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

Vlaar, L.E.

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Chapter 7

General discussion

Plants are successful chemists, together producing an estimated 200,000 to 1 million different metabolites [1]. This thesis revolves around one of them, called solanoeclepin A (solA), which acts as a rhizosphere signalling molecule and is the most potent hatching factor (HF) for Potato Cyst Nematode (PCN). This thesis studies solA signalling along the plant-nematode axis. Firstly, I show that solA – so far only known to function as nematode HF – affects gene expression in plants. The results, showing a decrease in immune response and increased growth, suggest that solA prepares the plant for colonization by microorganisms and that it affects root growth. Secondly, genetic variation in solA production was mapped in wild tomato as well as commercial potato varieties, and solA-related, new HFs were identified through machine learning. Lastly, it was shown that the dauer signalling pathway is conserved in PCN and that it is activated during solA-induced hatching, leading to hypobiosis exit. In the current chapter, I will discuss several questions that have arisen over the course of writing this thesis, and I will give an outlook on the scientific and practical implications of this work.

What is the role of solA in the plant?

Hitherto, eclepins were solely known to function as nematode HF and solA is no exception [2–4]. However, from an evolutionary point of view, it is to be expected that it has an additional, beneficial role for the plant, since it is unlikely that plants produce a compound that only has a negative effect (that is, to induce PCN hatching). Indeed, in Chapter 2 I showed that solA affects gene expression in plants, pointing at a possible signalling role.

Intriguingly, solA not only affects gene expression in tomato, but in Arabidopsis as well. Notwithstanding the possible production of some kind of 'arabidoeclepin' by Arabidopsis, it is unlikely that it produces solA itself, since this compound appears to be Solanaceae-specific. Hence, since solA is produced by tomato and changes gene expression in Arabidopsis, it serves as a plant-plant signalling molecule. Since we assume that the solA effect on tomato is beneficial, and that the transcriptional response to solA in Arabidopsis almost entirely overlaps with that in tomato, it must be beneficial for both species. Cooperative signalling is common in the plant kingdom: for example, many plants emit volatile organic compounds (VOCs) upon herbivory, and these induce reduced susceptibility in their neighbours which thereby have an advantage upon herbivore attack [5]. Underground signalling via roots has been reported as well, and it can alter plant physiology and development, also aboveground. For example, allelochemicals in root exudates can manipulate root growth into intrusive, avoidance or unresponsive patterns, and it can stimulate or delay flowering, both intra- and interspecifically [6]. Cooperative signalling also exists between different species; then it is called interspecific facilitation. For instance, peanut is triggered to induce the production of ethylene by cassava-produced cyanide. Ethylene, in turn, alters the composition of rhizosphere microbiota, thereby providing nutrients to the plant [7]. In another example, intercropping of maize and faba bean leads to higher yields and enhanced nodulation in faba bean, mediated by maize root exudates [8]. The fact that solA affects Arabidopsis transcriptionally, probably to the benefit of the latter, may be a new example of interspecific facilitation [9]. In examples of interspecific facilitation, the identity of the signalling metabolite is often not known. Hence, the discovery that solA is perhaps not only a HF but also rhizosphere signalling molecule that allows for interspecific facilitation, is exciting.

The most profound solA-induced changes in the transcriptome of tomato and Arabidopsis are the downregulation of the immune and hypoxia response, and ethylene biosynthesis and signalling, and the upregulation of (root) growth (Chapter 2). These results seem to suggest that solA prepares the plant for colonization by beneficial microorganisms and affects the root architecture. The fact that these transcriptional changes are strongest upon nutrient starvation, hints at a role for solA in enhancement of nutrient uptake, aided by microorganisms and/or altered root architecture. Clearly, this needs further confirmation with additional experiments, but the findings in Chapter 2 seem to fit with the nature of above-described other examples of interspecific facilitation.

A possible role for solA in the attraction of beneficial microorganisms under nutrient stress could be similar to the attraction of AM fungi and Oxalobacteraceae by flavones, under N deprivation, which can help with nutrient uptake [10,11], or similar to the strigolactones. Under P starvation, plants produce strigolactones that not only affect root architecture, but also attract AM fungi that aid in P uptake [12–14]. Alternatively, solA could function as a cry for help, attracting microorganisms that strengthen the defence against soil-borne pathogens, to compensate for the downregulation of immune-related genes in the plant. Under nutrient stress, plants are known to lower their immune response, which could be compensated by the attraction of defence-enhancing microorganisms.

