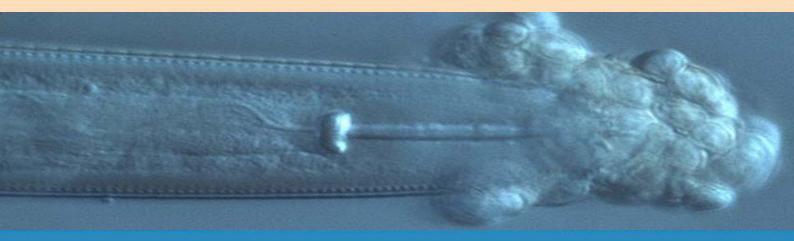


Matthias Daub (Hrsg.)

### 45. Jahrestagung des DPG-Arbeitskreises Nematologie 2017



Zusammenfassungen der Arbeitskreisbeiträge PI (Persistent Identifier): urn:nbn:de:0294-jb-ak-2018-nem-5



#### 46. Tagung des DPG Arbeitskreises Nematologie

13. und 14. März 2018

**Tagungsstätte** 

Rheinische Friedrich-Wilhelms-Universität Bonn Hörsaal IV (Meckenheimer Allee 176, 53115 Bonn)

Kurzfassungen

Herausgeber:Matthias DaubJulius Kühn-InstitutBundesforschungsinstitut für KulturpflanzenInstitut für Pflanzenschutz in Ackerbau und GrünlandDürener Strasse 7150189 Elsdorf (Rheinland)

#### 159 years after Schacht - what we know and still not know about cyst nematodes

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In 1859, German Botanist Hermann Schacht, Professor at Bonn University, gave a detailed description of a nematode parasitizing sugar beet. The description was later accomplished and placed into the then existing phylogenetic system by Schmidt in 1871. The basis for the scientific work on Heterodera schachtii and cyst nematodes in general was laid. In the following years, a focus was placed on deeper understanding the nematode's biology and developing control measures. Kämpfe and his coworkers performed first very detailed analyses on the interaction of *H. schachtii* with its host plant in the 1950s and 1960s. A breakthrough was the detailed dissection of nematode behaviour and plant responses during invasion and feeding presented by Wyss and coworkers in the beginning of the 1990s. Almost at the same time, Arabidopsis thaliana was established as a model host setting the ground to apply the rapidly developing genetic and molecular tools for functional analyses of plant responses occurring during the susceptible interaction with H. schachtii. This model, however, has its limitations: whereas pattern-triggered immunity is elicited by nematodes, effector-triggered immunity (ETI) has not yet been observed. On the other hand, studies in host plants with well-established ETI, such as sugar beet, mustard, and oil seed radish are less suited to perform, functional gene analyses. Compared with other nematodes, our knowledge about genetic resistance against H. schachtii is far from being conclusive. Nematode tolerance, a trait that is extensively used in sugar beet breeding and production, is, however, still a black box, waiting to be discovered. The interaction between plants and nematodes is influenced by numerous environmental factors with microbes playing an important role. Certain bacteria and fungi reducing nematode performance and enhancing plant growth are have been used to develop environmentally friendly control agents - so far with only limited success. Recently developed technologies enable scientists to study not only single microbes, but also the structure and dynamics of microbial communities. It will be intriguing to unravel the mechanisms by which plants, nematodes, and microbes interact and control each other.

