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Characterisation of mechanisms involved in hatching of the potato cyst nematode



by

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Front picture: Field attacked by potato cyst nematodes. Photographer: Stig Andersson, SLU Alnarp.

1. Summary

The potato (*Solanum tuberosum*) is one of the most important foods in the world. Estimates of yield losses in potato production have concluded that potato cyst nematode (PCN) is responsible for tuber losses of 9 % per annum. Attack of PCN is without doubt one of the most serious pest problems in the Swedish potato cultivation and also in other places around the world. Because of this it is of great importance to try to develop a control method. This can be done by studying nematodes complex hatching mechanism and find a way that alters the nematodes hatching mechanism.

In Sweden there are currently two species of PCN, the golden potato cyst nematode (*Globodera rostochiensis*) and the white potato cyst nematode (*G. pallida*), both of which are obligate parasites of the family *Solanaceae*. Potato is the most important host plant, followed by tomato (*Lycopersicon esculentum*), aubergine (*Solanum melongena*) and different weeds in the family *Solanaceae*, which ensures that nematodes survive even if the potato is not present.

This review of the literature examined some of the factors affecting PCN hatching factors, e.g. potato root diffusate (PRD), temperature, soil, potato cultivars, biotic factors and signals between plant and organism. According to the literature the white PCN hatches at lower temperatures than the yellow. This may be an advantage for the white PCN in a competitive situation in cold climates. It is important to note that the white and yellow PCN differ in some hatching factors even if they are closely related species. This enables them to develop differently and have different reproductive abilities. The hatching mechanism of the white and yellow is equal. Both PCN- species must be stimulated with the PRD to hatch. PRD is the most important factor in the hatching mechanism because it initiates a response in juveniles.

2. Sammanfattning

Potatis, *Solanum tuberosum*, är en av de viktigaste livsmedelkällorna i världen. Uppskattningar har blivit gjorda på avkastningsförluster i produktion av potatis där det kom fram att PCN svarat för årliga förluster på ungefär 9 %. Angrepp av potatiscystnematoder är utan tvivel ett av de allvarligaste växtskyddsproblemen i svensk potatisodling och även på andra ställen runtom i världen. På grund av detta är det av stor vikt att försöka utveckla en kontrollmetod. Detta kan bli gjort genom att studera nematodernas komplexa kläckningsmekanism och finna ett sätt som förändrar nematodernas kläckningsmekanism.

I Sverige finns i dag två arter av potatiscystnematoden (PCN), *Globodera rostochiensis*, den gyllene potatiscystnematoden och *G. pallida*, den vita potatiscystnematoden. Båda arterna av PCN är förplikta parasiter av familjen *Solanaceae*. Potatis (*Solanum tub.*) är den viktigaste värdväxten följt av tomat (*Lycopersicon esculentum*), äggplanta (*Solanum melongena*) och olika ogräs i familjen *Solanaceae*. Att livnära sig på olika arter garanterar nematoden att överleva även om den viktigaste värdväxten inte är närvarande.

I denna litteraturstudie undersöks några av de olika kläckningsfaktorer, PRD (potatis rot diffusat), temperatur, jordförhållanden, potatissorter, biotiska faktorer och signaler mellan växter och organismer. Enligt den genomgångna forskningen har det visat sig att den vita potatiscystnematoden kläcker vid lägre temperaturer än den gula. Detta kan vara en fördel för den vita potatiscystnematoden i en konkurrenssituation i kalla klimat. Det är viktigt att tänka på att den vita och den gula potatiscystnematoden skiljer sig åt vid vissa kläckningsfaktorer fast att de kommer från samma familj. Detta gör att de utvecklas olika och har olika reproduktionsförmågor. I kläckningsmekanismen är den vita och den gula likvärdiga då de kläcks på samma sätt. Båda potatiscystnematodarterna måste bli stimulerade med PRD för att kläckas. PRD är den viktigaste faktorn i kläckningsmekanismen.

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4. Introduction

The potato (*Solanum tuberosum*) is one of the most important food sources in the world (FAO, 2008). It was introduced from Peru to Spain around 1550 but at that time it was acclimatised to the day length in the tropics and could not produce tubers in Europe, so it was grown as an ornamental plant. Around 1720, Jonas Alströmer imported potatoes to Sweden and also published a book about potato growing. Commercial potato production started first in 1800 and by around 1850 potatoes had become an important food source in Sweden (Olsson, 2005).

