# UNIVERSITY OF AMSTERDAM

# UvA-DARE (Digital Academic Repository)

## The role of rhizosphere signalling in the plant-cyst nematode interaction

Vlaar, L.E.

**Publication date**
2022

**Citation for published version (APA):**
Vlaar, L. E. (2022). *The role of rhizosphere signalling in the plant-cyst nematode interaction*. [Thesis, fully internal, Universiteit van Amsterdam].

# Chapter 1

General introduction

Humans are highly dependent on plants, since all our food originates from them. Of several hundred thousand plant species known, however, only about 150 are cultivated for human food, and 50% of primary crop production is accounted for by just four species: sugar cane, maize, wheat and rice [1]. The global human population is increasing towards an estimated 9.3 billion in 2050 [2] and therefore, agricultural yields will need to increase. To achieve that, a profound knowledge of plant biology is necessary as it will enlarge the toolbox with which plant growth and yield can be optimized. Intuitively, it seems effective to focus research on edible or green parts of the plant, since these are directly related to growth (photosynthesis) and yield. However, this approach leaves a plant organ with a high potential to improve plant yield unexplored: the root system.

Although belowground and hence not directly visible to the human eye, the roots of plants are at least as important as the aboveground organs: they anchor the plant in the soil and allow the plant to take up nutrients and water. Furthermore, some plants, such as potato, form edible, belowground storage organs, tubers, although these are technically stem-derived. The roots of plants also exude molecules into the soil and thereby engage in interactions with organisms that surround their roots. The rhizosphere is the area of the soil directly adjacent to the plant roots. Apart from plants, other organisms reside in the rhizosphere, such as bacteria, fungi, protists, oomycetes, arthropods, algae, and nematodes. Some of these organisms can help the plant with nutrient uptake or improve their ability to cope with stress. Others are pathogens or parasites and result in damage to the plant. Some do not directly interact with the plant, but all together, they form an ecosystem of species that are interdependent and of which population sizes usually remain within a sustainable range. Plants can manipulate this so-called rhizobiome by exuding specific molecular signals or macronutrients from their roots, which can stimulate the growth of beneficial micro-organisms in the rhizosphere. For example, maize flavones are exuded into the rhizosphere, and attract bacteria that stimulate growth and nitrogen (N) acquisition [3], and strigolactones are exuded upon phosphate (P) starvation, and attract specific fungi that improve P uptake [4]. It is estimated that plants exude up to 20% of their photosynthetically fixed carbon into the rhizosphere.

However, some pathogens have evolved the capacity to eavesdrop on these signals, and use them as cues for the presence of a suitable host. One of the pathogens with this strategy is the parasitic Potato Cyst Nematode (PCN), an umbrella term for two species of the genus *Globodera*: *G. pallida* and *G. rostochiensis* [5]. These species use a plant molecule as a cue for the presence of a suitable host, a member of the *Solanaceae*, and adapt their behaviour accordingly: dormant eggs will hatch upon perception of the cue. This is to the detriment of the plant, since juvenile nematodes will migrate towards the plant root once they are hatched, and start to feed on it. In my thesis, I study this chemical communication between plant and parasitic nematode, specifically the *Solanaceae* and PCN, focussing on a rhizosphere signalling molecule, presumably synthesized in the plant, solanoeclepin A (solA). With this work I aim to get a better understanding of the plant-nematode interaction, which could then be used to improve management and control strategies to combat PCN infection.

## The phylum of the Nematoda

Nematodes are the most abundant metazoa on Earth, both in abundance as well as in biodiversity [6]. It is estimated that the phylum harbours around 100,000 to 10 million species, even though only 20,000 are so far described, and that each hectare of soil contains around 1.2 billion nematode individuals [7]. They inhabit a large variety of habitats, ranging from deep sea

to fresh water and from soil to inside other organisms, as parasites. The phylum Nematoda is not closely related to any extant group, and the lineage they arose from is probably extinct [6]. They have a simple, hollow tube-like body plan, with a mouth on one side and an anus on the other, and the digestive system in between. The phylum is divided into 12 clades (Fig. 1) [8]. It is argued that nematodes have a marine origin, and only later evolved to inhabit fresh water and soils [9]. Although there has been a lot of attention for parasitic nematodes, since they are relevant for humans both in terms of health as well as economically, most species are non-parasitic and are free-living in aquatic sediments and soil [10]. There, they reside mostly in the top soil layer at less than 15 cm deep [11], and feed on bacteria, fungi, algae and other nematodes, and hence fulfil many important ecological roles. Some of these roles are the control of populations of soil microorganism, the sequestering and redistribution of minerals, carbon and energy, and the degradation of toxins [12]. Because of their abundance, variety in feeding guilds - that is, bacterivores, fungivores, carnivores, omnivores and herbivores - and biodiversity, nematodes are a good indicator of soil biological activity [13].