#### Arabidopsis HIPP27 is a host susceptibility gene for the beet cyst nematode *Heterodera schachtii*

<u>Radakovic ZS</u>, Anjam MS, Escobar E, Chopra D, Cabrera J, Silva AC, Escobar C, Sobczak M, Grundler FMW, Siddique S

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Sedentary plant-parasitic cyst nematodes are obligate biotrophs that infect the roots of their host plant. Their parasitism is based on modification of root cells to form a hypermetabolic syncytium from which the nematodes draw their nutrients. The aim of this study was to identify nematode susceptibility genes in *Arabidopsis thaliana* and to characterize their roles in supporting the parasitism of *Heterodera schachtii*. By selecting genes that were most strongly upregulated in response to cyst nematode infection, we identified HIPP27 (HEAVY METAL-ASSOCIATED ISOPRENYLATED PLANT PROTEIN 27) as a host susceptibility factor required for beet cyst nematode infection and development. Detailed expression analysis revealed that HIPP27 is a cytoplasmic protein and that HIPP27 is strongly expressed in leaves, young roots and nematode-induced syncytia. Loss-of-function Arabidopsis hipp27 mutants exhibited severely reduced susceptibility to *H. schachtii* and abnormal starch accumulation in syncytial and peridermal plastids. Our results suggest that HIPP27 is a susceptibility gene in Arabidopsis whose loss-of-function reduces plant susceptibility to cyst nematode infection without increasing susceptibility to other pathogens or negatively affecting plant phenotype.

# In vitro life cycle and transcriptome of *Heterodera sacchari*

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*Heterodera sacchari*, a cyst nematode is an important parasite of sugarcane and rice crop. The preparasitic infective juveniles (J2) penetrate the host root and induce a metabolically active syncytial feeding cell system in the stele. Initiation and maintenance of syncytium is achieved by injecting protein effectors into the affected root cells. In order to gain more detailed insights, we established an in vitro culture of *H. sacchari* on nipponbare rice in pluronic gel. We found that nematodes successfully completed life cycle in 7-8 weeks at 25 °C. From in vitro material, we accomplished a transcriptomic analysis of *H. sacchari* by using J2s and 15 days post infective (dpi) old female juveniles in order to find effector protein candidates which are involved in parasitism. Comparative analyses of *H. sacchari* with *Globodera pallida*, *G. rostochiensis* and *H. avenae* transcriptomes revealed the absence of CAZymes family (GH53) in monocot parasitic nematodes (*H. sacchari* and *H. avenae*). We also analyzed two other effector families, SPRYSEC and CLE, which are involved in host defense suppression and host cell modification, respectively. Each family consists of 6 candidates. Based on their expression level, we selected 2 most important candidates for localization using in situ hybridization and Expression analysis was performed using eggs, pre-parasitic J2, 15 and 25 dpi stages. Such studies will generate more information which helps to understand the *H. sacchari*-rice interaction.

#### A proteinaceous molecule from plant parasitic nematodes activates defense responses in *Arabidopsis*

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Plant defense against pathogens relies on recognition of pathogen-associated molecular patterns (PAMPs) by surface-localized receptors leading to the activation of PAMP-triggered immunity (PTI). The role of PTI during plant-nematode interaction is not well known. Here we show that treatment of *Arabidopsis* seedlings with a nematode aqueous diffusate (NemaWater) triggers PTI-like responses including ROS burst, gene expression, and seedling growth inhibition in a manner dependent on the common immune co-receptor BAK1. Treatment of NemaWater with proteinase K or heating abolished the ROS burst activation of NemaWater, indicating that the eliciting capacity of NemaWater is due to the presence of a heat-sensitive proteinaceous ligand. The results showed that a leucine-rich repeat receptor-like kinase, termed NILR1, is essential for the induction of immune responses by NemaWater and for immunity to nematodes. A proteome analysis of NemaWater showed the presence of nematode surface proteins that are involved in triggering PTI through NILR1. These results demonstrate the relevance of PTI during plant-nematode interaction. The role of various conserved signalling molecules present in NemaWater will be discussed.

