg masses produced at different stages of plant growth illustrates the influence of the plant on development and subsequent hatching. As Perry & Gaur (1996) pointed out, this reflects a change of priority for the

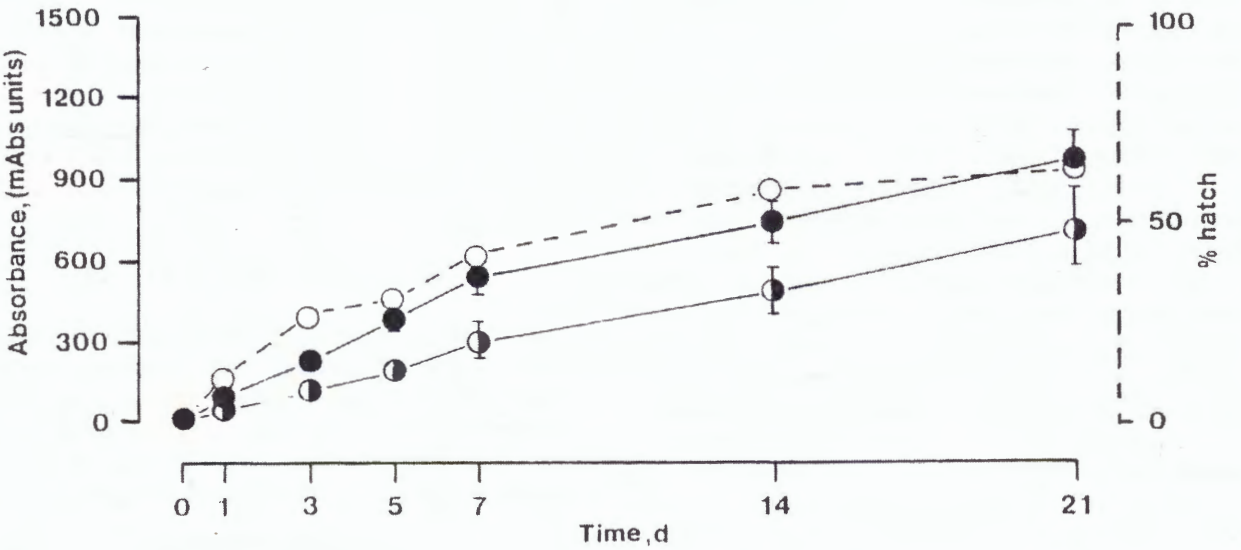


**Fig. 1.** Cumulative lipase activity, assayed at pH5 ( ● ) and 8 ( ◗ ), and percentage hatch ( ○ ) of *Meloidogyne incoginita* in glass distilled water over a three-week period. Enzyme activity is expressed in absorbance units as mean ± standard error of three separate analyses. (From Perry *et al.*, 1992.)

species during the host plant growing season from rapid re-infection of young plants to survival after host senescence.

The hatching response of species that co-exist may also differ. *Meloidogyne chitwoodi* and *M. fallax* cause severe damage to economically important crops and, in 1998, both species were listed as quarantine pests in Europe. Comparative studies were made on the effects of root diffusates and host age on the *in vitro* hatching of *M. chitwoodi* and *M. fallax* (Wesemael *et al.*, 2006). There is a marked contrast in the hatching response of the two species. Hatching of J2 of *M. chitwoodi* produced on young plants did not require host root diffusate stimulus, whereas at the end of the plant growing season, egg masses contained a percentage of unhatched J2 that require host root diffusate to cause hatch. This form of obligate dormancy at the end of the host growing season was not found in *M. fallax*. This species hatched well in water and did not require hatch stimulation from root diffusate, irrespective of the age of the plant on which the egg masses were produced. The factors causing the change in hatching response of species such as *M. triticoryzae* and *M. chitwoodi* are unknown.

In the research on *M. chitwoodi* and *M. fallax*, egg masses were exposed to optimum *in vitro* conditions for hatch, including diffusate from 6-week-old plants that had maximum hatching activity (Wesemael *et al.*, 2006). Work on cyst nematode hatching has demonstrated that the production of active diffusate declines with the onset of plant senescence (Perry, 1997) and, in several plant species, it is confined to a short period of plant growth. This is an additional factor ensuring that J2 do not hatch at a time when their food source is senescing but carry-over to the next crop growing season; research on this aspect is needed for *Meloidogyne* species.