## Parasitism

Although it was deduced that nematode ancestors were free-living, parasitism is now widespread throughout the phylum, with 8 clades, out of 12, containing parasitic species, 4 of which contain plant parasitic species (Fig. 1). Parasitism has evolved independently at least 18 times in the phylum [9,14]. Nematodes parasitise herbaceous plants, trees, insects and vertebrates, including humans. Hence, they use organisms from virtually all domains of life as an energy source. The economic consequences are large: damage caused by nematodes in livestock reaches tens of billion of dollars annually; six of the 13 core neglected tropical human diseases are caused by nematodes; and in agriculture, plant-parasitic nematodes cause up to $US100 billion in losses annually [15–17].

Parasitic nematodes can be divided into two groups. The first group consists of obligate parasites, which implies that they require a host to feed on and if they are unable to locate one, they will die. The other group, the facultative parasites, can survive one or more life cycles without a host, feeding on alternative food sources. For example, *Strongyloides stercoralis*, the threadworm that causes strongyloidiasis in humans, can survive for one full generation outside the body of a host, feeding on bacteria [18]; the plant-parasitic nematode, *Bursaphelenchus xylophilus*, which usually parasitises pine trees, can survive without the host feeding on fungi [19]. Many species parasitizing on plant roots are obligate parasites, and some feed on the root while remaining outside, whereas others spend part of their life cycle, usually late juvenile and adult stages, inside the host. In any case, eggs of, for example, plant parasitic cyst and root-knot nematodes hatch outside the host, and juveniles need to re-enter their host in a later life stage. This is a critical stage, as the juvenile needs to locate the host and migrate to it successfully or it will die.

To increase the chance of success in this critical life stage, almost all parasitic nematode species have evolved a hypobiotic dispersal stage in their life cycle [20]. During this stage, the nematode does not feed and is often resistant to various types of stress. Moreover, it does not age and can remain in this life stage for a long time (up to two decades for some cyst nematodes [21]), waiting for the appearance of a suitable host. In some cases, parasitic nematode species use a vector, often an insect, to be transported to their host. This is the case, for example, in *B. xylophilus*: upon the death of a pine tree, the juveniles of this species are transported to a new tree using a beetle (*Monochamus* sp.) as vector, which it enters and exits through the spiracles

[19,22]. The filarial nematode *Brugia malayi*, causing elephantiasis in humans, is transmitted by mosquitoes (*Mansonia* sp.) that transmit juveniles while taking a blood meal [23]. Usually, species that use a vector for dispersal are in a hypobiotic stage inside their vector, while waiting for a suitable host.

In Clade 9 and 10 (Fig. 1), many studies showed that the hypobiotic state of parasites evolved from a similar state in species with a free-living lifestyle, called dauer [24–27]. Dauer was first identified in *Caenorhabditis elegans*, a free-living species that is established as a model organism [28]. Dauer is a facultative, alternative L3 (third larval) stage, that is only entered upon unfavourable circumstances, such as crowding, lack of food and high temperature (Fig. 2). In dauer, *C. elegans* can survive for up to five months, whereas its normal life span is just a few weeks. Whether a larva enters dauer or not is determined by the perception of environmental cues, which are perceived through the dauer signalling pathway. This pathway was found to be conserved in many parasitic Clade 9 and 10 species [27].

In *C. elegans*, the cue to enter dauer is a change in the composition of the ascaroside blend. These are acyl sugars that are secreted and perceived by nematodes, usually exclusively con-specifically [29]. The specific mixture that induces dauer is called the dauer pheromone. A high concentration of ascarosides can, for example, indicate crowding, which then induces dauer entry [30]. Once L2 (larvae of the second stage) perceive the dauer pheromone, they will enter the dauer stage.
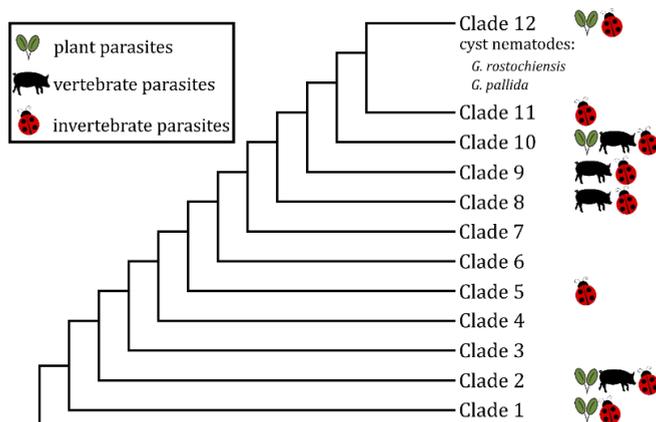


**Fig. 1: phylogenetic tree showing the 12 Nematoda Clades and the type of parasitism found in each Clade**. *Adapted from* [9,14].

