Involvement of pectate lyases in the formation of feeding structures induced by cyst and root-knot nematodes

Krzysztof Wieczorek, Abdelnaser Elashry, Michael Quentin, Florian M.W. Grundler, Bruno Favery, Georg J. Seifert, Holger Bohlmann

a field is found to be infested, management options are prescribed to suppress the nematode. Primary options are resistant cultivars. In Germany, these are registered by the Julius Kühn-Institut in collaboration with the Federal Plant Variety Office. Changes in the production chain need careful attention to manage the risk with these nematodes.

IMPROVED GROWTH OF *BETA VULGARIS* AFTER DIGESTATE FERTILIZATION IN *HETERODERA SCHACHTII*-INFESTED SOIL. **Westphal[1], A., M. Kücke[2] and H. Heuer[3].** [1]Institute for Plant Protection in Field Crops and Grassland, Julius Kühn-Institut, Messeweg 11/12, 38104 Braunschweig, Germany; [2]Institute for Crop and Soil Science, Julius Kühn-Institut, Bundesallee 50, 38116 Braunschweig, Germany; [3]Institute of Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut, Messeweg 11/12, 38104 Braunschweig, Germany.

Heterodera schachtii is one of the most important pests of sugar beet. It occurs in many sugar beet producing areas. Management of *H. schachtii* relies on crop rotation with sugar beet planted not more than every three years, and the planting of resistant cover crops.. Recent policy changes have resulted in increased amounts of organic waste materials from biogenic energy production. Digestate is one such product from bio-energy fermenters. The aim of this study was to determine if digestate improved growth of sugar beet (*Beta vulgaris saccharifera*) and mangold (*Beta vulgaris vulgaris*). In sugar beet production, *H. schachtii*-infested microplots were fertilized with equivalents of 60, 120, or 240 kg nitrogen (N)/ha contained in digestate or with calcium ammonium nitrate fertilizer. Phosphate (P) and potassium (K) were adjusted correspondingly with mineral fertilizers. In mangold, an additional treatment applied only 30 kg N/ha. Controls were unfertilized plots and those that received the 120 kg N/ha rate plus the nematicide Fosthiazate. In both crops, digestate amendments were followed by lower nematode penetration rates and improved early growth; yield data were less distinct between the two fertilizer forms but there were increased yields with increasing fertilizer rates. In sugar beet, increased digestate amounts resulted in reduced nematode penetration and improved early growth, but yields were highest in the nematicide-treated plots. In both crops, unfertilized plots had high penetration rates, low initial growth, and poor yields. This data indicate that fertilizing sugar beet and mangold with digestate was beneficial in nematode suppression and concomitant nutrient supply.

DISTRIBUTION AND GENETIC DIVERSITY OF *BAKERNEMA INAEQUALE* (CRICONEMATIDAE) IN NORTH AMERICA. **Whitlock[1], K.J., E.C. Bernard[1] and T.O. Powers[2].** [1]Entomology & Plant Pathology Department, The University of Tennessee, 2505 E. J. Chapman Drive, 370 Plant Biotechnology Building, Knoxville TN 37996-4560 USA; [2]Plant Pathology Department, The University of Nebraska, 406 Plant Science Hall, Lincoln, NE 68583-0722 USA.

Bakernema inaequale is a widespread criconematid nematode species endemic to eastern North America. Because this nematode is unique in appearance, easily recognizable under a stereo microscope, and known only from forest sites, it has potential as an indicator species for environmental health and climate change. Many criconematid species actually are species complexes; therefore, extensive sampling for *B. inaequale* was carried out and specimens were characterized morphologically and molecularly (COI). We hypothesized that northward migration of *B. inaequale* following the last ice age caused the population to split into western and eastern populations separated by the Appalachian Mountains. Soil samples were collected from diverse habitats of non-agricultural land from northern Pennsylvania to the Florida Panhandle and Alabama, and added to samples already obtained from more northern U.S. states. After extraction with a sugar flotation method, up to five live specimens from each sample were imaged and measured, then prepared for DNA amplification. Although much more sampling needs to be done, *B. inaequale* appears to be a distinct lineage with little haplotype diversity observed so far. This nematode radiated rapidly from a southern Pleistocene refugium of taiga and northern hardwoods without significant genetic drift or east-west speciation. *Bakernema inaequale* is amphimictic and spread of genes through the mountains via mating probably has maintained genetic stability within the species.