### **Tricky parasites: How nematodes take their vitamins from plants**

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Vitamin B5 (VB5) is an essential nutrient that is synthesized via a three-step process in plants. In Arabidopsis, AtPANB1 and AtPANB2 encode the enzyme for the first step and AtPANC the enzyme for the last step of the pathway. In comparison to plants, multicellular animals absorb VB5 from their diet. Cyst nematodes are biotrophs, and parasitism is based on the formation of a syncytium in the roots from which nematodes withdraw their nutrients. Here we investigated the role of VB5 during cyst nematode interaction with *Arabidopsis*. We found that expression of AtPANB1 and AtPANB2 is strongly induced upon infection, and this upregulation is essential for nematode development. In comparison to AtPANB, AtPANC is not upregulated and does not play a role in parasitism. Notably, we identified a nematode PANC gene (HsPANC), and showed that the nematodes are able to perform the last step of VB5 biosynthesis using HsPANC. A comprehensive, biochemical, molecular, and genetic analysis revealed that compartmentalization of VB5 biosynthesis between plants and nematodes is necessary to avoid feedback/feed-forward inhibition and ensures a continuous supply of VB5 to rapidly developing nematodes.

# Die Zukunft von *H. schachtii* - Szenarien im Rahmen der Klimaveränderung

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Untersuchungen zur nicht-invasiven Dichteschätzung von *H. schachtii* Populationen anhand hyperspektraler Reflexionsdaten erforderten die Anwendung eines Simulationsmodells, welches eine quantitative Zuordnung der Nematodenstadien in der Pflanze zu jedem Messzeitpunkt liefert. Bei der Modellanwendung auf die Jahre 2011 bis 2014 verlief die Nematodendynamik ausschließlich in Größenordnungen, die früher nur als seltene Ereignisse auftraten, dann aber zu sehr hohen Vermehrungsraten geführt haben. Daraus ergibt sich die Frage, in welcher Größenordnung sich die Nematodendynamik in Folge zukünftig entwickeln könnte. Zur Klärung dieser Frage hat das PIK einen Temperaturdatensatz von 1901 bis 2100 erstellt. In der Regel liegen die Temperatursummen zur Basis 8 (TS8) im Bereich von 1200°C bis 1600°C, was bei einem gegebenen Pi-Wert von z.B. 1000 E&L/100 ml einer mittleren Vermehrungsrate von 2 bis 5 entspricht. Bis zum Jahr 2000 treten vereinzelt wesentlich höhere TS8 auf, aber mit einer geringeren Wahrscheinlichkeit als im Vergleich zum aktuellen Jahrhundert. Diese "Ausreißer" oberhalb von 1600°C sind von besonderem Interesse. Die Pf/Pi Verhältnisse erreichen schon mal Werte von 7-10, ab 1700°C sind Verhältnisse bis 40 möglich. Statistisch zusammengefasst steigt das Pf/Pi Verhältnis um 1 mit jeder Erhöhung der Temperatursumme um 10°C sobald eine kritische Temperatursumme von ca. 1620°C überschritten ist.

#### PARES – Neue Resistenzquellen gegenüber Globodera pallida in Stärkekartoffeln

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Die Produktion von Stärkekartoffeln hat in einigen Regionen Deutschlands ein grosse wirtschaftliche Bedeutung für die landwirtschaftliche Produktion. Neben anderen Schaderregern verursachen die Kartoffelzystennematoden G. rostochiensis und G. pallida bereits jetzt grosse Probleme. Im Rahmen der amtlichen EU-Erhebungen zeigte sich, dass Kartoffelzystennematoden und insbesondere G. pallida weiterverbreitet sind als bisher vermutet. Des Weiteren wurde ein neuer Virulenztyp von G. pallida festgestellt, der sich an Sorten mit Pa3-Resistenz vermehren kann. Somit stehen zurzeit für diese Befallsflächen keine Optionen zur Bekämpfung, im Rahmen der Verordnung zur Bekämpfung von Kartoffelkrebs und Kartoffelzystennematoden, zur Verfügung. Ziel des PARES-Projektes ist es daher neue Resistenzen gegen G. pallida Pa3 (EU-Prüfpopulation Charvornay) und den neuen Virulenztyp "Emsland" zu identifizieren. Durch die Entwicklung diagnostischer Marker unterstützt, sollen die neuen Resistenzen in neue Sorten überführt werden. Vorläufige Ergebnisse zeigen, dass in verschiedenen Quellen (Wildarten) Resistenzen gegen beide Virulenztypen von G. pallida vorhanden ist. Das Screening solcher Pflanzen bedeutet einen hohen Aufwand, da vorerst die Vermehrungsrate zur Bewertung der Resistenz herangezogen wird. Um den Zeit- und Ressourcenbedarf für das Screening verschiedener Resistenzquellen zur reduzieren, wurde die Eignung von Gewebekulturpflanzen (GK) zur Phänotypisierung von G. pallida Resistenz überprüft. Es zeigte sich, dass GK Pflanzen sehr gut geeignet sind, um neue Resistenzquellen zu selektieren und entsprechende Marker zu entwickeln. In der zweiten Phase des PARES Projektes wird nun der Focus auf eine Feinkartierung der Resistenz gelegt.