Dauer exit occurs upon improvement of the environmental conditions, such as upon appearance of food or a change in temperature. In parasitic nematodes, the cue that induces dauer-like stage exit is related to the host. For example, in the Clade 9 parasites *Ancylostoma caninum* and *Nippostrongylus brasiliensis*, host serum and an increase in temperature up to 37°C, signifying the presence of their host, result in an exit of the dauer-like stage [25,31]. Similar mechanisms play a role in the hypobiosis of parasites in other clades. In Clade 2, for example, *T. spiralis*, which resides in the digestive system of mammalian hosts, exits its hypobiotic state after exposure to trypsin, bile or a temperature increase [32]. Analogously, in Clade 12, the larvae of the plant-parasitic cyst nematodes exit their dormant state and start to actively search for and

migrate towards their plant host after perception of a plant-produced molecule, exuded into the soil [33,34].

## Plant-parasitic nematodes

Plant parasitic nematodes (PPN) are one of the biggest competitors of humans for agricultural crops. About 4,000 species, or 15% of all documented nematode species, are PPN [35]. PPN are found in Clade 1, 2, 10 and 12 and evolved at least 12 times independently [14] (Fig. 1). Most PPN species attack and feed on plant roots, but some exceptions exist where humidity is high enough to allow above-ground nematode life. For example, the pine wood nematode, *B. xylophilus*, attacks pine aboveground, and there are several species of the genera *Ditylenchus* and *Aphelenchoides* that feed on stems and leaves, crawling from the soil up the stem in a water film.

The common feeding organ of PPN is the stylet or, in case of Clade 1, a protrusible tooth, a long needle-like organ, with which they can puncture plant cell walls. Although all PPN have this organ, it is not limited to this group, since also some carnivorous nematodes have a similar structure [36]. PPN species are divided in feeding groups according to their parasitic strategy. Ectoparasites do not enter the plant, but use their stylet to puncture plant cells and to extract nutrients from the outside of the plant. The longer the stylet, the deeper into the root the nematode can feed. Ectoparasites usually stay motile. Semi-endoparasites enter the root, but only with their anterior part, while the posterior part of their body remains outside. Endoparasites, finally, completely enter the plant root. Migratory endoparasites like *Pratylenchus* do not induce a fixed feeding site, and hence keep migrating through the plant while feeding and completing their life cycle. Sedentary endoparasites will, after a migratory phase, establish a feeding site, called syncytium, which allows for highly efficient extraction of nutrients from the plant [37]. For example, in cyst nematodes, only J2s are migratory, and once they moult into J3, they become sedentary. Adult females become obese and can hence produce many eggs.

Attempts to control PPN species in the 20th century mostly focused on fumigation methods with methyl bromide, chloropicrin and 1,3-dichloropropene, especially for cash crops such as coffee and banana. They are still used, but these compounds pose serious threats to soil, environmental and human health. Hence, most of the early fumigants are now banned, and the remaining are strictly regulated. Currently, strategies focus on management rather than control, using sanitation, crop rotation, suppressive soils, resistance breeding and biological control agents. In the latter group, the focus lies on nematophagous fungi, endophytic fungi such as arbuscular mycorrhiza, which suppress nematode infections, and bacteria such as *Pasteuria* that infect nematodes [38].

## Cyst nematodes

Cyst nematodes are the second most economically important PPN species, after the root-knot nematodes [5]. They are present in many countries worldwide and infect a range of crops: cereals, soybean, potato, pea, tobacco and sugarbeet [39]. The two most important genera are *Heterodera* (82 species) and *Globodera* (12 species) [39]. Cyst nematodes are called after the structure that encapsulates the eggs while in the soil, dormant, awaiting the presence of a host. This cyst is a hard shell, containing up to 500 eggs, that protects the eggs from desiccation, toxins and other adverse environmental conditions. The eggs, protected by the cyst, can remain viable in the soil for years, adding to the persistency of the problem for agriculture.