In conclusion, solA may be considered, apart from its long-known role as HF, as plant-plant, as well as, possibly, plant-microorganism semio-chemical. Furthermore, since our results imply that solA affects growth, it may be considered a plant hormone. SolA is not the only known eclepin; kidney bean produces three glycinoeclepins [2] and in this thesis I show that potato produces another solA/eclepin-like compound, coined solanoeclepin B, that correlates with PCN hatching (Chapter 4). Eclepins are produced in extremely low concentrations (picomolar range) (Chapter 3 and 4), depending on the nutrient status of the plant, and are therefore difficult to detect and identify. Hence, it is possible, or even likely, that other plant families produce, so far unidentified, eclepins as well, possibly originating from the same biosynthetic pathway. Whether these act as HF for other (cyst) nematodes and present a means for interspecific facilitation, cry-for-help and hormone signalling as well, will have to be determined.

Are the two effects of solA, induction of PCN hatching and plant tolerance to nutrient starvation, connected?

The SolA content of the root exudate varies strongly between species and even between cultivars. In Chapter 3, we showed that solA content of several tomato genotypes and wild tomato relatives ranges from 0 to 50 pmol/g FW with an average of 16 pmol/g FW [15]. In Chapter 4 we showed that solA content in the root exudate of a series of potato (*S. tuberosum*) cultivars ranged from 0 to 110 pmol/g FW, with an average of 28 pmol/g FW. Hence, variation is high and potato tends to produce more solA than tomato and its relatives. This variation in solA content depends not only on genotype, but on age of the plant as well. SolA production is at its highest between three and five weeks of age [15].

The consequence of this age-dependent solA production is beneficial for PCN. It is unfavourable to hatch in large numbers when only a young plant is close by, since this will not provide enough food for the nematodes to fulfil their lifecycle [16]. The reason that a plant starts to produce solA only later in life might be due to the fact that it downregulates the immune response; younger plants might not be able to survive such a vulnerability, whereas older plants are sturdier. The genotype-dependency of solA content is less easily explained and probably it does not serve a purpose for PCN. However, this could be a strategy that wild Solanaceous species have developed to reduce the PCN burden on the plant. Completely losing the ability to biosynthesize solA is probably not favourable for the plant, because of its beneficial role under N and P deficiency (Chapter 2).

Following this reasoning, it is not likely that the two effects of solA, induction of PCN hatching and enhanced plant tolerance to nutrient starvation, are connected. It is likely that solA is synthesized because of its beneficial role for the plant, and that PCN hijacked this signalling relation by evolving the ability to perceive this compound as a hatching cue. A similar course of events has been demonstrated for the strigolactones: this group of plant hormones is released into the rhizosphere to attract arbuscular mycorrhizal fungi, which aid in P uptake [12,13]. Parasitic plants such as Striga have hijacked this signalling relation, and use strigolactones as a cue to germinate and attach to the host plant, from which they will extract nutrients [17].

Is solA crucial for hatching of PCN?