#### Sensitivity of Ditylenchus dipsaci to fluopyram

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Sugar beet stem nematode affect 5% of the Swiss sugar beet area. As of 2016, no nematicide is authorized to control *D. dipsaci* in Switzerland. However, fluopyram, a SDHI fungicide, is known to possess nematicide properties against other nematode species. This study therefore aims to determine the influence of fluopyram on *D. dipsaci* population development.

In greenhouse experiment, *D. dipsaci* infectivity was reduced only at high a.i. concentrations (10  $\mu$ g/ml). Conversely, field experiments showed a positive effect of fluopyram at reducing *D. dipsaci* infectivity. Greenhouse experiments confirmed the poor nematicide effect of fluopyram observed in laboratory conditions, showing no reduction of *D. dipsaci* population development in sugar beet treated with fluopyram. In the field, despite a reduction of *D. dipsaci* infectivity in spring, fluopyram was not effective at reducing *D. dipsaci* population development until harvest. In field conditions, fluopyram was effective at reducing rotting severity. In greenhouse conditions, *D. dipsaci* pressure was higher and no fungicide effect of fluopyram was observed.

Thus, fluopyram may influence *D. dipsaci*. However, its effect is not sufficient to control the development of *D. dipsaci* in the long term. Although fluopyram is effective at reducing *D. dipsaci* infectivity and rotting symptoms, the extremely high reproduction capacity of *D. dipsaci* inhibits long-term successful control of the sugar beet stem nematode in highly infested fields.

#### Investigations into nematode resistance of grapevine rootstocks within the joint project "MureViU"

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Plant parasitic nematodes are an underestimated source of crop loss in viticulture each year. Not only aggressive root feeding causes plant damage, but also a possible transmission of so called nepoviruses. The ectoparasitic dagger nematode *Xiphinema index* vectors grapevine fanleaf virus (GFLV). Among others, this virus is responsible for fanleaf degeneration, one of the most severe viral disease in viticulture. The lack of treatment possibilities shifts research interest to resistance breeding programs of rootstocks. Within the nationwide joint BMEL/BLE project "multiresistant Vitis rootstocks" (MureViU), the institute of plant protection at the DLR Rheinpfalz participates in the identification of novel *X. index* resistances in rootstocks. Therefore, available genetic resources of all project partners are screened for preferably lowest nematode reproduction rates by a so called glass tube test.

First results show strong variations in *X. index* reproduction on multiple wild Vitis species and their F1 crosses, and thus indicate variant host suitabilities of potential rootstocks. Those identified candidates are analyzed for a concomitant virus resistance. Furthermore, gene expression analyses of known plant resistance genes are performed to identify potential marker genes for the development of faster screening methods.