Potato production in Sweden is concentrated to three main regions, Skåne, Västergötland and Halland, but potatoes are also produced in Blekinge and Östergötland. In 2003, the commercial acreage was 21 752 ha of table potatoes and 8 587 ha of potatoes for processing (Manduric, 2004).

There are two species of potato cyst nematode (PCN) in Sweden, the golden potato cyst nematode (*Globodera rostochiensis*) and the white potato cyst nematode (*G. pallida*). Both species are obligate parasites of the family *Solanaceae*. Potato is the most important host plant, followed by tomato (*Lycopersicon esculentum*), eggplant (*Solanum melongena*) and different weeds in the family *Solanaceae* (Andersson & Eriksson, 2001). Feeding on different plant species allows the nematode to survive even if the host plant is not present (Perry, 1998). Factors in the high survival of PCN in soil are its high reproductive potential, its ability to live in cold climates and the ability of juveniles to survive in cyst form for 20-25 years (Turner & Evans, 1998).

PCN has most likely been present in Europe since 1600-1700, arriving with infested potato tubers from South America. Now it has spread over almost all the world (Andersson & Eriksson, 2001). Estimates of yield losses in potato production have concluded that PCN is responsible for tuber losses of 9% per annum (Devine & Jones, 2001).

In Sweden, *G. rostochiensis* is the most common species (Anderson & Eriksson, 2001). It has been endemic in Sweden since 1922, when it was found in Högsjö, Södermanland (Kemner, 1927), while *G. pallida* has been present since 1965 (Manduric, 2004). The two species of PCN are divided into different phatotypes according to multiplication ratio on different potato species. In Europe there are five phatotypes (Ro 1-5) of *G. rostochiensis* and three (Pa 1-3) of *G. pallida* (Kort *et al.*, 1977). Because of the difficulty in classifying them into phatotypes, they are normally divided into virulence groups. Phatotypes

of *G. rostochiensis* comprise three different virulence groups (Ro1, 4; Ro2, 3; and Ro5) while phenotypes of *G. pallida* comprise two groups (Pa1, Pa2 and 3) (Trudgill, 1985).

4.1 Symptoms caused by PCN

Symptoms that occur within plants infested with PCN include disruption of water and nutrient uptake, mechanical injury to cells and tissues, cell death, avenues of ingress for other organisms and modified cell development and function. The visual symptoms of plant are stunting, slow growth, chlorosis, nutrient deficiencies, wilting, reduced yield and various root symptoms such as root tip stunting, root tip thickening and lesions on the root caused by secondary parasites (Andersson & Eriksson, 2001).



Figure 1. Stunted potato foliage growth caused by PCN (Photo: Stig Andersson).

4.2 Distribution

PCN can be spread by potato tubers, soil, equipment and machinery used in the field. They can also be spread by wind and water (Evira, 2006).

Problems with PCN are prevalent in many continents and countries (Canadian Food Inspection Agency, 2007):

- **Africa:** Algeria, Egypt, Libya, Morocco, Sierra Leone and South Africa.
- **Asia:** India, Japan, Pakistan and Sri Lanka.
- **Central America and Caribbean:** Costa Rica and Panama.
- **Europe:** All countries except Turkey.
- **Middle East:** Lebanon.
- **North America:** Canada, USA and Mexico.
- **South America:** Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Peru and Venezuela.

PCN are quarantine pests and are regulated by different directives specific for different regions. Within the European Union, the occurrence of PCN must be reported according to Commission Directive 2007/33/EC, which requires each Member State to send an annual report on PCN status to the Commission and to the other Member States (EU, 2007).