The eclepins are potent cyst nematode hatch inducers: solA can induce hatching up to 80% (Chapter 3, Fig. 4B). Hatched PCN juveniles need to locate and enter a suitable host soon after hatch, or they will die. Hence, the high dependence of hatching on a host-derived hatching cue in the host root exudate is a convenient strategy to prevent hatching without a suitable host nearby. SolA is a highly potent HF, active in as low as picomolar concentrations, that can, in some cases, account for most of the hatch-inducing potency of root exudates (Chapter 3). For example, root exudates of *S. pimpinellifolium* and *S. habrochaites* induce around 65% hatching, whereas pure solA in the same concentration, induces around 45% hatching (Chapter 3, Fig. 4). However, root exudates of other species contain much less, or even no solA, and still induce considerable amounts of hatching. Hence, hatch-inducing potency of root exudates does not only rely on solA. Indeed, solA content of root exudates and hatching percentage induced by root exudates do not correlate strongly (Chapter 3, Fig. 4C; Chapter 4, Fig. 1B) and there is a whole series of known and unknown HF, hatching stimulants (HS) and hatching inhibitors (HI) described in the literature [16]. However, hitherto no other PCN HFs with the same hatch-inducing potency as solA were identified, since other HFs, such as glycoalkaloids, need higher concentrations to be active [18]. Intriguingly, we identified a solA-like compound, solanoeclepin B (solB), in potato root exudate that highly correlates – more than solA itself – with hatching (Chapter 4). SolB is probably structurally related to solA, but its hatch-inducing activity could not be analysed since we do not have a standard. From the glycinoeclepins, of which three are known, it is known that related structures are not necessarily equally active on cyst nematode eggs: whereas glycinoeclepin A (glyA) is active at 10^{-11} to 10^{-12} g/mL, glyB and glyC are active only at higher concentrations, around 10^{-8} to 10^{-9} g/mL [2,3].

Notwithstanding the uncertainty whether solB induces hatching, solA and solB were consistently the two features that predict hatching of PCN the best, tested both by RF feature selection and Pearson's correlation test (Chapter 4). Other compounds scored high as well, but the two solanoeclepins were the only compounds that were present in fractions of root exudate that induced high hatching rates (Chapter 4). It is therefore tempting to conclude that these two compounds account for most of the hatch-inducing activity of Solanaceous root exudates. Nevertheless, also root exudates devoid of these two compounds can still induce considerable hatching rates. This might mean that more related structures exist, as is the case for the glycinoeclepins [2]. Because of their low concentrations, they might not be detected using untargeted metabolomics approaches. This diversification of the eclepins suggests an arms race similar to the strigolactones. More than 25 strigolactones exist, and they have both beneficial as well as detrimental effects on plant survival, as described above [19]. Their diversification might be driven by the selection pressure to evade the perception of strigolactones by parasitic plants, but to keep their useful role as attractant for AM fungi and as plant hormone affecting tillering and root architecture [19,20]. Similarly, eclepins are suggested to carry out a beneficial role for the plant in the attraction of microorganisms under nutrient stress and the adjustment of growth patterns, but it is in the plant's interest that eclepins are not perceived by their parasites, the cyst nematodes. The result is an arms race, in which the eclepins diversify in order to evade perception by harmful organisms.

Is hatching analogous to dauer exit?

In this thesis, it was shown that during hatching of PCN, the conserved dauer signalling pathway is active (Chapter 6). In animal parasitic nematodes (APN) of Clade 9 and 10, which are relatively closely related to *C. elegans*, the dauer pathway regulates the exit from the dauer-analogous infective stage (iL3) (Chapter 5, [21]). Hence, it is tempting to conclude that in PCN, hatching marks the end of the dauer-analogous hypobiosis.

However, there are some crucial differences between hatching and the end of the infective stages of APN. Firstly, many APN can be forced to resume reproductive behaviour when treated

with dafachronic acid (DA) [22–24], whereas DA does not stimulate PCN hatch (Chapter 6). Moreover, in APN species *S. strongyloides*, it was shown that the pattern of endogenous DA levels of iL3 (infective stage larvae) and L3+ (L3 larvae with reactivated development that have entered the host) is very similar to the analogous *C. elegans* dauer and L3 larvae: from low in iL3/dauer to high in L3+/L3 larvae [25,26]. In PCN, endogenous DA could not (yet) be detected in q-J2 (quiescent, unhatched larvae) or pre-J2 (hatched J2 in search of a host) (Chapter 6). This leaves the possibility that par-J2 (J2 inside the host) or J3 produce DA endogenously, but this stage is located inside the host and therefore difficult to sample. Secondly, iL3 of APN have entered their hosts before they exit their quiescence, and the resumption of feeding can begin immediately, but for PCN, hatching only marks the onset of the host search. Following these two discrepancies, it could be argued that the hypobiosis of PCN only ends when the juvenile has entered a host root and moulted into J3; hence, the entire J2 stage, from the J1-J2 moult inside the egg, diapause, quiescence and hatching, to migrating through the soil in search of a host, up to the establishment of a feeding site in the plant root, could potentially still be hypobiosis.