The life cycle of all nematodes is divided into four juvenile stages, called J1-J4, or in the case of animal parasitic nematodes, larval stages, L1-L4 (Fig. 2). These stages are separated by moulting. In cyst nematodes, the first moult happens inside the egg, after which the stylet is formed, which will be used for penetrating the eggshell upon hatch. Depending on the species, the resulting J2 can remain dormant up to twenty years [21], until the hatch. This is when the active stage of the life cycle commences: the juvenile now must find a host in a limited amount of time, or it will die of starvation. To spread the risk, not all eggs hatch simultaneously. In order to locate a host, nematodes primarily depend on chemotaxis. The host root exudes species-specific secondary metabolites into the soil, which are used as cues. Volatiles can act as long distance cues, for nematodes to migrate towards root-occupied soil, whereas water-soluble cues lead nematodes to individual roots [40]. For root knot nematodes (*Meloidogyne sp.*), a range of attractants and repellents have been identified, but for cyst nematodes only some plant hormones (salicylic acid, methyl jasmonate, indole-3-acetic acid) and metabolites of the ethylene pathway have been shown to attract juveniles [41–43]. Nematodes can perceive molecules with their amphids, which are located in the head region and are the principal olfactosensory organ of the worm.
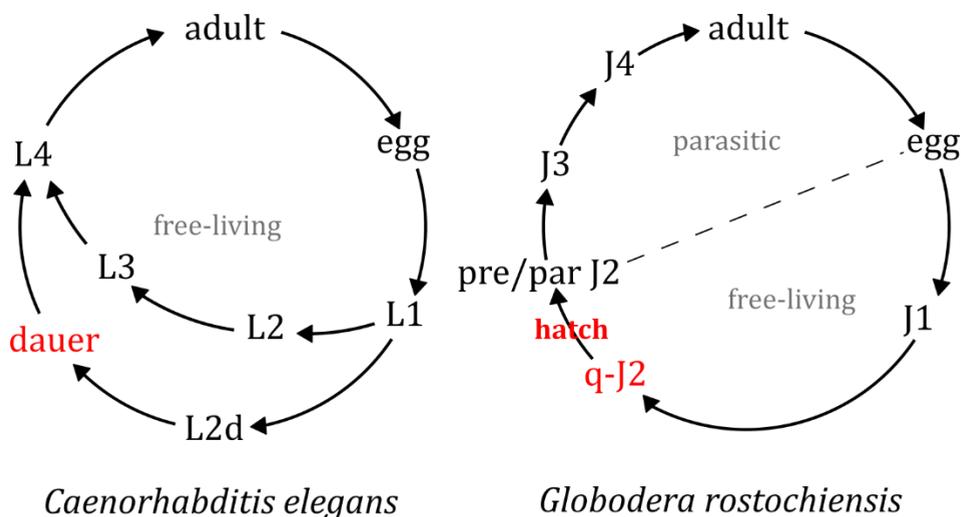


**Fig. 2: schematic representation of the lifecycles of** C. elegans *and potato cyst nematode* G. **rostochiensis.** *Dauer and dauer-like q-J2 stage and hatch are indicated in red. Adapted from* [44]. *L2d: second larval stage leading to dauer; q-J2: quiescent J2 (inside egg); pre/par-J2: pre-parasitic (in soil) and parasitic (in host plant) J2.*

Once the nematode has arrived at the root, the J2 pierces the epidermis with its stylet, usually just behind the growing root tip. Subsequently, the worm migrates to the pericycle and selects a cell that will become the syncytium, from which it takes up nutrients. After several days, J2 moult into J3. The female becomes round because of the developing ovaries. She will continue to grow and rupture the root cortex as she moults into J4 and adult stages, while she is producing eggs. The males migrate through the root and are non-feeding, but fertilize the eggs, which will develop into J2 while inside the living female's body. Once the female dies, her body will harden and become the cyst. At some point, the cyst will detach from the root and remain in the soil until a next host grows nearby.

# Potato Cyst Nematode: diapause, quiescence and hatching

Yield losses in potato in Europe as a result of PCN infection are estimated to about 9% of the total production [39], which translates to 10 million tonnes in 2020 in Europe alone or 32 million tonnes worldwide [2]. However, this is probably an underestimation, since symptoms are generic and many uneducated farmers in developing countries might not recognize and hence, report PCN infection [5].

PCN were introduced from the Andes in South America in the nineteenth century, together with the import of their hosts, potato, tomato and eggplant, from the same region. It is believed that Europe became a secondary distribution centre and that cysts in root material were distributed worldwide with improved crop varieties [45]. Now, they are reported in almost all countries where potato is cultivated. The symptoms of a PCN infection in potato are wilting, reduced number and size of tubers, chlorosis and poor growth, thus, not very specific. *G. pallida* and *G. rostochiensis* are closely related and distinguishable by the colour of the maturing cyst, which is pale/white in the former and yellow in the latter.

One of the features that makes this pest difficult to manage is its persistence in the soil. Cyst nematodes go through two types of dormancy, called diapause and quiescence. Diapause is an inactive state based on season and temperature. It will not be relieved by the perception of chemicals, that would otherwise induce hatching, but lasts a set time, depending on the environmental conditions such as temperature. In PCN, the diapause of J2 lasts a minimum of three months and is a means to survive winter. Once temperatures increase in spring, diapause is released. From that time on, the juveniles are responsive to host cues, but will remain quiescent until perception thereof. This quiescence can last up to two decades.

Hatching occurs upon the perception of a host cue that is present in root exudates. This mechanism effectively synchronizes the hatch with the vicinity of suitable host plants, and therefore, is critical for survival. PCN are almost completely dependent on root exudates for hatching, as opposed to some other cyst nematodes. Species with a broader host range, such as *Heterodera schachtii*, hatch more easily in water [46]. *Solanaceae* root exudate is a very potent hatching agent for PCN, and it can induce hatch over a distance of up to 80 cm [47]. Root exudates remain active in inducing hatching for at least 100 days [48].