4.2.1 Management of PCN

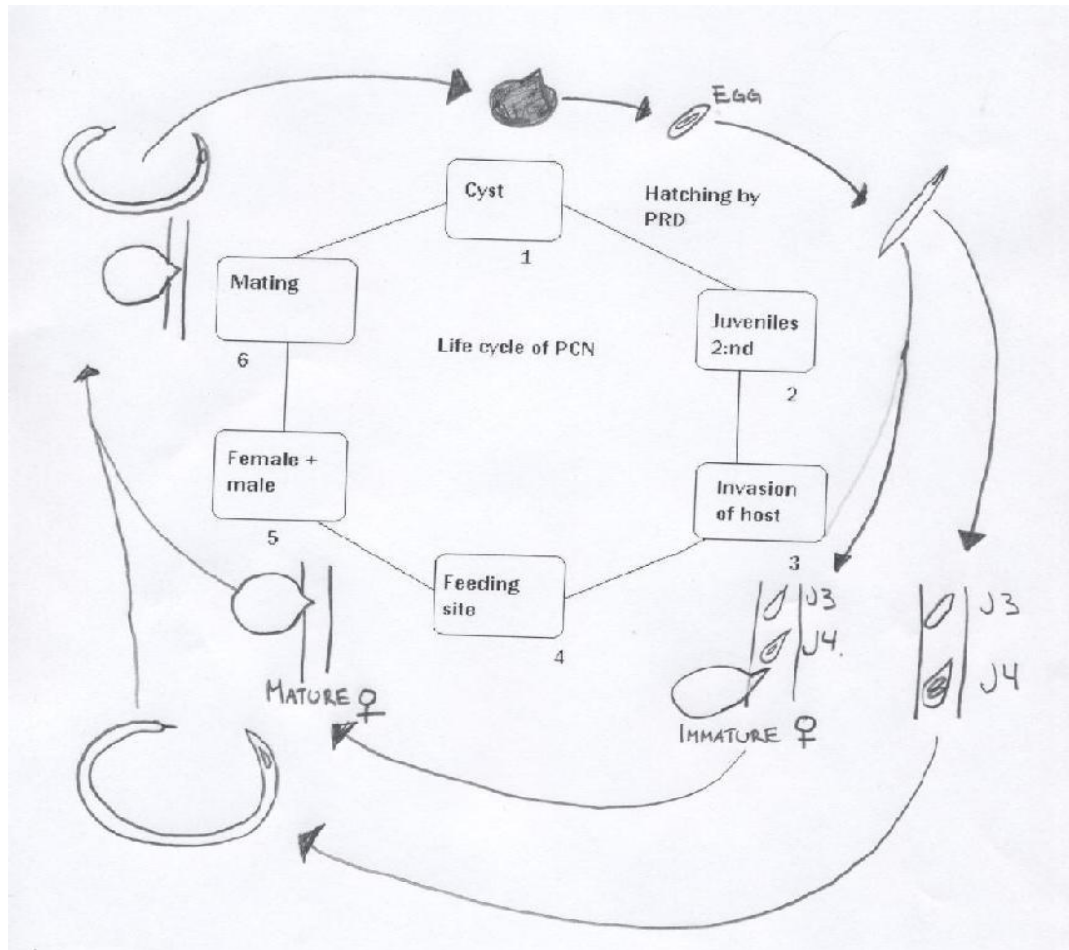
Management of PCN is difficult. Various control strategies have been developed, including chemical control, crop rotation (Evans & Haydock, 2000) and nematode resistance in different potato cultivars. From an economic point of view the resistant cultivars are the best option in managing PCN, but are not always available. Some control methods such as chemicals and hot water treatment are recommended to eradicate nematodes in the soil. To prevent nematode spread by machinery, it is important to have compulsory cleaning of all machinery after use (Evira, 2006). To reduce the risk of heavier infestations, use of an appropriate crop rotation is recommended. The crop rotation must include resistant cultivars so that adult nematodes die of starvation (Karssen and Moens, 2006).

4.3 Aim

The aim of this study was to carry out a systematic review of the literature on factors involved in the hatching of the golden potato cyst nematode (*Globodera rostochiensis*) and the white potato cyst nematode (*G. pallida*).

5. Biology

Both *G. rostochiensis* and *G. pallida* juveniles hatch from cysts containing eggs (Figure 2), in response to hatching factors around the cyst. When the potato plant starts to develop roots, the production of root diffusate (PRD) begins. PRD is at its peak within the two to three weeks after the start of root production, but the production of small concentrations of PRD continues for the rest of the growing period (Robinson *et al.*, 1987).



6.

Figure 2. Life cycle of potato cyst nematodes (modified from Lee, 2002).