#### **Approaches in the search for entomopathogenic nematodes with enhanced longevity and virulence**

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Despite its efficacy against insect pests, the use of the entomopathogenic nematode Heterorhabditis bacteriophora in large scale agriculture is limited by environmental stresses. This limitation implies high costs for the end user. Several approaches to enhance the longevity and virulence of this EPN are thus being applied. Concerning DJ-longevity, a significant correlation between oxidative stress tolerance and survival during normal storage conditions has been found. A collection of H. bacteriophora WT strains has been characterized for their shelf life under oxidative stress, and this property has been proposed as predictor to select for longer living nematodes. Hybrid strains and EMSmutants with extended survival time have been tested for trait-off effects. Concerning virulence, environmental- and host-selective experimental procedures have enabled to determine and generate H. bacteriophora lines with enhanced efficacy. Basic genomic tools have been also applied to gain insights into the mechanisms of Virulence and DJ-longevity in H. bacteriophora. Sequence information has been generated on this species, comprising the expression of more than 20.000 different transcripts under infective- and stress-conditions. This phenotypic and genotypic information will enable the search for molecular markers for breeding and selection approaches in H. bacteriophora. In this way the application costs of EPNs will be reduced, making EPN more accessible to large-scale farmers.

#### Einsatz entomopathogener Nematoden im Apfelanbau

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Neben dem Apfelwickler ist auch die Apfelsägewespe ein wichtiger Schädling im Apfelanbau. *Steinernema feltiae* eignet sich gut zur Reduktion überwinternder Apfelwicklerlarven im Stamm und im Boden. Versuche, die Sägewespe im Larvenstadium zu bekämpfen, ergaben sehr variable Wirkungsgrade. Dagegen ist der Einsatz gegen erwachsene Wespen zum Zeitpunkt des Schlupfs aus der Puppe erfolgreich.

### African nightshade and African spinach decreases root-knot nematode and potato cyst nematode infestation in soil

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African nightshade (*Solanum* spp) and African spinach (*Amaranthus* spp) are important leafy vegetables in many parts of Africa as a rich source of nutrition and income. However, their host status to parasitic nematodes remains largely speculative. Our survey revealed severe root galling on *S. villosum* whereas on *S. scabrum*, *A. cruentus* and *A. dubius* root galling was rare or very low. Moreover, soil collected from *S. villosum* and *S. scabrum* rhizosphere contained few cysts of potato cyst nematodes (PCN) and no developing females were observed on the roots of growing plants. Therefore, we studied the dynamics of RKN and PCN on *A. dubius*, *A. cruentus*, *S. scabrum* and *S. villosum* over 2 years in an experimental station. The effects of the crop species on RKN and PCN soil infestation was evaluated using susceptible crop species. After the cultivation of *A. dubius* and *S. villosum* our results show that RKN soil infestation decreased by 85%, whereas *S. scabrum* and *S. villosum* decreased PCN by more than 80%. After cultivation of these crops as trap crops, the galling index and number of developing PCN females measured on susceptible *S. esculentum* and *S. tuberosum* decreased by more than 75%. Here, we show for the first time the design of a cropping system using African spinach and African nightshade as trap crops for RKN and PCN. This approach creates dynamics in cropping systems allowing more diversity to control RKN and PCN in an environmentally friendly, effective and productive way.

## The new Plant Health Regulation: what will be the effect on the quarantine status of nematodes?

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From December 2019 new phytosanitary regulations will be effective: The Plant Health Regulation and the Official Control Regulation. Until that time, the different quarantine statuses as described in the annexes belonging to the current council directive 2000/29/EC apply, such as the IAII status of *Meloidogyne chitwoodi*, *M. fallax*, *Globodera pallida* and *G. rostochiensis*. The status of these quarantine organisms will be reassessed. The old IIAII organisms are going to get the new status of RNQPs, regulated non quarantine pest, e.g. *Radopholus similis*, *Ditylenchus dipsaci* and *D. destructor*. However the organisms that now belong to categories IAI, IIAI and IAII are reassessed and might change status, with three options. The largest group will remain EU quarantine organism. In case of a finding, always action should be taken such as reporting and eradication. The second option is the priority pest status. In this category action must be taken even before a finding has taken place: an annual survey must be carried out, an elimination scenario must be ready, a trial run of these scenarios must be carried out and an immediate report must be submitted to the EU when such an organism is found. The third option is the RNQP status, where requirements are set for propagation material..