Nevertheless, there is a strong physiological argument in favour of the quiescence ending with hatching. Pre-J2 cannot be qualified as hypobiotic, since they can only survive for a short period of time without finding a host. The exit of this enduring state is therefore likely happening, or at least initiated, with hatching. In addition, upon hatching, a release of a stressful condition is taking place, just like in dauer exit: osmotic stress, which is high in the egg, decreases dramatically, caused by the change in eggshell permeability and subsequent leakage of trehalose. Moreover, specific inhibitors of dauer pathway proteins have a strong inhibiting effect on hatching (Chapter 6). Among these are dafadine A and ketoconazole, which both inhibit DAF-9, which is the last enzyme in the biosynthetic pathway of DA. Although ketoconazole is a general CYP450 inhibitor and the result can therefore be aspecific for the dauer signalling pathway, dafadine A specifically inhibits DAF-9 and reduces hatching induced by root exudates or solA by ~50% (Chapter 6). From these results, it is clear that DAF-9, and hence the dauer signalling pathway, is involved in and necessary for hatching. If DAF-9 is active, it is to be expected that DA is produced upon hatching, even though this could not yet be shown (Chapter 6). The fact that addition of exogenous DA does not hatch PCN eggs, could be due to the impermeability of the eggshell, through which it cannot reach the amphids of the dormant nematode. In contrast, for the APN iL3's, that exit the infective stage under DA treatment, there is no eggshell to be crossed, hence, DA can readily reach their amphids. Another explanation for the fact that DA does not hatch PCN is that PCN synthesises a slightly modified version of DA, possibly produced from a plant steroid.

In conclusion, more research is necessary to irrefutably answer this question, as convincing arguments for both scenarios can be offered. Untargeted metabolomics detecting an endogenous steroid – DA or a related molecule produced from a plant steroid – at a specific life stage could be one way to answer this question. However, to that end, sampling of large numbers of par-J2 and J3, life stages that take place inside the host, would be needed. The concluding step of the dauer signalling pathway, which is the biosynthesis of DA(-like) and its binding to DAF-12, will probably take place at some point in the life of the nematode, since PCN harbour a *daf-9* copy in their genome that is active *in vitro*. However, it is still unclear in which life stage this happens.

How does PCN perceive solA?

Much is unknown about the hatching mechanism of PCN and how HFs stimulate the larvae to become active remains unclear. For example, in the phylogenetically related root knot nematodes (*Meloidogyne sp.*), the metabolic activation of the nematode happens prior to the change in eggshell permeability, that will ultimately lead to eclosion, but for *Globodera spp.*, the order of events in the hatching cascade is not known [16]. It has been proposed that hatching is mediated by a receptor, since a five-minute exposure to root exudate is sufficient to induce

hatching, which is a process that takes multiple days [27]. What type of receptor this is, or whether this receptor resides on the eggshell or in the larva, remains hitherto elusive.

Since hatching was linked to the dauer signalling pathway, (Chapter 6), it makes sense to start the search for the solA receptor there. The *C. elegans* dauer signalling pathway contains several receptor proteins. Firstly, several GPCRs perceive, in the case of *C. elegans*, the dauer pheromone and other ascarosides [28]. Furthermore, GPCRs are involved in host seeking behaviour in many parasitic nematode species [29]. Secondly, the Insulin-Like Peptides (ILPs) are perceived by DAF-2, a receptor tyrosine kinase [30]. Thirdly, the TGFβ homologue, DAF-7, is perceived by the TGFβ-specific receptors DAF-1 and DAF-4. Lastly, the product of the dafachronic acid (DA) signalling pathway is perceived by the nuclear hormone receptor (NHR) DAF-12. From these types of receptors in the dauer signalling pathway, it is unlikely that HFs are perceived by DAF-2, DAF-1 or DAF-4 homologues, since these are peptide receptors specific for ligands from the insulin and TGFβ families, that are conserved throughout the animal kingdom. It is assumed that HF and HS are non-protein metabolites. The two remaining options, GPCRs or NHRs, are both plausible HF receptors.