There are several compounds present in root exudates that are known to induce hatching (discussed below). Remarkably, the perception of these compounds by the nematode has not been studied, while it is a relevant, and even crucial, event in the parasitic life cycle of PCN. Since a five-minute exposure to root exudate is enough to initiate hatching, it is believed that a receptor-ligand interaction starts the hatching mechanism [49]. This receptor could possibly induce activation of the dauer exit signalling pathway, as happens in Clade 9 and 10 parasites.

The hatching mechanism is based on the fact that, in an unhatched egg, osmotic pressure is high because the perivitelline fluid contains trehalose. The high concentration of this sugar achieves cryoprotection, protection against desiccation and allows limited movement of the juvenile inside [50]. Through a change in the permeability of the egg shell, caused by the release of $Ca^{2+}$ ions, the pressure is released [51]. This allows the juvenile to start moving, puncture the eggshell with its stylet, and subsequently hatch.

# The Solanaceae and their rhizosphere

The *Solanaceae* are a monophyletic group, including some of the world's most important agricultural crops: potato, tomato, eggplant, sweet and chilli pepper and tomato. Potato is the most important non-grain staple crop in the world, with an annual production of 370 MT in 2021 [2], and its popularity in developing countries is increasing [52]. Potato as well as tomato originate from the Andes in South America, from where they were taken to Europe by the Spanish in the 16th century. Potatoes were regarded as food for the destitute and animals, but later, famines made potatoes more popular [52]. The majority of the tuber consists of starch, but protein content of potato is high compared to other tubers, and it is rich in several micronutrients, such as dietary fiber, vitamin C, vitamin B6, potassium, magnesium, iron, carotenoids and phenolic acids [53].

## Glycoalkaloids

The *Solanaceae* produce a type of secondary metabolites that is specific for this family, called steroidal glycoalkaloids (SGAs). They are composed of cholesterol-derived alkaloids with an attached sugar. SGAs taste bitter and can be toxic to humans in high concentrations. However, their concentration is highest in leaves and sprouts, which are not consumed. SGA production is evolutionarily conserved since they serve as anti-fungal agents and repel herbivores [54,55]. It is usually not the total amount of SGAs that determines the degree of resistance, but the specific composition of the SGA cocktail, since some SGAs are more potent than others [56].

SGAs are exuded via the roots into the rhizosphere and some of them can induce hatching of PCN. For example, potato SGAs, α-solanine and α-chaconine, and tomato SGA α-tomatine, induce hatching of *G. rostochiensis* [57,58]. These SGAs are produced by commercial varieties, whereas many more types are found in wild potato and tomato species. Furthermore, some of the alkaloid aglycones, show hatching activity as well, such as solanidine and solasodine, but to a lower extent than their glycosides [58,59]. Hence, the sugars seem to play an important hatch-stimulating role. Moreover, some SGAs can, in specific concentrations, act as hatch inhibitors [60].

## Eclepins

A group of compounds that induces hatching of cyst nematodes in much lower concentrations than SGAs are the eclepins. Glycinoeclepin A was the first eclepin to be isolated; it was purified from kidney bean and characterized [34,61]. Soon after, two closely related eclepins, glycinoeclepin B and C, were identified in kidney bean as well [62]. These three compounds induce hatching of Soybean Cyst Nematode (SCN), *Heterodera glycines*, at pM to nM concentrations. In the nineties, another eclepin was identified, purified from potato, that induces hatching of PCN in similar concentrations, called solanoeclepin A (solA) [33,63].

Whereas for the SGAs their anti-fungal and anti-herbivore effect present a clear evolutionary advantage, the evolutionary relevance of the eclepins is unknown. However, it is likely they have some sort of selective advantage that surpasses the detrimental effect eclepin production has on PCN parasitism, or eclepin production would have been lost.

## Suicide hatch

The mechanism with which eclepins, SGAs and their aglycones stimulate PCN hatching is unclear. It was shown that μM (SGAs) or pM (eclepins) concentration ranges are effective, and that concentrations above physiological levels are inhibitory [57]. Probably, HFs destabilize the membrane, thereby inducing an increase in permeability of the eggshell membrane. This causes trehalose to leak, and the osmotic pressure to drop, rehydrating the juveniles and thereby allowing them to start moving [57]. It was suggested that different factors in root exudates have different roles in initiating hatching, that is, a separate effect on the egg shell and on the nematode [57]. However, recent experiments with standard solutions containing only one hatching molecule, that induce high hatching, disprove this theory [58,59,64].

The goal of identifying new hatching factors (HF) and stimulants (HS) is to find a compound that could be used to induce suicide hatching. Treatment of fields with a hatching factor prior to planting potato could induce hatching, but the absence of a host would lead to massive starvation of the juveniles [65]. However, there are two difficulties in this approach. Firstly, the chemical nature of the hatching factors thus far identified is complex [61,64], which makes industrial-scale synthesis difficult and costly. Secondly, even though eclepins induce more hatching at a lower concentration than SGAs, PCN eggs never hatch all at the same time and a large percentage of eggs needs multiple stimulations in order to hatch, which may make the suicidal hatching strategy complicated [66].

