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Nematodes



5ICN

Down Under

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SESSION EIGHT – RESISTANCE BREEDING AGAINST NON-SEDENTARY NEMATODES

CONVENORS: DANNY COYNE & JOHN THOMPSON

Evaluating Wheat for Tolerance and Resistance to Root-lesion Nematodes

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Root-lesion nematodes are estimated to cost the Australian wheat industry AUD\$260 million/year. *Pratylenchus thornei* is the dominant species in the north and *P. neglectus* in the south and west. We have tested wheat lines for tolerance to *P. thornei* on a dedicated 10-ha site of vertisolic soil near Jondaryan 170 km west of Brisbane. The site has been managed in a 4-year rotation of fallow-sorghum-wheat-wheat test plots, such that 2.5 ha are available each year with high *P. thornei* population and negligible other soil-borne wheat pathogens. About 2000 early-generation wheat lines are sown in unreplicated plots of 3 rows each 5 m long which are rated twice during the growing season for symptoms of nematode damage. Pre-release lines and varieties for the northern region are sown in plots of 7 rows by 8 m long in replicated experiments on two sowing dates. Yield of the lines/varieties determined from machine harvest is expressed as percentage of site mean yield (SMY) and averaged across trials and years. Tolerance indices derived from SMY have proved very predictive of varietal yield at independent sites infested with *P. thornei*, and are published annually in extension brochures. Resistance of wheat lines against *P. thornei* and *P. neglectus* has been assessed in replicated glasshouse experiments. The wheat lines are tested in pots of vertisolic soil inoculated with cultured nematodes and grown with optimum nutrition, water supply and temperature for nematode multiplication. After 16 weeks, nematodes are extracted from roots and soil from the bottom half of the pot and enumerated under a compound microscope. These methods have been used successfully to: (1) identify sources of resistance in wild relatives, landrace and synthetic hexaploid wheats, (2) characterise breeders' lines and varieties for resistance, (3) screen segregating progeny from backcross and topcross programs, and (4) characterise mapping populations for development of molecular markers to resistance genes.

Distribution, Virulence and Genetic Management of Root Lesion Nematodes in the Pacific Northwest USA

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Root lesion nematodes (RLNs) have been identified in more than 90% of dryland cropping fields in the Pacific Northwest (PNW; Oregon, Washington, Idaho) USA. *Pratylenchus neglectus* dominates but *P. thornei* and mixtures of both species occur commonly. Until recently, RLNs were only considered to cause significant yield loss in annual spring crops with traditional winter wheat-summer fallow rotations considered unsuitable for damaging RLN populations to develop. Recent research has shown that spring wheat and barley yields increased up to 98% and 15% respectively in response to aldicarb (4.2 kg a.i./ha) applied at planting in *P. neglectus* infested soil and 49% and 14% respectively in *P. thornei* infested soil. Yields of winter wheat and barley cultivars grown at the same locations also responded up to 21% and 27% respectively in *P. neglectus* infested soil and 15% and 20% respectively in *P. thornei* infested soil. Glasshouse resistance screening showed that all tested PNW wheat cultivars were susceptible to both RLNs. Barley cultivars were generally more resistant than wheat cultivars but still moderately susceptible to *P. neglectus* and moderately resistant to moderately susceptible to *P. thornei*. Crop species identified as resistant to *P. neglectus* and/or *P. thornei* and suitable for crop rotation are either not well adapted to PNW cropping systems or have marginal economic viability. Breeding cereal cultivars with tolerance and resistance to RLNs has proven an effective management strategy in similar international situations and has been undertaken collaboratively in the PNW. Characterisation of commercial cultivars and advanced breeding lines for tolerance and resistance has begun as has the introduction of elite sources of tolerance and resistance genes through a targeted crossing program. Molecular techniques are also being developed to identify RLNs to species from soil samples and resistance gene markers to assist breeding selection.

Community Structure of Plant-Parasitic Nematodes under Different Soil Type and Latitude in Soybean Fields

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Plant-parasitic nematodes are important as a potential yield limiting factor in agriculture production, they cause estimated annual crop losses \$78 billion worldwide. Soybean is major economic crop in China, there are many genera of plant-parasitic nematodes in soybean rhizosphere. The test was conducted in major soybean production area of China in 2005 to confirm community structure, occurrence frequency and population density of plant-parasitic nematodes in soybean rhizosphere, which will offer data for assessment on agriculture soil health, control of soybean root diseases and study on soybean rhizosphere microbial ecosystems in the future. In total 86 soil samples were collected from soybean fields of 9 provinces (HeiLongjiang, Neimenggu, Jilin, Liaoning, Shandong, Shanxi, Hebei, Henan, Jiangsu), soil samples include 8 soil types (black soil, meadow soil, aquic brown soil, alkaline saline soil, brown soil, white puddled soil, sandy soil, aquic soil) from 32°05'N to 52°50'N. Baermann funnel technique was used to extract nematodes from soil samples.