EFSA and EPPO are involved in the risk assessment of all potential EU quarantine organisms and RNQPs. Decisions on the new statuses will be taken at EU level by the end of 2019.

# The role of the soil microbiota in reduction of *Meloidogyne hapla* in suppressive soils

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The inability of current measures to significantly reduce the yield losses caused by plant-parasitic nematodes has urged on alternative attempts to solve this problem. The microbiome of suppressive soils in this matter has led to a reduction of nematode populations and has resulted in the discovery of important microbial antagonists. However, it is still unclear which processes and microbial species cause the suppressive effect. In our study, we investigated the attachment of soil microbes to second-stage juveniles (J2) of Meloidogyne hapla in order to test its involvement in suppression. Several soils were tested against the performance of *M. hapla* on tomato plants. The root invasion of J2 and the parameters directly associated to the nematode reproduction (number of galls, egg masses and eggs) were highly reduced in some of the soils. Relying on the fact that the J2 are directly exposed to the soil microbiota prior to their entrance into the roots, thus presenting a target for microbial attachment, we incubated J2 in soil suspensions of the tested soils and harvested the bacterial colonies attached to their cuticle by a culture-dependent approach. The isolates with a unique BOX-PCR pattern were used in re-attachment assays, and the best "attachers" are to be tested for their binding to different nematode species and populations. The final aim is to determine if the single bacterial isolates are able to significantly reduce the nematode propagation on its host and to unravel their mode of action, either through a direct antagonism or by mediating plant defence responses.

### Impact of plant parasitic nematodes and root pathogens on root architecture: biotic-abiotic interactions impacting food security in Southern Africa

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The STIAS program on sustainable intensification of agricultural production and food security in Southern Africa is looking at technologies and processes that will lead to improved food production in small to medium size family farms in the semi-arid and dry sub-humid climatic zones that characterize this region. The poor yields obtained by farmers in Southern Africa are caused by low levels of fertility, soil degradation, lack of quality seed and insufficient plant protection. Yield is also greatly impacted by climate change induced hotter and drier soils. The STIAS program on sustainable intensification will be outlined. Plant parasitic nematodes are present in all small scale farming systems in Africa. However, root health problems usually go unseen or are not recognized as serious constraints. Nematodes cause tap root deformation, tissue necrosis, stunting, abnormal branching and reduced biomass. Damage leads to abnormal root architecture and a decrease in the roots' ability to function efficiently under the abiotic stress conditions existing in these climatic zones. Attempts to improve crop yield on small family farms usually stress: breeding improved cultivars and to a lesser extent improving soil fertility management. Little to nothing is known about the presence and importance of plant parasitic nematodes that negatively alter root architecture. Nematodes are often involved in synergistic interactions with abiotic stresses further reducing plant growth. If crop production is to be significantly improved upon in these climatic zones, crop management programs must include technologies that strengthen nematode and disease management. Because genetic resistance to nematodes is often not available additional efforts needs to be placed on integrated crop health management approaches. Breeding programs designed to select for higher yield need to be linked with selection of resistance/tolerance to plant parasitic nematodes and soil borne diseases. Bioassays designed to detect increased root biomass, root development and root depth penetration for improved water use-efficiency must be supplemented with tests for improved resistance/tolerance to soil borne pests and diseases. We need to look more closely at the impact of complex abiotic and biotic stresses on root architecture if yield and food security is to be improved in these climatic zones.