PCN have four juvenile stages and one adult stage (Figure 2). When the second stage juvenile (J2) hatches from the egg, the active part of the life cycle starts. It is the J2 that invade the host plant (Andersson, 1997), generally in spring/early summer (Evira, 2006). At the third juvenile stage (J3), females start to develop into a saccate shape (Figure 2). The adult females rupture through the root tissue so that the posterior body part lies exposed and ready for mating. After the J3 stage the males do not feed. They emerge from the root into the soil as adults (Andersson, 1997).

When the females are sexually mature, they release sex pheromones which attract the adult males in soil. After mating the female starts to produce up to 300-400 eggs. The female then dies and her body becomes chemically hardened to form a protective cyst (Awan & Hominick, 1982; Cronin *et al.*, 1997). Eventually the cyst becomes detached from the roots at the end of the growing season (Evira, 2006) and remains dormant in soil until presence of a suitable host crop is detected. Dormancy is a response to unfavourable conditions, associated with a lowered metabolic rate (Awan & Hominick, 1982; Cronin *et al.* 1997). The

dormant period allows the nematode to survive until favourable conditions return and nematodes can persist in soil in cyst form for more than 20 years (Karssen & Moens, 2006).

6. Hatching factors

There are three major factors that have an effect on nematode cyst hatching, namely host root diffusate, physical conditions before and during juvenile emergence, and hereditary preconditions of the cyst (Manduric, 2004).

The hatching mechanism of PCN has been shown to be stimulated by PRD and by different hatching factors such as temperature, oxygen availability, soil type and soil water content.

6.1 Potato root diffusate

Peak production of PRD is confined to a short period in the plant's life and in this short period the juveniles are stimulated to hatch. Maximum activity of PRD is reached two weeks after planting (Perry, 2002). Two classes of chemicals have been found in PRD, hatch inhibitors and hatch stimulants. Studies of PRD have indicated that it contains at least four to six different hatching agents (chemical stimulants). The inhibitors are produced at an earlier stage than the stimulants, protecting the plant during its most sensitive stage of growth (Byrne *et al.*, 1998). PRD is highly mobile in soil and studies have shown an effect on hatching 80 cm from the potato root (Turner and Rowe, 2006).

Recent research has concluded that the compound Solanoeclepin A, which occurs naturally in potatoes, is one of the agents involved in hatching of nematode cysts (Figure 3). Its structure has been determined by researchers in the Netherlands and it may be suitable for use in an environmental-friendly control method for nematodes (Blaauw *et al.*, 2001).

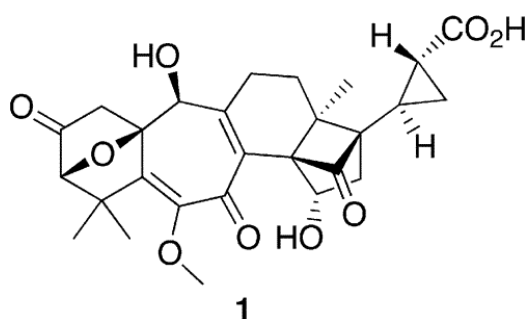


Figure 3. Solanoeclepin A - one of the hatching agents for PCN (Blaauw *et al.*, 2001).

6.2 Temperature

Temperature is an important factor in the hatching of PCN. PCN requires a relative stable temperature regime to hatch (Perry, 1998). According to Franco *et al.* (1998), the optimum hatching temperature in the field is 13.4 °C but substantial emergence can be observed at 10 °C.

The optimum temperature for *G. rostochiensis* hatching *in vitro* is 20 °C. This is a temperature that is unlikely to be reached in soil under the normal hatching period in Europe, which is generally in April. *G. pallida* is adapted to lower temperatures (16 °C) (Robinson *et al.*, 1987).

At the optimum temperature the fastest growth, reproduction, *etc.* occur. At temperatures below and above the optimum, the rates are slower or death occurs (Wharton, 2002).

6.3 Soil and water status

Soil structure and soil water status are important factors for maximum hatching of PCN and all other soil-borne nematodes. Coarse-textured soils favour hatching and when the water content is at field capacity the maximum hatching occurs. Drought and water-logging inhibit the hatching of plant parasitic nematodes (Perry, 2002).

6.4 Potato cultivar

Potato cultivars can be divided into fully susceptible, partially susceptible and resistant cultivars. A fully susceptible plant allows the nematode to multiply free on the roots while the partially susceptible plant allows less multiplication. For the resistant cultivars multiplication does not occur, but the larvae can still enter (Whitehead & Turner, 1998).