In total 13 genera and 6 species of plant-parasitic nematodes were identified, 8 genera and 4 species were first reported in soybean fields of HeiLongjiang Province. *Aphelenchus*, *Heterodera* and *Helicotylenchus* survive widely in soybean fields, the occurrence frequencies of them were 75%, 69.1% and 63.1%, respectively. There were 12 genera in black soil, 9 genera in meadow soil and 6 genera in brown soil. The result indicated that community structure of plant-parasitic nematodes were influenced by soil type in soybean field. Soil with high organic matter had better community structure and lower population density of plant-parasitic nematodes. Latitude had little effect on distribution of plant-parasitic nematodes range from 32°05'N to 52°50'N.

Crop Rotation to Replace Nematicide Treatments for Assessing Chickpea Varietal Tolerance to *Pratylenchus thornei*

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Field trials were conducted to determine if crop rotation would suppress soil populations of *Pratylenchus thornei* and eliminate the use of nematicide plots when assessing chickpea cultivars for tolerance. Assessing yield response to *P. thornei* is difficult due to problems associated with nematicide penetration in the sub-soil. In year one, plot treatments consisted of canola, linseed, canaryseed, wheat and bare fallow, applied with or without aldicarb at 10kg/hectare in a three randomized block design. The following year the same plots were re-cropped with four chickpea cultivars and one intolerant wheat cultivar and aldicarb re-applied to the nematicide plots. Results from the pre-crops revealed wheat produced significantly higher nematode numbers throughout the soil profile than the other treatments. *P. thornei* populations peaked at depths of 45-60cm but nematicide was only effective down to depths of 30cm. Canola and fallow resulted in significantly lower populations of mycorrhizal spores

than wheat. Canaryseed resulted in low nematode numbers in contrast to wheat and high mycorrhizal spores comparable to wheat so it was selected as the best control treatment for future use. All pre-crop treatments had similar moisture down to depths of 60cm. Chickpea yields on pre-cropped canary plots increased by 10-30% compared to yields on pre-cropped wheat plots. Nematicide on canary plots gave no additional yield of the following chickpea. Results demonstrated that using resistant canary and susceptible wheat plots is an effective alternative to plus nematicide and minus nematicide treatments when assessing tolerance in chickpea cultivars. This methodology has been successfully implemented in subsequent trials.

Molecular Markers for Resistance to Root-Knot Nematode on Egyptian Cotton

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Survey of nematodes in cotton fields at Fayoum Governorate indicated nine genera of plant-parasitic nematode associated with cotton root *Helicotylenchus*, *Hemicycliophora*, *Hoplolaimus*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus*, *Rotylenchulus*, *Tylenchorhynchus* and *Xiphinema*. The root-knot nematode (RKN) (*Meloidogyne* spp.) was widely distributed in the surveyed fields. Identification of RKN, *M. incognita* isolates revealed one race. Race 3. The rates of nematode build-up gradually increased with increased inoculation level up to 2000 juveniles / plant but later a gradual drop in numbers occurred. Relative susceptibility of cotton cultivars subjected to infection by, *M. incognita* race 3, revealed that 2 were rated as highly susceptible, Giza 88 and Menoufi, 5 were rated as susceptible, Giza 70, Giza 83, Giza 85, Giza 89 and Giza 90 and 4 were rated as resistant, Giza45, Giza 80, Giza 86 and Giza 91. Plant growth in susceptible cultivars was significantly affected by nematode infection, whereas plants of resistant cotton cultivars were not affected. In the susceptible cotton Giza 83, the life-cycle of *M. incognita* race 3 durated about 25 days. In the resistant cotton Giza 86, it lasted about 45 days. Plant cortical layer, stellar regions and pericycle tissues changed by RKN infection. Also, similarity matrix based on RAPD-PCR analysis was 67.6%, between the two resistant cotton cultivars and was 60.6%, between the two highly susceptible and susceptible cotton cultivars. RAPD-PCR showed some molecular markers related to RKN resistant and susceptible plants. For instance, primer OP-A16 gave specific markers with MW of 260 bp for (Giza70), 950bp; 700bp and 300bp for (Giza 90) and 250bp for (Giza 80).

Distribution of Known and New Migratory Endoparasitic Nematodes on Wheat Production in the Isparta Province of Turkey

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Migratory endoparasitic nematodes, especially *Pratylenchus* species are considered economically important nematodes on cereals. A survey was conducted from 8 locations of Isparta taking 63 random soil samples in wheat fields around post flowering between May and June of 2007. Nematodes were extracted soil by using modified Baermann Funnel

technique and samples examined under dissecting microscope and species identified using morphological characters. *Pratylenchus thornei*, *P. neglectus*, *P. scribneri* and *Pratylenchoides alkani* were identified by using morphological characters, and often these were found in mixed populations, and a single species were distributed in some locations. Of interesting not the species *Pratylenchoides alkani* was found in high populations in many samples and always in combination with *Pratylenchus*. *Pratylenchoides alkani* has not been reported on wheat, and this study would suggest it could be economically important.