### Poster

### Nematode inspections and surveys in the Netherlands in 2017

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One of the tasks of the NPPO is to safeguard the import and export of plant material into and out of the EU by performing inspections and surveys. In the Netherlands this task is partly performed by inspection bodies under the authority of the NPPO. For Nematology many inspections are performed by the inspection bodies, such as all inspections related to potato, export inspections of onions for *Ditylenchus dipsaci* and part of the ornamental import inspections for *Radopholus similis*. Depending on the inspection body, sample analysis is performed by them as well. For this reason the number of samples from inspections processed by the NPPO are relatively small. The nematode samples of flower bulb inspections on the other hand, are all processed by the NPPO. During these inspections only symptomatic material is collected. In 2017 from about 45.000 flower bulb lots 151 samples were analysed and *D. dipsaci* was found in 83 of them, in 6 of the samples *D. destructor* was present and in 25 samples *Aphelenchoides subtenuis* was found. Ornamental crops imported from China and Japan (especially bonsais) are inspected for adhering soil, which is analysed for the presence of *Xiphinema americanum* s.l. was found. In 7 samples other *Xiphinema* species were found, which did not belong to the *X. americanum* s.l. group.

A variety of surveys are performed every year, some are obligatory by the EU, such as *Bursaphelenchus xylophilus*, *Globodera pallida* and *G. rostochiensis*. Some surveys are performed to determine whether a certain nematode is present in NL, sometimes a scientific interest is the reason for performing a survey. Most of these surveys are conducted by the NPPO and samples are analysed at the NPPO nematology laboratory. In 2017 specific surveys were performed for *Meloidogyne mali* in avenue trees, *Rotylenchus buxophilus* in Boxwood, *Xiphinema americanum* s.l. in fruit tree production fields (nurseries), and nematodes in imported aquatic plants.

An overview of the nematode species that have been found in these surveys is presented.

#### Resistance and tolerance of different sugar beet genotypes against the beet cyst nematode *Heterodera schachtii*

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Sugar beet genotypes resistant or tolerant against the beet cyst nematode *Heterodera schachtii* can contribute to reduce nematode infestation levels and damage to the crop. Due to their high yield stability and partial resistance, tolerant sugar beet genotypes are increasingly cultivated. To compare the resistance and tolerance levels of different sugar beet genotypes (4 x susceptible, 3 x resistant, 5 x tolerant), single plants were cultivated in loess-filled pots in a greenhouse experiment with controlled watering. In early two-leaf stage plants were inoculated with different levels of second-stage juveniles of *H. schachtii* (0, 2000, 8000, 20 000, 40 000 juveniles/pot). About 6 weeks after inoculation (507°Cd to the basis of 8°C) plants were harvested and evaluated for nematode reproduction. Final nematode densities (eggs+juveniles/pot) were highest on susceptible sugar beet genotypes and lowest in resistant genotypes. In tolerant sugar beet genotypes reproduction rates were reduced by 50-75% compared to susceptible genotypes at the lowest inoculum level but differences decreased at higher inoculum levels. Shoot weights were significantly reduced at the highest inoculum level in the susceptible, resistant and one of the tolerant sugar beet genotypes, but were much less affected in the other tolerant genotypes. Root weights were more reduced at increasing inoculum levels than shoot weights. Results of the greenhouse test complemented previous findings from field experiments.

### Genome-Wide Association Study Dissects Genetic Diversity of Wheat against Cyst Nematode *Heterodera filipjevi* resistance