INVOLVEMENT OF PECTATE LYASES IN THE FORMATION OF FEEDING STRUCTURES INDUCED BY CYST AND ROOT-KNOT NEMATODES. **Wieczorek[1], K., A. Elashry[2], M. Quentin[3], F.M.W. Grundler[2], B. Favery[3], G.J. Seifert[4] and H. Bohlmann[1].** [1]Department of Crop Sciences, Division of Plant Protection, University of Natural Resources and Life Sciences, Konrad-Lorenz Straße 24, A-3430 Tulln, Austria; [2]Institute of Crop Science and Resource Conservation, Department Molecular Phytomedicine, University Bonn, Karlrobert-Kreiten-Str. 13, D-53115 Bonn, Germany; [3]Institut Sophia Agrobiotech UMR INRA 1355-CNRS 7254-Université de Nice Sophia Antipolis, F-06903, Sophia Antipolis, France; [4]Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Muthgasse 18, A-1190 Vienna, Austria.

Pectin in the primary plant cell wall is thought to be responsible for its porosity, charge density, and microfibril spacing and is the main component of the middle lamella. Plant-parasitic nematodes secrete a cocktail of cell wall degrading enzymes that macerate the plant tissue, facilitating the penetration and migration within the roots. In sedentary nematodes, these enzymes are released only during the migration of infective juveniles through the root. At later stages nematodes manipulate the expression of host plant genes including various cell wall enzymes in order to induce specific feeding sites. In this study we investigated pectin and pectate epitopes together with the expression of two *Arabidopsis thaliana* pectate lyase-like genes, *PLL18* (At3g27400) and *PLL19* (At4g24780), in both syncytia induced by the cyst nematode *Heterodera schachtii* and giant

cells induced by the root-knot nematode *Meloidogyne incognita*. We analyzed their expression in both types of feeding sites and confirmed their upregulation based on our previous GeneChip and microarray results by qRT-PCR and *in situ* RT-PCR. Furthermore, the functional analysis of mutants demonstrated the important role of both *PLL*s in the development and maintenance of syncytia but not giant cells. Our results show that both enzymes play distinct roles in different infected root tissues as well as during parasitism of different nematodes.

NEMATODE TOLERANCE ENHANCED USING SOIL AMENDMENTS ON TSG2-GUAVA TREES IN SOUTH AFRICA. **Willemse, A.S., M.S. Daneel, K. de Jager and W.P. Steyn.** Agricultural Research Council - Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, 1200, South Africa.

In the past the South African guava industry was mainly based on one commercial cultivar namely 'Fan Retief'. With the outbreak of Guava Wilt Disease which was first detected in the Mpumalanga Province in the early 1980s it became critically important for the guava industry to breed a cultivar with an adequate degree of tolerance against Guava Wilt Disease. In 1995, resistant cultivar, 'TS-G2' was released by the ARC-ITSC. While cultivar 'Fan Retief' seemed to be resistant to root-knot nematodes, nematodes were never perceived as a problem until 'TS-G2' was released, as this cultivar was highly susceptible to root- knot nematodes. Normal chemical nematode control practices do not seem to be sufficient and alternatives were investigated where enhanced root growth together with nematode control was evaluated. A greenhouse trial consisted of *Moringa* leaf extracts, Biostart, Gwamis, Nitrospray, mycorrhrizae, two nematicides Vydate and Nemacur and an untreated control. 'TS-G2' seedlings were inoculated with 9000 *Meloidogyne* spp. eggs collected from an infested guava orchard. Plant and root growth was observed with several of the treatments compared to the untreated control with Vydate, guano manure (Gwanis) and mycorrhizae showing the best growth enhancement, however nematode control was less obvious. It is therefore most likely that a combination of chemical nematicides and organic amendments might give sufficient nematode control over an extended period.