6.5 Signals between plants and organisms

Nematodes and other organisms are influenced by host signals as regards hatching, host location and selection, feeding-site location, sex determination and intra-host migration. Signals contain information, which react with a receptor and elicit a response. Most signals between organisms and their host plant/s are based on phytochemicals from the host plant, but the organism can also release chemicals to the host plant. The host plant then starts to defend itself (Hirsch *et al.*, 2003).

6.6 Biotic factors

Studies have shown that soil micro-organisms living in the rhizosphere play a significant role in the hatching of cyst nematodes. According to Ryan and Jones (2004), who tested 70 rhizobacterial isolates,

nine of the isolates had a significantly effect on hatching. Six of these affected the hatching of *G. pallida* and three affected *G. rostochiensis*. None of the isolates affected both species. The PCN attained optimum hatching, 43-100% more hatching, when treated with a potato isolate that had been soaked in PRD for 48 h, while no effect was found when treated with isolate without PRD (Ryan and Jones, 2004).

7. Response to hatching agents

The response to hatching has been shown to differ between the two PCN species. After hatch stimulation, a series of physical and metabolic changes occur in the unhatched juvenile (Robinson *et al.* 1987). The hatching response of PCN cysts to PRD is generally fast. After very short exposure (5 min) the PCN juveniles are triggered to start substantial hatching from the cyst (Forrest & Perry, 1980). Tests *in vitro* on *G. pallida* show that it has a much slower initial rate of hatch compared with *G. rostochiensis* (Robinson *et al.*, 1987).

7.1 Life inside the egg

In general, at least three requirements must be fulfilled for efficient hatch: the physiological mechanism of the nematode must be operating efficiently; the nematode must be highly mobile; and it has to possess the anatomical requirements for locomotion and penetration out of the egg (Wallace, 1973).

Movements inside the egg can be considered an adaptation that enables the nematode to reach a pitch of physiological efficiency at the time it hatches. The stylet, which is necessary for the escape out of the cyst, is produced. The juvenile of both PCN species makes a line of overlapping punctures with its stylet, forming a slit through which the larva escapes (Wallace, 1973).

7.1.1 Egg shell permeability

According to Perry *et al.* (1982), the effect of PRD stimulation is a change in metabolic activity that increases the permeability of the lipoprotein membrane of the eggshell, allowing the egg to take up water. Egg shell permeability is affected by a change in calcium ion concentration, which changes the structural lipids (Clarke *et al.* 1978). These PRD-induced changes in egg shell permeability and subsequent water uptake by the juvenile allow the passage of water and flourachrome through the unhatched juvenile, causing it to hatch (Perry & Feil, 1986). Water uptake by the juvenile also increases oxygen consumption inside the egg (Clarke & Perry, 1977; Atkinson & Ballantyne, 1977). The changes in egg shell permeability occur during the first 24 h after exposure to PRD, but it takes 3 days for juvenile hatching (eclosion) to take place (Perry & Feil, 1986).

7.2.1 Metabolic response

When the changes in the juvenile have occurred it starts the behavioural sequence leading to hatching, during which the juvenile goes through three different stages (Figure 4): widespread exploration when the juvenile becomes fully mobile inside the egg, local exploration when only the head of the juvenile is moving while the lip region is pressed to the eggshell, and the stage when the juvenile cuts its way out of the eggshell with the stylet (Ellenby, 1974). After hatching the juvenile takes up more water before it emerges from the cyst (Perry, 1998).