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Cereal cyst nematodes are obligate biotrophic parasites posing a serious threat to global wheat production. In the past, conventional approaches were applied in resistance breeding; however, these approaches are labor intensive and time consuming. In recent years, there has been enormous progress in wheat genomics by high density molecular markers and genome wide association studies (GWAS). Association mapping is a powerful tool to detect associations between phenotypic variation and genetic polymorphisms, in this way, favorable traits such as resistance to nematode can be detected. In this study, 161 wheat accessions were screened for resistance against H. filipjevi under growth room condition. The results of infection assay ranked 1% of the wheat accessions as resistant, 16% as moderately resistant, 41% as moderately susceptible, 26% as susceptible, and 15% as highly susceptible. These accessions were genotyped with 90K iSelect SNP chip and subsequently correlated with phenotype. Genotyping data analysis revealed high variation of SNP marker distribution on A, B, and D genomes indicating different rates of recombination in the three sub-genomes. Linkage disequilibrium across wheat genome was <3cM in 161 wheat accessions and principle component analysis explained very low genetic differentiation. GWAS identified 9 novel quantitative trait loci (QTLs) on chromosome 1BS, 2BS, 2BL, 3BS, 4AL, 5BL and 7BL linked to H. filipjevi. In-silico annotation of significant markers revealed that six QTLs on chromosomes 1BS, 2BS, 2BL, 4AL, and 5BL were linked to putative genes known to be involved in biotic stress, while three other QTLs on chromosomes 3BS and 7BL linked to putative genes supposed to be involved in response to abiotic stress.

### Nematode infection redirects hormonal homeostasis via Rboh-mediated ROS to facilitate parasitism of host roots

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Cyst nematodes are obligate parasites that establish syncytial-feeding sites in roots of their host plants. Their invasion and feeding causes tissue damage in the host roots triggering an oxidative burst. In plants, ROS is mainly produced by plasma membrane-bound NADPH oxidases, named respiratory burst oxidase homolog (Rboh). Surprisingly, *Arabidopsis* mutants lacking ROS production by Rboh (rbohD/F) have been shown to be less susceptible to cyst nematode attack. A comprehensive microscopic, biochemical and molecular analysis has demonstrated that Rboh-dependent ROS are not required for *Arabidopsis* root invasion by cyst nematodes; however, the absence of Rboh-mediated ROS impairs syncytial establishment and development. To understand the underlying mechanistic details of Rboh-mediated ROS in syncytium formation, we performed a genome-wide transcriptome analysis between Col-0 and rbohD/F upon nematode infection. Several genes involved in auxin transport, synthesis and/or homeostasis were down regulated in rbohD/F as compared to wild type. Notably, we identified an auxin transporter as one of the downstream targets of ROS. Hormone quantifications, metabolic profiling, genetic complementation and mutant analysis suggest that it regulates the pathways linking Rboh-mediated ROS to downstream responses. In summary, our work provides a first mechanistic understanding of the role of ROS in promoting infection of nematodes and other pathogens.

### Proteomic profiling of syncytia induced by *Heterodera schachtii* reveals new candidate effector proteins

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The plant-parasitic cyst nematode, *Heterodera schachtii*, infects several crop species, causing yield loss worldwide. *H. schachtii* juveniles induce the formation of a syncytial nurse cell systems in the host plants' roots, which serve as the sole source of nutrients for these nematodes. Here, we have performed proteomic profiling of syncytia induced by *H. schachtii* in *Arabidopsis thaliana* roots at 5 and 15 days post inoculation using a Quadrupole-Orbitrap mass spectrometer. The nematodes were carefully detached from syncytia and analyzed separately. We found that proteins related to metabolic processes and energy production were particularly enriched in syncytia as compared to control roots. Notably, we identified a repertoire of 100 proteins of nematode origin within the proteome of the syncytium. These represent strong candidate effector proteins, and included a few effectors previously shown to play a role in parasitism, thus validating this approach. We further shortlisted these candidate effector proteins based on the following criteria: i) presence of a signal peptide; ii) absence of a transmembrane domain; iii) function not known; and iv) homologs conserved in cyst nematodes but absent in all other organisms. In this way, we selected 8 candidate effectors, which we termed SPEs (Syncytial-isolated Putative nematode Effectors), for further analysis. Further characterisation of SPEs is underway and will provide new insights into the nematode parasitism process.