GENETIC MAPPING AND PHYSICAL LOCALIZATION OF VIRULENCE TRAITS IN *MELOIDOGYNE HAPLA*. **Williamson[1], V.M., V.P. Thomas[1], J. Jimeno[1], S. Fudali[1], D. Bird[2,3] and D. Nielsen[4].** [1]Department of Plant Pathology, University of California, Davis, CA 95616, USA; [2]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA; [3]Bioinformatics Research Center, North Carolina State University, Raleigh, NC 27695, USA; [4]Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695, USA.

The root-knot nematode *Meloidogyne hapla* can reproduce on a wide range of crop species, but there is variability in host range and virulence both within and between isolates. The genome sequence of several strains of this species has been obtained, identifying an abundance of scorable DNA polymorphisms. Due to the unusual reproductive mechanism, that is, facultative meiotic parthenogenesis, controlled crosses can be carried out to produce F2 lines resembling recombinant inbred lines. These lines can be maintained as cultures and scored for both pathogenicity phenotypes and molecular markers to generate integrated genetic linkage maps. Progeny lines from a cross of two *M. hapla* isolates that reproduce poorly on the wild potato *Solanum bulbocastanum* SB22 differ dramatically in ability to reproduce on this host with some lines able to reproduce to higher levels than either parental strain. Three quantitative trait loci contributing to reproductive ability of the nematode on SB22 were identified and positioned on linkage groups. Similarly, analysis of progeny from a cross between two *M. hapla* strains that differ in their ability to reproduce on the resistant common bean variety NemaSnap identified a single, major genetic locus that mediates variety-specific virulence. The availability of genome sequence scaffolds together with the integrated genetic map have allowed us to physically localize this virulence trait to a region of <100 kb in the *M. hapla* genome providing an inroad for its future identification.

ASSESSMENT OF SOILS FOR THE PRESENCE OF THE NEMATOPHAGOUS FUNGUS *DACTYLELLA OVIPARASITICA* BY A PCR-BASED STRATEGY. **Witte[1], H., J. Yang[2], J. Borneman[3], J. Smith Becker[1] and J.O. Becker[1].** [1]Departments of Nematology, [2]Botany and Plant Sciences, [3]Plant Pathology and Microbiology, University of California, Riverside, California 92521, USA.

The nematophagous fungus *Dactylella oviparasitica* (syn. *Brachyphoris oviparasitica*) is considered the primary causal agent of a long-term *Heterodera schachtii* population suppression in a field of the Agricultural Operations, University of California-Riverside. This strain has been shown to successfully suppress sugarbeet cyst nematode populations after its introduction into various field soils. We hypothesize that parasitism of *Heterodera schachtii* by *D. oviparasitica* might be a major factor in regulating cyst nematode populations in the Imperial Valley, California's main sugarbeet production area. As little is known about the geographical distribution of this fungus, we developed an assay for the detection of *D. oviparasitica* and closely related organisms in soils. The fungus was baited by planting a soil sample with *H. schachtii*-infested Swiss chard. White *H. schachtii* females were picked from the roots and subjected to DNA extraction. A qPCR assay utilizing sequence-selective primers targeting the ITS region of the rRNA gene sequence of *D. oviparasitica* was used to amplify and detect the desired sequence. This procedure was used to screen soil samples from 77 different sugar beet fields in the Imperial Valley. Preliminary results indicate the presence of *D. oviparasitica* or closely related fungi in 15 samples. The obtained