The population dynamics of root lesion nematode were followed during one field season at three weekly intervals by taking from soil and root samples in different wheat cultivars (Ikizce, Kızıltan, Gün 91, Mirzabey). Root lesion nematodes were well developed in all wheat cultivars, and all cultivars were considered susceptible, with possibly two generations over one season. The number of lesion nematode in the root over the season increased whilst soil number declined. This trend was reversed at the end of the season, presumably due to exiting of nematodes from roots into the soil. More work is being conducted to determine the economic importance of both lesion and *Pratylenchoides* on wheat.

Survey for Root-lesion and Stunt Nematodes in the Northern Australian Grain Region

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The presence of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and stunt nematode (*Merlinius brevidens*) in some parts of the northern grain region has been known since the 1960's and yield loss in wheat caused by *P. thornei* has been demonstrated since the late 1970's. However, the distribution of *P. thornei* was considered restricted to the Darling Downs in Queensland and to a few localities in northern NSW. Following the diagnosis of *P. thornei* in a wheat crop in a newer cropping area around Goondiwindi in 1996 a more extensive survey of wheat fields was conducted. Soil samples (0-30cm) collected mainly from under wheat crops were processed manually and nematodes extracted by the Whitehead tray method and enumerated under a compound microscope. Out of 795 fields tested from 1996 to 2002, *Pratylenchus thornei* occurred in 67%, *P. neglectus* in 32% (both species occurred together in 26%) and no *Pratylenchus* spp. were detected in 27%. *Merlinius brevidens* occurred in 73% of fields. Edaphic factors controlling the incidence of these nematodes were tested on 833 samples collected in 1996-7. All three nematode species had a broad pH range, namely from 6.5 to 9.5 for both *Pratylenchus* spp. and from 6.0 to 9.5 for *Merlinius brevidens*. All species were detected in soil samples ranging from <20 to 80% clay and from <20 to >80% sand. However, within this range maximum incidence of *P. thornei* was in finer textured soils than for *P. neglectus*. Soil organic carbon had no clearcut effect on the incidence of the species. The incidence of *P. neglectus* appeared to increase with increasing concentration of DTPA-extractable zinc and bicarbonate-extractable phosphorus in the soil. Observations indicate that *P. thornei* was spreading in the region in run-off water and in soil on farm machinery and increasing under intense cropping to wheat.

showed a tendency of longer nematicidal activity in the soil than that of MCW-2. In microplot experiments, soil drench with an EC formulation at 2.0 kg a.i./ha had the same control level of fenamiphos at 4.0 kg/ha or cadusafos at 3.0 kg/ha, based on galling index of tomato roots caused by *M. javanica*. No phytotoxic symptoms were observed on tomato plants at the concentration. The compound has a far lower toxicity to rats (acute oral LD₅₀: >500 mg/kg) and non-target organisms (non-toxic to bees and earthworms) comparing with organophosphate or carbamate nematicides. MCW-2 has also a low leaching potential in the soil. The results indicate that MCW-2 has a great potential as a nematicide, which belongs to a new chemical group, and probably has a novel mode of action. Results from field experiments will be also presented.

Crop Rotation for the Management of Root-lesion Nematodes in the Northern Grain Region of Australia

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Root-lesion nematodes, *Pratylenchus thornei* and *P. neglectus* are widely distributed in the northern grain region of Australia which encompasses inland northern NSW and south-east Queensland. This sub-tropical region has a summer dominant rainfall and fertile clay soils. A broad range of both summer and winter crop species are grown and it is notable for its production of Australian Prime Hard wheat (high protein milling wheat) and grain sorghum for stock feed.

P. thornei is the most commonly found root-lesion nematode in this region and poses a significant economic threat to susceptible crops, such as wheat and chickpea. For example, intolerant wheat varieties can suffer yield losses as high as 70%. Marked differences are observed in the virulence of *P. thornei* and *P. neglectus* not only for crop species but also between crop varieties. For example, wheat cv. EGA Burke has a moderate level of resistance to *P. thornei* but is susceptible to *P. neglectus*; grain sorghum is susceptible to *P. neglectus* but resistant to *P. thornei*; mungbeans are susceptible to *P. thornei*, but resistant to *P. neglectus*. We have identified the resistance/ susceptibility of common crops grown in this region to *P. thornei* and *P. neglectus* in both glasshouse and field experiments. This information is complemented by work on tolerance/ intolerance of susceptible crop species of wheat and chickpea.

So, the management of root-lesion nematodes depends firstly on correct identification to species level, then tailoring crop rotation plans so that resistant crops and tolerant cultivars are dominant in the farming system. When this is combined with farm hygiene to limit the spread of nematodes between paddocks and on farm machinery, the production of valuable, but susceptible crops, such as wheat, can be optimised and root-lesion nematode populations will remain at low, manageable levels.