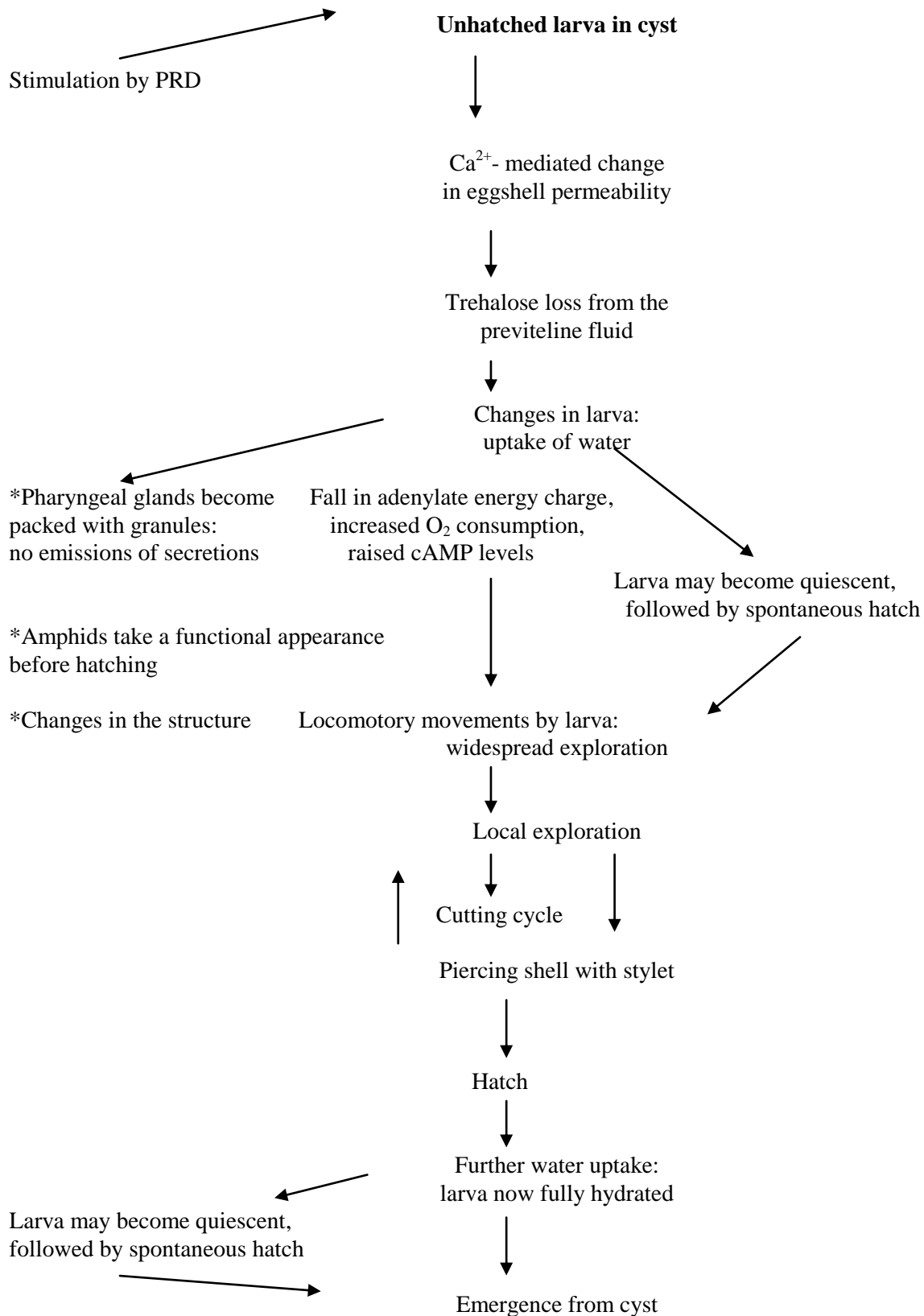


Figure 4: Sequence in hatching of PCN after stimulation with PRD (modified from Perry, 1998).

8. Discussion

Both species of potato cyst nematode (PCN), *Globodera rostochiensis* and *G. pallida*, are obligate parasites of the family *Solanaceae*, weeds and cultivated crops (Andersson & Eriksson, 2001). PCN are responsible for potato tuber losses of up to 9% per annum in Europe. In other countries or where no control strategies are employed, total crop losses can occur (Devine & Jones, 2001). Due to these great losses in food production it is important to find a control strategy against PCN, possibly by studying the hatching mechanism of PCN.

Because of the different survival strategies the nematode has developed, PCN are difficult to manage. Different control strategies have been developed, including non-environmental friendly methods (chemicals) and environmental friendly methods (crop rotation and resistance in different potato cultivars). From an economic point of view the resistant cultivars are the best option in managing the PCN, but are not always available (Evira, 2006).