## Outline of the thesis

The eclepins are a group of plant metabolites with a detrimental effect on their producers: the hatch of the parasitic cyst nematodes. From an evolutionary standpoint, it is expected that these metabolites also carry out a beneficial effect for these plants, or their synthesis would have gone extinct under selective pressure. In **Chapter 2**, I try to unravel this beneficial role through RNAseq on two species, tomato and Arabidopsis. There, I analyse the transcriptional response to the exogenous treatment with solA under N and P starvation treatments. Subsequently, the production of solA in tomato and potato cultivars and species is studied. In **Chapter 3,** the UPLC-MS/MS method for solanoeclepin A purification, analysis and quantification from root exudates is presented, together with a validation of its biological effect through hatching assays. In **Chapter 4**, I use this method, in combination with untargeted metabolomics, to study genotypic variation in solA production and PCN hatching of a range of commercial potato varieties. Through data analysis and machine learning, I detect several putative new hatching factors and possibly, a solA precursor.

PCN is a destructive parasite leading to large economic and societal problems. Hatching is one of the most critical events in the PCN lifecycle, and by manipulation of the hatching cue perception suicidal hatching may be induced. Therefore, it is important to study the structure of HFs and HSs, in order to determine which chemical moieties are responsible for hatching activity. To that end, a putative HF receptor in the nematode should be identified, to allow for receptor-ligand studies and potentially achieve the production of synthetic HFs. Therefore, in this thesis, the possibility of the involvement of the dauer signalling pathway in HF perception is further explored in **Chapter 5**. In that Chapter I review the regulation and occurrence of dauer in the Nematoda and the cues that can wake parasitic nematodes from their hypobiosis. I conclude there that the dauer pathway is probably involved in hatching factor perception in PCN. This hypothesis is experimentally tested in **Chapter 6** using transcriptomics, bioassays,

phylogenomics, worm extracts and in vitro enzyme assays. Lastly, in **Chapter 7**, which comprises the general discussion of this thesis, I discuss the chemo-ecological role of solA and the presence of new, hitherto unconfirmed HFs in *Solanaceae* root exudate. Furthermore, I consider the possible perception mechanisms of solA in PCN, including the dauer hypothesis for Clade 12.

## Acknowledgements

# References

1.  FAO. World Food and Agriculture - Statistical Yearbook 2020. Rome; 2020.
2.  FAO website, accession date 2021 Nov 12. http://faostat.fao.org/
3.  Yu P, He X, Baer M, Beirinckx S, Tian T, Moya YAT, et al. Plant flavones enrich rhizosphere *Oxalobacteraceae* to improve maize performance under nitrogen deprivation. Nat Plants. Springer US; 2021;7:481–99. Available from: http://dx.doi.org/10.1038/s41477-021-00897-y
4.  Andreo-Jimenez B, Ruyter-Spira C, Bouwmeester HJ, Lopez-Raez JA. Ecological relevance of strigolactones in nutrient uptake and other abiotic stresses, and in plant-microbe interactions below-ground. Plant Soil. 2015;394:1–19.
5.  Jones JT, Haegeman A, Danchin EGJ, Gaur HS, Helder J, Jones MGK, et al. Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol. 2013;14:946–61.
6.  Poinar GO. Phylum Nemata. In: Thorp JH, editor. Thorp Covich's Freshw Invertebr. Academic Press; 2016. p. 169–80.
7.  Poinar GOJ. The evolutionary history of nematodes: as revealed in stone, amber and mummies. Leiden, The Netherlands: Brill; 2011.
8.  Holterman M, Van Der Wurff A, Van Den Elsen S, Van Megen H, Bongers T, Holovachov O, et al. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Mol Biol Evol. 2006;23:1792–800.
9.  Blaxter M, Koutsovoulos G. The evolution of parasitism in Nematoda. Parasitology. 2015;142:S26–39.
10. Moens T, Braeckman U, Derycke S, Fonseca G, Gallucci F, Gingold R, et al. 3. Ecology of free-living marine nematodes. In: Schmidt-Rhaesa A, editor. Vol 2 Nematoda. De Gruyter; 2013. p. 109–52.
11. Jobbágy EG, Jackson RB. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol Appl; 2000;10:423–36.
12. Ferris H. Contribution of nematodes to the structure and function of the soil food web. J Nematol. Society of Nematologists; 2010;42:63.
13. Neher DA. Role of nematodes in soil health and their use as indicators. J Nematol. Society of Nematologists; 2001;33:161.
14. Holterman M, Karegar A, Mooijman P, Van Megen H, Van Den Elsen S, Vervoort MTW, et al. Disparate gain and loss of parasitic abilities among nematode lineages. PLoS One. 2017;12:1–18.
15. Roeber F, Jex AR, Gasser RB. Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance - An Australian perspective. Parasites and Vectors. 2013;6:1–13.
16. Payne L, Fitchett JR. Bringing neglected tropical diseases into the spotlight. Trends Parasitol. 2010;26:421–3.
17. Nicol JM, Turner SJ, Coyne DL, den Nijs L, Hockland S, Tahna Maafi Z. Current nematode threats to world agriculture. Genomics Mol Genet Plant-Nematode Interact. 2011. p. 369–93.
18. Page W, Judd JA, Bradbury RS. The Unique Life Cycle of *Strongyloides stercoralis* and Implications for Public Health Action. Trop Med Infect Dis. 2018;3.
19. Zhao L, Zhang S, Wei W, Hao H, Zhang B, Butcher RA, et al. Chemical signals synchronize the life cycles of a plant-parasitic nematode and its vector beetle. Curr Biol. 2013;23:2038–43.
20. Gibbs HC. Hypobiosis in parasitic nematodes – an update. Adv. Parasitol. 1986.
21. Hunt DJ, Luc M, Manzanilla-López RH. Plant parasitic nematodes in subtropical and tropical agriculture. In: Identification, morphology and biology of plant parasitic nematodes. Luc M, Sikora RA, Bridge J, editors. 2nd ed. Wallingford, UK: CABI publishing; 2005. p. 11–52.
22. Wu Y, Wickham JD, Zhao L, Sun J. $CO_2$ drives the pine wood nematode off its insect vector. Curr Biol. 2019;29:R619–20.
23. Anderson RC. The origins of zooparasitic nematodes. Can J Zool. 1984;62:317–28.
24. Grant W, Viney M. The dauer phenomenon. In: Perry RN, Wharton D, editors. Molecular and physiological basis of nematode survival. 1st ed. Cambridge, USA: CAB International; 2011. p. 311.
25. Hotez P, Hawdon J, Schad GA. Hookworm larval infectivity, arrest and amphiparatenesis: the *Caenorhabditis elegans* Daf-c paradigm. Parasitol Today. 1993;9:23–6.
26. Sudhaus W. Preadaptive plateau in Rhabditida (Nematoda) allowed the repeated evolution of zooparasites, with an outlook on evolution of life cycles within Spiroascarida. Palaeodiversity. 2010;3:117–30.
27. Crook M. The dauer hypothesis and the evolution of parasitism: 20 years on and still going strong. Int J Parasitol. 2014;44:1–8.
28. Cassada RC, Russell RL. The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev Biol. 1975;46:326–42.
29. Ludewig AH, Schroeder FC. Ascaroside signaling in *C. elegans*. Wormbook. Ed. the *C. elegans* Research