G. pallida and *G. rostochiensis* harbour 103 and 87 G-Protein Coupled Receptors (GPCRs), and 54 and 65 Nuclear Hormone Receptors (NHRs) in their genomes, respectively [29,31]. Probably, GPCR's are involved in the process *after* hatching, when the juvenile needs to find a host as soon as possible to start feeding [29]. GPCRs are located on the cell membrane located in the amphids (nematode sensory organs), and therefore suitable to perceive exogenous cues [32]. Atropine, a GPCR antagonist, inhibited hatching of PCN (Chapter 6), which suggests that HFs are perceived by a GPCR. In contrast, NHRs are located in the nucleus and influence gene transcription based on the presence of a ligand. The closest human homologue of DAF-12 is the vitamin D receptor and typically, their ligands are steroids and terpenoids [33]. Since solA is a complex terpene, it is not unthinkable that it is perceived by an NHR. Alternatively, HF could be perceived by receptors located on the eggshell, and from there, the dauer signalling pathway in the juvenile is activated [16].

There are several examples of nematode species that exit their infective stage when stimulated with host-specific cues. For example, *Ancylostoma duodenale* and *Ancylostoma caninum* (Clade 9) need host serum to progress development into the adult reproductive stages [34,35]. In animal parasites, *daf-9* homologues were not found, which led to the suggestion that animal hosts of parasitic nematodes produce DA or related molecules, which then enforce infective stage exit [36]. Indeed, it was found that a mammalian NHR, CYP27A1, is able to catalyse the synthesis of DA [37,38]. However, this is clearly not the case for PCN since they harbour a copy of *daf-9* in their genome that encodes a protein that is active *in vitro* (Chapter 6), but it is possible that the precursor of DA is of plant origin, for example a plant sterol or steroid. In that case, DA biosynthesis may not start before the J2 has reached and entered a host root, since then, it has access to the precursor compound, unless it is exuded. Thus, the dauer signalling pathway would only be concluded - that is, DA would bind to DAF-12 - upon the start of feeding, which would make that event the exit of the hypobiotic phase. However, at this point, contrasting results make it impossible to say whether PCN hypobiosis ends at hatching, or at the moment when the juvenile enters the plant and moults into J3.

SolA is not the only HF: besides the eclepins, glycoalkaloids and their aglycones induce hatching [39]. Since only a select group of metabolites exuded by Solanaceous species is able to induce PCN hatch and metabolites from other species cannot, the ligand affinity of the HF receptor must be very specific. Therefore, probably multiple HF receptors exist – different for glycoalkaloids and eclepins, for example - although it is possible that HF with a similar structure, such as solA and solB, use the same receptor, probably with varying affinities.

In conclusion, with the work of this thesis, we are a step closer to unravelling the HF perception mechanism in PCN, but at this point, it is still impossible to say how the nematode perceives

solA and other HF. Probably, hatching can be initiated through several different receptors, specific for one HF or a group of structurally similar HFs. The localization of these receptors, on the eggshell membrane or in the larva, is uncertain. Furthermore, although the dauer signalling pathway is active during hatching, suggesting it leads to exit of hypobiosis, it remains to be revealed whether a HF is directly perceived by a component of that pathway, or if the pathway is activated through a cascade of other signals.

Outlook: science

The down-regulation of the immune response and the upregulation of growth, especially under N and P starvation in tomato and Arabidopsis upon solA treatment (Chapter 2) suggest that solA is hereby preparing the plant to be colonized by beneficial microorganisms, possibly aiding in nutrient uptake under starvation conditions. Furthermore, the upregulation of growth could point at a change in root architecture, which, as well, can promote nutrient uptake and/or beneficial microbe recruitment. However, these hypotheses must be tested experimentally for example by the analysis of plant phenotypes under N and P starvation and solA treatment, as well as nutrient uptake experiments after solA treatment, preferably with and without symbionts. Additionally, the root microbiome with and without solA should be mapped to determine which species is/are responsible for the enhanced nutrient uptake. The limiting factor for these experiments is the availability of solA: it can be synthesized, but this process is extremely laborious [40], and since it is produced by plants in very low amounts, purification from root exudates will be arduous as well. Moreover, biosynthesis genes specific for solA have not been identified yet, and therefore, the creation of knockouts is not yet feasible.