PCN has excellent abilities allowing it to survive for a long time, e.g. high reproductive potential, ability to live in cold climates (Turner & Evans, 1998) and adaptation to a dormant period, which ensures nematode survival until favourable conditions return (Karszen & Moens, 2006). The dormant period are divided into two, quiescence and diapause. The quiescence phase prevents the juveniles from hatching under unfavourable conditions and is an important survival mechanism. The diapause ends only when specific requirements have been fulfilled (Perry, 1998). The favourable conditions to start hatching after quiescence dormancy consist of stable temperature (Perry, 1998), specific soil structure and soil water status (Jones, 1975) and presence of a host plant.

There are at least three factors with an effect on hatching: host root diffusate, physical conditions before and during the juvenile emergence, and hereditary preconditions of the cyst (Manduric, 2004).

Juveniles of PCN species hatch from cysts when stimulated by potato root diffusate (PRD). The cyst is a protected resting stage before the juvenile hatch. PRD is produced by the potato when it starts developing roots. Production of PRD is confined to a short period in the plant's life with the maximum activity reached two or three weeks after planting, but production of small concentrations of PRD can continue for the rest of the growing period (Robinson *et. al.*, 1987; Perry, 2002). The PRD is produced along the entire

root but the most active diffusate is produced in the root tip (Rawsthorne and Brodie, 1986). In addition to PRD, several factors in the surrounding environment must also be fulfilled.

PRD contains at least four to six different chemical hatching agents that induced hatching. Two classes of chemicals have been found in PRD, hatch inhibitors and hatch stimulants. The inhibitors are produced at an earlier stage than stimulants, protecting the plant during sensitive stages (Byrne *et al.*, 1998).

After 5 minutes of exposure to PRD, the PCN juveniles start substantial hatching from the cyst (Forrest & Perry, 1980). To reach a peak of physiological efficiency when the nematode hatches, it has made an adaptation with movements with water inside the egg (Wallace, 1973). The effect of PRD stimulation is evident as a change in metabolic activity, which increases the permeability of the lipoprotein membrane of the eggshell, allowing the egg to take up water (Perry *et al.*, 1982). Water uptake by the juvenile also increases oxygen consumption inside the egg (Clarke & Perry, 1977; Atkinson & Ballantyne, 1977).

The mechanism affecting egg shell permeability is the calcium ion concentration, which changes the structural lipids through a mediated change (Clarke *et al.*, 1978). The egg shell permeability increases during the first 24 h after exposure to PRD, but juvenile hatching takes 3 days (Perry & Feil, 1986).

When the changes in the juvenile have occurred, it starts the three-stage behavioural sequence leading to hatching: widespread exploration when the juvenile gets fully mobile inside the egg, local exploration when only the head of the juvenile is moving while the lip region is pressed to the eggshell, and cutting through the eggshell with the stylet (Ellenby, 1974). Physiological factors of importance for hatching are temperature, oxygen availability, soil type and soil water content (Perry, 2002).

PCN requires a relative stable temperature regime to hatch (Perry, 1998). *Globodera pallida* is more adapted to lower temperatures than *G. rostochiensis* (Robinson *et al.*, 1987), which is an advantage in cold soils. The differences between the species may be important in relation to competition and habitat adaptation (Clarke & Perry, 1977).

Most studies of PCN have been carried out under laboratory conditions and may be different under field conditions. Soil structure and soil water status are important factors for movement and maximum hatch of

PCN and all other soil-borne nematodes (Jones, 1975). Coarse-textured soils favour hatching (Perry, 2002). The nematodes are dependent on a film of water in order to move through the soil (Jones, 1975).

Due to the severity of PCN in potato growing in Sweden and elsewhere, effective control methods are essential. By studying the hatching mechanism, researchers hope to find the most efficient hatching agent that can be used in a future control method. Recent studies have concluded that Solanoeclepin A is one of the main hatching agents for PCN. Solanoeclepin A is derived from potatoes and, as a natural product, is suitable for an environmentally friendly nematode control method (Blaauw *et al.*, 2001). By studying the hatching mechanism researchers hope to find the most efficient hatching agent that can be used in a future control method to (Blaauw *et al.*, 2001).

Further studies of the hatching mechanism are required, in order to increase the knowledge about the factors and host searching. This thesis reviews the literature on the hatching mechanism of potato cyst nematodes. Improved understanding of hatching and contributing factors in potato cyst nematodes is an urgent current research challenge towards the development of future, sustainable management strategies.

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