Community; 2013.

30. Butcher RA, Fujita M, Schroeder FC, Clardy J. Small-molecule pheromones that control dauer development in *Caenorhabditis elegans*. Nat Chem Biol. 2007;3:420–2.

31. Huang SCC, Chan DTY, Smyth DJ, Ball G, Gounaris K, Selkirk ME. Activation of *Nippostrongylus brasiliensis* infective larvae is regulated by a pathway distinct from the hookworm *Ancylostoma caninum*. Int J Parasitol. 2010;40:1619–28.

32. Stewart GL, Despommier DD, Burnham J, Raines KM. *Trichinella spiralis*: behavior, structure, and biochemistry of larvae following exposure to components of the host enteric environment. Exp Parasitol. 1987;63:195–204.

33. Mulder JG, Diepenhorst P, Plieger P, Brüggemann-Rotgans IEM. Hatching agent for the potato cyst nematode. 1996.

34. Masamune T, Anetai M, Takasugi M, Katsui N. Isolation of a natural hatching stimulus, glycinoeclepin A, for the soybean cyst nematode. Nature. 1982;297:495–6.

35. Decraemer W, Hunt DJ. Structure and classification. In: Perry RN, Moens M, editors. Plant Nematology CABI; 2006. p. 3–32.

36. Yeates GW, Bongers T, de Goede RGM, Freckman DW, Georgieva SS. Feeding habits in soil nematode families and genera - an outline for soil ecologists. J Nematol. Society of Nematologists; 1993;25:315–31.

37. Perry RN, Moens M. Plant nematology. 2nd ed. Perry RN, Moens M, editors. CABI publishing; 2006.

38. Viaene N, Coyne DL, Davies KG. Biological and cultural management. In: Perry RN, Moens M, editors. Plant Nematol. 2nd ed. CABI publishing; 2006. p. 542.

39. Turner SJ, Subbotin SA. Cyst nematodes. In: Perry RN, Moens M, editors. Plant Nematol. 2nd ed. CABI publishing; 2006. p. 542.

40. Sikder MM, Vestergård M. Impacts of root metabolites on soil nematodes. Front Plant Sci. 2020;10:1–18.

41. Fleming TR, Maule AG, Fleming CC. Chemosensory responses of plant parasitic nematodes to selected phytochemicals reveal long-term habituation traits. J Nematol. 2017;49:462–71.

42. Hu Y, You J, Li C, Williamson VM, Wang C. Ethylene response pathway modulates attractiveness of plant roots to soybean cyst nematode *Heterodera glycines*. Sci Rep. Nature Publishing Group; 2017;7:1–13.