A second item that requires more work is the confirmation of the molecular structure and hatch inducing potency of solB (Chapter 4). For identification, it is necessary to purify this compound from root exudate, and to use NMR to determine its structure. Furthermore, when the compound is purified, it can be applied to PCN eggs to test its hatch inducing activity. Since the concentration of solB did not only correlate with hatching, but also with solA content, it cannot be ruled out that solB does not induce hatching at all, and is just correlating with hatching *because of* its correlation with solA. Nonetheless, this seems unlikely because the correlation of solB with hatching is in fact higher than that of solA (Chapter 4, Fig. 3). Since solA and solB are positively correlated and are structurally similar, they probably originate from a similar biosynthetic pathway. The productivity of this pathway depends on genotype, since the amounts of solA and solB vary greatly between genotypes of potato (Chapter 4, Fig. 1A, Fig. S3).

Lastly, the HF receptors in PCN should be identified, since this can pose interesting new methods for their control via synthetic ligands that result in suicide hatch. Since solA is the most potent HF, the receptor for this ligand should be prioritized. Recently, a genome sequence of *G. pallida* of good quality has been published, which can benefit the search for the receptor [41]. Above I described that GPCRs or NHRs are the most likely HF receptor candidates, but narrowing down candidates further is difficult. Therefore, I propose a ligand binding screen of all *G. pallida* GPCRs and NHRs. Since this is practically impossible, this could be done virtually through 3D structural modelling using computational approaches. For example, the network approach used in AlphaFold2 allows the modelling of uncharacterised proteins and the subsequent docking of ligands, and takes into account the 3D interaction between distant residues. It is claimed to outperform traditional approaches that model the folding process [42]. Results of these *in silico* studies hopefully narrow down the candidate list, and these should be confirmed using a wet lab approach. For GPCRs, this can be done using sensor-based screening of ligands, which relies on a change in refractive index upon interaction of receptor and ligand [43]. Alternatively, GPCRs are often evaluated using GPCR desensitization/cAMP assays [44]. For both NHRs and GPCRs, AlphaScreen, a chemiluminescence technique, could be used, which is the method with which DA was shown to be the ligand for DAF-12 in *C. elegans* [37]. This method is based on the binding of two molecules to beads, that, in case of binding of the

molecules, and thus close proximity, induce an energy transfer. This results in a chemiluminescent signal that can be observed and quantified.

Outlook: agriculture

Although hatching is a crucial process in the cyst nematode lifecycle, it is often overlooked as a factor in resistance breeding. Instead, much attention is given to the penetration of the host root, the subsequent establishment of a syncytium (feeding site) and the interaction between nematode effectors and the plant's defence system. However, when the objective is to decrease infection and subsequent yield losses, hatching should also be considered. A crucial element here is timing: if the juvenile hatches too early while no host plant is near, it will die of starvation, since the pre-J2 can only survive without a host for up to a few weeks [45]. In contrast, staying quiescent (unhatched) is a safe strategy, since encysted eggs can remain viable for up to two decades [46]. Hence, luring the quiescent J2 (q-J2) out of their eggs prematurely is an effective control strategy that is already used in the form of trap-cropping. In this system, a species that produces *Globodera* HFs such as solA, but does not allow the nematode to fulfil its lifecycle, is planted to clear a field of dormant cysts. One of the main trap crops used in agriculture is *Solanum sisymbriifolium* (sticky nightshade), which can reduce PCN infection in potato by up to 97% [47]. As an additional benefit, trap cropping bypasses any of the chemical soil treatments such as bromide fumigation. Moreover, when destroyed mechanically and incorporated into the soil, the trap crop can serve as green manure. Nevertheless, it is labour intensive and time consuming, while the cover crop itself does not render any profit, and it is therefore not ubiquitously used.