**Egg numbers and embryogenesis.** Other differences between *M. chitwoodi* and *M. fallax* were noted by Wesemael *et al.* (2006). The number of eggs per egg mass for *M. fallax* collected on senescing plants was significantly greater than the number of eggs per egg mass for *M. chitwoodi*, and the number of eggs per egg mass of *M. chitwoodi* decreased with plant age. Together with the fact that 90% of J2 of *M. fallax* from egg masses on senescing plants hatched in soil leachate this might indicate that a greater number of J2 of *M. fallax* remains in the soil after crop harvest compared with *M. chitwoodi*. However, survival as hatched J2 requires energy (Van Gundy, 1985), and it will be interesting to determine the comparative rate of lipid depletion during a winter period; Robinson *et al.* (1987) showed that if *Globodera* spp. utilised 50% of their energy reserves, there was insufficient left to enable successful invasion of plant roots.

Although no quiescent unhatched J2 were found in egg masses of *M. fallax* obtained from senescing plants, there was a small proportion (4–14%) of unembryonated eggs that did not contain developed J2. Unembryonated eggs were also

found in *M. chitwoodi*, and in both species the percentage of these eggs increased with plant age, although the increase was not significant. This type of arrest in development is a well-documented survival strategy of nematodes, and is evident in many species of animal-parasitic nematodes (Perry & Clarke, 1982). However, cessation of development during embryogenesis does not appear to be a major component of the survival strategy of cyst nematodes, where all the evidence points to continued development to fully formed infective J2, which is the 'survival' stage. The arrest in development during embryogenesis of species of *Meloidogyne* was investigated in detail by de Guiran & Villemin, (1980) and reviewed by Evans (1987), who concluded that it could not be considered as a diapause as there was no element of periodicity linked to season.

## CONCLUSIONS

The differences between the hatching responses of species of *Meloidogyne* such as *M. chitwoodi* and *M. fallax* may be linked to different survival strategies. *Meloidogyne chitwoodi* seems to have a strategy for survival in the inter-crop period that is centred on quiescent J2, which hatch only in the presence of root diffusates, and delayed development of unembryonated eggs. By contrast, survival of *M. fallax* seems to be based on delayed development of embryonating eggs and, presumably, the ability of hatched J2 to survive in the soil. The greater number of eggs per egg mass of *M. fallax* collected on senescing plants indicates that a large number of J2 will be in the soil.

It is clear that some species of *Meloidogyne* have hatching attributes that are similar in certain respects to those of some cyst nematodes. Cyst nematodes show a spectrum of reliance on root diffusates to stimulate hatch (Perry, 2002), from almost total dependence in species such as *G. rostochiensis*, to an absence of any need for diffusate stimulation in temperate populations of *H. schachtii*. Although, in general, species of *Meloidogyne* are less dependent on root diffusates, it is interesting to note the increased dependence of species such as *M. chitwoodi* and *M. triticoryzae* on diffusate stimulation as plants senesce. The variations in hatching in relation to generation and plant age require more investigation to expand knowledge of the survival strategies of *Meloidogyne*.

The variations in hatching response have important implications for effective detection of plant-parasitic nematodes by advisory and statutory bodies. Soil sampling and subsequent incubation has to take into account delayed hatch, and the presence of dormant J2 and unembryonated eggs. Knowledge about the condition of the host plant can help to optimise the incubation conditions and to avoid an underestimation of the nematode population. This is clearly of paramount importance when assessing the viability of quarantine species of plant-parasitic nematodes.

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**Perry R. N., Wesemael W. M. L.** Воздействие растения-хозяина на вылупление яиц галловых нематод.

**Резюме.** Способность пребывать в состоянии покоя и вылупляться лишь при воздействии соответствующих внешних сигналов позволяет нематодам растений синхронизировать их жизненный цикл с растением-хозяином. Имеющиеся данные показывают, что растение-хозяин, особенно в конце вегетативного сезона, оказывает значительное влияние на физиологию развивающихся и еще не вылупившихся личинок галловых нематод. Вылупление личинок *Meloidogyne* инициируется, в первую очередь, температурой среды, и зачастую может происходить без какого-либо стимулирующего эффекта корневых диффузатов. Однако, в некоторых случаях, корневые диффузаты стимулируют данный процесс. Некоторыми исследованиями также было доказано воздействие растений-хозяев на вылупление отдельных видов *Meloidogyne*. Таким образом, можно отметить некоторые аналогии между галлообразующими и цистообразующими нематодами во взаимосвязи между стимулами, оказывающими влияние на вылупление, и общей стратегией выживания. Понимание особенностей вылупления нематод составляет основу для разработки стратегии контроля этих вредителей.