43. Kammerhofer N, Radakovic Z, Regis JMA, Dobrev P, Vankova R, Grundler FMW, et al. Role of stress-related hormones in plant defence during early infection of the cyst nematode *Heterodera schachtii* in Arabidopsis. New Phytol. 2015;207:778–89.

44. Ma G, Wang T, Korhonen PK, Hofmann A, Sternberg PW, Young ND, et al. Elucidating the molecular and developmental biology of parasitic nematodes: Moving to a multiomics paradigm. Adv Parasitol. 2020;1–55.

45. Evans K, Stone AR. A Review of the distribution and biology of the Potato Cyst-Nematodes *Globodera rostochiensis* and *G. Pallida*. PANS. 1977;23:178–89.

46. D'Addabbo T, Sasanelli N, Greco N, Stea V, Brandonisio A. Effect of water, soil temperatures, and exposure times on the survival of the sugar beet cyst nematode, *Heterodera schachtii*. Phytopathology. 2005;95:339–44.

47. Rawsthorne D, Brodie BB. Movement of potato root diffusate through soil. J Nematol. 1987;19:119–22.

48. Tsutsumi M. Conditions for collecting the potato root diffusate and the influence on the natural hatching of potato cyst nematode. Japanese J Nematol. 1976;6:89–95.

49. Perry RN, Beane J. The effects of brief exposures to potato root diffusate on the hatching of Globodera rostochiensis. Rev Nématologie. 1982;5:221–4.

50. Clarke AJ, Perry RN, Hennessy J. Osmotic stress and the hatching of *Globodera rostochiensis*. Nematologica. 1978;24:384–92.

51. Clarke AJ, Perry RN. Egg-shell calcium and the hatching of *Globodera rostochiensis*. Int J Parasitol. 1985;15:511–6.

52. Lutaladio NB, Castaldi L. Potato: The hidden treasure. J Food Compos Anal. 2009;22:491–3.

53. Beals KA. Potatoes, Nutrition and Health. Am J Potato Res. 2019;96:102–10.

54. Fewell AM, Roddick JG. Interactive antifungal activity of the glycoalkaloids α-solanine and α-chaconine. Phytochemistry. 1993;33:323–8.

55. McKee RK. Factors affecting the toxicity of solanine and related alkaloids to *Fusarium caeruleum*. J Gen Microbiol. 1959;20:686–96.

56. Pelletier Y, Horgan FG, Pompon J. Potato resistance against insect herbivores: resources and opportunities. In: Giordanengo P, Vincent C, Alyokhin A, editors. Insect pests potato Glob Perspect Biol Manag. Academic Press; 2013. p. 439–55.

57. Devine KJ, Byrne J, Maher N, Jones PW. Resolution of natural hatching factors for golden potato cyst nematode, *Globodera rostochiensis*. Ann Appl Biol. 1996;129:323–34.

58. Shimizu K, Kushida A, Akiyama R, Lee HJ, Okamura Y, Masuda Y, et al. Hatching stimulation activity of

steroidal glycoalkaloids toward the potato cyst nematode, *Globodera rostochiensis*. Plant Biotechnol. 2020;37:319–25.

59. Ochola J, Cortada L, Ng'ang'a M, Hassanali A, Coyne D, Torto B. Mediation of potato – Potato Cyst Nematode, *G. rostochiensis* interaction by specific root exudate compounds. Front Plant Sci. 2020;11.

60. Byrne J, Twomey U, Maher N, Devine KJ, Jones PW. Detection of hatching inhibitors and hatching factor stimulants for golden potato cyst nematode, *Globodera rostochiensis*, in potato root leachate. Ann Appl Biol. 1998;132:463–72.

61. Fukuzawe A, Furuskari A, Ikura M, Masamune T. Glycinoeclepin A, a natural hatching stimulus for the soybean cyst nematode. Nature. 1984;297:495–6.

62. Fukuzawa A, Matsue H, Ikura M, Masamune T. Glycinoeclepins B and C, Nortriterpenes related to Glycinoeclepin A. Tetrahedron Lett. 1985;26:5539–42.

63. Schenk H, Driessen R a J, Gelder R De. Elucidation of the structure of solanoeclepin A, a natural hatching factor of potato and tomato cyst nematodes, by single-crystal X-ray diffraction. Croat Chem Acta. 1999;72:593–606.

64. Tanino K, Takahashi M, Tomata Y, Tokura H, Uehara T, Narabu T, et al. Total synthesis of solanoeclepin A. Nat Chem. Nature Publishing Group; 2011;3:484–8.

65. Devine KJ, Jones PW. Response of *Globodera rostochiensis* to exogenously applied hatching factors in soil. Ann Appl Biol. 2000;137:21–9.

66. Perry RN, Clarke AJ. Hatching mechanisms of nematodes. Parasitology. 1981;83:435–49.