A much less labour and time intensive method would be to use a synthetic or purified HF, that can be applied on the soil and induces PCN hatching. After a couple of weeks, without any extra labour, the field would be ready for potato planting. This was demonstrated by Devine and Jones, who showed that application of root exudates in the field reduces the number of viable larvae by 50% [48]. Possibly, the reduction rate could increase even further by using pure HFs, so that hatch inhibitors (HI) are absent. However, eclepins are complicated chemicals: solA can be synthesized in the lab but it takes 32 chemical reactions to do so [40]. Notwithstanding its activity at extremely low concentrations (picomolar), it is at this point not feasible to use solA commercially. In order to develop simpler, synthetic HFs, it is necessary to study the perception mechanism in PCN, and in particular, the solA receptor. This knowledge is necessary to develop, for example, derivatives of solA that interact with the receptor, but are easier to synthesize.

If our hypothesis that solA facilitates colonization of beneficial microorganisms is true, this could be used to make plants more resilient against N and P starvation. We showed that transcription in a species that does not synthesize solA itself, is altered through solA treatment (Chapter 2). Moreover, the changes in gene expression in these plants suggest preparation for the recruitment of beneficial microbes, and altered root architecture, under N and P starvation. The fact that these changes are achieved by a molecular cue or signal from another species, would qualify this as interspecific facilitation. This can be exploited in agriculture through intercropping in areas where N and/or P are scarce: planting high solA producers in between other crops, that are sensitive to N and P shortage, may enhance their ability to cope with these sub-optimal conditions and offer a good yield nonetheless. This would be a cheap and low-tech solution, especially suitable for low-income countries.

Another application of solA, one that requires more extended research, is to use a solA-derived molecule and spray this on crops. Since producing synthetic solA is not commercially viable, the plant solA receptor should be identified, in order to develop synthetic ligands with a similar structure, exerting the same effect. SolA-derivatives can then be used harmlessly on non-Solanaceous crops – it might induce PCN hatch but there will be no host available – to induce the downregulation of the immune response and alteration of the root architecture, that could

result in the recruitment of a beneficial microbiome and improve nutrient uptake. If this can be achieved on crop species, it can increase yield and reduce the need to use chemical fertilizers.

The Netherlands is one of the countries that suffers heavy economical damage from PCN-related yield loss [49]. In Chapter 4, we showed that especially several potato cultivars are high solA producers, and this is probably one of the reasons that PCN places such a high burden on worldwide potato production, especially in species where high solA production is combined with a genetic predisposition towards PCN susceptibility, such as cultivar Desiree (Chapter 4). Therefore, in countries where N and/or P are not limiting factors for crop growth, breeding could aim at developing cultivars with a reduced level of HF biosynthesis. However, even potato cultivars that produce very low amounts of solA, still induce considerable amounts of hatching (Chapter 4). For example, the cultivar Seresta contains only trace amounts of solA and seems to contain almost no solB either, while still inducing a considerable hatch of 35% (Chapter 4, Fig. 1A, Fig. 3A, Fig. S3). Thus, focusing only on solA and solB for the reduction of PCN hatch is not sensible. In this light, it would be unwise to breed for cultivars that produce a low amount of solA to decrease PCN hatch induction, since it would also cut its beneficial role in coping with nutrient deficiencies. Possibly, breeding can benefit from the structural diversity in the eclepins; within the strigolactones, is was shown that a change in the composition of the variants leads to a sustained attraction of AM fungi but reduced perception by Striga [50]. Hence, eclepins might vary in hatch inducing activity, as was already shown for the glycinoeclepins [2], and in their role in enhanced nutrient uptake. The key for effective breeding towards yield improvement and reduced fertilizer needs is to find the eclepins that induce only low PCN hatching but do stimulate nutrient uptake (through the microbiome), and then to establish cultivars that produce mainly these.

Concluding remarks

In conclusion, this thesis has established new insights in the biological relevance of solanoeclepin A, a rhizosphere signalling molecule, in plants as well as in the parasitic potato cyst nematode. This poses new opportunities for a more sustainable agriculture, such as the use of intercropping with solA-producing plants or the development of synthetic solA derivatives to improve beneficial microbe recruitment and nutrient uptake in crops, and synthetic suicide hatch inducing compounds. Hence, this work brings a decrease in the extensive use in agriculture of fertilizer and nematicides one step closer. At the same time, it offers intriguing new insights in the role of the dauer signalling pathway in the evolution of cyst nematodes, and the role that rhizosphere compounds can have in the maintenance of plant fitness.

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